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PI3K pathway alterations in cancer: variations on a theme

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Abstract

The high frequency of phosphoinositide 3-kinase (PI3K) pathway alterations in cancer has led to a surge in the development of PI3K inhibitors. Many of these targeted therapies are currently in clinical trials and show great promise for the treatment of PI3K-addicted tumors. These recent developments call for a re-evaluation of the oncogenic mechanisms behind PI3K pathway alterations. This pathway is unique in that every major node is frequently mutated or amplified in a wide variety of solid tumors. Receptor tyrosine kinases upstream of PI3K, the p110α catalytic subunit of PI3K, the downstream kinase, AKT, and the negative regulator, PTEN, are all frequently altered in cancer. In this review, we will examine the oncogenic properties of these genetic alterations to understand whether they are redundant or distinct and propose treatment strategies tailored for these genetic lesions.

Keywords

PI3K; PTEN; HER2; tumorigenesis

Introduction

Phosphoinositide 3-kinases (PI3Ks) belong to a conserved family of lipid kinases that phosphorylate the 3′-hydroxyl group of phosphoinositides. The most well-characterized product of this reaction is phosphatidylinositol-3,4,5-trisphosphate or PIP_3, a critical second messenger that recruits AKT for activation of growth, proliferation and survival signaling (Cantley, 2002). PIP_3 is negatively regulated by dephosphorylation by the tumor suppressor, PTEN. The production of PIP_3 is a unique feature of the class I subclass of PI3Ks, which can be further divided into class IA and class IB. Thus far, only the class IA PI3Ks have been implicated in human cancer, but frequent genetic alterations in these enzymes and their pathway effectors have made the PI3K pathway one of the most frequently dysregulated pathways in cancer. Currently, a significant effort is being made to develop pan-specific and isoform-specific PI3K inhibitors for the treatment of cancer.

Class IA PI3Ks are heterodimers comprised of a regulatory subunit (p85α, p55α, p50α, p85β, p55γ) and a catalytic subunit (p110α, p110β, p110δ) and are activated downstream of receptor tyrosine kinases (RTKs) (Engelman *et al.*, 2006). Activating mutations in *PIK3CA*, the gene encoding the p110α catalytic subunit of PI3K, were recently identified as novel mechanisms of inducing oncogenic PI3K signaling (Samuels *et al.*, 2004). These mutations join *PIK3CA* amplification, PTEN loss, AKT mutations and RTK amplification in a class of

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frequent genomic aberrations that promotes tumorigenesis through upregulation of the PI3K/AKT signaling axis.

It is perplexing that every member of this signaling axis is frequently altered in cancer, when ostensibly they serve the same purpose. Tumors can be considered Darwinian microcosms in which cancer cells are constantly under selection for growth or proliferative advantages. In this way, there should be no selection for absolute redundancy, which would impede the momentum inherent in evolution. In this review, we explore the oncogenic mechanisms of these various PI3K pathway alterations to elucidate the unique ways in which they confer oncogenicity. We will also speculate on ways to use novel PI3K inhibitors as single agents and in combination with other targeted therapies to attack cancer cells and the tumor microenvironment.

PI3K inhibitors

Like the majority of protein kinase inhibitors, all existing PI3K inhibitors bind competitively in the ATP-binding pocket of the catalytic domain. This strategy has enabled the development of both pan-PI3K- and isoform-specific inhibitors. Loosely discriminate inhibitors that target multiple PI3K isoforms may more thoroughly shut down PI3K signaling for the treatment of acute life-threatening diseases (Crabbe *et al.*, 2007). The potential toxic side effects on glucose metabolism and the immune response may be tolerated with short-term use of pan-specific inhibitors. Isoform-specific inhibitors, which have been more difficult to develop because of the highly conserved nature of the ATP-binding pocket (Walker *et al.*, 1999), are promising alternatives for treatment of cancers with known mutations or chronic diseases. In addition, these selective inhibitors may eschew off-target effects on the related PIKKs, mTOR, DNA-PK, ATM, ATR, SMG-1 and the class III PI4Ks.

The discovery of wortmannin and LY294002 as competitive ATP binders revealed what we now know to be the vast potential of targeting the ATP-binding site of p110. Wortmannin is a potent pan-specific inhibitor that occupies the ATP-binding site of p110 by forming a covalent bond between C20 of the wortmannin furan ring and K802 of p110α (Wymann *et al.*, 1996). However, wortmannin has a half-life of only a few minutes in serum due to the highly reactive C20 position. Wortmannin derivatives such as PX-866 (Oncothyreon, Bellevue, WA, USA) have been shown to be significantly more stable *in vivo*, with cytostatic effects when used as monotherapy (Howes *et al.*, 2007). A stabilized wortmannin prodrug also shows promise for treating cancers (Yuan *et al.*, 2007; Barnes *et al.*, 2008).

LY294002 (Lilly, Indianapolis, IN, USA), a reversible synthetic compound, makes a key hydrogen bond between the morpholino oxygen in the compound and the backbone amide of V882 of p110γ, mimicking the interaction made by the adenine of ATP (Walker *et al.*, 2000). Variations in this key interaction are conserved in all existing PI3K inhibitors. SF1126 (Semafore, Indianapolis, IN, USA) is a LY294002 prodrug that utilizes an RGDS peptide to increase plasma half-life and target drug delivery to the tumor vasculature (Garlich *et al.*, 2008). PI-103 (Piramed, Slough, USA) and ZSTK474 (Zenyaku Kogyo, Tokyo, Japan) share LY294002's arylmorpholine structure and are potent pan-PI3K inhibitors. ZSTK474 inhibits all class I PI3Ks (IC_{50} = 16–49 nM) and has antitumorigenic effects on a wide variety of xenografts (Yaguchi *et al.*, 2006; Kong and Yamori, 2007). PI-103 preferentially inhibits p110α (IC_{50} = 11 nm) as well as mTORC1/2 (IC_{50} = 2/83 nM, respectively), and in combination with erlotinib or radiation therapy has been effective in the treatment of xenografts of glioblastoma cell lines (Fan *et al.*, 2006, 2007; Chen *et al.*, 2008).

Other PI3K inhibitors occupy the ATP-binding site and extend into an affinity pocket where it makes hydrophobic interactions that increase the affinity of the compound for the enzyme

(Knight *et al.*, 2006). These compounds are pan-specific, yet extremely potent. PIK-90 (Bayer, Leverkusen, Germany), PIK-93 (Novartis, Basel, Switzerland) and the aforementioned PI-103 are all members of this class of compounds. Interestingly, PIK-90 inhibits p110α (IC_{50} = 8.2 nM) and to a limited extent, mTOR (Fan *et al.*, 2006). Only in combination with rapamycin does PIK-90 mimic the potent proliferation block induced by PI-103 (Fan *et al.*, 2006).

Several PI3K inhibitors are now progressing from the preclinical phase to phase I clinical trials in patients. Exelixis compounds, XL147 and XL765, are currently in phase I trials for the treatment of solid tumors. Both compounds inhibit multiple PI3K isoforms with preference for p110α, -δ and -γ (see www.exelixis.com). Two Novartis compounds, BGT226 and the pan-PI3K/mTOR inhibitor, NVP-BEZ235, are in ongoing trials as monotherapy for breast and other solid tumors and are producing promising results (reviewed by Sellers and Garcia-Echeverria, in this issue).

Known mutations in the pathway

The PI3K pathway is unique, in that all of the major elements of this pathway have been found mutated or amplified in a broad range of cancers. Numerous tumor types are thus likely to benefit from the development of PI3K inhibitors. The axis of PI3K signaling in cancer begins with engagement of growth factors by RTKs (Figure 1). PI3K is then recruited to plasma membrane-anchored receptors and is activated and phosphorylates PIP_2 to generate PIP_3. Through its pleckstrin homology (PH) domain, the nodal kinase AKT (also known as PKB) binds to PIP_3, where it is activated by two phosphorylation events, and triggers a complex cascade of signals that regulate growth, proliferation, survival and motility. The lipid phosphatase, PTEN, antagonizes this process by dephosphorylating PIP_3 to inhibit activation of AKT. Unlike any other major signaling pathway in the cell, every member of this signaling axis is frequently altered in cancer.

Receptor tyrosine kinases

PI3K is activated downstream of numerous RTKs that directly or through adaptor proteins bind and activate PI3K. PI3K activity is thus carefully regulated by growth factor–receptor interactions. In fact, the vast majority of PI3K remains inactive in the cytoplasm, far from its plasma membrane-associated substrates, and only a small percentage of PI3K becomes activated upon growth factor stimulation. Therefore, even slight modulations in receptor activity can lead to many-fold increases in PI3K activity.

EGFR

Epidermal growth factor receptor (EGFR, ERBB1) is an upstream activator of PI3K that is frequently altered in cancer. Malignant gliomas often exhibit EGFR amplifications with copy numbers increased by as much as 20-fold (Sauter *et al.*, 1996). Mutations that confer constitutive kinase activity include the tissue nonspecific EGFRvIII deletion of exons 2–7 (Narita *et al.*, 2002) and non-small cell lung cancer (NSCLC)-specific deletions in exon 19 or L858R substitutions in exon 21 (Arteaga, 2006). In addition, exon 20 insertions and T790M mutations have been identified in NSCLC, which confer resistance to the EGFR inhibitors gefitnib and erlotinib (Arteaga, 2006).

HER2

HER2 (EGFR-2, ERBB2) is another member of the EGFR family and possesses the strongest catalytic kinase activity of all other family members. HER2 is overexpressed through gene amplification or transcriptional deregulation in 25–30% of invasive breast and ovarian cancers and is associated with poor prognosis (Moasser, 2007). Rare (4% of NSCLC

and 10% of lung adenocarcinomas) somatic mutations have been found in the kinase domain of HER2 and occur predominantly in people of Asian descent (Stephens *et al.*, 2004; Moasser, 2007).

KIT, PDGFRα and MET

Activating mutations in KIT and PDGFRα can result in the formation of gastrointestinal stromal tumors. Mutations in KIT occur most frequently in exons 9 and 11 and confer constitutive kinase activation through induction of dimerization, whereas mutations in PDGFRα occur most frequently in exon 18, encoding the activation loop (Tornillo and Terracciano, 2006). The PI3K, mitogen-activated protein kinase (MAPK) and STAT pathways are activated downstream of each of these RTKs; however, only inhibition of PI3K results in robust growth arrest and induction of apoptosis in imatinib-resistant tumors (Heinrich *et al.*, 2003; Bauer *et al.*, 2007). Finally, focal MET amplifications have been found in 22% of acquired gefitnib-resistant lung cancers and have been shown to restore AKT activity via MET-dependent phosphorylation of ERBB3 and consequent activation of PI3K (Engelman *et al.*, 2007).

p110α

PIK3CA, the gene encoding the p110α catalytic subunit of PI3K, was found to be frequently mutated in breast (27%), endometrial (23%), colorectal (14%), urinary tract (17%) and ovarian (8%) cancers (http://www.sanger.ac.uk/genetics/CGP/cosmic). These mutations cluster in two conserved regions of the gene, which encode the kinase and helical domains of the protein. These ‘hot spot’ mutations, H1047R, E545K and E542K, are non-synonymous missense mutations that confer constitutive kinase activity (Samuels *et al.*, 2004, 2005). The PIK3CA gene is also amplified at high frequencies in head and neck, squamous cell lung carcinoma, gastric and cervical cancers (Engelman *et al.*, 2006).

PTEN

The PTEN tumor suppressor is mutated or lost in both heritable and spontaneous cancers. Germline mutations in PTEN cause autosomal dominant hamartoma tumor syndromes, whereas sporadic missense mutations occur frequently in central nervous system (20%), endometrial (39%), colorectal (9%), skin (17%), prostate (14%) and breast (6%) cancers (http://www.sanger.ac.uk/genetics/CGP/cosmic). Monoallelic loss of PTEN contributes to tumor growth in the context of other somatic mutations, and PTEN protein levels correlate with disease severity, suggesting that PTEN is functionally haploinsufficient (Salmena *et al.*, 2008).

AKT

Amplifications in multiple AKT isoforms have been reported in pancreatic, ovarian, and head and neck cancers (Engelman *et al.*, 2006). Recently, a somatic missense mutation in the PH domain of AKT1 (E17K) was identified in breast, colorectal and ovarian cancers (Carpten *et al.*, 2007). The PH domain mutation results in constitutive association with the plasma membrane and prolonged AKT activation that is sufficient to transform cells in culture (Carpten *et al.*, 2007).

Same or different?

The critical question is whether alterations in ERBB1/2, p110α, PTEN and AKT confer oncogenicity in redundant ways. Ostensibly, if this were a linear pathway, these genetic alterations should behave identically by hyperactivating the PI3K pathway, suggesting that first-line therapy for this ‘hyper-PI3K’ class of tumors should be inhibitors of downstream targets such as PI3K or AKT. This would also imply that none of the mutations confer a

significant selective advantage over another. In this review, we will argue that this scenario is unlikely. Rather, growth and survival signaling involves complex networks with redundancies, additive and synergistic effects. Modulation of the various nodes in the PI3K network may affect non-linear pathways including negative feedback loops, non-overlapping pathways and autocrine loops. We will attempt to more closely understand the oncogenic mechanisms of PI3K pathway alterations and will propose strategies to treat PI3K-driven tumors with targeted therapy.

Mutual exclusivity and coexisting mutations

One clue to resolving the possibility of absolute redundancy in PI3K pathway mutations is the presence or absence of mutual exclusivity. Coexistence of two or more PI3K pathway mutations in a single tumor would suggest differences in oncogenic mechanisms, given that there would be no selective advantage for cells bearing redundant mutations. Table 1 illustrates how frequently mutations arise singly and in combination in the breast, colon and endometrium, three of the most targeted tissues for PI3K pathway mutations. Compiled from studies of tumors analysed for PIK3CA, PTEN, RAS and HER2 status via DNA sequencing or immunohistochemistry, these data will help to elucidate mechanisms of oncogenicity within specific tissue types.

To make assumptions from these comparative studies, it is important to note that, as expected, mutual exclusivity exists between PIK3CA double hotspot mutations as well as between PIK3CA mutations and PIK3CA amplifications. Hollestelle *et al.* (2007) show that 2 (5%) of 40 breast cancer cell lines are doubly mutant in PIK3CA; however, in both cases only one of the two mutations was a hotspot mutation (P539R/H1047R; E545K/K567R). Similarly, Saal *et al.* (2005) identify 2 (0.7%) double mutant tumors (H1047R/E418K; T1025S/E545K) out of 292 primary breast tumors and in both cases, a hotspot mutation was combined with an infrequent mutation. In these few cases, it is possible that the less oncogenic mutation arose early and that the tumor acquired the more potent hotspot mutation later. In addition, Ollikainen *et al.* (2007) show that in 160 colon and endometrial cancers, PIK3CA mutations and amplifications arise at a frequency of 9.4 and 10.4%, respectively, but never concomitantly within the same tumor. However, it is possible that if amplification of the gene occurred first, one could miss a mutation in one of the amplified alleles.

Interestingly, Zhao and Vogt (2008) artificially engineered double hotspot mutations into chicken embryo fibroblasts and show that double helical domain mutations on the same allele (E545K/E542K) confer a small additive effect as measured by phospho-AKT levels and foci formation, whereas helical domain–kinase domain double mutations on the same allele (E545K/H1047R) confer a robust synergistic effect. This suggests that helical and kinase domain mutations may have different mechanisms of inducing constitutive PI3K activity. The relatively low frequency of multiple PIK3CA mutations observed in real tumors further suggests that only one mutation is sufficient for tumorigenesis and that double mutations may indeed have negative effects. In fact, the identification of PI3K as an oncogene was hindered by early experiments showing that overexpression of PIK3CA in human or mouse fibroblasts resulted in suppression of growth. This likely reflects a senescence phenotype that prevents double hotspot mutations from occurring in human tumors.

PTEN loss and PIK3CA mutations

Overall, PIK3CA mutations and PTEN loss coexist in breast, endometrial and colon cancers. In the breast, the observed frequency of tumors with coexisting PTEN loss and PIK3CA mutations is 8.7% (Table 1 and references therein). If these two alterations occurred

randomly and were independently selected for (coexistent), then the expected frequency of coexistence should be the product of the frequency of PTEN loss and the frequency of PIK3CA mutation. In the breast, the expected frequency of coexistence is 8.9%, which is in agreement with the observed data, and strongly argues for coexistence. Similarly, in endometrial cancer, the observed frequency (15%) equals the expected frequency (15%), and in the colon, the observed frequency (5.6%) is within a reasonable margin of error from the expected frequency (7.0%) (Table 1 and references therein). It should be noted that whereas these numbers represent a compilation of the data in the literature, individual studies do not all come to the same conclusion. This discrepancy may be due to differences in how PTEN status was determined. In Table 1, $PTEN^{-}$ represents abrogation of PTEN through deletions and mutations found by sequencing and decreased expression as shown through immunohistochemistry. It is possible that mutated PTEN functions differently or to varying degrees compared to loss of PTEN protein. In addition, Table 1 includes both tumor cell lines and primary tumors, which may have varying degrees of stromal cell contamination.

Nevertheless, when taken together, the data from Table 1 indicate that concomitant PIK3CA and PTEN alterations indeed occur, suggesting that the two genetic aberrations are not completely redundant. Although both activate the PI3K pathway, tumors bearing both alterations must have an additional selective advantage. One possibility is that PTEN and/or p110α are involved in negative feedback loops that regulate pathway activity. Alterations at these nodes may circumvent negative feedback, leading to an additive effect on PI3K pathway activation.

RAS mutations and PI3K alterations

RAS and PIK3CA mutations are mutually exclusive in endometrial cancers but coexistent in colorectal cancers. The number of breast carcinomas analysed in the literature is too low to make a definitive conclusion, though one study demonstrates a trend toward mutual exclusivity (Table 1) (Hollestelle *et al.*, 2007). In the endometrium, 232 primary endometrial tumors were analysed, and the observed frequency of concomitant RAS and PIK3CA mutations was 0.4%—significantly lower than the expected frequency of 2.5% (Table 1 and references therein). This suggests that the two mutations were not randomly and independently selected for and argues for mutual exclusivity.

In this scenario, it is possible that an early RAS mutation committed or addicted the cell to a network independent of the PI3K network, as appears to be the case for most K-RAS mutant pancreatic and lung cancers. This would render a secondary PIK3CA mutation ineffectual and potentially disadvantageous if overactivation of mitogenic signaling pathways leads to oncogene-induced senescence (Sarkisian *et al.*, 2007). In addition, RAS-driven tumors appear to be highly dependent on PI3K signaling during tumor initiation and less so during tumor maintenance (unpublished results from this laboratory). During tumor initiation, PI3K signaling could be activated by autocrine factors or chemokines secreted by the tumor stroma. During tumor maintenance, reliance on PI3K signaling decreases and accumulation of PIK3CA mutations would be rare.

Unlike endometrial cancers, the observed frequency of RAS and PIK3CA mutations in the colon is 7.3%, which is slightly higher than the expected frequency of 5.4% (Table 1 and references therein). This suggests that constitutively active RAS and PIK3CA may function synergistically in the colorectal epithelium to confer an important selective advantage. It is possible that PIK3CA mutations arise first in these tumors and that RAS facilitates activation of the mutant PIK3CA (see below). This mechanism for maintaining activation of mutant PI3K may be more critical in intestinal epithelial tissues than in breast or endometrium. Previous studies have indicated that the ability of PIK3CA to bind to

activated RAS is critical for initiation of lung tumors by mutant K-RAS in a mouse model (Ramjaun and Downward, 2007).

The differences we see between tissue types with regard to mutations in PIK3CA and PTEN may reflect the possibility that in certain tissues PTEN loss alone or PIK3CA mutations alone are insufficient to enhance cell growth or survival. This could be due to higher expression of redundant negative regulators of this pathway such as the lipid phosphatases, SHIP and INPP4B, or due to the fact that mutations in this pathway without prior loss of p53 result in senescence (Chen *et al.*, 2005).

PI3K and HER2

In the breast, HER2 is amplified in 30% of tumors (Downward, 2003) and appears to coexist with both PIK3CA mutations and PTEN loss (Table 1). In fact, the coexistence of PTEN loss and HER2 amplification was critical to understanding trastuzumab resistance in HER2-positive breast cancers. Three studies showed that loss of PTEN expression occurred at high frequencies in HER2-overexpressing breast tumors (11/55 (21.7%), 17/47 (36.2%) and 8/17 (47.1%)) and led to resistance due to incomplete shut off of PI3K signaling (Nagata *et al.*, 2004; Fujita *et al.*, 2006; Berns *et al.*, 2007). This suggests that PTEN loss and HER2 overexpression have redundant abilities to activate PI3K, but in order for the double mutation to confer a selective advantage, HER2 overexpression may also circumvent negative feedback at the level of receptor recycling. In addition, other mitogenic pathways downstream of HER2 may be active and advantageous for tumor growth. Finally, HER2 overexpression may also provide PIK3CA mutants and $PTEN^{-/-}$ cells with sufficient growth factor stimulation to achieve the high levels of PIP_3 needed for AKT activation and transformation.

Possible models of oncogenicity

This analysis of coexistence and mutual exclusivity of PI3K alterations gets us closer to answering the question posed in the beginning of this review of whether oncogenic alterations in the same pathway behave identically. The answer to this question will be critical in identifying patients who will respond to PI3K inhibitors alone and those who will benefit from combination therapy. Table 2 summarizes the data analysed above. The coexistence of PIK3CA mutations and PTEN loss in all the three tissues examined provides striking evidence that the two genetic alterations have both redundant and non-overlapping mechanisms of oncogenicity. HER2 overexpression and PI3K pathway alterations likely also have divergent but additive mechanisms, whereas RAS mutations and PI3K pathway alterations may drive tumorigenesis by completely different mechanisms in certain tissues. The following models, illustrated in Figure 2, attempt to explain the data described above and will be helpful in identifying the best treatment for tumors bearing PIK3CA mutations, PTEN loss or RTK overexpression.

Model 1: redundancy

Model 1 illustrates a scenario in which two genetic alterations would be mutually exclusive if their functions are absolutely redundant. Under such circumstances, cells would gain no selective advantage by harboring two mutations that simply activate the same pathway. It is possible that absolutely redundant mutations could amplify signaling through the pathway additively, if downstream effectors were not limiting or if negative feedback loops, such as the loop between S6K and IRS adaptor proteins (Harrington *et al.*, 2004), were disrupted. However, given that we rarely see mutual exclusivity between PI3K pathway alterations, it is unlikely that PI3K pathway alterations function with absolute redundancy.

Model 2: activation of non-overlapping pathways

Model 2 illustrates a more likely explanation for the data described above. Coexisting mutations can arise if each targeted gene activates non-overlapping pathways that (1) also induce tumorigenesis (growth/proliferation/motility) or (2) relieve negative feedback on the PI3K pathway. These additional pathways, along with redundant activation of PI3K signaling, would justify retention of multiple PI3K alterations within the same tumor.

PIK3CA and PTEN alterations may coexist if PTEN loss results in deregulation of other lipids or proteins that confer a growth advantage or disrupt a negative feedback loop. Similarly, RAS mutations may coexist with PIK3CA mutations/PTEN loss because in addition to the PI3K pathway, RAS activates the RAF and RalGDS pathways, which are also known to be critical for tumor growth (Downward, 2003). HER2 overexpression and PIK3CA mutations/PTEN loss may coexist because HER2 overexpression can lead to reshuffling of ERBB family members at the plasma membrane, causing amplification of numerous pathways. HER2–HER3 heterodimers are robust activators of the PI3K pathway, through binding of the p85 subunit to pY-X-X-M motifs in ERBB3/HER3 (Soltoff *et al.*, 1994; Engelman *et al.*, 2005; Hirata *et al.*, 2005). However, an increase in HER2–EGFR heterodimers at the membrane activates RAS-MAPK and PLCγ in addition to PI3K. Importantly, HER2–EGFR heterodimers have prolonged signaling compared to EGFR homodimers because HER2 induces recycling of its EGFR-binding partner as opposed to degradation, thereby relieving negative feedback at the level of receptor internalization (Hendriks *et al.*, 2003). Finally, HER2-overexpressing conditions may also disrupt cell adhesion and polarity through modulation of PAR6, aPKC and related signaling pathways (Moasser, 2007).

Coexisting mutations between PTEN loss and other RTKs may also be important for amplifying signaling through PI3K. Unlike constitutively active p110α, which is at least partially active in serum-starved conditions in mammary cells, the oncogenic potential of PTEN loss is dependent on ligand-induced or autoactivation of receptors (Isakoff *et al.*, 2005). The coexistence of RTK amplifications/mutations and PTEN loss would thus serve to induce PI3K activity in conditions of low ligand availability such as the tumor core.

Model 3: addiction to different networks

The single case of mutual exclusivity between RAS and PIK3CA mutations in the endometrium may reflect a scenario in which RAS is in a network independent of PI3K. In model 2, PTEN/PIK3CA/AKT/RTK/RAS are elements in a single network that is highly committed to PI3K signaling to drive tumor formation and maintenance. In the endometrium, it is possible that if RAS mutations occurred first, cells become addicted to RAS signaling via a pathway independent of PI3K and are posed to gain no advantage by acquiring a PIK3CA mutation.

Alternatively, it is possible that the two mutations coexisted during tumor formation, when PI3K is critical, but selection for one of the two mutations was lost as the microenvironment changed. This scenario was depicted in a study by Lim and Counter (2005) of oncogenic RAS signaling. The authors show that oncogenic RAS signaling to MAPK, RalGDS and PI3K is necessary for tumor initiation; however, in established tumors cells, constitutive PI3K signaling replaced the need for RAS signaling, presumably because the stroma and vasculature supply factors that activate the remaining RAS effector pathways (Lim and Counter, 2005). Given that the patient samples in these studies were obtained from well-established tumors, it is possible that the mutual exclusivity seen between RAS and PIK3CA mutations in the endometrium does not reflect the dispensability of certain pathways, but simply an alternate source of pathway activation.

By fitting the data from Table 2 into models 1, 2 or 3, we propose that PI3K pathway alterations signal nonlinearly through a complex network of redundant and non-overlapping pathways. This emphasizes the important fact that tumors with different PI3K pathway alterations may not respond successfully to PI3K inhibitors alone and will benefit more from combination therapy. Finally, we also propose models 4 and 5 as alternative explanations for the data from Tables 1 and 2, which shed light on how coexisting mutations may cooperate in notable but less conventional ways to induce tumorigenesis.

Model 4: overcoming growth arrest

The selective pressure for mutations to coexist with PTEN loss may have less to do with additive activation of PI3K and other mitogenic pathways, and more to do with overcoming growth arrest. Acute loss of PTEN was observed to cause p53-induced cellular senescence in prostate cancer mouse models and primary mouse embryonic fibroblasts (Chen *et al.*, 2005). Chen *et al.* (2005) show that PTEN loss-induced AKT activity led to p19ARF accumulation, p53 stabilization and upregulation of p21, correlating with the fact that $PTEN^{-/-}$ invasive carcinomas of the prostate often show concomitant loss of p53. Recently, Kim *et al.* (2007) have shown that PIK3CA mutations in human mammary epithelial cells can also induce p53 upregulation, but retain the ability to grow in soft agar. p53 stabilization is AKT-dependent in both cases, suggesting that there are spatial-temporal or intensity differences in AKT activation in PIK3CA mutant and $PTEN^{-}$ cells that cause functional differences, such as senescence. This suggests a model in which $PTEN^{-}$ cells undergo p53-dependent growth arrest but later acquire PIK3CA mutations, which amplify AKT activity past a certain threshold to reverse growth arrest. The coexisting mutations may then cooperate to enhance proliferation and survival of cancer cells as described in model 2.

Model 5: synergism through protein interaction

Mutations in RAS and PIK3CA are interesting, in that they are mutually exclusive in the endometrium, but coexist in the colon. This may be a result of tissue-specific expression of negative regulators as described previously or differences in hormone sensitivity or proliferative index. In the colon, where the two alterations coexist, model 2 may apply, reflecting an increased dependency on MAPK and RalGDS activation in the colon. However, as seen in Table 1, there may be a preference for coexistence of the two mutations, suggesting that they function synergistically. RAS mutants may increase PI3K signaling by increasing the rate of translocation of PI3K to the plasma membrane or direct activation of the enzyme itself. RAS is known to bind to p110, which contributes to PI3K activity in the cell (Rodriguez-Viciana *et al.*, 1994). One mechanism is through recruitment of PI3K to membrane-anchored receptors that do not directly bind p85 but rely on adaptor proteins to help recruit p85. Gupta *et al.* (2007) show that AKT activation is attenuated in cells expressing a mutant RAS that does not bind p110 under conditions of epidermal growth factor and fibroblast growth factor stimulation, but not platelet-derived growth factor stimulation. This can be explained by the fact that platelet-derived growth factor binds p85 directly, whereas EGFR and fibroblast growth factor receptor bind PI3K through adaptor proteins. RAS may thus function to help recruit PI3K to receptors that do not directly bind p85. Importantly, the PI3K–RAS interaction is critical for KRAS-driven tumorigenesis (Gupta *et al.*, 2007). In addition, RAS has been shown to increase the enzymatic activity of $p110\gamma$ through allosteric interactions, which could also apply to $p110\alpha$ (Suire *et al.*, 2002).

An added layer of complexity exists, in that helical and kinase domain p110 mutants have unique oncogenic properties caused by differential interactions with RAS and p85. The charge reversal of glutamic acid to lysine in the E545K and E542K helical domain mutants disrupts an inhibitory interaction between p110 with p85, leading to constitutive kinase activity (Huang *et al.*, 2007; Miled *et al.*, 2007). However, the transforming potential of

helical domain mutants is dependent on interactions with RAS. Zhao and Vogt (2008) show that mutation of the RBD of E545K and E542K mutants blocks p110-RAS binding and that these mutants fail to transform chicken embryo fibroblasts. This interaction with RAS is dispensable in the H1047R kinase domain mutant, which conversely is dependent on p85 binding for full transforming potential (Zhao and Vogt, 2008). This suggests that perhaps RAS mutations and p110α helical domain mutations coexist to synergistically activate the PI3K pathway.

Summary

PI3K signaling drives tumorigenesis in cancers with genetic alterations in p110α, PTEN and ERBBs. However, these genetic lesions should not be treated as a single class of hyper-PI3K cancers. Evidence of coexistence between two alterations suggests that individual mutations are not absolutely redundant but bear the capability to also induce PI3K-independent signaling, cooperate to varying degrees with RAS and modulate negative feedback loops (reviewed by Carracedo and Pandolfi, in this issue). This suggests that mutations in the PI3K pathway will require and likely be very responsive to PI3K inhibitors in combination with other targeted therapies.

Strategies for treatment with PI3K inhibitors

Single-agent therapy

Oncogene-addicted tumors should respond extremely well to single-agent therapies that target the oncogene to which the cell is addicted. Although oncogene addiction is difficult to prove, the frequency of PI3K pathway mutations and the dependence on PI3K signaling in RTK-addicted tumors (Engelman, 2007) strongly suggest that tumors with oncogenic PIK3CA mutations will indeed be addicted to PI3K as the primary source of growth, proliferation and survival signaling. However, tumors with both PI3K and RAS mutations may not respond to single-agent therapy. As more PI3K inhibitors enter the clinic for phase I trials, it will be especially interesting to see if p110α isoform-specific inhibitors will be successful single-agent therapies for tumors with PIK3CA mutations.

Combination therapy

The coexistence of multiple PI3K pathway mutations within a single tumor suggests that these genetic lesions are not redundant. As illustrated in Figure 2, mutations or amplifications in RTKs, PTEN or RAS are likely to activate PI3K and other important pathways and to shut off negative feedback loops. These other pathways must confer a selective advantage and thus will be important to shut off, in addition to PI3K, to fully inhibit tumor growth. This suggests that these tumors will respond well to PI3K inhibitors in combination with other targeted therapy. Combination therapy also has the potential to overcome drug resistance or escape from oncogene addiction. Tumors that develop resistance to RTK inhibitors, for example, would maintain inhibition of the PI3K pathway under combination therapy. The following section reviews recent studies supporting the use of combination therapy for PI3K-dependent tumors.

PTEN and trastuzumab resistance

Trastuzumab (Herceptin) is a humanized monoclonal antibody against HER2, frequently used in the treatment of HER2-positive metastatic breast cancer and in adjuvant therapy of HER2-amplified early stage disease. Trastuzumab's antitumor activity is thought to be conferred through receptor downregulation (Nagata *et al.*, 2004). Two major drawbacks in the use of this antibody are frequent adverse side effects and the development of drug resistance. Nagata *et al.* (2004) and Berns *et al.* (2007) demonstrate that loss of PTEN or activating mutations in PIK3CA are major determinants of trastuzumab resistance in breast

cancer due to their antagonist affects on trastuzumab-mediated phospho- AKT downregulation. Treatment of cultured $PTEN^{-}$; $HER2^{+}$ breast cancer cells with LY294002 or wortmannin in combination with trastuzumab restored antiproliferative effects and decreased the growth of xenografts, but either treatment alone did not (Nagata *et al.*, 2004).

These studies confirm that HER2-overexpressing cells induce the PI3K pathway in addition to PI3K-independent pathways and that PTEN loss cooperates with HER2 to maximally activate AKT. Given that a trastuzumab-containing chemotherapy regimen is now the standard of care in the treatment of HER2-positive breast cancer, it will be interesting to see if similar positive effects can be achieved through combination therapy with trastuzumab and PI3K inhibitors for the treatment of $PTEN^{-}$; $HER2^{+}$ breast cancers.

Receptor tyrosine kinase coactivation

Glioblastomas multiforme are often extremely PI3K-dependent tumors that upregulate PI3K signaling through loss of PTEN or activation of RTKs. Like $PTEN^{-}$; $HER2^{+}$ breast cancers that are resistant to trastuzumab, $PTEN^{-}$;EGFRvIII glioblastomas are resistant to the EGFR inhibitors, erlotinib and gefitinib, due to the persistence of EGFR-independent PI3K signaling (Mellinghoff *et al.*, 2005). Another means of PI3K activation found in glioblastoma cell lines is concurrent ligand-independent activation of multiple RTKs, including EGFR, ERBB3, PDGFRα and MET. Stommel *et al.* (2007) show that expression of EGFRvIII and constitutive MET in glioblastoma cell lines renders cells resistant to EGFR, MET and PDGFRα inhibitors alone, but not in combination. Interestingly, only partial rescue of viability under triple combination therapy was achieved by restoring constitutive PI3K activity, again indicating that PI3K is not the only critical pathway activated downstream of RTKs. In such cases of multiple overactivated RTKs, it might be more efficient and perhaps less toxic to replace RTK inhibitors for combination therapy with inhibitors of major downstream targets like PI3K and MEK.

Rapamycin and EGFR inhibitors

The TORC1 signaling complex of the mTOR protein-Ser/Thr kinase is activated downstream of both PI3K and ERK, and the activation of this complex regulates protein synthesis and cell growth, the two essential functions in cancer cells. It is still unclear which pathways downstream of PI3K are critical for tumor growth and survival. The critical pathways may vary by tissue type, but recent studies have shown that mTOR may be critical especially in tumors with hyperactivation of PI3K. Wang *et al.* (2006) show that inhibition of the TORC1 complex with rapamycin increases the sensitivity of PTEN-deficient glioblastoma cell lines to erlotinib to induce growth arrest. These inhibitory growth effects were corroborated *in vivo* by Buck *et al.* (2006), who treated mice with rapamycin and erlotinib and showed deceleration of tumor growth in an erlotinib-resistant xenograft model. In a mouse model of bronchial and peripheral lung cancer, Li *et al.* (2007) demonstrated that tumors induced by the EGFR T790M/L858R compound mutation can be shrunk by a combination of rapamycin and an irreversible EGFR/HER2 inhibitor, HKI-272, but not with either therapy alone. These studies suggest that the TORC1 complex is a critical component of downstream PI3K activity and that dual mTOR/PI3K inhibitors in combination with EGFR inhibitors may produce a more pronounced cytotoxic effect.

Isoform-specific PI3K inhibitors emerge

Until recently, there were few PI3K inhibitors available for studies. With the emergence of isoform-specific inhibitors, we are beginning to see the potential of PI3K inhibition for the treatment of cancer. Some of the PI3K inhibitors entering phase I clinical trials broadly target the class IA PI3Ks (p110α, p110β and p110δ), whereas others inhibit both class IA PI3Ks and the catalytic site of mTOR. This latter category is particularly interesting as

inhibition of the catalytic site of mTOR results in inhibition of both the TORC1 and TORC2 signaling complexes (as opposed to rapamycin and analogs, which only inhibit the TORC1 complex). Thus, catalytic-site inhibitors of mTOR are expected to be more potent in blocking tumor growth and survival as, in addition to inhibiting TORC1 signaling, they also prevent TORC2-dependent phosphorylation of Thr-473 of AKT, among other functions of this complex. Fan *et al.* (2006) showed that dual inhibition of p110α and the catalytic site of mTOR induced striking antitumorigenic effects in glioblastoma cell lines and xenografts using a novel dual PI3K/mTOR inhibitor, PI-103. Importantly, these effects were apparent regardless of PTEN, p53 or EGFR status, emphasizing the unique ability of combined PI3K/mTOR inhibitors to circumvent the complications of upstream signaling. In a second study, Fan *et al.* (2007) show that inhibition of p110α, mTOR and EGFR using PI-103 and erlotinib induced a more dramatic growth arrest in EGFRvIII;PTEN^{-} glioblastoma cells, than dual treatment with rapamycin and erlotinib or a p110α inhibitor and erlotinib. As more PI3K and mTOR inhibitors are validated as effective single agents, it will be exciting to see their effects in combination.

Exciting combinations

As depicted in Figure 2, mutations in RTKs, RAS and PTEN activate PI3K in addition to other pathways. The RAS–MAPK pathway is likely to be a major contributor to tumorigenesis in this setting, making inhibition of the PI3K and MAPK pathways a potentially powerful combination therapy. Targeting these downstream kinases in which multiple signaling events converge may also overcome resistance mechanisms like those described above.

Until now, the dearth of clinically available PI3K inhibitors has forced scientists and clinicians to design clinical trials that only indirectly explore this combination. Sorafenib, a multikinase inhibitor of RAF, BRAF, VEGFR-2/3 and others, has antitumorigenic and antiangiogenic effects on various solid tumor types (Wilhelm *et al.*, 2004). Although these effects are generally attributed to disrupting angiogenesis, numerous ongoing clinical trials are evaluating the response to sorafenib in combination with temsirolimus/RAD001 (mTOR inhibitor), erlotinib, cetuximab (EGFR inhibitor) and imatinib (Gleevec) in a variety of solid tumors (www.cancer.gov). It will be important to see if inhibition of mTOR or PI3K via EGFR inhibitors synergizes with RAF inhibition to induce robust antitumorigenic effects in EGFR- or RAS/RAF-driven tumors and if PI3K inhibitors will be more effective than RTK inhibitors in combination with sorafenib.

MEK inhibitors are also attractive candidates for use in combination with PI3K inhibitors. Like PI3K inhibitors, MEK inhibitors are relatively new, and compounds such as AZD6244 (Array BioPharma, Boulder, CO, USA), XL518 (Exelixis, San Francisco, CA, USA), RDEA119 (Ardea Biosciences, San Diego, CA, USA) and PD0325901 (Pfizer, New York, NY, USA) are in ongoing clinical trials as monotherapy and in combination with cytotoxic chemotherapy for blood and solid tumors (www.cancer.gov).

Combination-targeted therapy with PI3K inhibitors and RAF/MEK inhibitors or RTK inhibitors will be important trials to conduct, especially for the treatment of PI3K-driven tumors. We have reviewed how single and double mutations in RTKs, PI3K, PTEN and RAS occur in a broad spectrum of tumors. Signaling downstream of these alterations converge on PI3K, often activate non-redundant pathways and may utilize other less conventional mechanisms to induce oncogenesis. This emphasizes the importance of using combination therapy to target signaling in cancer cells at multiple nodes.

PI3K inhibitors in the tumor microenvironment

Although it is clear that PI3K inhibitors will be promising treatments for cancer cells, an exciting added benefit to the development of these compounds is the potential to affect PI3K-dependent cell types in the microenvironment of solid tumors. The tumor microenvironment is a heterogeneous compartment comprised of stromal fibroblasts, immune cells, adipocytes and blood vessels. During chronic inflammation, wounding and cancer, the complex communication between these cell types is disrupted through persistent overproduction of chemokines and matrix metalloproteinases, leading to structural disorder of the tissue and abnormal proliferation (Bissell and Radisky, 2001). PI3K plays a critical role in signaling within endothelial and immune cells, and inhibition of PI3K may help to normalize the stroma, thereby indirectly inhibiting tumor growth (Figure 3).

Endothelial cells

Endothelial cells line the lumen of blood and lymphatic vessels. They are responsible for internalizing secreted factors from the tissue and initiating local vascular remodeling to accommodate changes in nutrient and oxygen supplies. Angiogenesis is critical in tumorigenesis because *de novo* blood vessel formation must occur to maintain oxygen and nutrient exchange between the tumor periphery and the hypoxic core (Folkman, 2007). As critical signaling intermediaries, endothelial cells express numerous cell-surface receptors to integrate the vascular growth factors secreted by tumor and stromal cells. Importantly, among the key receptors expressed by endothelial cells are the VEGFR1–3, TIE-1/2, FGFR1–2, PDGFR-β and ERBB1–4 RTKs (Hofer and Schweighofer, 2007). PI3K is activated downstream of each of these receptors and acts as a master regulator of angiogenic signaling in the endothelium. Several studies have recently elucidated the role of PI3K signaling in normal and tumor angiogenesis and provide encouraging support for targeting PI3K for antiangiogenic therapy.

Studies in mice using conditional or germline knockouts of PI3K effector genes illustrate the importance of the PI3K pathway in angiogenesis. FOXO1 germline deletion results in underdeveloped arteries and early vessel remodeling defects (Furuyama *et al.*, 2004). Complete loss of PTEN in the endothelium results in abnormal vascular remodeling, bleeding and embryonic lethality (Sun *et al.*, 2005). Constitutive AKT activity in the endothelium results in abnormal vessel patterning, vessel congestion and breaching (Hamada *et al.*, 2005). These studies strongly support the use of PI3K inhibitors for antiangiogenic therapy. Schnell and co-workers have recently demonstrated that treatment with the pan-PI3K/mTOR inhibitor, NVP-BEZ235, affects multiple aspects of tumor angiogenesis, including endothelial cell proliferation and vascular permeability to confer marked antitumor activity (paper submitted).

Until recently, it was unclear which PI3K isoforms were important for the effects seen in these studies, which will be critical in choosing the optimal PI3K inhibitor in the clinic. We have utilized a conditional mouse model with deletion of the class 1A PI3Ks in the endothelium to show that loss of this class of PI3Ks causes a defect in vessel integrity in tumor allografts. This defect decreases the rate of tumor growth and limits tumor size (Yuan *et al.*, 2008, paper accepted, *Proceedings of the National Academy of Sciences of the United States of America*). Graupera *et al.* (2008) have also recently shown that p110α plays an isoform-specific role in endothelial cell migration. These data further support the use of PI3K inhibitors in the clinic and may be especially effective in patients with PIK3CA mutations by attacking the tumor on multiple fronts.

Immune cells

The environment surrounding a malignant tumor resembles an environment under chronic inflammation. Not only does the tumor recruit immune cells, but also do they produce the chemical factors that regulate inflammation. Among the immune cells that are recruited to the tumor stroma are macrophages, dendritic cells, T cells, mast cells and natural killer cells. Anti-inflammatory drugs can reduce the risk of certain cancers, and it will be interesting to see if PI3K inhibitors can have a similar effect by modulating inflammatory responses in the tumor stroma (reviewed by Hirsch *et al.* (2008)).

Class 1A PI3K is activated in monocytes, mast cells, natural killer cells, and B and T cells (Koyasu, 2003). In particular, the class 1A enzyme, p110δ, is essential for B-cell development, and the class 1B enzyme, p110γ, is a key regulator in macrophages (Rommel *et al.*, 2007). Macrophages have matrix-remodeling capabilities that allow tumor cells to invade into the surrounding stroma and migrate toward blood vessels (Hirsch *et al.*, 2000). They also produce proangiogenic factors to induce vascularization. Inhibition of PI3K may therefore dampen the inflammatory response in the tumor stroma and help prevent infiltration of cancer cells further into the microenvironment and into the vasculature.

Metastasis

Although tumor cells may frequently escape the primary colonization site, the steps to successful secondary site colonization are arduous and many (Gupta and Massague, 2006). To intravasate into the circulation, metastatic cells may undergo an epithelial-to-mesenchymal transition that relinquishes the cells from intrinsic polarization and adhesion to the extracellular matrix. Mobilization to blood vessels then requires activation of Rho, Rac, Cdc42 and other motility pathways. Upon intravasation into the vasculature, strong survival mechanisms must be in place to combat nutrient and oxygen deprivation, acidic environments and immune surveillance. Finally, if cells manage to survive and extravasate from the vasculature, they are faced with the challenge of initiating tumor growth. Due to the inhospitable nature of most sites of extravasation, metastatic cells commonly lie dormant or die because of the inability to initiate tumor growth.

As discussed above, inhibition of PI3K in the tumor microenvironment may help impede metastasis by damaging the vasculature and inhibiting infiltration by immune cells. In addition, inhibition of PI3K in tumor cells may directly impair many of the steps required for metastasis. Beginning with intravasation, PI3K plays an important role in cell polarity by maintaining the delicate balance of phosphoinositide localization. Disruption of the PI3K pathway through oncogenic alterations such as PTEN ablation has been shown to disrupt apical-basal membrane segregation in epithelial cells, which could contribute to epithelial-to-mesenchymal transition (Martin-Belmonte and Mostov, 2008). The PI3K pathway could also contribute to metastatic cell motility through activation of Rac and Cdc42 (Kolsch *et al.*, 2008) and circumvent cell death in the harsh vascular environment by activating AKT-mediated survival pathways.

However, perhaps the most critical contribution PI3K could make to the establishment of metastases is facilitating tumor formation at the secondary site. It is currently unclear what drives certain disseminated cells back into the cell cycle; however, activation of the PI3K pathway may be an important factor because of its essential role in tumor initiation. Lim and Counter (2005) demonstrate that for oncogenic RAS-induced tumors, activation of the PI3K pathway is essential for tumor initiation. Immortalized cells with active RalGDS and MAPK alone did not initiate tumor formation; however, activation of PI3K signaling in these cells restored tumor-initiating capabilities. Engelman and co-workers have further narrowed the window of time when PI3K activity is required for RAS-driven tumorigenesis. They show

that in cancers driven by oncogenic PI3K, the Novartis pan-PI3K/mTOR inhibitor, NVP-BEZ235, induces striking regression of established tumors. Disruption of class IA PI3K simultaneously with RAS activation also prevented tumor formation. However, in cancers driven by oncogenic RAS, administration of NVP-BEZ235 after primary tumor establishment does not cause tumor regression (paper submitted). This suggests that although PI3K is required for tumor initiation, it is dispensable for tumor maintenance in RAS-driven tumors. Yet administration of the drug may inhibit tumor initiation in dormant disseminated cells that have escaped from the primary tumor. Given that metastases are the leading cause of death in patients with solid tumors, the ability to inhibit metastatic tumor initiation with PI3K inhibitors should be closely studied.

Outlook

With the development of more targeted therapies, it will be important to closely understand the oncogenic mechanisms of individual tumors. Studies like those reviewed here give us important clues as to which pathways are activated downstream of mutations in the PI3K pathway. This will allow us to predict sensitivity to patient-tailored combination therapies. It will also be critical to screen this new generation of drugs for toxicity and adverse effects, given their potential to be very potent compounds. Sequential as opposed to concurrent dosing of combinatorial drugs may be a favorable strategy. Although trials for adjuvant therapy are time consuming and expensive and not the preferred route for approval of PI3K inhibitors, in the end, these compounds may have the greatest impact in this setting. In the future, biopsies should be systematically screened not only for their driver mutations but also for important downstream activators. As we accumulate more targeted therapies in the clinic, the ability to completely and efficiently shut off major pathways in cancer cells will hopefully lead to less drug resistance and increased patient survival.

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References

Abubaker J, Bavi P, Al-Harbi S, Ibrahim M, Siraj AK, Al-Sanea N, et al. Clinicopathological analysis of colorectal cancers with PIK3CA mutations in Middle Eastern population. Oncogene. 2008; 27:3539–3545. [PubMed: 18193083]

Arteaga CL. EGF receptor mutations in lung cancer: from humans to mice and maybe back to humans. Cancer Cell. 2006; 9:421–423. [PubMed: 16766261]

Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol Ther. 2004; 3:772–775. [PubMed: 15254419]

Barault L, Veyrie N, Jooste V, Lecorre D, Chapusot C, Ferraz JM, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer. 2008; 122:2255–2259. [PubMed: 18224685]

Barnes KR, Blois J, Smith A, Yuan H, Reynolds F, Weissleder R, et al. Fate of a bioactive fluorescent wortmannin derivative in cells. Bioconjug Chem. 2008; 19:130–137. [PubMed: 17988080]

Bauer S, Duensing A, Demetri GD, Fletcher JA. KIT oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway. Oncogene. 2007; 26:7560–7568. [PubMed: 17546049]

Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell. 2007; 12:395–402. [PubMed: 17936563]

Bissell MJ, Radisky D. Putting tumours in context. Nat Rev Cancer. 2001; 1:46–54. [PubMed: 11900251]

Buck E, Eyzaguirre A, Brown E, Petti F, McCormack S, Haley JD, et al. Rapamycin synergizes with the epidermal growth factor receptor inhibitor erlotinib in non-small-cell lung, pancreatic, colon, and breast tumors. Mol Cancer Ther. 2006; 5:2676–2684. [PubMed: 17121914]

Cantley LC. The phosphoinositide 3-kinase pathway. Science. 2002; 296:1655–1657. [PubMed: 12040186]

Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007; 448:439–444. [PubMed: 17611497]

Chen JS, Zhou LJ, Entin-Meer M, Yang X, Donker M, Knight ZA, et al. Characterization of structurally distinct, isoform-selective phosphoinositide 3′-kinase inhibitors in combination with radiation in the treatment of glioblastoma. Mol Cancer Ther. 2008; 7:841–850. [PubMed: 18413797]

Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature. 2005; 436:725–730. [PubMed: 16079851]

Crabbe T, Welham MJ, Ward SG. The PI3K inhibitor arsenal: choose your weapon! Trends Biochem Sci. 2007; 32:450–456. [PubMed: 17920279]

Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer. 2003; 3:11–22. [PubMed: 12509763]

Engelman JA. The role of phosphoinositide 3-kinase pathway inhibitors in the treatment of lung cancer. Clin Cancer Res. 2007; 13:s4637–s4640. [PubMed: 17671156]

Engelman JA, Janne PA, Mermel C, Pearlberg J, Mukohara T, Fleet C, et al. ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. Proc Natl Acad Sci USA. 2005; 102:3788–3793. [PubMed: 15731348]

Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006; 7:606–619. [PubMed: 16847462]

Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007; 316:1039–1043. [PubMed: 17463250]

Fan QW, Cheng CK, Nicolaides TP, Hackett CS, Knight ZA, Shokat KM, et al. A dual phosphoinositide-3-kinase alpha/mTOR inhibitor cooperates with blockade of epidermal growth factor receptor in PTEN-mutant glioma. Cancer Res. 2007; 67:7960–7965. [PubMed: 17804702]

Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, et al. A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell. 2006; 9:341–349. [PubMed: 16697955]

Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov. 2007; 6:273–286. [PubMed: 17396134]

Fujita T, Doihara H, Kawasaki K, Takabatake D, Takahashi H, Washio K, et al. PTEN activity could be a predictive marker of trastuzumab efficacy in the treatment of ErbB2-overexpressing breast cancer. Br J Cancer. 2006; 94:247–252. [PubMed: 16404430]

Furuyama T, Kitayama K, Shimoda Y, Ogawa M, Sone K, Yoshida-Araki K, et al. Abnormal angiogenesis in Foxo1 (Fkhr)-deficient mice. J Biol Chem. 2004; 279:34741–34749. [PubMed: 15184386]

Garlich JR, De P, Dey N, Su JD, Peng X, Miller A, et al. A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. Cancer Res. 2008; 68:206–215. [PubMed: 18172313]

Graupera M, Guillermet-Guibert J, Foukas LC, Phng LK, Cain RJ, Salpekar A, et al. Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration. Nature. 2008; 453:662–666. [PubMed: 18449193]

Gupta GP, Massague J. Cancer metastasis: building a framework. Cell. 2006; 127:679–695. [PubMed: 17110329]

Gupta S, Ramjaun AR, Haiko P, Wang Y, Warne PH, Nicke B, et al. Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. Cell. 2007; 129:957–968. [PubMed: 17540175]

Hamada K, Sasaki T, Koni PA, Natsui M, Kishimoto H, Sasaki J, et al. The PTEN/PI3K pathway governs normal vascular development and tumor angiogenesis. Genes Dev. 2005; 19:2054–2065. [PubMed: 16107612]

Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, et al. The TSC1-2 tumor suppressor controls insulin–PI3K signaling via regulation of IRS proteins. J Cell Biol. 2004; 166:213–223. [PubMed: 15249583]

Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. Science. 2003; 299:708–710. [PubMed: 12522257]

Hendriks BS, Opresko LK, Wiley HS, Lauffenburger D. Coregulation of epidermal growth factor receptor/human epidermal growth factor receptor 2 (HER2) levels and locations: quantitative analysis of HER2 overexpression effects. Cancer Res. 2003; 63:1130–1137. [PubMed: 12615732]

Hirata A, Hosoi F, Miyagawa M, Ueda S, Naito S, Fujii T, et al. HER2 overexpression increases sensitivity to gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor, through inhibition of HER2/HER3 heterodimer formation in lung cancer cells. Cancer Res. 2005; 65:4253–4260. [PubMed: 15899817]

Hirsch E, Ciraolo E, Ghigo A, Costa C. Taming the PI3K team to hold inflammation and cancer at bay. Pharmacol Ther. 2008; 118:192–205. [PubMed: 18420279]

Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, et al. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. Science. 2000; 287:1049–1053. [PubMed: 10669418]

Hofer E, Schweighofer B. Signal transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thromb Haemost. 2007; 97:355–363. [PubMed: 17334501]

Hollestelle A, Elstrodt F, Nagel JH, Kallemeijn WW, Schutte M. Phosphatidylinositol-3-OH kinase or RAS pathway mutations in human breast cancer cell lines. Mol Cancer Res. 2007; 5:195–201. [PubMed: 17314276]

Howes AL, Chiang GG, Lang ES, Ho CB, Powis G, Vuori K, et al. The phosphatidylinositol 3-kinase inhibitor, PX-866, is a potent inhibitor of cancer cell motility and growth in three-dimensional cultures. Mol Cancer Ther. 2007; 6:2505–2514. [PubMed: 17766839]

Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, et al. The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. Science. 2007; 318:1744–1748. [PubMed: 18079394]

Ikeda T, Yoshinaga K, Suzuki A, Sakurada A, Ohmori H, Horii A. Anticorresponding mutations of the KRAS and PTEN genes in human endometrial cancer. Oncol Rep. 2000; 7:567–570. [PubMed: 10767369]

Isakoff SJ, Engelman JA, Irie HY, Luo J, Brachmann SM, Pearline RV, et al. Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. Cancer Res. 2005; 65:10992–11000. [PubMed: 16322248]

Jhawer M, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, et al. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res. 2008; 68:1953–1961. [PubMed: 18339877]

Kang S, Seo SS, Chang HJ, Yoo CW, Park SY, Dong SM. Mutual exclusiveness between PIK3CA and KRAS mutations in endometrial carcinoma. Int J Gynecol Cancer. 2008 e-pub ahead of print.

Kato S, Iida S, Higuchi T, Ishikawa T, Takagi Y, Yasuno M, et al. PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. Int J Cancer. 2007; 121:1771–1778. [PubMed: 17590872]

Kim JS, Lee C, Bonifant CL, Ressom H, Waldman T. Activation of p53-dependent growth suppression in human cells by mutations in PTEN or PIK3CA. Mol Cell Biol. 2007; 27:662–677. [PubMed: 17060456]

Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, et al. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell. 2006; 125:733–747. [PubMed: 16647110]

Kolsch V, Charest PG, Firtel RA. The regulation of cell motility and chemotaxis by phospholipid signaling. J Cell Sci. 2008; 121:551–559. [PubMed: 18287584]

Kong D, Yamori T. ZSTK474 is an ATP-competitive inhibitor of class I phosphatidylinositol 3 kinase isoforms. Cancer Sci. 2007; 98:1638–1642. [PubMed: 17711503]

Koyasu S. The role of PI3K in immune cells. Nat Immunol. 2003; 4:313–319. [PubMed: 12660731]

Li D, Shimamura T, Ji H, Chen L, Haringsma HJ, McNamara K, et al. Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKI-272 and rapamycin combination therapy. Cancer Cell. 2007; 12:81–93. [PubMed: 17613438]

Lim KH, Counter CM. Reduction in the requirement of oncogenic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance. Cancer Cell. 2005; 8:381–392. [PubMed: 16286246]

Martin-Belmonte F, Mostov K. Regulation of cell polarity during epithelial morphogenesis. Curr Opin Cell Biol. 2008; 20:227–234. [PubMed: 18282696]

Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med. 2005; 353:2012–2024. [PubMed: 16282176]

Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science. 2007; 317:239–242. [PubMed: 17626883]

Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene. 2007; 26:6469–6487. [PubMed: 17471238]

Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell. 2004; 6:117–127. [PubMed: 15324695]

Narita Y, Nagane M, Mishima K, Huang HJ, Furnari FB, Cavenee WK. Mutant epidermal growth factor receptor signaling down-regulates p27 through activation of the phosphatidylinositol 3-kinase/Akt pathway in glioblastomas. Cancer Res. 2002; 62:6764–6769. [PubMed: 12438278]

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]

Ollikainen M, Gylling A, Puputti M, Nupponen NN, Abdel-Rahman WM, Butzow R, et al. Patterns of PIK3CA alterations in familial colorectal and endometrial carcinoma. Int J Cancer. 2007; 121:915–920. [PubMed: 17471559]

Perez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjold B, Rutqvist LE, et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res. 2007; 13:3577–3584. [PubMed: 17575221]

Ramjaun AR, Downward J. Ras and phosphoinositide 3-kinase: partners in development and tumorigenesis. Cell Cycle. 2007; 6:2902–2905. [PubMed: 17993782]

Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, et al. Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature. 1994; 370:527–532. [PubMed: 8052307]

Rommel C, Camps M, Ji H. PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? Nat Rev Immunol. 2007; 7:191–201. [PubMed: 17290298]

Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, et al. 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]

Salmena L, Carracedo A, Pandolfi PP. Tenets of PTEN tumor suppression. Cell. 2008; 133:403–414. [PubMed: 18455982]

Samuels Y, Diaz LA Jr, Schmidt-Kittler O, Cummins JM, Delong L, Cheong I, et al. 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, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004; 304:554. [PubMed: 15016963]

Sarkisian CJ, Keister BA, Stairs DB, Boxer RB, Moody SE, Chodosh LA. Dose-dependent oncogene-induced senescence *in vivo* and its evasion during mammary tumorigenesis. Nat Cell Biol. 2007; 9:493–505. [PubMed: 17450133]

Sauter G, Maeda T, Waldman FM, Davis RL, Feuerstein BG. Patterns of epidermal growth factor receptor amplification in malignant gliomas. Am J Pathol. 1996; 148:1047–1053. [PubMed: 8644846]

Soltoff SP, Carraway KL III, Prigent SA, Gullick WG, Cantley LC. ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. Mol Cell Biol. 1994; 14:3550–3558. [PubMed: 7515147]

Stephens P, Hunter C, Bignell G, Edkins S, Davies H, Teague J, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature. 2004; 431:525–526. [PubMed: 15457249]

Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science. 2007; 318:287–290. [PubMed: 17872411]

Suire S, Hawkins P, Stephens L. Activation of phosphoinositide 3-kinase gamma by Ras. Curr Biol. 2002; 12:1068–1075. [PubMed: 12121613]

Sun JF, Phung T, Shiojima I, Felske T, Upalakalin JN, Feng D, et al. Microvascular patterning is controlled by fine-tuning the Akt signal. Proc Natl Acad Sci USA. 2005; 102:128–133. [PubMed: 15611473]

Tokunaga E, Oki E, Kimura Y, Yamanaka T, Egashira A, Nishida K, et al. Coexistence of the loss of heterozygosity at the PTEN locus and HER2 overexpression enhances the Akt activity thus leading to a negative progesterone receptor expression in breast carcinoma. Breast Cancer Res Treat. 2007; 101:249–257. [PubMed: 17006756]

Tornillo L, Terracciano LM. An update on molecular genetics of gastrointestinal stromal tumours. J Clin Pathol. 2006; 59:557–563. [PubMed: 16731599]

Velho S, Oliveira C, Ferreira A, Ferreira AC, Suriano G, Schwartz S Jr, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer. 2005; 41:1649–1654. [PubMed: 15994075]

Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, et al. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. Mol Cell. 2000; 6:909–919. [PubMed: 11090628]

Walker EH, Perisic O, Ried C, Stephens L, Williams RL. Structural insights into phosphoinositide 3-kinase catalysis and signalling. Nature. 1999; 402:313–320. [PubMed: 10580505]

Wang MY, Lu KV, Zhu S, Dia EQ, Vivanco I, Shackleford GM, et al. Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN-deficient and PTEN-intact glioblastoma cells. Cancer Res. 2006; 66:7864–7869. [PubMed: 16912159]

Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004; 64:7099–7109. [PubMed: 15466206]

Wymann MP, Bulgarelli-Leva G, Zvelebil MJ, Pirola L, Vanhaesebroeck B, Waterfield MD, et al. Wortmannin inactivates phosphoinositide 3-kinase by covalent modification of Lys-802, a residue involved in the phosphate transfer reaction. Mol Cell Biol. 1996; 16:1722–1733. [PubMed: 8657148]

Yaguchi S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, et al. Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. J Natl Cancer Inst. 2006; 98:545–556. [PubMed: 16622124]

Yuan H, Barnes KR, Weissleder R, Cantley L, Josephson L. Covalent reactions of wortmannin under physiological conditions. Chem Biol. 2007; 14:321–328. [PubMed: 17379147]

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 USA. 2008; 105:2652–2657. [PubMed: 18268322]

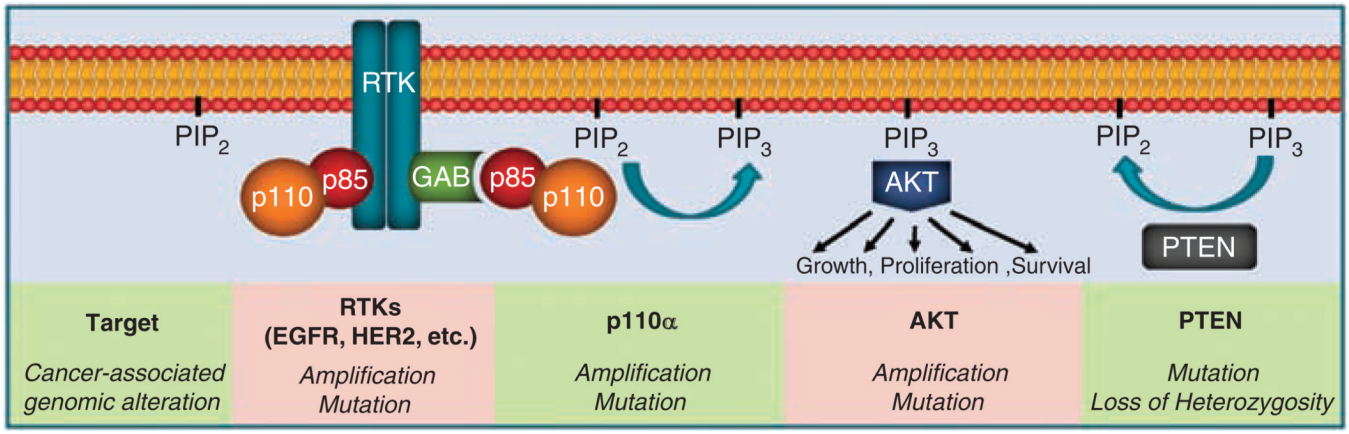

Figure 1.

The PI3K signaling axis. Activation of RTKs recruits PI3K directly or through adaptor proteins such as the GAB proteins. PI3K phosphorylates PIP2 to generate PIP3, which leads to AKT activation and activation of numerous effectors that regulate critical cellular functions in cancer cells. PTEN negatively regulates this process through dephosphorylation of PIP3. All major members of this signaling axis are frequently altered in cancer. PI3K, phosphoinositide 3-kinase; RTKs, receptor tyrosine kinases.

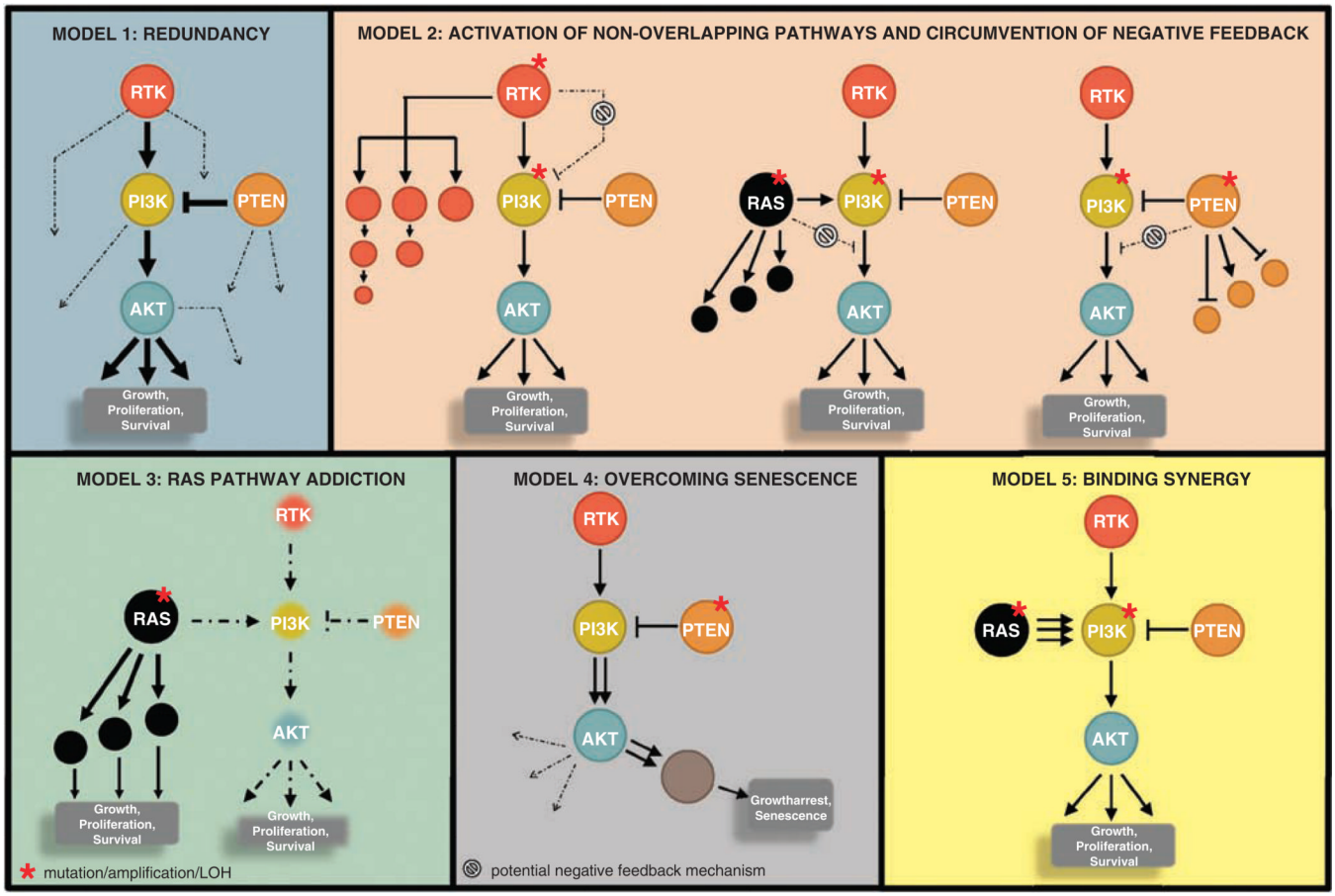

Figure 2.

Possible models of oncogenicity. In model 1, genetic alterations in any of the PI3K pathway members lead to amplification of PI3K signaling. In model 2, alterations of RTKs, RAS or PTEN in addition to mutant PIK3CA lead to activation of non-overlapping pathways or disrupt negative feedback loops to enhance PI3K signaling. In model 3, oncogenic RAS leads to tumorigenesis via a mechanism independent of PI3K. In model 4, loss of PTEN leads to p53-dependent cellular senescence, which may be overcome by acquisition of an additional mutation in the pathway. In model 5, activation of PI3K is enhanced through interactions with oncogenic RAS. PI3K, phosphoinositide 3-kinase; RTKs, receptor tyrosine kinases.

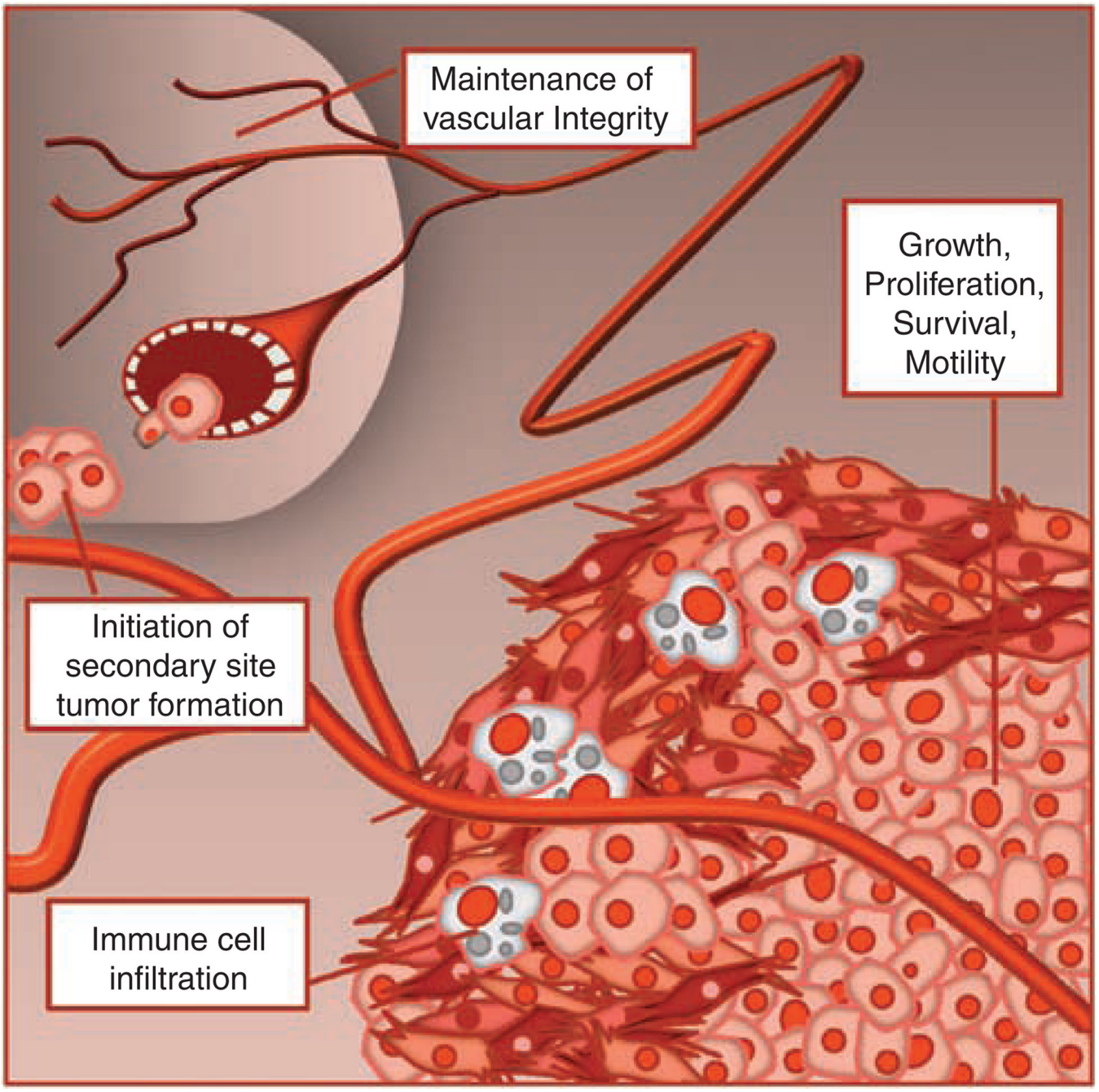

Figure 3.
PI3K in the tumor microenvironment. In tumor cells, PI3K plays an important role in tumor initiation, growth and proliferation. PI3K also plays a major role in endothelial and immune cells that support tumor growth, intravasation and invasion. Inhibition of PI3K may thus have the potential to inhibit formation of secondary-site metastases. PI3K, phosphoinositide 3-kinase.

Table 1

Frequency of PI3K-related genetic alterations in solid tumors

Genetic variants	Breast	Endometrium	Colon	References
PTEN^{-}	131/392 (33%)	126/233 (54%)	261/482 (54%)	Breast: *1–3*; Endometrium: *4–7*; Colon: *6*, *8*, *9*
PIK3CA^{mut}	104/392 (27%)	65/233 (28%)	63/482 (13%)	
PTEN^{-} + PIK3CA^{mut}	**34/392 (8.7%)**	**34/233 (15%)**	**27/482 (5.6%)**	
Expected if independent	**35/392 (8.9%)**	**35/233 (15%)**	**34/482 (7.0%)**	
RAS^{mut}	7/40 (18%)	26/232 (11%)	325/939 (35%)	Breast: *3*; Endometrium: *5–7*; Colon: *6*, *8*, *10–12*
PIK3CA^{mut}	14/40 (35%)	52/232 (22%)	146/939 (16%)	
RAS^{mut} + PIK3CA^{mut}	**1/40 (2.5%)**	**1/232 (0.4%)**	**69/939 (7.3%)**	
Expected if independent	**2.5/40 (6.3%)**	**5.7/232 (2.5%)**	**51/939 (5.4%)**	
RAS^{mut}	7/40 (18%)	18/134 (13%)	17/70 (24%)	Breast: *3*; Endometrium: *6*, *13*; Colon: *6*
PTEN^{-}	8/40 (20%)	65/134 (49%)	22/70 (31%)	
RAS^{mut} + PTEN^{-}	**0/40 (0.0%)**	**7/134 (5.2%)**	**5/70 (7.1%)**	
Expected if independent	**1.4/40 (3.6%)**	**8.5/134 (6.3%)**	**5.3/70 (7.5%)**	
HER2^{+}	150/489 (31%)	NA	NA	Breast: *1*, *2*, *14*
PIK3CA^{mut}	118/489 (24%)	NA	NA	
HER2^{+} + PIK3CA^{mut}	**36/489 (7.4%)**	NA	NA	
Expected if independent	**37/489 (7.5%)**	NA	NA	
HER2^{+}	69/315 (22%)	NA	NA	Breast: *15*, *16*
PTEN^{-}	94/315 (30%)	NA	NA	
HER2^{+} + PTEN^{-}	**25/315 (7.9%)**	NA	NA	
Expected if independent	**21/315 (6.6%)**	NA	NA	

Abbreviations: NA, not applicable; PI3K, phosphoinositide 3-kinase. Two mutations are independently selected (coexistent) if the frequency of the double mutation equals the product of the frequencies of the single mutations.

1 Saal *et al.* (2005);

2 Perez-Tenorio *et al.* (2007);

3 Hollestelle *et al.* (2007);

4 Oda *et al.* (2005);

5 Velasco *et al.*, 2006;

6 Ollikainen *et al.* (2007);

7 Kang *et al.* (2008);

8 Jhawer *et al.* (2008);

9 Abubaker *et al.* (2008);

10 Velho *et al.* (2005);

11 Kato *et al.* (2007);

[12]Barault *et al.* (2008);

[13]Ikeda *et al.* (2000);

[14]Bachman *et al.* (2004);

[15]Fujita *et al.* (2006);

[16]Tokunaga *et al.* (2007).

Table 2

Summary of mutually exclusive and coexisting genetic alterations

Genetic variants	Breast	Endometrium	Colon
$PTEN^{-}$ + $PIK3CA^{mut}$	Coexistent	Coexistent	Coexistent
RAS^{mut} + $PIK3CA^{mut}$	Seemingly exclusive[a]	Mutually exclusive	Coexistent
RAS^{mut} + $PTEN^{-}$	Seemingly exclusive[a]	Coexistent	Coexistent
$HER2^{+}$ + $PIK3CA^{mut}$	Coexistent	NA	NA
$HER2^{+}$ + $PTEN^{-}$	Coexistent	NA	NA

Abbreviation: NA, not applicable.

[a]Numbers are too low to make a definitive conclusion about exclusivity.