Phosphoinositide 3-Kinase and INPP4B in human breast cancer

Micka C. Bertucci,1 and Christina A. Mitchell1

1Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria.

Address for correspondence: Christina A. Mitchell, Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800. ChristinaMitchell@monash.edu

The PI3K/Akt signaling pathway is frequently increased in many human cancers, including breast cancer. Recent studies have identified INPP4B, which inhibits PI3K signaling, as an emerging tumor suppressor in breast cancer.

Keywords: PI3K; breast cancer; PTEN; 4-phosphatase; INPP4B

Introduction

In response to extracellular stimuli, phosphoinositide 3-kinase (PI3K) is activated resulting in the phosphorylation of membrane-bound phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2] to transiently generate phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P3] which binds the pleckstrin homology (PH) domain of many proteins, including phosphoinositide dependent kinase 1 (PDK1) and the proto-oncogene serine/threonine kinase Akt.1 Phosphoinositide binding of these PI3K effectors leads to their allosteric activation and in turn the activation of many downstream targets including the mammalian target of rapamycin (mTOR) complex, which promotes cell growth and protein translation, serum glucocorticoid regulated kinase (SGK), and signaling pathways that promote cell cycle progression and cell survival. PtdIns(3,4,5)P3 is rapidly dephosphorylated by inositol polyphosphate 5-phosphatases leading to the production of a second signaling lipid PtdIns(3,4)P2. This phosphoinositide also binds the PH domain of Akt with high affinity and with PtdIns(3,4,5)P3 facilitates full activation of Akt.1 PI3K signaling is negatively regulated by several phosphoinositide phosphatases including the tumor suppressor PTEN, which dephosphorylates the 3-position phosphate from the inositol ring of PtdIns(3,4,5)P3 to form PtdIns(4,5)P2. More recently a novel tumor suppressor INPP4B has been identified, which dephosphorylates the 4-position phosphate from the inositol ring of PtdIns(3,4)P2 forming PtdIns(3)P, which also inhibits PI3K-dependent Akt activation. Enhanced PI3K/Akt signaling has been identified in many human cancers. In primary human breast cancer, ~70% of cases exhibit alterations within one or more components of the PI3K pathway,2 including mutation or amplification of the gene encoding the catalytic subunit of class I PI3K.
activation of oncogenic tyrosine kinase receptors such as HER2, which in turn activate PI3K/Akt, loss of function of the tumor suppressors PTEN and/or INPP4B, or mutation and/or amplification of the proto-oncogene Akt. The focus of this review is the role of PI3K signaling and its regulation by INPP4B in human breast cancer.

**Phosphoinositide 3-Kinase mutations in breast cancer**

Invasive breast cancer is a heterogeneous disease that is generally classified into distinct subtypes, luminal A, luminal B, HER2-positive and triple-negative on the basis of the expression of specific receptors and clinicopathological markers. Luminal A and luminal B breast cancers are hormone-dependent and express the estrogen receptor (ER) alpha and/or progesterone receptor (PR), and are responsive to treatments that block ER signaling. HER2-positive breast cancers exhibit amplification or overexpression of the proto-oncogene ERBB2 (HER2) and respond to blockade of HER2 receptor signaling. Triple-negative breast cancers do not express ER, PR or HER2, and for this subtype chemotherapy is the main treatment option and in general these cancers exhibit a worse prognosis. Basal-like breast cancers can be distinguished from triple-negative cancers through the assessment of expression of basal markers such as cytokeratin 5/6 and epidermal growth factor receptor (EGFR).

It is estimated that approximately 27% of human breast tumors carry gain-of-function mutations in the *PIK3CA* gene (as reviewed in Liu et al. 2008). Class I PI3K family members are heterodimeric lipid kinases comprising a catalytic p110 and regulatory p85 subunit. Somatic missense mutations in *PIK3CA* are clustered into two ‘hotspot’ regions in exon 9 and exon 20, corresponding to the helical and kinase domains of p110α, respectively. More than 80% of *PIK3CA* mutations occur at E542K, E545K or H1047R and these mutations are oncogenic resulting in increased Akt activation. *PIK3CA* mutations are most frequently observed in hormone-receptor-positive breast tumors and HER2-positive tumors and rarely in the triple-negative/basal-like cancers. In some studies *PIK3CA* mutations occur in a mutually exclusive manner from PTEN loss of function. However, concordance of *PIK3CA* mutational events with PTEN loss in breast cancer has also been described. The association between *PI3K* mutations and long term survival is complex, some studies have shown a surprising correlation of *PI3KCA* mutations with good prognosis. Gene expression and protein data from ~1800 breast cancers revealed *PIK3CA* mutations in ER-positive/HER2-negative breast cancers were associated with Akt pathway activation, but a relatively low mTORC1 signaling profile, and predicted for better clinical outcomes with
tamoxifen (which blocks ER signaling) therapy. Mouse models that express the PIK3CA-H1047R mutant in luminal mammary epithelium recapitulate the heterogeneity of breast cancer, with induction of carcinomas containing cells that express luminal or basal markers or both, with ER expression also observed. Liu and colleagues also generated transgenic mice expressing mutant human PI3KCA-H1047R under the control of a tetracycline-inducible promoter in mammary tissue. Heterogeneous breast cancer occurred in 95% of mice within 7 months, and tumors regressed following switching off of the transgene, but this was not complete and most tumors reoccurred, associated with Myc amplification. Therefore mutations PIK3CA that lead to activation of Akt signaling are common in ER-positive breast cancer, and appear to initiate breast cancer in mouse models, however their prognostic significance is still emerging.

**Role of PTEN in breast cancer**

PTEN is a tumor suppressor gene located at 10q23, a region frequently mutated in human cancers. PTEN is a negative regulator of PI3K activity, that hydrolyzes PtdIns(3,4,5)P\(_3\) to PtdIns(4,5)P\(_2\). PTEN contains an amino-terminal phosphatase domain, with a conserved catalytic CX\(_5\)R motif and a carboxyl-terminal domain containing a C2 domain. Loss of PTEN function increases PtdIns(3,4,5)P\(_3\) signals leading to unrestrained Akt activation resulting in increased cell survival, growth and proliferation.

In cancer reduced PTEN function can occur via mutation, loss of heterozygosity (LOH), protein instability and/or epigenetic modifications. Germline mutations of PTEN are present in 80% of families with autosomal dominant Cowden’s syndrome, which is associated with an elevated risk of breast cancer. Somatic and biallelic mutations of PTEN are common in high grade glioblastoma, melanoma, as well as prostate and endometrial cancers however, PTEN mutations in sporadic breast cancer are less frequent at 2.3% incidence and appear limited to ER/PR-positive tumors. LOH at the PTEN locus, 10q23, is found in 24.9% of breast carcinomas. PTEN protein expression is lost in approximately 28% of primary breast carcinomas. Loss of PTEN protein expression may be associated with enhanced lymph node metastasis and disease-related death. However, in contrast, a study of 292 breast cancer patients found no association between low PTEN levels and metastasis or disease-related death, but observed a correlation with the poor prognosis basal-like breast cancer subtype. Pten-null mice exhibit early embryonic lethality due to developmental defects. Pten\(^{+/-}\) mice develop spontaneous tumors of the thyroid, colon and gonado-stromal
tissues. Notably a *Pten* hypermorphic mouse, *Pten*<sup>hy/+</sup> has been generated that expresses 80% wild type levels of Pten. These mice spontaneously develop a range of tumors, with mammary tumors at highest prevalence. Significantly, the spectrum of tumors exhibited by *Pten*<sup>hy/+</sup> mice are distinct from those observed with the *Pten*<sup>+/−</sup> mouse, suggesting that subtle alterations in Pten expression may promote tumorigenesis in a tissue-specific manner.

**The role of the inositol polyphosphate 4-phosphatase INPP4B in breast cancer**

Recent studies have identified that the inositol polyphosphate 4-phosphatase, INPP4B, may function as a tumor suppressor in breast cancer. INPP4B, and the related INPP4A, hydrolyze PtdIns(3,4)P<sub>2</sub> producing PtdIns(3)P and also display catalytic activity towards the inositol phosphates Ins(1,3,4)P<sub>3</sub> and Ins(3,4)P<sub>2</sub>, however, PtdIns(3,4)P<sub>2</sub> is the preferred *in vivo* substrate. INPP4A and INPP4B share 37% amino acid identity, with N-terminal C2 domains and a C-terminal catalytic CX<sub>5</sub>R motif. INPP4A is critical for normal neuronal function. A spontaneous *Inpp4a* mutation in a mouse strain called Weeble (*Inpp4awbl*) results from a single base pair deletion (Δ74). The *weeble* mouse dies 2–3 weeks postnatally with cerebellar ataxia and growth retardation. Gene targeted deletion of *Inpp4a* in mice leads to a similar phenotype with evidence of glutamate neuroexcitatory cell death. INPP4A also regulates PI3K/Akt dependent signaling and cell survival. *Weeble* mouse embryonic fibroblasts (MEFs) exhibit Akt activation and anchorage independent cell growth. SV40-transformed *weeble* MEFs form tumors in nude mice. However there is limited evidence of changes in INPP4A expression in human cancers.

There is emerging evidence that INPP4B functions as a putative tumor suppressor in several human cancers, including breast cancer, prostate cancer and melanoma. INPP4B regulates PI3K/Akt signaling in breast cancer cell lines. shRNA-mediated INPP4B protein knockdown in ER-positive MCF-7 breast cancer cells results in increased Akt activation with enhanced colony formation in soft agar and increased xenograft tumor growth in nude mice. INPP4B overexpression in SUM149 cells, a *BRCA1* mutated human invasive ductal carcinoma cell line, decreases tumor growth in a xenograft mouse model. Knockdown of both INPP4B and PTEN in human mammary epithelial cells induces cell senescence, a phenotype rescued by p53 depletion. In the normal breast, INPP4B protein expression is limited to ER-positive mammary ductal luminal epithelial cells. Consistent with this INPP4B protein expression is restricted to ER-positive but not ER-negative breast cancer cell lines. However INPP4B expression does not appear to be regulated by ER signaling. In
human breast cancers, INPP4B expression is strongly associated with both ER and PR expression. Notably immunohistochemical INPP4B antibody staining of 374 primary human breast carcinomas revealed frequent loss of INPP4B protein expression in aggressive basal-like breast carcinomas (84% of cases). LOH at 4q31.21, the chromosomal region containing INPP4B, is also reported to occur in triple-negative/basal-like tumors (55% of cases). The lower frequency for LOH compared to loss of protein expression of INPP4B in triple-negative carcinomas suggests differences in the rates of allelic compared to INPP4B protein loss. INPP4B LOH is most commonly detected in BRCA1 mutant tumors (60% of cases). BRCA1 mutant and basal-like carcinomas are typically triple-negative for ER/PR and HER2 expression. INPP4B LOH also occurs in ovarian cancers (39.8% of cases). Reduced INPP4B protein expression correlates with decreased overall patient survival for both breast and ovarian cancer. The triple-negative/basal-like breast tumor subtype in which INPP4B LOH and decreased protein expression is observed are typically more metastatic with an unfavorable patient outcome. In a breast carcinoma patient cohort, with few BRCA1 mutant and basal-like subclasses, loss of INPP4B protein expression significantly correlated with reduced overall patient survival. Therefore, INPP4B expression in breast cancer may play a role in predicting long term survival. As loss of INPP4B is associated with enhanced PI3K-dependent Akt signaling in both cell lines and tissues it is likely these tumours may be response to treatment with PI3K and/or pathway inhibitors. No association has been reported between INPP4B protein expression and PIK3CA mutation/amplification. However, in one report INPP4B protein loss was significantly associated with PTEN-loss of function and high pAkt tumor levels. In contrast INPP4B loss of protein expression by itself is not associated with high pAkt levels. Both PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ are required for the full and sustained activation of Akt. Therefore, concurrent loss of INPP4B and PTEN, which degrade PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ respectively, may lead to the development of more aggressive forms of breast cancer with Akt hyperactivation.

INPP4B is also implicated as a tumor suppressor in prostate cancer with only one study to date. In primary prostate tumors, INPP4B and PTEN show reduced expression compared with normal tissue. Decreased expression of INPP4B is associated with reduced time to biochemical evidence of prostate cancer recurrence. INPP4B LOH has also been reported in a high proportion of melanomas (21.6% of cases).

Recently, the first Inpp4b knockout mouse was generated and characterized. Inpp4b is a significant regulator of osteoclast differentiation that can significantly impact on bone
mass. Inpp4b-deficient mice display osteoporosis, due to increased osteoclast cell populations and enhanced bone resorption. Interestingly, ablation of Inpp4b in mice did not result in spontaneous tumor development in mice up to 4 months of age. Therefore, INPP4B loss alone may not be sufficient to cause tumor development, with additional PI3K pathway aberrations required. Alternatively, INPP4B loss of function may be critical at later stages of tumor development.

Summary

Recent studies have identified INPP4B as a novel tumor suppressor that inhibits PI3K/Akt signaling and proliferation in ER-positive mammary cancer cells. Loss of INPP4B expression has been observed predominantly in basal-like/triple negative breast cancers and may be an additional predictive marker of poor outcome. Furthermore there is evidence that INPP4B is concurrently lost with PTEN in these poor prognosis breast cancer subtypes and it is likely these tumors may be candidates for treatment with PI3K pathway inhibitors. In addition several recent studies suggest INPP4B expression may be altered in melanomas and prostate cancer. Therefore it is likely that this newly identified tumor suppressor gene may be an important regulator of PI3K/Akt signaling in many human cancers.

Acknowledgments

This work was supported by a grant from the National Health and Medical Research Council (606621) (CAM).

Conflicts of interest

The authors declare no conflict of interest.

References

1. Ma, K., et al. 2008. PI(3,4,5)P3 and PI(3,4)P2 levels correlate with PKB/Akt phosphorylation at Thr308 and Ser473, respectively; PI(3,4)P2 levels determine PKB activity. Cellular Signalling. 20: 684-694.

Figure 1. The PI3K signaling pathway. Activation of PI3K at the plasma membrane by receptor tyrosine kinase (RTK) results in the transient production of phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P3] which is hydrolyzed by the inositol polyphosphate 5-phosphatases (5-phos) to generate PtdIns(3,4)P2. Both PtdIns(3,4,5)P3 and PtdIns(3,4)P2 facilitate the activation of Akt. PI3K signaling can be terminated through the actions of the 3-phosphatase, PTEN, which hydrolyzes PtdIns(3,4,5)P3 to produce PtdIns(4,5)P2. PtdIns(3,4)P2 can be hydrolyzed by the actions of the 4-phosphatase, INPP4B, to generate PtdIns(3)P. Alterations that have been reported in these enzymes in breast cancer are indicated in the boxed areas.
- ~27% breast cancers harbor gain-of-function mutations in the PIK3CA gene (Liu et al. 2009).
- Over 80% of PIK3CA mutations are clustered into hotspots at E542K, E545K and H1047R.

- Loss of PTEN protein expression occurs in ~28% of primary breast cancers (Lopez-Knowles et al. 2010).
- PTEN LOH occurs in ~25% of breast cancers (Liu et al. 2009).

- INPP4B protein loss occurs in 84% basal-like breast cancers (Fedele et al. 2010).
- INPP4B LOH occurs in 55% of triple-negative/basal-like cancers (Gewinner et al. 2009).
- INPP4B LOH occurs in 60% of BRCA1 mutant tumors (Gewinner et al. 2009).