

Review

Phosphoinositide 3-kinase signalling in breast cancer: how big a role might it play?

Michael John Fry

School of Animal and Microbial Sciences, University of Reading, Reading, UK

Correspondence: Michael John Fry, School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ, UK. Tel: +44 118 987 5123, ext 7028; fax: +44 118 931 0180; e-mail: M.J.Fry@reading.ac.uk

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Abstract

Phosphoinositide 3-kinase (PI3K) was first identified as a lipid kinase activity associated with the products of viral oncogenes and with activated protein-tyrosine kinases. Since those early studies, the PI3K superfamily has grown to embrace at least 12 structurally and functionally related enzymes present in the human genome, some of which have protein kinase activity but not lipid kinase activity. Evidence is emerging that PI3K superfamily members, and components of PI3K signalling, play a role in the development of many human cancers. In this review, the PI3K family of enzymes and their signalling is reviewed, with particular reference to possible involvement in breast cancer.

Keywords: Akt, ataxia telangiectasia mutated, BRCA1, phosphoinositide 3-kinase, PTEN

Introduction

Phosphoinositide 3-kinase (PI3K) was first detected in the 1980s as a novel lipid kinase activity found associated with several oncogene products (*v-Ros*, *v-Src* and polyoma virus middle T antigen [mT]) and subsequently with many activated growth factor receptor complexes [1]. It is surprising, given this beginning, that it was not until the late 1990s that a PI3K was isolated, first as a retroviral oncogene and then implicated in several human cancers [2]. It is now clear that alterations in PI3K signalling cassettes can lead to changes in a number of cell functions that contribute to the transformed phenotype, including cell growth and proliferation, differentiation, cell survival, adhesion and cell motility [2,3]. The PI3K family of enzymes, their targets, and regulators are thus now considered important potential therapeutic targets [4]. In this review, the PI3K superfamily will be briefly described with respect to how these enzymes function and are regulated. The emerging data implicating PI3K signalling in

human cancer, with special reference to possible roles in breast disease, will be considered.

The PI3K superfamily

The PI3K superfamily is defined by sequence motifs present in the catalytic domain of these enzymes [1,5]. There are at least 12 members of this family present in the human genome that can be divided into two main groups. The true PI3Ks display lipid kinase and some protein kinase activity, and are further subdivided into Class I–III enzymes. The PI3K-related enzymes (Class IV) are large proteins that possess protein kinase activity only [6].

Class I PI3Ks

Class I PI3Ks have been studied most extensively and are best understood. It was a Class I PI3K that was originally found associated with oncogene products, and a member of this family was the first to be cloned [1]. Class I PI3Ks are activated by diverse cell surface receptors including

Akt = protein kinase; ATM = ataxia telangiectasia mutated; ATR = ataxia telangiectasia related; DNA-PK = DNA-dependent protein kinase; EGF = epidermal growth factor; ER = oestrogen receptor; mT = middle T antigen; PDK = phosphoinositide-dependent protein kinase; PH = pleckstrin homology; PI = phosphoinositide; PI3K = phosphoinositide 3-kinase; PtdIns = phosphatidylinositol; PTEN = phosphatase and tensin homologue deleted on chromosome 10; PTK = protein-tyrosine kinase; TOR = target of rapamycin.

G-protein-coupled receptors, by receptors with either intrinsic or associated protein-tyrosine kinase (PTK) activity, by association with tyrosine phosphorylated proteins (e.g. insulin-regulated substrate 1) and, in addition, by the action of small G proteins (e.g. Ras) [1]. Class I PI3Ks can phosphorylate phosphatidylinositol (PtdIns), PtdIns4P and PtdIns(4,5)P₂ *in vitro* to generate PtdIns3P, PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃, respectively. PtdIns(4,5)P₂, on activation *in vivo*, is believed to be the preferred substrate. PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ are found at low levels in resting cells and increase dramatically on stimulation [1]. Class I PI3Ks also possess some protein kinase activity towards specific substrates, but the significance of this remains controversial [1,5]. Class I is subdivided into Class IA and Class IB.

Class IA PI3Ks are heterodimeric, consisting of a 110 kDa catalytic subunit and a 55–85 kDa regulatory subunit [1,5]. There are three Class IA catalytic subunits in the human genome: p110 α , p110 β and p110 δ (Fig. 1). There are also three regulatory subunits (p85 α , p85 β and p55PIK), although additional forms exist owing to alternative splicing. All of the regulatory subunits contain two Src homology 2 domains flanking a p110-binding site. The p85 isoforms also possess an N-terminal Src homology 3 domain. These Src homology 2 and Src homology 3 domains mediate the recruitment and regulation of Class IA PI3Ks by a multitude of activated PTKs, with the PI3K usually being recruited either directly to the receptor via binding to autophosphorylation sites or via substrate proteins such as insulin-regulated substrate 1 [1].

A single Class IB heterodimeric enzyme exists, consisting of the 110 kDa catalytic subunit, p110 γ , and a 101 kDa regulatory subunit. Unlike the p85 subunits, p101 contains no obvious signalling modules or motifs. The Class IB enzyme is subject to regulation by G-protein signalling and thus is linked to activation by G-protein-coupled receptors [1,5].

Class II PI3Ks

The Class II PI3Ks are structurally related to the Class I enzymes, but lack the p85/p101 binding motifs and instead possess additional regulatory domains including the phox homology and C2 domains (Fig. 1) [5]. Class II PI3Ks can utilise PtdIns and PtdIns4P, but not PtdIns(4,5)P₂ *in vitro*. Like Class I enzymes, Class II PI3Ks are linked to diverse receptor-mediated signalling processes, including integrin signalling in platelets, chemokine signalling, insulin, and epidermal growth factor (EGF) and platelet-derived growth factor signalling (see [7] and references therein). There are three human Class II PI3Ks: PI3KC2 α and PI3KC2 β are widely expressed in mammalian tissues [8,9], and PI3K-C2 γ is restricted to a few tissues including the liver, breast and prostate [10,11]. The function(s) and *in vivo* substrates of the Class II PI3Ks remain to be determined.

Class III PI3Ks

Class III PI3Ks are PtdIns-specific enzymes generating only PtdIns3P *in vitro* or *in vivo*, the prototype being the *Saccharomyces cerevisiae* protein Vps34p (the only PI3K present in this yeast genome), which is involved in vesicle sorting and trafficking [5,12]. A single mammalian homologue has been identified and is suggested to play a similar role in intracellular trafficking processes. The human and yeast Vps34 proteins both associate with an N-terminally myristoylated serine/threonine kinase (Vps15/p150), which recruits Vps34 to cell membranes and enhances its lipid kinase activity [5,12]. While this Class of PI3K is not normally considered when looking at cell proliferation and cancer (PtdIns3P levels are comparable in resting and growth factor stimulated cells), it cannot be completely ruled out as inhibitory antibodies specific for hVps34 can block insulin-stimulated DNA synthesis [13].

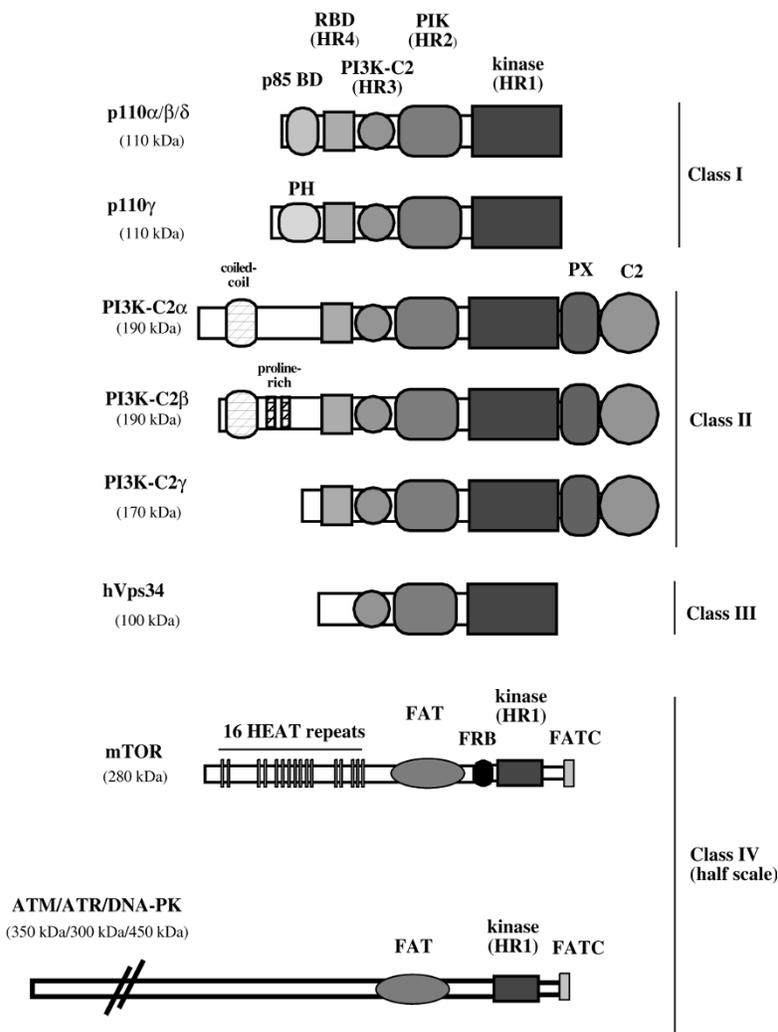
Class IV: PI3K-related enzymes

This family of high molecular weight proteins includes the target of rapamycin (TOR)/FKBP rapamycin-associated protein and the ataxia telangiectasia mutated/ataxia telangiectasia related/DNA-dependent protein kinase (ATM/ATR/DNA-PK) family of enzymes [6,14]. TOR proteins were cloned as targets of rapamycin, a fungicide and immunosuppressant [14]. They have subsequently been linked to the integration of mitogenic and nutritional signals through the regulation of initiation of translation via the substrates p70 S6 kinase and eukaryotic initiation 4E binding protein (4E-BP-1 or PHAS-1) and entry into the G1 phase of the cell cycle [14]. ATM, ATR and DNA-PK are linked to meiotic and V(D)J recombination, repair and maintenance of chromosomes (in response to various damaging agents including ionising radiation), and cell-cycle checkpoint control [6]. While Class IV enzymes possess some of the conserved motifs present in the catalytic domain of the true PI3Ks, they lack others such as the PIK domain (Fig. 1). Although initial studies suggested that Class IV enzymes might have lipid kinase activity, the consensus is that they are all exclusively protein kinases. Class IV enzymes are considered here for two reasons: they are sensitive to the widely used PI3K inhibitors (see next section), and they are linked to diverse human diseases ranging from immunological disorders to cancer, including that of the breast.

PI3K family inhibitors

There are two commonly used, structurally unrelated, cell-permeable PI3K inhibitors: wortmannin and LY294002 [4,5]. Much of the work linking PI3K activity to various cellular processes has been carried out using these PI3K inhibitors, and this has led to many processes being linked to PI3K activity. However, there is less information as to which of the classes of enzyme described in the preceding sections are involved in specific processes. These PI3K inhibitors do not distinguish between the different

Figure 1



The phosphoinositide 3-kinase (PI3K) superfamily. Schematic representation of the catalytic subunits of the PI3K superfamily of enzymes. The names of the kinases are given on the left, together with their size (kDa). Classification by class is indicated by the bars on the right. Recognised domains are indicated. ATM/ATR/DNA-PK, Ataxia telangiectasia mutated/ataxia telangiectasia related/DNA-dependent protein kinase; FAT, domain present in FKBP rapamycin-associated protein/target of rapamycin (TOR), ATM and TRRAP (ATM-related) proteins; FATC, C-terminal domain found only in FAT domain containing proteins; FRB, FKBP12/rapamycin binding domain; HEAT, domain found in Huntington, EF3, a subunit of protein phosphatase 2A and TOR; HR1–HR4, homology region; p85 BD, p85 binding domain; PH, pleckstrin homology; PX, phox homology; RBD, Ras binding domain.

PI3K isoforms or Classes I–III and, in addition, when used at higher concentrations (>100 nM wortmannin, >50 μM LY294002) they inhibit the Class IV PI3K-related protein kinases. Both agents have been shown experimentally to inhibit tumour growth and to sensitise cells to radiation treatment, and this has enhanced the idea that PI3Ks are potential therapeutic targets [4,15–18].

PI3K signalling

The molecular basis of PI3K signalling has emerged in the past few years and is dependent on the presence of lipid recognition modules found in target proteins. The two key domains so far identified are the pleckstrin homology (PH) domain and the FYVE domain [3,5]. The FYVE domain

seems to be involved predominantly in the recognition of PtdIns3P. PH domains with specificity for PtdIns(3,4,5)P₃, PtdIns(3,4)P₂, PtdIns(4,5)P₂ and PtdIns3P have been reported [3,5]. On activation of an appropriate PI3K, 3-phosphorylated inositol lipids are produced in the target membrane and proteins containing high-affinity binding sites for these lipids are subsequently recruited. Key PH domain proteins involved in signalling via PI3Ks include the phosphoinositide-dependent protein kinase 1 (PDK1), protein kinase B (Akt) and a number of exchange factors for small G proteins of the Ras superfamily [3,5]. Recruitment of these signalling proteins to membranes brings them into close contact with their substrates or target proteins, thus potentiating further signalling events.

Termination of PI3K signalling is caused by the action of phosphoinositide (PI) phosphatases that work on distinct phosphates of the inositol ring (Fig. 2). The 5-PI phosphatases can attenuate Class I PI3K signalling by removing the 5' phosphate of PtdIns(3,4,5)P₃. This results in the production of PtdIns(3,4)P₂, which is also a signalling molecule and the preferred binding lipid for several PH domains [3]. PI3K signalling can be completely terminated by the action of a recently identified family of dual-specificity protein phosphatases/3-PI phosphatases. The prototype member of this family is the tumour suppressor gene product PTEN, the phosphatase and tensin homologue deleted on chromosome 10 (also known as MMAC-1 and TEP-1) [19]. Because this phosphatase acts specifically on the 3' phosphate, it has the potential to inactivate all possible PI3K products.

The best understood PI3K target is Akt, a serine/threonine kinase [20]. Akt is recruited, on PI3K activation and 3-PI production, to cell membranes via its PH domain. This interaction also appears to expose a phosphorylation site in the activation loop that is then phosphorylated by other kinases stimulated by 3-PIs including PDK1 and PDK2. Once activated, Akt phosphorylates a number of proteins leading to regulation of metabolism (via glycogen synthase kinase 3), translational control (via p70 S6 kinase and PHAS-1), and cell survival (via forkhead family transcription factors, BAD and caspase 9) [20]. Akt is also linked to regulation of the cell cycle through cyclin D₁ and E2F [20].

PI 3-kinase family signalling in cancer

PI3K activity has, from the start, been linked with many aspects of the cell transformation process, including increased cell growth, proliferation and survival, adhesion, metastasis and angiogenesis [2]. The first reports of PI3K activity saw it linked to transforming gene products of the acutely transforming retroviruses, such as v-Src and v-Ros, and to polyoma virus mT [1]. It was subsequently shown that the presence of PI3K activity is critical for the transforming potential of these oncogenes. Mutant forms of mT, Src and Abl viral oncoproteins, which fail to bind and activate PI3K, are greatly impaired in transforming ability. This activity is believed to be a Class I PI3K.

Class I PI3Ks

In 1997, a Class IA PI3K catalytic subunit was isolated as the transforming gene of a chicken sarcoma virus, *v-p3k* [2]. This virus is fused with the retroviral *gag* gene, resulting in a *gag-v-p3k* fusion protein that is N-terminal myristoylated and targeted to cell membranes. Experimental manipulation of Class I PI3K by the addition of membrane targeting sequences leads to the formation of constitutively active PI3K that is able to induce many aspects of cell transformation [2].

The first direct reports that PI3K catalytic subunits might be altered in human cancer came with the reports that amplification of 3q26 is seen in 40% of ovarian cancer and in many other cancers. The PIK3CA gene (encoding the Class I p110 α) is located at this locus, and ovarian and cervical tumours were found to have elevated protein and PI3K activity levels [2,21]. Surprisingly, given the evidence for the activation of Class IA catalytic subunits in cancer, it has recently been reported that the loss of the Class IB enzyme PI3K γ results in colorectal adenocarcinomas in mice. It has also been reported that this is also observed in primary colorectal adenocarcinoma and in colon cancer cell lines in humans. A possible tumour suppressor role for some PI3Ks therefore also needs to be considered [2]. There is no strong evidence to date in support of any role for Class II or III PI3Ks in human cancer, although there have so far been few studies in this area.

Target of rapamycin

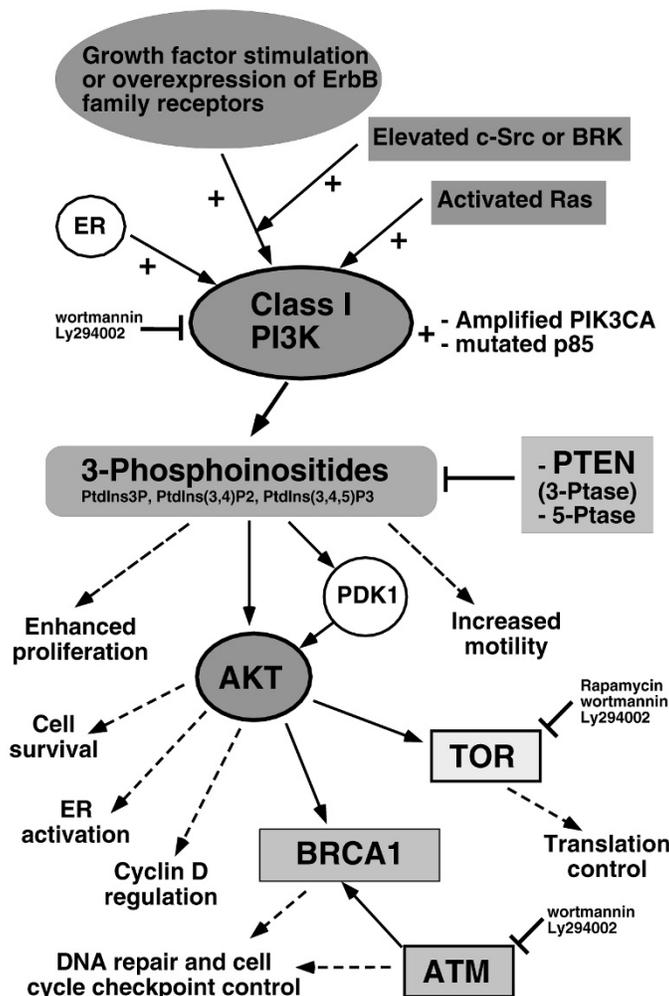
The ability of TOR to act as an oncogene remains to be determined, but TOR activity is required for PI3K-mediated and Akt-mediated cell transformation [22]. This fits with experimental data that places TOR downstream of PI3K/Akt in signalling cascades and points to the importance of this pathway to aspects of the fully transformed phenotype. A number of human tumour cell lines have been reported sensitive to growth inhibition by rapamycin, thus the TOR proteins may provide a more selective target for a chemotherapeutic agent. A derivative of rapamycin with antitumour activity, CCI-779, is currently in phase I trials [23].

PI3K family in normal and malignant breast

A polymerase chain reaction based screen has been used to identify PI3Ks in the human breast using mRNA derived from cells and epithelial tissue from reduction mammoplasty. Using this approach, all eight Class I-III PI3K isoforms were identified [10]. Of the eight PI3Ks, all but the Class IB PI3K γ were expressed in the epithelial component of the tissue. Most PI3Ks are widely expressed in human tissues, but the PI3K-C2 γ isoform is expressed to significant levels only in the breast, prostate and liver [10,11]. Detailed studies of PI3K catalytic subunit expression in breast cancer are rare. However, a recent study on a series of tumour and adjacent mammary glands from 33 patients observed increased levels of p85 regulatory subunit in the tumour over adjacent tissues in 79% of their pairs, which was not linked to other prognostic factors [24]. Further studies focusing on the catalytic subunits and lipid products are clearly necessary to assess the relevance of this finding.

Evidence for PI3Ks playing a role in mammary carcinogenesis arises primarily from animal model studies and cell-based studies. Amundadottir and Leder [25] studied five different transgenic mouse models of malignant transfor-

Figure 2



Phosphoinositide 3-kinase (PI3K) signalling and cancer. Schematic signalling pathway indicating the interactions that occur between known breast cancer oncogenes (ErbB2, Src) and tumour suppressors (PTEN, BRCA1) with components of the PI3K signalling cassette. Direct physical interactions are shown with solid arrows. Processes involving several steps are indicated with a dashed arrow; +, an activation step. Blunt arrows indicate inhibitory effects. Akt, Protein kinase B; ATM, ataxia telangiectasia mutated; ER, oestrogen receptor; 3-Ptase and 5-Ptase, phosphoinositide 3-phosphatase and phosphoinositide 5-phosphatase activities; PTEN, phosphatase and tensin homolog deleted on chromosome 10; TOR, target of rapamycin.

mation of mammary epithelium (initiated by *ErbB2/neu*, *heregulin/NDP*, *TGF α* , *v-Ha-ras* and *c-myc*) and found, on the basis of inhibitor studies using LY294002, that PI3K activity made a contribution to all the transformed phenotypes (in particular, to anchorage-independent growth). PI3K is also implicated in transformation of the murine mammary gland mediated by polyoma virus mT, where studies with mutants of mT defective in binding PI3K where shown to induce high levels of apoptosis [26].

Activation of PI3K signalling by PTKs in breast cancer

The best-studied mechanism of Class I PI3Ks activation is via their recruitment to activated PTKs [1]. PI3Ks can be

activated via signalling through ErbB family receptors (mainly via association with ErbB3, which has six PI3K binding sites). It is therefore likely that, in the many breast tumours where there is amplification of ErbB family members, in particular of ErbB2, that there will also be enhanced PI3K signalling (Fig. 2) [4,27,28]. Activation of PI3K through either growth factor activation or overexpression of ErbB family receptors is linked to enhanced migration of breast cancer cells [29,30]. The cytoplasmic PTK Src is overexpressed in a high percentage of breast cancers, and this kinase is capable of sensitising cells to signalling via EGF. One of the effects of Src overexpression is to enhance coupling of the EGF receptor to PI3K signalling [31]. Another cytoplasmic PTK, BRK, has

recently been implicated as possibly playing a role in breast cancer. BRK has a similar domain structure to the Src family non-receptor PTKs. Whereas BRK expression is low or undetectable in normal mammary tissue or benign lesions, 27% of breast tumours overexpress BRK fivefold or more [32]. Overexpression of BRK in mammary epithelial cells enhances the ability of the cells to grow in an anchorage-independent manner and sensitises them to EGF [33]. BRK overexpression has recently been shown to enhance the coupling of EGF signalling to PI3K/Akt via ErbB3 receptor phosphorylation [34]. These effects on PI3K and Akt activities may account for the ability of BRK to enhance mammary epithelial cell mitogenesis, and raises the possibility that breast tumours overexpressing BRK may have enhanced resistance to apoptotic signals. Taken together, these facts suggest that overexpression, or activation, of various PTKs in breast cancer may lead to PI3K signalling in a high percentage of breast tumours. This clearly deserves further study.

PTEN, negative regulator of PI3K signalling

Deregulation of PI3K signalling can also arise from the loss of the normal regulatory proteins. PTEN is clearly a major regulator of the 3-PIs produced by PI3Ks [19]. PTEN maps to 10q23, a chromosomal region that frequently displays loss of heterozygosity. Mutation of PTEN is one of the more common somatic mutations in human cancer and is found particularly in ovarian, prostate and glioblastoma cancers. Germ-line mutations in the PTEN gene are responsible for Cowden syndrome, of which breast cancer is a major feature. Loss of heterozygosity at the PTEN locus 10q23 is observed in 30–40% of sporadic breast cancers, but reports of PTEN mutations suggest that this occurs only in <5% of breast tumours. Evidence for a complete lack of PTEN protein in cases with hemizygous deletions of PTEN, however, suggests that an epigenetic mechanism may also play a role [35]. Loss of PTEN activity, whether by point mutation or deletion, results in elevated levels of 3-PIs and activation of PI3K targets such as Akt [2,19]. Studies of PTEN-deficient mice have found that the loss of this gene alone only rarely results in mammary carcinomas. However, the loss of PTEN accelerates the development of mammary oncogenesis by other mammary oncogenes not known to target PI3K signalling, such as Wnts [36]. Conversely, overexpression of PTEN in MCF-7 breast cancer cells induces apoptosis and cell-cycle arrest through a combination of PI3K/Akt-dependent and PI3K/Akt-independent pathways [37].

PI3K, Akt and cyclin D₁

There are three Akt proteins (proteins 1–3, i.e. PKB α , PKB β and PKB γ) that are widely expressed in most human tissues [20]. Prior to identification as a PI3K target, Akt had been found as a retroviral oncogene (gag-akt). Akt genes are amplified, or the protein is overexpressed, in a

number of human cancers including gastric, ovarian, breast, pancreatic and prostate cancer [20]. Interestingly, Akt3 is found to be upregulated in both oestrogen receptor (ER)-deficient breast cancers and in androgen-independent prostate cancer cell lines, suggesting this enzyme may contribute to the more aggressive clinical phenotype of these diseases [38].

D-type cyclins are targets for a number of growth-factor signalling pathways and their expression is elevated in a number of human tumours including those of the breast. D-type cyclins associate with cyclin-dependent kinases to form active complexes involved in the regulation of the retinoblastoma protein, and thus are involved in cell-cycle regulation. Several lines of evidence suggest that PI3K signalling is important in the regulation of cyclin D₁ at a post-transcriptional level [39,40]. Coexpression of an activated form of Akt with a transformation-defective polyoma virus mT mutant incapable of interacting with PI3K led to upregulation of cyclin D₁ and a dramatic acceleration of mammary tumourigenesis that correlated with reduced apoptotic cell death [41]. Activated Akt was unable, however, to restore wild-type metastasis levels.

BRCA1 and PI3K signalling

Links have also been forged between PI3K superfamily members, PI3K signalling pathways, and other gene products involved in breast cancer, notably BRCA1. Heregulins, a group of growth factors that regulate growth, differentiation and survival of a number of breast cancer cell lines, bind to members of the ErbB family of receptors and initiate signalling via Ras/MAPK and PI3K pathways. Heregulins have been shown to induce phosphorylation of BRCA1 in breast cancer cells in a PI3K/Akt-dependent manner [42]. A constitutively active PI3K was sufficient to induce BRCA1 phosphorylation, which could be blocked by PI3K inhibitors or by dominant negative Akt expression. Akt was shown to directly phosphorylate BRCA1 *in vitro* at a site close to the nuclear localisation signal sequence. Several lines of evidence have suggested that BRCA1 phosphorylation during cell-cycle progression and in response to a DNA damaging agent may affect its function. While the significance of this phosphorylation is currently unclear, its site close to the nuclear localisation sequence suggests that it may affect nuclear translocation and hence BRCA1 function.

ATM and cancer

Loss of ATM results in the hereditary syndrome ataxia telangiectasia [6]. Carriers who harbour single germ-line mutations in ATM are reported to be at increased risk of breast cancer (see [43] and references therein). This link between ATM and breast cancer remains controversial, since ATM mutations have not been found in large breast cancer case–control studies [44]. It has been hypothesised, however, that this may be owing to the presence of

more than one type of ATM heterozygote, and to the approaches taken to identify ATM mutations in large-scale studies that have largely focused on looking for truncating mutations rather than missense types of mutation [45].

Biochemical data also implicates ATM in breast cancer. BRCA1 is a target for phosphorylation by the Class IV PI3Ks, ATM and ATR, in response to infrared-induced and ultraviolet-induced DNA damage, respectively (Fig. 2) [46–48]. ATM also phosphorylates a BRCA1-associated protein, CtIP, which may modulate BRCA1-dependent regulation of the DNA damage response gene GADD45 [49]. Both ATM and ATR kinases are able to phosphorylate another breast cancer predisposition gene p53, the cause of Li–Fraumeni syndrome [6]. If one role of ATM/ATR is to transmit cell cycle or DNA damage signals to BRCA1 and p53, then this may explain why loss (or partial loss) of ATM function may lead to an increase in susceptibility to breast cancer.

PI3K signalling and oestrogen-mediated responses in breast tumour cells

A PIK activity was first reported associated with the ER in 1986 [50]. A recent report suggests that, in vascular endothelial cells, this interaction involves a Class I PI3K and a non-nuclear ER [51]. PI3K activity has also been linked to both proliferative and anti-apoptotic effects of oestrogen in breast and other tissues [52–54]. Further complications in this area arise from two recent reports that PI3K/Akt can activate the ER in the absence of oestrogen [55,56]. This might help to explain how anti-oestrogen resistant growth of ER-positive tumours, a significant clinical problem, may arise. This is clearly an area in need of further research.

Conclusions

PI3K activity is associated with a broad range of cancer-related functions, including cell proliferation, survival, and migration, that has resulted in increased interest in the PI3K family as drug targets [4]. Given the number of tumours in which a component of the PTK/PI3K/PTEN/Akt cassette is altered (Fig. 2), it is probable that this pathway will make a significant contribution to a high percentage of cancers, which at present can only be guessed at. What is also clear is that this pathway, and those regulated by the Class IV PI3K-related enzymes such as ATM, can also interact with BRCA1 and p53, suggesting that all these proteins may be functionally linked in the maintenance of normal breast tissue status. What percentage of breast cancers might have activated PI3K signalling is not clear. However, given that PI3Ks are activated by ErbB family PTK receptors as well as by the cytoplasmic PTKs Src and BRK (which together are overexpressed in >25% of all breast tumours), and given that Ras mutations and PTEN loss are each found in another ~5% of breast tumours, it may be that a significant number of breast

tumours would be targets for small molecules directed at components of PI3K signalling.

How can this be clarified? There is currently no suitable test for looking at changes of 3-PIs in clinical samples and sections. Various PH and FYVE domain reporters coupled to detection systems, currently in use to study changes in lipid levels and location in cell-based assays, may soon provide the tools to look at these changes. At present, the best indication of PI3K activation (by any means), or PTEN loss, is probably to screen tumours with antibodies that recognise the phosphorylated (active) form of Akt.

Do more specific PI3K inhibitors have potential as useful inhibitors of breast tumour growth or as sensitising agents for use with radiotherapy? Given the widespread commercial interest in these drugs, it is hoped that second-generation PI3K inhibitors should start to see the light of day in phase I trials and in research laboratories in the next few years [4]. Preliminary studies have shown that, at high concentrations (high nanomolar–low micromolar), even the low specificity PI3K inhibitors, such as wortmannin, have *in vitro* and *in vivo* anti-tumour activity against a variety of tumours including mammary tumours [15,18]. While demonstrating the potential of wortmannin as a chemotherapeutic agent, these effects do not correlate well with inhibition of PI3K activity and thus may be owing to the inhibition of other PI3K-related enzymes such as TOR or ATM/ATR/DNA-PK [18]. Possible uses of PI3K inhibitors can be envisaged when administered as part of a combinatorial therapy with other agents. Wortmannin has been shown to have a number of potential effects on tumours, including sensitising them to the effects of radiation [16] and enhancing the toxicity of receptor-directed-toxin chimeras [17]. What is needed now is some way of specifically delivering them to the tumour. There appears to be a lot of potential here and further work is clearly needed to fully assess the contribution that the PI3Ks and their signalling cassettes play in the development of breast disease.

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