

REVIEW

Recent advances in the genetic analysis of PTEN and PI3K innate immune properties

Philipp Günzl, Gernot Schabbauer*

Institute for Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology, Medical University Vienna, A-1090 Vienna, Austria

Received 23 July 2008; accepted 23 July 2008

Abstract

The role of the PI3-kinase pathway and its antagonist PTEN in the regulation of innate immune responses has only recently attracted the attention of the scientific community. The PI3K/PTEN signaling axis is most renowned for its critical involvement in the malignant transformation of cells leading to tumorigenesis. PI3K function in the regulation of innate immunity, either pro-inflammatory or anti-inflammatory, is still a controversial issue. Undoubtedly, PI3K serves as an essential pro-inflammatory signaling molecule to activate leukocytes, initiate migration and facilitate phagocytosis. Nevertheless, it is less clear how PI3K and PTEN modulate the amplitude of immune responses. Here, we review recent advances on the immune biology by means of reverse genetics analyzing the role of the PI3K/PTEN signaling cascade in innate immunity.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: PTEN; PI3K; Innate immunity; TLR

Contents

Introduction	760
PI3K – lipid kinase family	760
PTEN – lipid phosphatase	761
The controversy on innate immune functions of PI3K	761
Genetic analysis of PI3K properties in TLR-mediated signaling	761
Genetic analysis of PTEN in innate immunity	762
Concluding remarks	762
Acknowledgement	763
References	763

Abbreviations: ERK, extracellular signal-regulated kinases; GPCR, G-protein coupled receptor; GSK3 β , glycogen synthase kinase 3 β ; IFN, interferon; IKK, I κ B kinase; IL, interleukin; IRF, interferon regulatory factor; JNK, C-jun n-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF κ B, nuclear factor κ B; PDK, phosphoinositide-dependent kinase; PI3K, phosphoinositide-3 kinase; PtdIns, phosphatidylinositol; PTEN, phosphatase and tensin homologue on chromosome 10; RTK, receptor tyrosine kinase; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adapter-inducing interferon- β ; VSV, vesicular stomatitis virus.

*Corresponding author. Tel.: +43 1 4277 62508; fax: +43 1 4277 9625.

E-mail address: gernot.schabbauer@meduniwien.ac.at (G. Schabbauer).

Introduction

Phosphoinositide-3 kinase (PI3K) is a key enzyme governing a plethora of cellular processes (Cantley, 2002). The most prominent among these are cell survival, site-directed migration and phagocytosis, all of which are tightly regulated by different PI3Ks in inflammatory reactions and infectious diseases.

PI3K and downstream effector kinases, such as AKT, also known as protein kinase B, were initially described for attenuating apoptosis, promoting cell survival and, therefore, contributing to tumorigenesis (Burgering and Coffey, 1995; Staal, 1987). Some time later a tumor suppressor was found to counteract the PI3K/AKT signaling axis. This phosphatase, called “phosphatase and tensin homologue deleted on chromosome 10” or abbreviated PTEN, counter-regulates PI3K activity by dephosphorylating PI3K targets (Li et al., 1997; Liaw et al., 1997; Marsh et al., 1997).

PI3K phosphorylates the plasma membrane phospholipid phosphatidylinositol (PtdIns) on the 3-position of the inositol-ring to generate various forms of phosphorylated PtdIns, such as PtdIns(3,4,5)P₃. These phospholipids are bound by proteins such as AKT and phosphoinositide-dependent kinase (PDK)1 via their pleckstrin homology domains, to direct these proteins to the plasma membrane and activate subsequent kinase signaling activity (Vanhaesebroeck et al., 2001; Fig. 1).

PI3K – lipid kinase family

The PI3K family can be divided into three subclasses. Of those, only class I family members have been studied extensively. Little is known about the class II and class III enzymes, which have different PtdIns substrates. These PI3K subclasses are less well described and will not be a topic of this review.

Class I PI3K is subdivided into class IA, which is the major PI3K group, and class IB. Class IA enzymes consist of a regulatory subunit (p85 α , p85 β), which is required for correct regulation and spatial localization of the active hetero-dimer, and a catalytic subunit (p110 α , p110 β , p110 δ), harboring the catalytically active lipid kinase domain (Cantley, 2002). Class IA PI3Ks are activated by receptor tyrosine kinases (RTKs) or respective adaptor proteins. The latter is the case for Toll-like receptor (TLR)-mediated PI3K activation. Disruption of genes for either one of the major regulatory subunits p85 α (including all splice variants) or catalytic subunits, p110 α or p110 β , results in a lethal phenotype in mice. Importantly, there are two different p85 α -deficient mouse strains available to date. The first mouse strain, developed by the group of Lewis Cantley, shows a lethal phenotype, exhibiting extensive liver necrosis, with only a fraction of animals surviving the first couple of weeks postnatally. These data prove that p85 β cannot entirely substitute for the loss of p85 α (Fruman et al., 2000). Moreover, p85 α and p85 β double-deficient mice die even earlier in embryonic development, suggesting that there is at least some redundancy between the regulatory subunits (Brachmann et al., 2005). On the other hand, the group of Shigeo Koyasu generated a p85 α -deficient mouse, which still expresses smaller splice variants of p85 α , namely p50 α and p55 α . This mouse line displayed a normal gross phenotype without any overt patho-physiologic changes, proving that these isoforms can compensate for the deletion of the full gene product (Terauchi et al., 1999). This strain was among the first to be described for clear pathologic alterations in innate immune responses (see below for more details) (Fukao et al., 2002a, b).

Mice deficient for p110 δ and p85 β are viable, which indicates cell-type-specific properties for these subunits. In particular, p110 δ seems to be primarily expressed in the hematopoietic cell lineage.

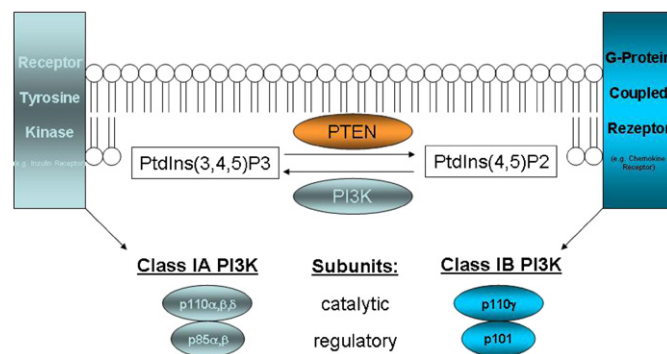


Fig. 1. PI3-kinase subclasses comprise different subunits and are activated by specific receptor families. The active class IA or IB PI3-kinase consists of a heterodimeric protein complex made up of a catalytic subunit and a regulatory subunit. Class IA PI3K is activated by RTKs, while Class IB PI3K is activated by GPCRs. The regulatory subunit harbors a SH2-domain, which directs the kinase to phosphorylated domains within activated receptors or adaptor molecules. Subsequently, the catalytic domain phosphorylates e.g. PI(4,5)P₂ to PI(3,4,5)P₃. PTEN antagonizes the PI3K catalytic function by dephosphorylating specifically the 3 position of PIP₃.

In contrast, class IB PI3K, which consists of a single regulatory (p101) and catalytic subunit (p110 γ), is activated by G proteins and G protein-coupled receptors. These receptors are typically involved in chemotactic signaling and important for leukocyte diapedesis (Hirsch et al., 2000). Class IB PI3K-deficient mice are viable, probably due to the restricted expression of p110 γ to the hematopoietic cell lineage. This is similar to the class IA p110 δ knock-out mentioned above. The class IB p110 γ $-/-$ mice are among the most extensively studied PI3K gene-targeted animals (Hirsch et al., 2000; Li et al., 2000; Sasaki et al., 2000).

PTEN – lipid phosphatase

The primary function of PTEN is to antagonize PI3K. It is a lipid phosphatase with only limited potential to dephosphorylate protein substrates. PTEN specifically dephosphorylates the 3-position on PtdIns, predominantly PtdIns(3,4,5)P₃, to generate PtdIns(4,5)P₂. This phospholipid is inert and cannot attract proteins to the plasma membrane (Vanhaesebroeck et al., 2001).

PTEN is regulated at multiple levels. Phosphorylation of PTEN itself is one key feature in the regulation of the phosphatase, which is mediated by kinases such as casein kinase II. Phosphorylated PTEN is supposed to be the inactive form, whereas dephosphorylation leads to activation and hydrolysis of PtdIns(3,4,5)P₃. PTEN regulation has been reviewed recently (Gericke et al., 2006).

One obstacle to study the patho-physiologic functions of PTEN is the fact that PTEN deficiency in mice leads to early embryonic lethality. Even in PTEN heterozygous animals, the incidence of tumor development is very high, which prevents appropriate analysis of immune-modulatory PTEN properties (Suzuki et al., 1998). Fortunately, the group of Tak Mak and others have created cell-type-specific, conditional PTEN knock-out mice using the cre lox-p system. Crossing LysM cre transgenic mice, which leads to cre expression in granulocytes and monocytes, with lox-p PTEN animals allows the study of PTEN functions in cells implicated in innate immunity (Suzuki et al., 1998). Akira Suzuki, who is one of the leading scientists in elucidating PTEN biology, has recently reviewed scientific advances using these particular conditional PTEN knock-out mice (Suzuki et al., 2008).

The controversy on innate immune functions of PI3K

Before genetically modified mice became available, pharmacologic inhibition of PI3K has been the method of choice. Inhibition of PI3K could be achieved by using

the fungal metabolite wortmannin (in nM range) or the synthetic inhibitor LY294002 (in μ M range). Furthermore, constructs for the artificial overexpression of wildtype or mutated PI3K family members were generated to dissect the pathway and prove pro- or anti-inflammatory effects.

In vitro studies using these compounds suggested an involvement of PI3K in the modulation of innate immune responses, though the role of PI3K still remained controversial. Some studies showed direct or indirect TLR-mediated activation of I κ B kinase (IKK)/nuclear factor κ B (NF κ B) signaling via PI3K/AKT. Inhibition of PI3K by pharmacologic inhibitors or dominant negative PI3K constructs led to diminished NF κ B activation and reduced inflammatory gene expression (Arbibe et al., 2000; Ojaniemi et al., 2003; Rhee et al., 2006). In contrast, others could show an enhancing effect of pharmacologic PI3K inhibition on pro-inflammatory gene expression, which was dependent on a number of signaling pathways such as p38, C-jun n-terminal kinase (JNK), extracellular signal-regulated kinases (ERK) and IKK (Aksoy et al., 2005; Az-Guerra et al., 1999; Guha and Mackman, 2002).

Furthermore, we and others provided *in vivo* evidence for a down-regulatory role on innate immunity of PI3K in models of acute inflammation and sepsis, using the potent PI3K inhibitor wortmannin (Schabbauer et al., 2004; Williams et al., 2004; Zhang et al., 2007).

Differential results using both inhibitors wortmannin and LY294002 led to speculations that these inhibitors have additional targets, which are unrelated to PI3K. This was confirmed thereafter, when a study demonstrated that an analogue of LY294002, which has no activity on PI3K, did still block the NF κ B-dependent signaling (Kim et al., 2005). Taken together, these data suggest that some of the observed effects are cell-type specific and true, but others are dependent on the non-specific inhibition of targets other than PI3K. Thus, these findings have to be reviewed carefully, especially in light of recent data based on reverse genetics (see below).

Genetic analysis of PI3K properties in TLR-mediated signaling

The first genetic reports on the role of PI3K in the regulation of innate immune responses were published by the group of Shigeo Koyasu. It could be shown *in vitro* that ablation or down-modulation of PI3K activity had strong impact on the innate immune response in dendritic cells. In particular, interleukin (IL)-12 expression upon pathogen-associated molecular pattern (PAMP) stimulation (TLR2, TLR4 and TLR9) was elevated in p85 α -deficient cells (Fukao et al., 2002a).

Another study could show *in vivo* that p85 α deficiency led to an enhanced response of gene-deficient mice to the

TLR5 agonist flagellin (Yu et al., 2006). These data indicate that PI3K is involved in the down-regulation of pro-inflammatory responses.

More recently, we could provide compelling evidence for a role of PI3K in the modulation of the amplitude of inflammatory signals. In contrast to the above-mentioned studies, we used the complete p85 α -deficient animals generated by Lewis Cantley's laboratory. Several pro-inflammatory signaling pathways activated by TLR4, such as the MAP kinases p38, JNK and ERK1,2, are affected by p85 α deficiency in macrophages. Activation of mitogen-activated protein kinases (MAPKs) upon lipopolysaccharide (LPS)/TLR4 activation was enhanced and persisted over a prolonged period of time in p85 α -deficient macrophages, as compared to wildtype control cells (Luyendyk et al., 2008). Interestingly, we could only detect minor effects on NF κ B signaling, quite in contrast to the conclusions drawn by Fukao and Koyasu (2003). In this respect, Martin et al. (2005) found direct effects of AKT and glycogen synthase kinase 3 β (GSK3 β) on NF κ B activity.

We could demonstrate that as a functional consequence of reduced PI3K activation, expression levels of secondary inflammatory mediators such as tumor necrosis factor (TNF) α and IL-6 were strongly elevated *in vitro* and *in vivo* due to p85 α deficiency (Luyendyk et al., 2008).

As mentioned above, downstream of AKT, GSK3 β is a crucial target in the PI3K signaling cascade. GSK3 β is important for a variety of cellular processes, such as glucose/glycogen metabolism, protein synthesis and apoptosis (Frame and Cohen, 2001). Phosphorylation of GSK3 β by AKT renders GSK3 β inactive. Recently, Martin et al. (2005) could prove genetically that AKT-mediated GSK3 β inhibition led to down-modulation of TLR2, 4, 5 and 9-mediated inflammation *in vitro* and *in vivo*. These findings confirmed previous reports using unspecific GSK3 β inhibitors *in vitro*, which indicated that GSK3 β is required for PI3K/AKT-dependent down-modulation of LPS-mediated expression of pro-inflammatory mediators (Guha and Mackman, 2002).

Genetic analysis of PTEN in innate immunity

Reports on PTEN function in innate immune responses are sparse. To date, PTEN has not been extensively investigated with respect to innate immune signaling. Susheela Tridandapani's group took a glance at TLR4 effects and Fc γ R signaling in PTEN-deficient macrophages. They noted that Fc γ R-mediated expression of cytokines was enhanced, while TLR-mediated expression of cytokines was inhibited by PTEN deficiency in macrophages (Cao et al., 2004). This may indicate that PI3K's pro- versus anti-inflammatory

functions strongly depend on specific receptor-mediated signal transduction.

In our recent study, we compared PI3K- and PTEN-dependent effects on LPS/TLR4-mediated expression of cytokines and pro-coagulant tissue factor. As we expected, data obtained in PTEN $-/-$ macrophages inversely correlated to results derived from p85 α $-/-$ macrophages. These findings were a proof of principle that the genetic manipulation of PI3K activity led to either enhanced (p85 α deficiency) or diminished (PTEN deficiency) cytokine expression in response to the TLR4 agonist LPS.

Moreover, we found that PTEN deficiency led to constitutively enhanced PI3K signaling activity, which could not be restored by endogenous SHIP phosphatase activity. This is noteworthy, since SHIP is a lipid-phosphatase dephosphorylating PI3K-generated PtdIns(3,4,5)P $_3$ at the 5 position, which is also implicated in innate immune regulation (Sly et al., 2004). Simultaneously, the amplitude and sustained activation of MAPK signaling by LPS were significantly reduced in PTEN-deficient macrophages (Luyendyk et al., 2008).

Furthermore, in collaboration with Philippe Georgel, we could detect TLR-specific anti-viral, cyto-protective properties of PTEN deficiency against vesicular stomatitis virus (VSV) infection in macrophages. Cellular virus resistance was conferred by reduced virus production and increased survival.

The VSV glycoprotein G has been shown to utilize the TLR4 signaling complex. In this particular case CD14/TLR4 activation is TIR-domain-containing adapter-inducing interferon- β (TRIF)/TRIF-related adaptor molecule (TRAM) dependent, but MyD88 independent (Georgel et al., 2007). TLR4/TRAM-induced interferon regulatory factor (IRF)7 and the type I interferon IFN β were found to be elevated in VSV-infected PTEN-deficient macrophages (Schabbauer et al., 2008).

Taken together, our findings suggest that applying a conditional PTEN knock-out strategy in macrophages leads to sustained activation of PI3K with simultaneous down-regulation of several NF κ B-dependent cytokines, in particular TNF α and IL6, but increase of other IRF-dependent factors, such as IFN β , in response to TLR4 activation.

Nevertheless, there are still numerous missing links in the PI3K/PTEN signaling cascade leading to down-modulation of pro-inflammatory signaling (see Fig. 2). To unravel the mystery of those factors will be a challenge in the near future.

Concluding remarks

The PI3K/PTEN signaling pathway is extremely pleiotropic, because this important signaling axis affects numerous cellular processes.

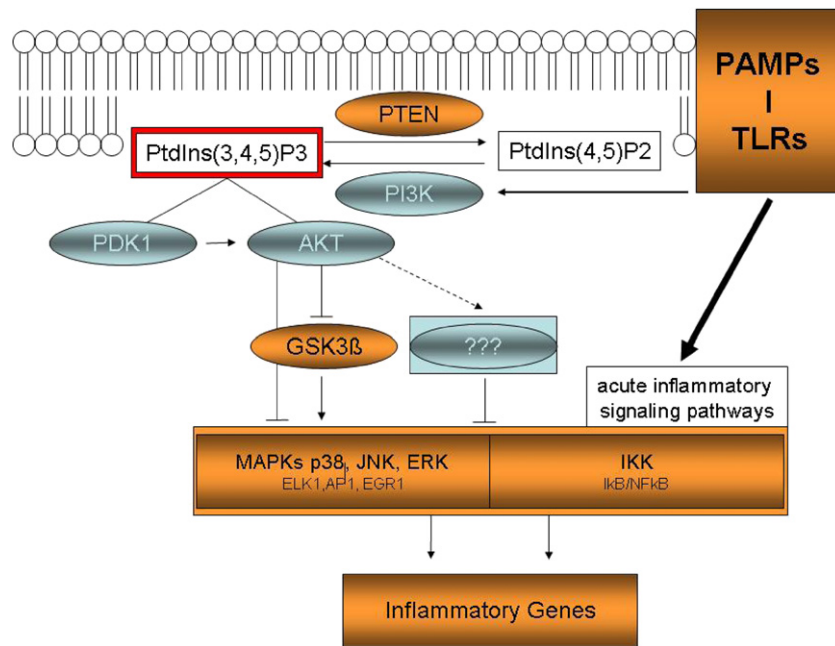


Fig. 2. Role of PI3K and PTEN in the modulation of acute inflammatory signaling processes. PI3-kinase is activated rapidly by TLRs that encounter pathogen-associated molecular patterns (PAMPs). PI3K activates downstream signaling, such as PDK1 and AKT, by PtdIns(4,5)P2 phosphorylation. PTEN antagonizes PI3K. Subsequently, AKT is activated by PDK1. AKT can either directly decrease the activity of pro-inflammatory signalling or can do so indirectly via GSK3 β inhibition. To date, intermediate factors, regulated by PI3K/AKT, that are putatively involved in the down-modulation are not known. Pro-acting molecules are depicted in orange. Counter-acting molecules are depicted in blue. Activating processes are depicted as arrows. Interaction is depicted as solid line. Putative activation or inhibition is depicted as dashed line. Please note that this scheme simplifies the complexity of signaling pathways shown.

Still, the role of PI3K and PTEN in innate immunity is not completely resolved. Remarkable progress over the last couple of years using reverse genetics unravelled PI3K's immune-modulatory features, which are antagonized specifically by PTEN. Downstream factors, such as GSK3 β and possible unidentified molecules, highlight the importance of TLR-mediated PI3K/AKT signaling on the regulation of pro-inflammatory signaling pathways such as the MAP kinases.

Acknowledgement

This work is supported by the Austrian Science Fund FWF P19850-B12 (G.S.).

References

- Aksoy, E., Vanden, B.W., Detienne, S., Amraoui, Z., Fitzgerald, K.A., Haegeman, G., Goldman, M., Willems, F., 2005. Inhibition of phosphoinositide 3-kinase enhances TRIF-dependent NF-kappa B activation and IFN-beta synthesis downstream of toll-like receptor 3 and 4. *Eur. J. Immunol.* 35, 2200–2209.
- Arbibe, L., Mira, J.P., Teusch, N., Kline, L., Guha, M., Mackman, N., Godowski, P.J., Ulevitch, R.J., Knaus, U.G., 2000. Toll-like receptor 2-mediated NF-kappa B activation requires a Rac1-dependent pathway. *Nat. Immunol.* 1, 533–540.
- Az-Guerra, M.J., Castrillo, A., Martin-Sanz, P., Bosca, L., 1999. Negative regulation by phosphatidylinositol 3-kinase of inducible nitric oxide synthase expression in macrophages. *J. Immunol.* 162, 6184–6190.
- Brachmann, S.M., Yballe, C.M., Innocenti, M., Deane, J.A., Fruman, D.A., Thomas, S.M., Cantley, L.C., 2005. Role of phosphoinositide 3-kinase regulatory isoforms in development and actin rearrangement. *Mol. Cell. Biol.* 25, 2593–2606.
- Burgering, B.M., Coffey, P.J., 1995. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 376, 599–602.
- Cantley, L.C., 2002. The phosphoinositide 3-kinase pathway. *Science* 296, 1655–1657.
- Cao, X., Wei, G., Fang, H., Guo, J., Weinstein, M., Marsh, C.B., Ostrowski, M.C., Tridandapani, S., 2004. The inositol 3-phosphatase PTEN negatively regulates Fc gamma receptor signaling, but supports toll-like receptor 4 signaling in murine peritoneal macrophages. *J. Immunol.* 172, 4851–4857.
- Frame, S., Cohen, P., 2001. GSK3 takes centre stage more than 20 years after its discovery. *Biochem. J.* 359, 1–16.

- Fruman, D.A., Mauvais-Jarvis, F., Pollard, D.A., Yballe, C.M., Brazil, D., Bronson, R.T., Kahn, C.R., Cantley, L.C., 2000. Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 alpha. *Nat. Genet.* 26, 379–382.
- Fukao, T., Koyasu, S., 2003. PI3K and negative regulation of TLR signaling. *Trends Immunol.* 24, 358–363.
- Fukao, T., Tanabe, M., Terauchi, Y., Ota, T., Matsuda, S., Asano, T., Kadowaki, T., Takeuchi, T., Koyasu, S., 2002a. PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat. Immunol.* 3, 875–881.
- Fukao, T., Yamada, T., Tanabe, M., Terauchi, Y., Ota, T., Takayama, T., Asano, T., Takeuchi, T., Kadowaki, T., Hata, J.J., Koyasu, S., 2002b. Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. *Nat. Immunol.* 3, 295–304.
- Georgel, P., Jiang, Z., Kunz, S., Janssen, E., Mols, J., Hoebe, K., Bahram, S., Oldstone, M.B., Beutler, B., 2007. Vesicular stomatitis virus glycoprotein G activates a specific antiviral toll-like receptor 4-dependent pathway. *Virology* 362, 304–313.
- Gericke, A., Munson, M., Ross, A.H., 2006. Regulation of the PTEN phosphatase. *Gene* 374, 1–9.
- Guha, M., Mackman, N., 2002. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. *J. Biol. Chem.* 277, 32124–32132.
- Hirsch, E., Katanaev, V.L., Garlanda, C., Azzolino, O., Pirola, L., Silengo, L., Sozzani, S., Mantovani, A., Altruda, F., Wymann, M.P., 2000. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 287, 1049–1053.
- Kim, Y.H., Choi, K.H., Park, J.W., Kwon, T.K., 2005. LY294002 inhibits LPS-induced NO production through a inhibition of NF-kappaB activation: independent mechanism of phosphatidylinositol 3-kinase. *Immunol. Lett.* 99, 45–50.
- Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliareis, C., Rodgers, L., McCombie, R., Bigner, S.H., Giovanella, B.C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M.H., Parsons, R., 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943–1947.
- Li, Z., Jiang, H., Xie, W., Zhang, Z., Smrcka, A.V., Wu, D., 2000. Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. *Science* 287, 1046–1049.
- Liaw, D., Marsh, D.J., Li, J., Dahia, P.L., Wang, S.I., Zheng, Z., Bose, S., Call, K.M., Tsou, H.C., Peacocke, M., Eng, C., Parsons, R., 1997. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat. Genet.* 16, 64–67.
- Luyendyk, J.P., Schabbauer, G.A., Tencati, M., Holscher, T., Pawlinski, R., Mackman, N., 2008. Genetic analysis of the role of the PI3K–Akt pathway in lipopolysaccharide-induced cytokine and tissue factor gene expression in monocytes/macrophages. *J. Immunol.* 180, 4218–4226.
- Marsh, D.J., Dahia, P.L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R.J., Eng, C., 1997. Germline mutations in PTEN are present in Bannayan-Zonana syndrome. *Nat. Genet.* 16, 333–334.
- Martin, M., Rehani, K., Jope, R.S., Michalek, S.M., 2005. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat. Immunol.* 6, 777–784.
- Ojaniemi, M., Glumoff, V., Harju, K., Liljeroos, M., Vuori, K., Hallman, M., 2003. Phosphatidylinositol 3-kinase is involved in toll-like receptor 4-mediated cytokine expression in mouse macrophages. *Eur. J. Immunol.* 33, 597–605.
- Rhee, S.H., Kim, H., Moyer, M.P., Pothoulakis, C., 2006. Role of MyD88 in phosphatidylinositol 3-kinase activation by flagellin/toll-like receptor 5 engagement in colonic epithelial cells. *J. Biol. Chem.* 281, 18560–18568.
- Sasaki, T., Irie-Sasaki, J., Jones, R.G., Oliveira-dos-Santos, A.J., Stanford, W.L., Bolon, B., Wakeham, A., Itie, A., Bouchard, D., Koziarz, I., Joza, N., Mak, T.W., Ohashi, P.S., Suzuki, A., Penninger, J.M., 2000. Function of PI3Kgamma in thymocyte development, T-cell activation, and neutrophil migration. *Science* 287, 1040–1046.
- Schabbauer, G., Tencati, M., Pedersen, B., Pawlinski, R., Mackman, N., 2004. PI3K–Akt pathway suppresses coagulation and inflammation in endotoxemic mice. *Arterioscler. Thromb. Vasc. Biol.* 24, 1963–1969.
- Schabbauer, G., Luyendyk, J., Crozat, K., Jiang, Z., Mackman, N., Bahram, S., Georgel, P., 2008. TLR4/CD14-mediated PI3K activation is an essential component of interferon-dependent VSV resistance in macrophages. *Mol. Immunol.*
- Sly, L.M., Rauh, M.J., Kalesnikoff, J., Song, C.H., Krystal, G., 2004. LPS-induced upregulation of SHIP is essential for endotoxin tolerance. *Immunity* 21, 227–239.
- Staal, S.P., 1987. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc. Natl. Acad. Sci. USA* 84, 5034–5037.
- Suzuki, A., de la Pompa, J.L., Stambolic, V., Elia, A.J., Sasaki, T., del Barco Barrantes, I., Ho, A., Wakeham, A., Itie, A., Khoo, W., Fukumoto, M., Mak, T.W., 1998. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr. Biol.* 8, 1169–1178.
- Suzuki, A., Nakano, T., Mak, T.W., Sasaki, T., 2008. Portrait of PTEN: messages from mutant mice. *Cancer Sci.* 99, 209–213.
- Terauchi, Y., Tsuji, Y., Satoh, S., Minoura, H., Murakami, K., Okuno, A., Inukai, K., Asano, T., Kaburagi, Y., Ueki, K., Nakajima, H., Hanafusa, T., Matsuzawa, Y., Sekihara, H., Yin, Y., Barrett, J.C., Oda, H., Ishikawa, T., Akanuma, Y., Komuro, I., Suzuki, M., Yamamura, K., Kodama, T., Suzuki, H., Yamamura, K., Kodama, T., Suzuki, H., Koyasu, S., Aizawa, S., Tobe, K., Fukui, Y., Yazaki, Y., Kadowaki, T., 1999. Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nat. Genet.* 21, 230–235.
- Vanhaesebroeck, B., Leevers, S.J., Ahmadi, K., Timms, J., Katso, R., Driscoll, P.C., Woscholski, R., Parker, P.J., Waterfield, M.D., 2001. Synthesis and function of 3-phosphorylated inositol lipids. *Annu. Rev. Biochem.* 70, 535–602.

- Williams, D.L., Li, C., Ha, T., Ozment-Skelton, T., Kalbfleisch, J.H., Preiszner, J., Brooks, L., Breuel, K., Schweitzer, J.B., 2004. Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. *J. Immunol.* 172, 449–456.
- Yu, Y., Nagai, S., Wu, H., Neish, A.S., Koyasu, S., Gewirtz, A.T., 2006. TLR5-mediated phosphoinositide 3-kinase activation negatively regulates flagellin-induced proinflammatory gene expression. *J. Immunol.* 176, 6194–6201.
- Zhang, W.J., Wei, H., Hagen, T., Frei, B., 2007. Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Proc. Natl. Acad. Sci. USA* 104, 4077–4082.