Review

The Essential Role of Phosphoinositide 3-Kinases (PI3Ks) in Regulating Pro-Inflammatory Responses and the Progression of Cancer

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Phosphoinositide 3-Kinases (PI3Ks) are proteins coupled to a variety of cell surface receptors and play a key role in signal transduction cascade regulating fundamental cellular functions such as transcription, proliferation, and survival. PI3Ks also are important in disease processes such as inflammation and cancer. The aim of this review is to outline current understandings of the PI3K family, mechanism of their activation, their role in inflammatory responses and the development of malignant tumors. *Cellular & Molecular Immunology*. 2005;2(4):241-252.

Key Words: PI3K, signal transduction, inflammation, malignant tumor

Introduction

Homeostasis of the organisms is maintained by precisely orchestrated functions at system, organ, cell and molecular levels. The organisms "sense" stimulants in the environment with numerous and functionally divergent cell surface receptors. Once activated, these receptors transduce signals operating cellular metabolism, growth, and programmed death. Cell surface receptors also recognize and interact with "noxious" stimuli, which may disrupt normal cell function thereby causing diseases. In mammals, the cell receptor system has evolved in a manner such that they are grouped and specialized in mediating essential cell functions to maintain homeostasis by reacting to hormones, growth factors, adhesion molecules, and proteins that direct cell trafficking and homing. These functions are also crucial for host defense in fending off pathogens and for elimination of transformed cells.

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Received Aug 17, 2005. Accepted Aug 22, 2005.

Cell surface receptors, regardless of their structures, share a variety of intracellular proteins that catalyze signaling pathways. Phosphoinositide 3-kinases (PI3Ks) belong to such intracellular proteins and play a major role in the development of immune response, inflammation and tumor growth. These kinases therefore have been considered as important targets for the design of therapeutic agents for human diseases. In this article, we will review progress in studies of PI3K family members and their function with emphasis on their roles in inflammation and malignant tumor.

An overview of PI3K family

Based on their structural characteristics and substrate specificity, PI3Ks can be divided into three classes, referred to as I-A, I-B, II and III (Figure 1).

The class I PI3K family includes four different enzymes that are subdivided into I-A and I-B on the basis of their molecular structure and activation mechanisms. Class I-A PI3Ks are heterodimeric kinases consisting of a regulatory subunit and a catalytic subunit. There are three genes for the class I-A catalytic isoforms and each encodes a protein product named p110α, p110β or p110δ, which form stable heterodimers with class I-A regulatory subunits. There are five members of the I-A regulatory subunits p85α, p85β, p55α, p55γ and p50α. The p85α, p55α, and p50α proteins are alternative splicing products of the same gene locus, *Pik3r1*. P85β and p55γ are encoded by distinct genes. Class I-A catalytic and regulatory isoforms have broad and

Abbreviations: GPCR, G-protein coupled receptor; PI3Ks, phosphoinositide 3-kinases; TLR, Toll-like receptor; ROS: reactive oxygen species; PTEN, phosphatase and tensin homologue deleted on chromosome ten.

Mammalian PI3Ks

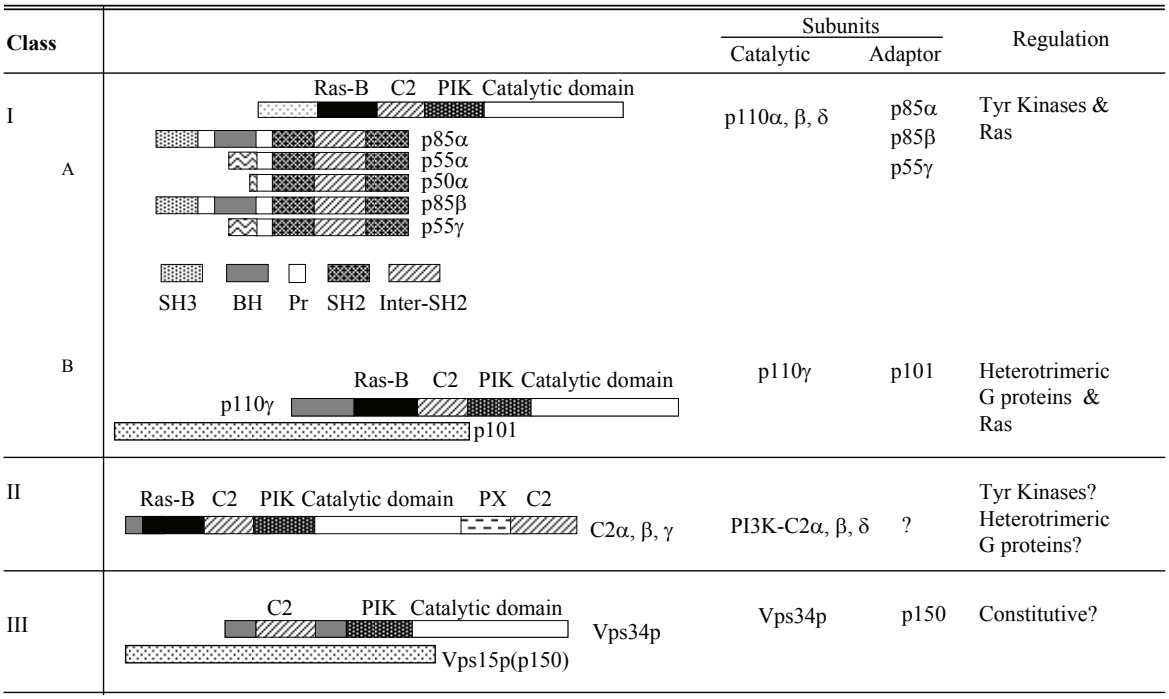

Figure 1. Structure of the PI3K family. Mammalian PI3Ks are composed of I-A, I-B, II and III. C2 domains in PI3Ks bind phospholipids. The inter-SH2 domain of p85α, p55α, p50α, p85β and p55γ subunits interacts with the N-terminal domain of p110α, β and δ. Dual SH2 domains bind to tyrosine-phosphorylated adaptor proteins, catalizing the kinase activity of the p110 subunits. p101 specifically interacts with the N-terminal domain of p110γ. PX domains bind PI(3)P and PI(3,4)P2 and may recruit class II PI3Ks to the cell membrane. Functions of the Ras-binding domain (Ras-B), SH-3 and Bcr domains and the proline-rich region (Pr) in PI3Ks are not fully known. Vps34p, the class III PI3K, forms a complex with a serine/threonine kinase, Vps15p.

overlapping tissue distribution with an apparent exception of p110δ, which is found mainly in leukocytes. The class I-B enzyme is composed of p110γ only and interacts with the regulatory subunit p101. The enzyme is expressed preferentially in mammal leukocytes (1-7). For G-protein-coupled receptors (GPTR), activation of PI3K class I-B is mediated by Gβγ complex of the heterotrimeric G-proteins, after the receptor binding of agonists. Class I PI3K enzymes use phosphatidylinositol (PI), phosphatidylinositol(4)momophosphate (PI(4)P) and phospatidylinositol(4,5)bisphosphate (PI(4,5)P2) as substrates. Unlike other classes of PI3K, class I-A PI3Ks can phosphorylate PI(4,5)P2 to generate PI(3,4,5)P3 *in vivo*.

The class II PI3K family contains three members: PI3K-C2α, PI3K-C2β and PI3K-C2γ. PI3K-C2α and PI3K-C2β are expressed ubiquitously, whereas PI3K-C2γ is expressed primarily in hepatocytes in mammals (1-4). A class II PI3K has two distinct domains at the C-termini, a phox homology (PX) domain and a C2. The PX domain binds PI(3)P and PI(3,4)P2 (2-11), and may recruit class II PI3Ks to the cell membrane. The cellular location of the class II PI3K family remains controversial, whereas some studies suggest that most class II PI3Ks are present in the nucleus, others imply Golgi apparatus and clathrin-coated pits being the location of class II PI3Ks (1, 3, 12).

Class III PI3K family contains only a single member and is the homologue of *Saccharomyces cerevisiae* Vps34p, which exclusively generates PI(3)P (1-4). Class III PI3K is constitutively active *in vitro* and its target proteins contain FYVE finger domains and PX domains (2-11). In yeast, Vps34p forms a heterodimer complex with the seine/threonine kinase Vps15p and regulates vesicle trafficking through proteins containing FYVE finger domains that bind PI(3)P.

The mechanisms of PI3K activation

Each member of the PI3K family is activated in a distinct manner based on the cell type or cell surface receptors.

Class I-A PI3Ks are activated *via* cytokine receptors, T cell receptor (TCR), B cell receptor (BCR), natural killer cell (NK) stimulatory receptors and Fc receptors. Costimulatory receptors such as CD28 on T cells, CD19 on B cells, cell adhesion molecules, and some receptors lacking an apparent association with protein tyrosine kinases (PTKs), such as IL-1, TLRs and TNF receptors also activate class I-A PI3K

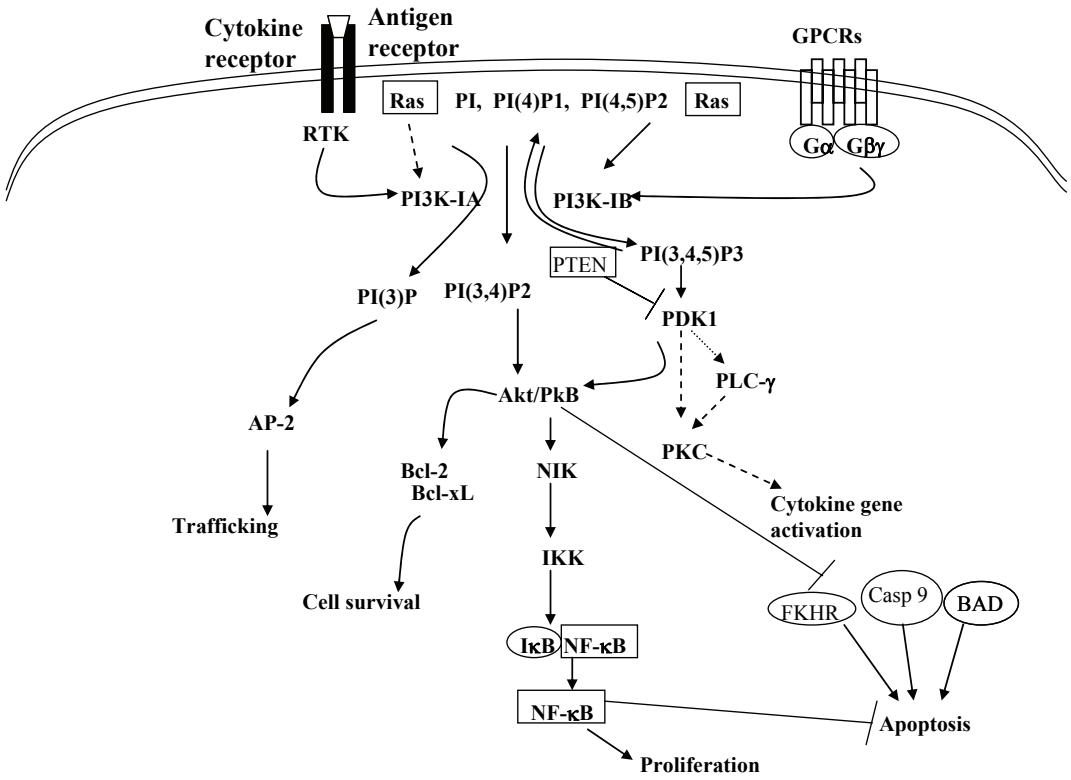

Figure 2. Signal transduction pathways involving PI3Ks. PI3Ks are activated by a number of cell surface receptors when they interact with agonists. Proteins containing PH domains (Akt/PKB, PDK1, PLC-γ) are present downstream of PI(3,4)P2 and PI(3,4,5)P3. PI3K/Akt pathway triggers a variety of functional proteins and is turned off by phosphatase and tensin homologue (PTEN).

(Table 1). In contrast, class I-B PI3Ks are activated mostly through GPCRs, such as receptors for chemokines and chemotactic formyl peptides.

PI3Ks regulate various signaling molecules including G proteins and members of the protein kinase C (PKC) family. Proteins containing pleckstrin homology (PH) domains such as protein kinase B (PKB/Akt), phosphoinositide-dependent kinase 1 (PDK1), Vav and phospholipase C-γ (PLC-γ) are thought to function directly downstream of PI3K because their PH domains bind PI(3,4)P2 or PI(3,4,5)P3. In fact, PKB/Akt is recruited to plasma membrane by binding to PI(3,4)P2 or PI(3,4,5)P3 and then is activated by PDK1 recruited by PI(3,4,5)P3. In this context, PKB/Akt is considered as a primary downstream target of PI3K (Figure 2).

Insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), integrins, and at least one chemokine, MCP-1, are capable of activating class II PI3Ks (13). All class II PI3Ks phosphorylate PI and PI(4)P *in vitro* in cell-free system to generate PI(3) and PI(3,4)P2. However, it is not clear whether the same process occurs in a living cell. One remarkable feature of some class II PI3Ks (PI3K-C2α and β) is that they utilize Ca^{2+} and ATP for their *in vitro* lipid kinase activity (13-16). Whether an increase in cellular Ca^{2+} generates PI(3,4,5)PI3 in living cells remains to be determined.

A single class III PI3K catalytic subunit, which is the homologue of the yeast vesicular protein-sorting protein Vps34p, has been identified in all eukaryotic species. In yeast and mammals, this catalytic subunit exists as a complex with a Ser/Thr protein kinase (Vps15p in yeast and p150 in mammals), and is myristoylated at the N-terminus. The class III PI3K solely uses PI as a substrate *in vitro,* and is most likely to generate a large fraction of PI(3)P in cells. Cellular PI(3)P is maintained at a relatively consistent level suggesting that the physiological processes in which class III PI3K participates are important for homeostasis. Nevertheless, how class III PI3K is at a constantly activated state in cells is unclear.

The role of PI3Ks in inflammatory responses

PI3Ks in Toll-like receptor (TLR) signaling

To date, 10 human and 9 murine Toll like receptors (TLRs) have been identified, which play key roles in initiating innate and adaptive immune responses in a variety of species. TLR family members recognize pathogen-associated molecular patterns (PAMPs). Some TLRs also interact with host-cell-derived molecules (17). Upon bacterial infection, antigen-presenting cells (APCs), such as macrophages and dendritic

Table 1. Agonists and receptors associated with class I-A PI3K activation

Agonists and receptors	Cells
Agonists	
Interleukin (IL)-2	
IL-3	
IL-4	
IL-6	
IL-7	
IL-15	
Granulocyte colony-stimulating factor (G-CSF)	
Erythropoietin	
Oncostatin M	
Receptors	
Cytokine receptors with intrinsic PTK activity	
Macrophage colony-stimulating factor receptor	monocytes/ macrophages
c-Kit	mast cells
Antigen recognition receptors	
T cell receptor (TCR)	T cells
B cell receptor (BCR)	B cells
Natural killer (NK) stimulatory receptors	NK cells
Fc receptors	
High-affinity IgE receptors (FcεRI)	
High-affinity IgG receptors (FcγRI)	
Costimulatory molecules	
CD28	T cells
CD19	B cells
Cytokine receptors without apparent association with PTKs (13-16)	
IL-1 receptors	many types of cells
Toll-like receptors (TLRs)	many types of cells
Tumor necrosis factor receptor family	many types of cells

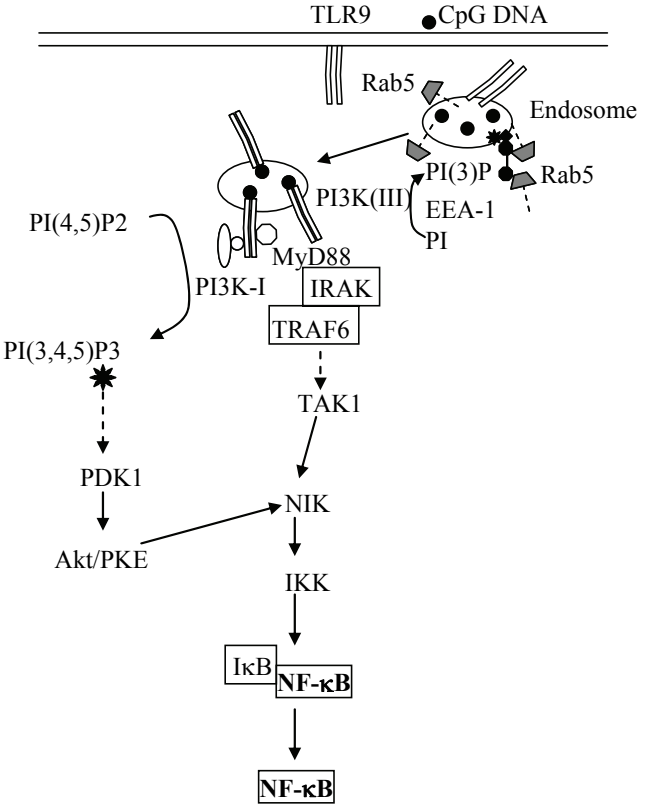

Figure 3. The role of PI3Ks in CpG ODN/TLR9-mediated signal transduction. Class III PI3Ks, EEA-1, and Rab5 mediate the trafficking and maturation of endosomes that capture complexes by CpG containing DNA and TLR9. This process is essential for TLR9 to transduce signals leading cell activation. An alternative pathway mediated by class I PI3K is also involved in TLR9 signaling.

cells (DCs), capture PAMP by using cell surface TLRs and the ensuing signaling pathways stimulate the production of reactive oxygen and nitrogen intermediates. Activation of TLRs on APCs also promotes adaptive immune responses by enhancing the production of pro-inflammatory cytokines and the expression of costimulatory molecules. Furthermore, activated APCs express unique chemokine receptors that direct cell trafficking to lymphoid organs where they present processed antigens to T- and B-lymphocytes (18-20). PI3Ks are important components in the signal transduction cascade of TLRs. Since the signaling pathways linked to TLRs share many common characteristics, we will use TLR2, TLR4 and TLR9 and representatives to illustrate their relationship with PI3K.

It has been reported that the PI3K-Akt axis plays a central role in TLR2-induced activation of neutrophils (21). Activation of Akt in neutrophils stimulated with the TLR2 ligands peptidoglycan (PGN), a product of Gram-positive bacteria, and the lipopeptide tri-palmitoyl-S-glyceryl-Cys-Ser-(Lys) (PAM) was of greater magnitude than that stimulated by the TLR4 agonist LPS. The release of the pro-inflammatory mediators TNF-α and macrophage inflammatory protein-2 (MIP-2) by TLR2 agonist-activated-neutrophils was inhibited by blockade of PI3K. Interestingly, PI3K blockade did not inhibit nuclear translocation of NF-κB in TLR2 ligand-activated neutrophils, but did prevent Ser (536) phosphorylation of its p65 subunit, an event required for maximal transcriptional activity of NF-κB. Inhibition of PI3K also prevented activation of p38 MAPK and extracellular receptor-activated kinase 1/2 in TLR2-stimulated neutrophils (21). Therfore, TLR2 on neutrophils participates in Gram-positive bacteria induced acute inflammation, which is mainly mediated by PI3K pathway.

In contrast to its role in TLR2 activation, PI3K may act as a limiting factor in TLR4-signaling cascade. In sepsis, LPS induces the production of tumor necrosis factor-α (TNF-α) and tissue factor (TF) by monocytic cells *via* activation of

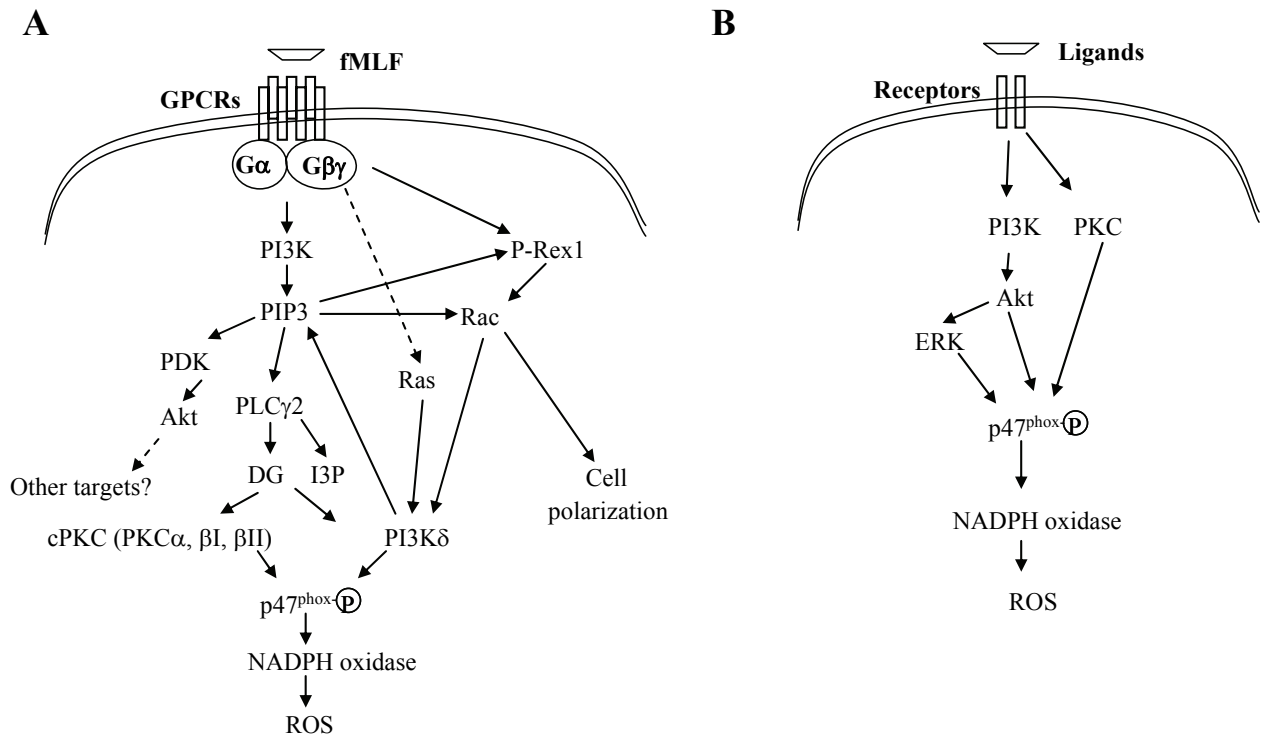

Figure 4. The role of PI3Ks in neutrophil polarization and production of reaction oxygen species (ROS). (A) The chemotactic peptide fMLF activates its GPCRs on neutrophil cell surface, leading to PI3K activation and subsequent PIP3 production. PIP3 activates phosphoinositide-dependent protein kinase (PDK) which in turn phosphorylates and activates Akt. However, Akt activated by this pathway plays little role in $p47^{phox}$ phosphorylation and NADPH oxidase activation. Rather, PLCγ2 is directly activated by PIP3 that triggers DG-dependent PKCs, cPKC, and PKCδ. PKCs phosphorylate $p47^{phox}$ with resultant NADPH oxidase activation. In fMLF-stimulated neutrophils, PI3Kδ can be activated by the components of heterotrimetric G proteins (Gαi and Gβγ), the Ras superfamily of G proteins as well as SFKs. Activated PI3Kδ leads to a positive feed back loop amplifying PIP3 production leading to cell polarization and chemotaxis. (B) Membrane receptors in neutrophil are activated by exogenous or host derived agonists. PKC is located downstream of a variety of receptors and its activation causes partial phosphorylation of $p47^{phox}$, which is also partially phosphorylated by Akt and ERK1/2. The phosphorylated $p47^{phox}$ translocates to the plasma membrane leading to the assembly of the NADPH oxidase that generates ROS.

TLR4 and transcription factors Egr-1, AP-1, and NF-κB. Inhibition of PI3K-Akt pathway enhances LPS-induced activation of MAPKs (ERK1/2, p38, and JNK) and the downstream transcription factors AP-1 and Egr-1 and nuclear translocation of NF-κB.

Since glycogen synthase kinase-β (GSK-β), which can be negatively regulated in PI3K-Akt-dependent manner, increases the transcriptional activity of NF-κB p65, inhibition of PI3K-Akt pathway prevents the inactivation of GSK-β in human monocytes (22). In animal model of endotoxemia. LPS induces a systemic inflammatory response and intravascular coagulation. When Wortmannin and LY294002, two classical PI3K inhibitors, were used in endotoxemic mice, LPS-induced Akt phosphorylation in leukocytes was diminished, while TF mRNA expression was increased. In addition, PI3K inhibitors increased TF antigen and thrombin-antithrombin III levels in the plasma, and fibrin deposition in the liver of endotoxemic mice. These mice showed markedly increased numbers of macrophages in the liver and kidney in association with reduced survival (23). Thus the PI3K-Akt pathway imposes a "braking" force on production of lethal levels of TNF-α and TF in endotoxemic mice.

PI3Ks also are important signaling molecules in TLR9 mediated pathways in mammalian cells. TLR9 recognizes unmethylated bacterial CpG-containing DNA and activates cells of the immune system (24, 25). Single-stranded oligodeoxynucleotides (ODNs) synthesized to contain unmethylated CpG motifs mimic the ability of bacterial DNA to stimulate cells that express TLR9, including B- and T-lymphocytes, natural killer cells, monocytes/macrophages and dendritic cells (26). CpG ODNs also promote the expression of a G-protein coupled receptor mFPR2/FPR1 by microglial cells, which are of the monocytic phagocyte lineage in the brain (19). Since mFPR2 and its human homologue FPR1 interact with the bacterial chemotactic peptide fMLF and amyloid β peptide ($A\beta_{42}$), a causative factor in Alzheimer's disease, TLR9 has been postulated to regulate pro-inflammatory response in brain infection and neuro-degeneration. CpG interacts with TLR9 present in the

Table 2. PTEN gene mutation in cancers

Cancer type	Mutations detected (%)
Primary melanoma	10
Sporadic breast cancer	5
Thyroid cancer	7
Head and neck cancer	12
Renal cell carcinoma	6
Lung cancer	9
Lymphoma	5
Hepatocellular carcinoma	6
Ovaarian cancer	9
Primary glioblastoma	>20
Prostate cancer	
Loss of heteozygosity at 10q23	50
Homozygous deletion of PTEN	10

endocytic vesicles and triggers activation of MAPKs (p38, JNK and ERK), and NF-κB-inducing kinase (NIK)-IKK-IκB (27-29) (Figure 3). Wortmannin treatment of macrophages reduced the size and number of endosomes containing both TLR9 and CpG, suggesting that PI3Ks are involved in vesicular trafficking of CpG (30). Rab (Ras-associated GTP-binding protein) 5-mediated recruitment of class III PI3K (PI3K III) leads to the production of PI(3)P in the endosomal membrane, which binds and recruits FYVE domain of early endosome antigen 1 (EEA1) into cell membrane. PI (3,4,5)P3, the product of class I PI3K (PI3K I), has been shown to activate a cascade consisting of 3'-phosphoino-sitide-dependent kinase-1 (PDK1) and Akt/PKB (31, 32). In DC, inhibition of PI3K pathway induced by CpG significantly reduces cell survival as well as IL-12 production, indicating that PI3Ks might be a crucial for baterial CpG DNA-induced cell survival as well as activation. In this regard, CpG DNA has been shown to up-regulate the anti-apoptotic proteins cIAPs, Bcl-2 and Bcl-xL, but down-regulate the active form of caspase-3. The pro-survival signals mediated by activated TLR9 in DC were abolished by PI3K inhibitors. Therefore, PI3Ks coupled to TLR9 are activated by bacterial DNA to stimulate and maintain host innate immune responses (33). Recent studies indicate that CpG DNA and other TLR ligands such as LPS (TLR4), PGN (TLR2) and R-848 (TLR7/8), all are effective in delaying spontaneous apoptosis and extending the functional life span of human neutrophils (34). The anti-apoptotic effect of these TLR agonists require the activation of NF-κB and PI3Ks and PI3K-dependent phosphorylation of Akt may be responsible for increased the levels of the anti-apoptotic protein Mcl-1 and A-1, both of which are members of the Bcl-2 family. In addition, TLR activation leads to PI3K-dependent phosphorylation of the pro-apoptotic protein Bad, which delays PMN apoptosis (34).

The role of PI3Ks in the extravasation, chemotaxis and phagocytosis of inflammatory cells

The blood-brain barrier (BBB) consists of endothelial tight junctions that permit stringent regulation of molecular transport and cell migration. During inflammatory conditions, endothelial cells become activated and express increased amounts of chemokines and cell-surface molecules, including cell adhesion molecules. These CAMs facilitate the interaction of brain endothelial cells and activated leukocytes and their subsequent migration into the tissue. For example, the endothelial cells express intercellular cell adhesion molelecules (ICAM-1 and 2) and vascular cell adhesion molecule-1 (VCAM-1), while the ligands (integrins) for ICAM-1 and VCAM-1 are expressed on leukocytes. PI3K/Akt signaling pathway regulates the interactions between endothelial CAMs and their ligands. TNF-α-stimulated endothelial cells increase their expression of endothelial CAMs and have the ability to bind more multiple monocytes. However, CAM expression is inhibited by lovastatin, an antagonist of the PI3K/Akt/CAM signaling cascade, resulting in decreased adhesion of monocytes (35). Thus, PI3Ks are important regulators of endothelium-monocyte interaction in inflammatory processes.

Leukocytes accumulate at the sites of bacterial infection, inflammation and tissue injury by directional migration (chemotaxis) in response to locally produced chemoattractants. The chemotactic capacity of leukocytes requires activation of class I PI3K members (36). p110δ and p110β are implicated in the chemotaxis of macrophages to colony-stimulating factor 1 in experiments using micro-injection of specific antibodies against the catalytic domains of the kinase subunits (37). On the other hand, class I-B PI3Ks are essential for neutrophil responses to a variety of chemoattractants associated with inflammatory responses (37). In chemotactic responses, leukocytes undergo several morphological changes. The cells first polarize to form leading and rear ends, then move rapidly towards the direction of the gradient formed by chemotactic stimuli. In this process, polarization indicates the adoption of a cell to a motile feature after detecting a chemoattractant gradient. Studies have demonstrated that PI(3,4,5)P3 plays a pivotal role in the establishment of cell polarity. For example, neutrophils can sense an extremely shallow gradient of chemoattractants and exhibit relatively robust chemotactic responses. The directional migration of neutrophils, and probably other motile cells as well, requires actin poly-merization that occurs predominantly in the leading edge of the polarized cells. PI3Kδ downstream of chemoattractant receptors controls PIP3 levels required for neutrophil polarization and directional migration (Figure 4A) (38). Selective inhibition of PI3Kδ with IC87114 results in the loss of neutrophil chemotaxis in response to the Gram-negative bacterial peptide fMLF (38). Studies with mice-deficient in PI3Ks reveal impaired neutrophil chemotaxis to fMLF, activate complement conponent 5 (C5a) and the chemokine IL-8. Mice lacking PI3Kγ show reduced neutrophil accumulation in the peritoneal cavity injected with both non-infectious (casein) and infectious *(Listeria* and *Escherichia coli*) stimuli (39-41). Thus, PI3Ks, PI3Kδ in particular are essential for neutrophils to respond to chemoattratants by rapid polarization and migration (Figure

4A) (42).

Microglial cells are of the monocytic cell lineage and actively participate in pro-inflammatory responses in the CNS. Upon stimulation with bacterial LPS and CpG, as well as the pro-inflammatory cytokine TNFα, mouse microglial cells express increased levels of a GPCR mFPR2 (18, 19, 43), which is a homologue of human FPRL1 and mediates cell migration in response to a variety of polypeptide agonists including the bacterial fMLF and Alzheimer's disease associated β Amyloid peptide $A\beta_{1-42}$. Interestingly, a type 2 cytokine, IL-4, markedly down-regulates the expression and function of mFPR2 in microglial cells induced by LPS and TNF-α (44, 45). The effect of IL-4 is dependent on activation of the PI3K pathway, in that the PI3K inhibitor LY294002 reversed the effect of IL-4 (44, 45). Further studies indicated that PI3K is crucial for IL-4 to enhance the activity of phosphatase PP2A that rapidly dephosphorylates MAPKs activated by LPS and TNFα, causing the attenuation of the signaling cascade coupled to TLR4 and TNF receptors. Thus, PI3Ks is also used by IL-4 to protect microglial cells from activation by pro-inflammatory stimulants in order to maintain CNS homeostasis (46).

Class I-A and class II PI3Ks are essential for the phagocytic processes of leukocytes. Leukocytes phagocytose antibody-coated particles *via* their Fcγ receptors and complement-coated particles and apoptotic cells *via* integrins ($\alpha_m\beta_2$ [CR3] and $\alpha_v\beta_3$, respectively). The primary role of PI3Ks in phagocysis of leukocytes appears to extend membrane components to the pseudopodia for engulfment of large particles. Subsequent accumulation of P(3)P in the phagosome, which is dependent on class II PI3K activity, seems to regulate the accumulation of proteins associated with maturation of the organelle and destruction of its biological contents (36).

The role of PI3Ks in the microbicidal activity of immune cells

Phagocytes such as neutrophils and macrophages constitute the first line host defense against bacterial infection. These cells eliminate bacteria by phagocytosing the invading pathogens, generation of reactive oxygen species (ROS), and release of bactericidal proteins into phagosomes that contain engulfed bacteria. In phagocytes, NADPH oxidase, consisting of a main catalytic component, flavocytochrome *b*558, is activated upon bacterial infection and generates reactive oxygen and nitrogen intermediates. In neutrophils intracellular production of superoxide is reduced by PI3K inhibitors and by targeted disruptions of specific PI3K isoforms (47, 48), suggesting that PI3Ks are important components of anti-bacterial responses.

PI3Ks also participate in chemoattractant induced anti-bacterial and pro-inflammatory responses in phagocytes. In human neutrophils Akt/PKB is activated by PI(3,4,5)P3 and PI(3,4)P2, and then phosphorylates the NADH component $p47^{phox}$ when the cells are stimulated by the bacterial chemotactic peptide fMLF which uses a GPCR (49). Inhibition of Akt in human neutrophils significantly attenuated fMLF-stimulated chemotaxis and superoxide release. Akt inhibitor also abrogates H_2O_2 production stimulated during phagocytosis process of neutrophils. Thus, Akt appears to mediate PI3K-dependent $p47^{phox}$ phosphorylation of the NADPH, which controls respiratory burst in human neutrophils (Figure 4B). It should be noted that pharmacological inhibition of PI3K attenuated both fMLF-stimulated $p47^{phox}$ phosphorylation and NADPH oxidase activity in human promyeloid HL-60 cells that have been differentiated to a neutrophil-like phenotype. Although fMLF activates Akt in a PI3K-dependent manner, an Akt inhibitor failed to inhibit the oxidase activity triggered by fMLF in these cells. In fact, in an *in vitro* kinase assay, Akt was unable to catalyze $p47^{phox}$ phosphorylation. Interestingly, cPKC and PKCδ activation is dependent on PI3Ks following fMLF stimulation and PI3K inhibitors reduced the activation of phospholipase Cγ2 without affecting its tyrosine phosphorylation. Therefore, PI3K regulates the phosphorylation of NADPH oxidase component $p47^{phox}$ by controlling diacylglycerol-dependent PKCs, but not Akt in HL-60 cells (Figure 4A) (50). Furthermore, addition of $p47^{phox}$ to the minimal core complex of NADPH enzyme promotes the capacity of a lipid product of PI3Ks phosphatidylinositol 3-phosphate (Ptdlns(3)P) to specifically enhance the formation of ROS (11). These results suggest that PI3Ks regulate anti-bacterial and pro-inflammatory innate host responses *via* both Akt dependent and independent pathways, exhibiting an orchestrated effect on cell responses to chemoattractants.

The role of PI3Ks in malignant tumors

Aberrant activation of PI3K pathway is associated with the development of cancer due to loss of the balance between cell proliferation and apoptosis. In fact, PI3Ks not only play a major role in promoting tumor growth but also affecting tumor responses to treatment.

HER2 (also known as erbB2) and its homologues HER1 (epidermal growth factor receptor; EGFR), HER3 and HER4 belong to the HER family of receptor tyrosine kinases (51). In normal cells, the receptors when activated control cell growth, differentiation, motility, and adhesion. Dysregulation of the function of these receptors occur in cancer cells. For instance, increased expression of HER2 in breast cancer cells (52) or HER1 in lung cancer cells (53) provides these cells with growth advantage over surrounding normal epithelial cells. Over-expression of HER2 has been reported in almost 30% of breast and ovarian cancers (54). HER2-HER3 forms dimer that activates PI3K/Akt-mediated survival pathway in tumor cells (55). HER3 possesses seven tyrosine residues that when phosphorylated, bind SH2 domains of the p85 regulatory subunit of PI3K (56). Hypoxia-inducible factors (HIFs), which are composed of α and β subunits, are constitutively activated in most human cancer cells and promote the production of angiogenic factors that favor tumor growth and metastasis. It has recently been reported that HER2 overexpression in breast cancer cells induces HIF activation *via* PI3K/Akt pathway suggesting that angiogenic processes involving HIF may also be elicited in a manner

independent of hypoxia (57).

Although overexpression of HER family receptors enables malignant tumor cells to more readily sense growth signals present in the microenvironment *via* PI3K-mediated pathway, many tumor cells also constitutively express higher levels of PI3K. The increased expression of PI3K3C, a gene encoding the p110α catalytic subunit of PI3K, has been detected in cancer cells of ovary (58), cervix (59), head and neck (60), stomach (61), and astroglia in the brain (62). Amplified expression of the gene for of Akt has also been found in human cancer cells. For instance in gastric carcinoma (63) and glioblastoma (62), Akt1 was considered as a potential oncogene. Akt2 gene amplification has been detected in malignant tumor cells of the ovary, pancreas, breast and stomach (64, 65). Moreover, immunohistochemical studies have detected constitutively phosphorylated Akt in melanoma and carcinoma cells of the head and neck, breast, colon, ovary, pancreas, bile duct and prostate (66-78). Thus, elevated activity of PI3K and the downstream Akt, Akt2 in particular, is a common feature in a variety of human malignant tumor cells.

In addition to increased expression by tumor cells of genes coding for proteins that are involved in PI3K signaling cascade, mutation in some genes also contribute to the aberrant activity of PI3K signaling cascade in tumor cells. For instance, mutation in *PIK3CA* gene leads to elevated levels of the p110α catalytic subunits of class I-A PI3Ks in many human carcinoma cells (79).

Phosphatase and tensin homologue (PTEN), which was originally identified as a tumor-suppressor gene, is a phosphatase with PI(3,4,5)P3, a PI3K product as its physiologic lipid substrate. PTEN dephosphorylates PI(3,4,5)P3 at 3 inositol positions and acts as a negative regulator for PI3K-mediated signaling. PTEN is frequently mutated in human tumor cells with high level of malignancy, notably in glioblastoma and carcinomas of the uterus, prostate, thyroid, breast, colon, and tumors of the soft tissue (Table 2) (80-87). In addition, PTEN mutation in germ line resulted in a rare hereditary syndrome, referred to as Cowden's disease, which is associated with a higher risk for development of malignant tumors, notably breast cancer (80). Mutations in PTEN cause its loss of function as a negative regulator of Akt activation through PI(3,4,5)P3 dephosphorylation, an important checker of maligent tumor progression.

Defects revealed by PI3K gene depletion

In mice depleted of PI3Kγ gene, DCs show reduced migratory ability in response to chemokines both *in vivo* and *in vitro*. When segments of dorsal ear skin from PI3Kγ$^{-/-}$ mice were placed in culture, the number of MHC-class II DCs emigrating spontaneously into the culture medium, was decreased in the presence of TNF or CCL21 in the incubation medium. *In vivo*, skin DCs of PI3Kγ$^{-/-}$ mice displayed an impaired ability to travel following local antigen administration as indicated by markedly reduced CD11c^{+} DCs recovered from inguinal lymph nodes, in association with diminished total cellularity of these lymph node and impaired adaptive immune responses. Thus, PI3Kγ plays a fundamental role in DC trafficking and the induction of specific immunity (88).

PI3Kγ$^{-/-}$ mice are *via*ble, with essentially normal leukocyte counts, suggesting that class I-B PI3K is not involved in the development and differentiation of hematopoietic cells. However, PI3Kγ$^{-/-}$ mice display a phenotype mainly affecting macrophages and neutrophils, with reduction in their migratory capacity in response to a variety of chemoattractants. PI3Kγ$^{-/-}$ neutrophils fail to produce PI(3,4,5)P3, to activate PKB, and to mount normal respiratory burst when stimulated by chemoattractants. Chemotaxis of PI3Kγ$^{-/-}$ macrophages was decreased in response to C5α, SDF-1 (stromal cell-derived factor-1), RANTES (regulated on activation, normal T cell expressed and secreted), MDC (macrophage-derived chemokine), and MIP-5 (macrophage inflammatory protein-5), suggesting that PI3Kγ is a crucial signaling molecule that controls proper cell responses in inflammation in which phagocyte infiltration is a hallmark (89-91).

In contrast to PI3Kγ, which appears not to be essential for mouse development, both p110α$^{-/-}$ and p110β$^{-/-}$ mice die early during embryogenesis due to profound defects in cell proliferation. Interestingly, heterozygous p110α$^{+/-}$ and p110β$^{+/-}$ mice grow normally (92-95). In p110δ$^{D910A/D910A}$ B-cells, which has been introduced a point mutation of Asp^{910}→Ala (D910A), anti-IgM-induced Akt/PKB phosphorylation was almost completely lost, and the calcium flux was attenuated and the proliferation of and p110δ$^{D910A/D910A}$ B-cells in response to anti-IgM stimulation was abrogated, suggesting that p110δ is the principal PI3K that signals downstream of the BCR. In p110δ$^{D910A/D910A}$ T-cells, Akt/PKB phosphorylation after cross-linking TCR by anti-CD3 antibodies, was almost ablated and calcium flux was attenuated, indicating that p110δ is also the main provider of PI3K activity downstream of the TCR (96). However, there are differences between p110δ$^{-/-}$ and p110δ$^{D910A/D910A}$ mice, in that the failure to proliferate in response to polyclonal anti-IgM F(ab')$_2$ antibody was less pronounced in p110δ$^{-/-}$ B-cells than p110δ$^{D910A/D910A}$ B-cells. Spleens of adult p110δ$^{-/-}$ mice contain nearly normal numbers of B- and T-cells, whereas the numbers of p110δ$^{D910A/D910A}$ spleen cells were reduced by approximately 50%. Compared with p110δ$^{D910A/D910A}$ mice, the apparently milder phenotype of p110δ$^{-/-}$ mice implies that abrogating that expression of the p110δ catalytic subunit might allow for some compensatory signaling through p110α and/or p110β (96, 97).

Mice depleted p85α, an adaptor protein of p110α, β and δ, develop B cell immunodeficiency, but they are viable with normal T cells, presumably due to compensatory overexpression of a splice variant of the adaptor 50α, which is not observed in B cells of wild type mice. The expression of both p50α and p55α splice variants was upregulated in muscle and adipose cells of p85α$^{-/-}$ mice (98, 99). Interestingly, p85α$^{-/-}$ mice develop hypoglycemia with decreased plasma insulin, whereas p85α$^{+/-}$ mice exhibit significantly

increased insulin sensitivity (100). These observations imply the possibility to target p85α as a therapeutic approach to type II diabetes.

Similar to p85α$^{-/-}$ mice, p85β$^{-/-}$ mice show hypo-insulinemia, hypoglycemia, and increased insulin sensitivity. The basis for this phenotype is attributed to significantly increased insulin-induced activation of Akt in muscle and enhanced insulin-dependent tyrosine phosphorylation of insulin receptor substrate-2. However, p85β$^{-/-}$ mice tend to be smaller than their wild-type littermates (101), suggesting a role for p85β in mouse development. It is interesting to note that depletion of both p85α and p85β genes is embryonic lethal (102).

Gene transfer and knockout studies have established the role of the PI3K regulator PTEN as a tumor suppressor. Restoration of PTEN expression in PTEN-deficient mutant human glioblastoma multiforme cell lines causes growth suppression. However, increasing PTEN expression in glioblastoma multiforme lines that retain normal PTEN expression does not suppress cell proliferation (103). In *Pten* heterozygous, Mlh1-null (mismatch repair deficient) mice, a majority of the animals develop polypoid lesions in the endometrium at 6 to 9 weeks of age. By 14 to 18 weeks, all double-mutant mice carry lesions histologically similar to those seen in *Pten*$^{+/-}$ mice, and some develop invasive disease. Moreover, the frequency of the loss of the wild-type *Pten* allele in double-mutant mice at 14 to 18 weeks is similar to lesions from 40-week-old *Pten*$^{+/-}$ mice. Thus, DNA mismatch repair deficiency can accelerate endometrial tumorigenesis in *Pten* heterozygous mice (104). *Pten*$^{+/-}$ mice show partially penetrant embryonic lethality associated with defects in both neural and placental development. The lethality is completely rescued by grb2 haplo-insufficiency. In contrast, grb2 heterozygosity did not alter tumorigenesis in either *Pten*$^{+/-}$ or T cell-specific *Pten*$^{-/-}$ mice (105). In addition, heterozygous *Pten*$^{+/-}$ mice develop gonadostromal, germ-line, and hemato-poietic tumors, and carcinormas of the endometrium, thyroid, prostate, breast, liver, and intestine (87). Reconstitution of PTEN into PTEN-deficient prostate, melanoma, or breast cancer cell lines inhibits tumor growth (87). These observations imply that lack of a limiting factor for PI3K activity promotes the malignant transformationnn of cells from a variety of tissues and organs. Yet it remains unclear whether the loss of protein phosphotase activity of PTEN also contributes to tumor development.

Perspectives

The role of PI3Ks in sustaining cell survival, proliferation, host immune responses, and the development of inflam-matory diseases and tumors has been increasingly appreciated during the past few years. The discovery of substrates for Akt has revealed novel functions of the PI3K-Akt/PKB pathway in inflammation and tumor progression. An important remaining task in this area of research is to understand the interaction between PI3Ks and other signaling pathways to determine potential positive and negative regulators under pathophysiologic conditions *in vivo*. For example, further characterization of the transcription factors involved in by PI3K-mediated regulation of Bcl-2 family members will be necessary to delineate the mechanisms of rescue of granulocytes cytokines from intrinsic apoptotic pathway. Studies of the role of PI3Ks in malignant tumors have indicated their potential as important therapeutic targets. In this regard, it has been shown that PI3K/Akt/mTOR pathway regulates several normal cellular functions but is also critical for tumorigenesis, including tumor cell survival, proliferation, and their mobility (106). On the other hand, termination of Akt signaling cascade is under the control of two key proteins, PTEN and PHLPP, a protein phosphatase, that inactivates Akt by direct dephosphorylation of its hydrophobic motif. PHLPP levels are markedly reduced in several colon cancer and glioblastoma cell lines that in contrast show elevated Akt phosphorylation. Reconstitution of PHLPP into glioblastoma cell lines results in marked suppression of tumor formation *in vivo* (107). These observations should prompt evaluation of drugs that regulate PI3K/Akt pathway in hope of developing novel anti-cancer therapeutics.

Acknowledgements

We thank Dr. J. J. Oppenheim for critical review of the manuscript, Ms. Cheryl. Fogle and Ms. Cheryl. Nolan for secretarial assistance.

This project has been funded in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12400, and by the Intramural Research Program of the National Cancer Institution.

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