

Review

Phosphatidylinositol-3,4,5-Triphosphate and Cellular Signaling: Implications for Obesity and Diabetes

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Key WordsPtdIns(3,4,5)P₃ • PI3K/PTEN equilibria • PH domain-containing proteins • Insulin resistance • Cardiovascular complications • Vascular inflammation**Abstract**

Phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P₃) is one of the most important phosphoinositides and is capable of activating a wide range of proteins through its interaction with their specific binding domains. Localization and activation of these effector proteins regulate a number of cellular functions, including cell survival, proliferation, cytoskeletal rearrangement, intracellular vesicle trafficking, and cell metabolism. Phosphoinositides have been investigated as an important agonist-dependent second messenger in the regulation of diverse physiological events depending upon the phosphorylation status of their inositol group. Dysregulation in formation as well as metabolism of phosphoinositides is associated with various pathophysiological disorders such as inflammation, allergy, cardiovascular diseases, cancer, and metabolic diseases. Recent studies have demonstrated that the impaired metabolism of PtdIns(3,4,5)P₃ is a prime mediator of insulin resistance associated with various metabolic diseases including obesity and diabetes. This review examines the current status of the role of PtdIns(3,4,5)P₃ signaling in the regulation of various cellular functions and the implications of dysregulated PtdIns(3,4,5)P₃ signaling in obesity, diabetes, and their associated complications.

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Introduction

Since their discovery in the mid 1950s by Hokin and Hokin [1], phosphoinositides (PI), the phosphorylated derivatives of membrane lipid phosphatidylinositol (PtdIns), have become established as important mediators of a number of signal transduction pathways [2]. They exert their function either indirectly as the precursor of second messengers, such as

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inositol-1,4,5-triphosphate (Ins(1,4,5)P₃) and diacylglycerol (DAG), or directly by interacting with downstream effector protein molecules and thereby orchestrating the spatio-temporal organization of intracellular signal transduction cascades [3-5]. Recent studies demonstrate the role of PI in a variety of physiological processes, including cell proliferation, survival, cytoskeletal regulation, intracellular vesicle trafficking, and cell metabolism [2, 4]. As a result, the enzymes involved in both their formation and their metabolism have become more popular as the subjects of biomedical research, since any alteration to them is linked to the development of pathophysiological disorders [6].

The inositol head group of PtdIns contains five free hydroxyl groups, of which only three have been found to be phosphorylated in mammalian cells [6]. PtdIns constitutes approximately 5-10% of the total lipid in eukaryotic cells membranes, 5% of which is phosphorylated at the 4'-position, and the other 5% of which is phosphorylated at the 4'- and 5'-positions. However, less than 0.25% is phosphorylated at the 3'-position, which indicates specific regulatory functions for these 3'-phosphorylated inositol lipid molecules inside the cell [7]. Phosphatidylinositol 3-kinases (PI3K) are a family of lipid kinases that catalyze the addition of a phosphate group to the 3'-position of the inositol ring of PtdIns or PI, resulting in the formation of four different lipid products: PtdIns(3)P, PtdIns(3,4)P₂, PtdIns(3,5)P₂, and PtdIns(3,4,5)P₃. According to how they use the substrate molecules, the nine members of the PI3K family are grouped into three classes: I, II, and III, as suggested by Domin and Waterfield [8]. Among the four different 3'-phosphorylated inositol lipid products, phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P₃) is emerging as an important signaling molecule in a myriad of cellular processes with implications in a wide range of human diseases (Fig. 1) [9]. PtdIns(3,4,5)P₃ plays a key role in insulin signaling and glucose metabolism; any alteration to this signaling pathway is associated with various metabolic diseases including diabetes and obesity, and their associated complications, such as cardiovascular complications, inflammation, etc [9]. In this review we will discuss the role of PtdIns(3,4,5)P₃ in the regulation of insulin signaling and glucose metabolism and the implications of PtdIns(3,4,5)P₃ dysregulation in diabetic pathophysiology.

Regulation of intracellular PtdIns(3,4,5)P₃ concentration

The majority of intracellular PtdIns(3,4,5)P₃ is synthesized by the phosphorylation of PtdIns(4,5)P₂ in response to extracellular stimuli, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin, and insulin-like growth factor I (IGF-I) [10]. The class I PI3K are the only enzymes that utilize PtdIns(4,5)P₂ as a substrate to synthesize PtdIns(3,4,5)P₃. Class I PI3K consist of the p110 catalytic subunit and p85 regulatory subunit. Activation of class I_A PI3K (p110 α , β , and δ) by growth factor stimulation is regulated either via the interaction of their SH2 domain with the tyrosine-phosphorylated receptor protein kinases, RTK [11], or by the GTP-bound form of the small G protein, Ras [12]. The class I_B PI3K (p110 γ) can be directly activated via the $\beta\gamma$ subunits of the heterotrimeric G proteins [13]. In addition to this, a PtdIns(4,5)P₂-independent pathway for PtdIns(3,4,5)P₃ synthesis has also been reported [14, 15]. Both Tolias et al. [15] and Zhang et al. [14] reported that PtdIns-4-Phosphate-5-kinases utilize PtdIns(3)P as substrate to form PtdIns(3,4,5)P₃ by phosphorylating the 4'- and 5'-positions of the inositol ring in a concerted reaction pathway. However, the contribution of this new pathway to intracellular PtdIns(3,4,5)P₃ concentration is still unknown. Under basal conditions, PtdIns(3,4,5)P₃ levels in plasma membrane are very low, constituting about 0.0001% of the total plasma membrane lipid content. However, in response to extracellular stimuli, the phosphorylation of PtdIns(4,5)P₂ by Class I PI3K causes a rapid increase in PtdIns(3,4,5)P₃ concentrations that is 500-fold greater than its basal levels [16]. The low levels of PtdIns(3,4,5)P₃ represent its potential regulatory effect on signal transduction pathways.

Dephosphorylation of PtdIns(3,4,5)P₃ is essential to prevent its accumulation and constitutive signaling processes. Several PtdIns(3,4,5)P₃ phosphatases have been isolated,

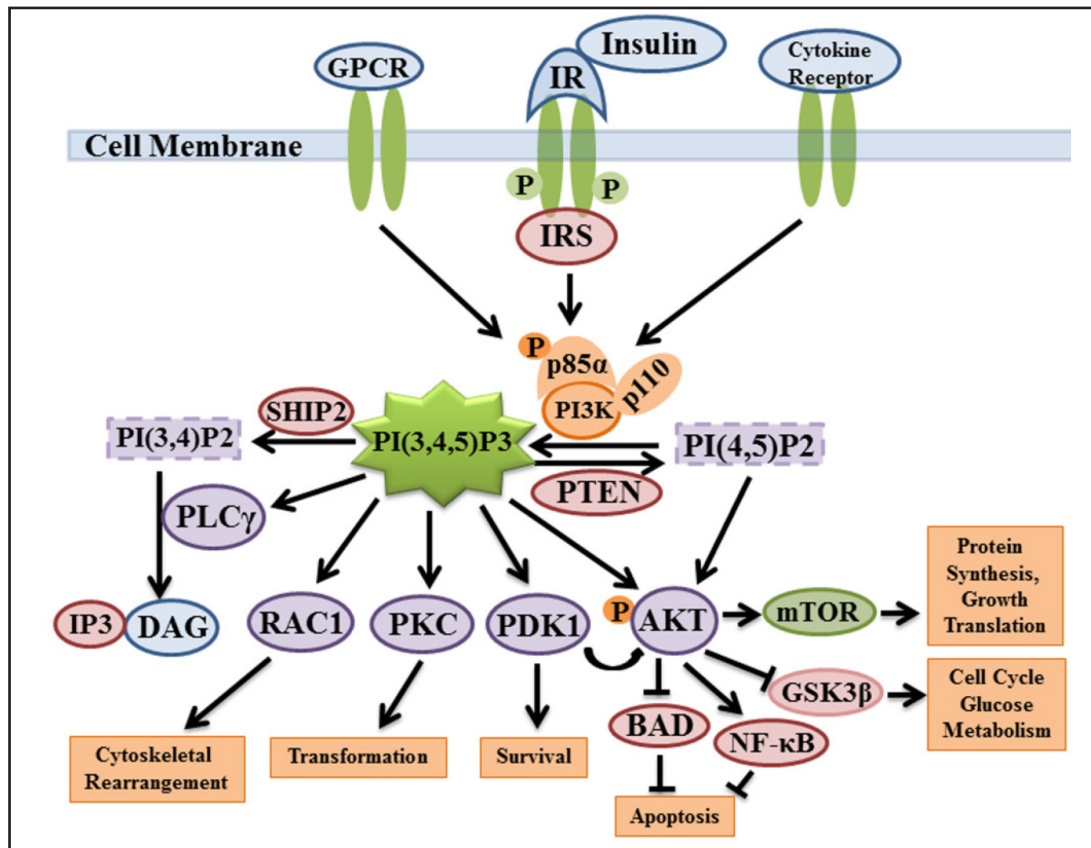


Fig. 1. The schematic diagram of PtdIns(3,4,5)P₃ formation and its downstream signaling. Activation of PI3K either by growth hormone receptor (IRS), G-protein coupled receptor (GPCR), or cytokine receptor phosphorylates PtdIns(4,5)P₂ to PtdIns(3,4,5)P₃ which recruits PH-domain containing effector molecules, such as AKT, PDK1, PKC ζ , small G-protein (RAC1), etc. Activation of these effector protein molecules regulates a myriad of cellular functions, including cell survival, proliferation, cytoskeletal rearrangement, intracellular vesicle trafficking, and cell metabolism. The phosphatases, PTEN and SHIP2 quench PtdIns(3,4,5)P₃ formation and its signaling effect.

such as phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and SH2-containing inositol 5-phosphatases (SHIP) [17]. PTEN, a tumor suppressor protein, can dephosphorylate PtdIns(3,4,5)P₃ at the 3'-position [18] and SHIP can dephosphorylate it at the 5'-position [19]. Christophe and colleagues reported that PTEN plays an important role in maintaining the basal levels of PtdIns(3,4,5)P₃, while SHIP inhibit the stimulus-induced increase in PtdIns(3,4,5)P₃ concentration [17]. Therefore, concerted action of PI3K and several phosphatases regulate the PtdIns(3,4,5)P₃ levels in the plasma membrane; this tight regulation is critical for the prevention of hyper- and hypo-responsiveness to many extracellular stimuli.

Targets of PtdIns(3,4,5)P₃

PtdIns(3,4,5)P₃ recruits its effector protein molecules from cytoplasm to the membrane surface to activate, stabilize, and propagate the downstream signaling cascade. The mechanism of action involves direct binding of the inositol head group of PtdIns(3,4,5)P₃ to the pleckstrin homology (PH) domain of proteins resulting in their recruitment to the plasma membrane [16]. About 40 of the approximately 250 known PH domain-containing proteins are found to interact with PtdIns(3,4,5)P₃ with varying degrees of specificity and

affinity. The most commonly studied PtdIns(3,4,5)P₃-specific downstream effector protein molecules include protein kinase B/Akt, phosphoinositide-dependent kinase-1 (PDK1), protein kinase ζ (PKCζ), guanine nucleotide-exchange factors, such as GRP1, VAV, and ARNO, and Bruton's tyrosine kinase (Btk). The PH domain of the effector protein molecules consists of approximately 120 amino acid residues arranged into a bowl-like binding pocket so that the inositol head group of PtdIns(3,4,5)P₃ can fit easily. The tertiary structure of the binding pocket is highly conserved among different classes of PtdIns(3,4,5)P₃-specific effector protein molecules; however, the primary structure varies rapidly, and this variation contributes to the specific affinity for PtdIns(3,4,5)P₃ versus other membrane bound PI [20]. The basic amino acid residues of the PH domain create a highly positive electrostatic environment capable of attracting the negatively charged PtdIns(3,4,5)P₃ in the interior of PH domain. The arrangement of the phosphate group on the inositol head also plays an important role in binding [16].

The serine/threonine protein kinase B, also known as Akt, is the most well-known target of PtdIns(3,4,5)P₃. Binding of PtdIns(3,4,5)P₃ to Akt produces conformational changes within the protein resulting in its phosphorylation (at residues Thr308 and Ser473) and full activation [21]. In most cases it has been observed that phosphorylation at the activation loop of these kinases is necessary for activation, but phosphorylation of other residues is also required for full activation. The phosphorylation of Akt on Thr308 is also catalyzed by another PtdIns(3,4,5)P₃-dependent protein, PDK1, which is recruited at the plasma membrane and aids in full activation of Akt. Mutation of the PH domain of PDK1 inhibits the full activation of Akt, which prevents the activation of downstream pathways [22]. PDK1 also phosphorylates the activation loop of p70S6K [23] and other PKC family members [24]. PtdIns(3,4,5)P₃ causes a marked stimulation of the autophosphorylation of PKCζ, which indicates that PKCζ may be a direct target of PtdIns(3,4,5)P₃ [25].

The presence of a PH domain in a wide range of guanine nucleotide-exchange factors for small G protein suggests that PtdIns(3,4,5)P₃ fulfills a regulatory function in the activation of these proteins [26]. Membrane localization of the GRP1 family of nucleotide-exchange factors via binding with PtdIns(3,4,5)P₃ stimulates their exchange activity toward small G protein, Arf, and Arf5 [27]. PtdIns(3,4,5)P₃ also stimulates the exchange activity of another nucleotide-exchange factor, VAV, towards the activation of small G protein, Rac [28]. PDGF-induced binding of GTP to the Rac depends on the activation of PI3K, suggesting a direct effect of PI3K lipid production on the activation of the exchange factor for Rac [29]. The PH domain of Btk was shown to interact with PtdIns(3,4,5)P₃ with high affinity [30]. Overexpression of Class I PI3K was shown to stimulate the autophosphorylation of Btk and increase its activity, which was inhibited by a PI3K inhibitor, wortmannin [31, 32]. Mutation in the PH domain of Btk also significantly affects its binding with PtdIns(3,4,5)P₃, causing X-linked immunodeficiency in mice [33]. The SH2 domains of the regulatory subunit of Class I PI3K, p85, have also been shown to interact with PtdIns(3,4,5)P₃, and the amount of PtdIns(3,4,5)P₃ is inversely correlated with the association between PI3K and tyrosine phosphorylated proteins in stimulated cells [34]. PtdIns(3,4,5)P₃ was also shown to bind with the SH2 domain of PCLy to enhance its activity toward PtdIns(4,5)P₂ *in vitro* [35, 36]. Another study reported that the PH domain of PCLy also binds with PtdIns(3,4,5)P₃ and thus mediates the translocation of PCLy to the plasma membrane in response to extracellular stimuli [37]. Altogether, these studies suggest that PtdIns(3,4,5)P₃ plays an important role in activating its effector protein molecules for the regulation of various physiological processes.

Cellular functions controlled by PtdIns(3,4,5)P₃

With the identification of several targets of PtdIns(3,4,5)P₃, many of the PI3K dependent cellular functions can be explained at the molecular level. Localization and activation of PtdIns(3,4,5)P₃ dependent effector proteins regulate a number of cellular functions including cell cycle progression, cell survival and apoptosis, cellular growth, cytoskeletal rearrangement, intracellular vesicle trafficking, and chemotaxis.

Cell cycle progression

The family of cyclin proteins plays an important role in the regulation of cell cycle progression via its interaction with cyclin-dependent kinase (CDK) inhibitors including p21^{Cip1} and p27^{Kip1} [38]. An increase in cyclin D1 and a reduction in p27^{Kip1} are required for the cells to transition from the G₁ checkpoint to the S phase of interphase of the cell cycle [39]. Binding of PtdIns(3,4,5)P₃ to Akt causes a translocation of active Akt to the nucleus where it phosphorylates Forkhead box Class O (FoxO) transcription factors on three different sites and thus promotes the release of FoxO from the nucleus [40]. Binding of export FoxO with 14-3-3 proteins causes the retention of the FoxO/14-3-3 complex in the cytosol, while the absence of FoxO from the nucleus increases the transcription of Cyclin D1 and reduces the transcription of CDK inhibitor p27^{Kip1}, triggering a G₁-S phase transition [41]. Activation of Akt again inhibits the activity of glycogen synthase kinase 3β (GSK3β), which also increases the accumulation of cyclin D1 [39].

Cell survival and Apoptosis

The activation of the PI3K/PtdIns(3,4,5)P₃/Akt pathway enables one of the most important cell survival signaling circuits in a wide range of cell types [41]. PtdIns(3,4,5)P₃ mediated activation of Akt phosphorylates the death promoter BAD at Ser136, leading to its retention in the cytosol via binding to 14-3-3 proteins, and inhibiting its translocation to mitochondria [42, 43]. The anti-apoptotic proteins, Bcl-2 and Bcl-X_L, are then released from the mitochondria, resulting in the inhibition of the mitochondrial step in apoptotic activation [43]. Akt can also activate and phosphorylate inhibitory-κB kinase (IKK) at Thr23, which in turn phosphorylates the inhibitor IκB, leading to the release of transcription factor NF-κB. The translocation of NF-κB to the nucleus activates the transcription of the anti-apoptotic proteins [44, 45]. Another important role played by Akt in apoptosis is mediated by the inactivation and cytosolic retention of FoxO transcription factors as mentioned above. FoxO factors stimulate the transcription and synthesis of a variety of proteins involved in apoptosis, including the TNFα family members, TRAIL and FasL, and the pro-apoptotic family members, BIM and PUMA [38].

Cellular growth

Regulation of cellular growth is one of the key functions of PtdIns(3,4,5)P₃. Nutrients and growth factors in the extracellular matrix activate the PI3K/ PtdIns(3,4,5)P₃ signal transduction pathway, which increases the biosynthesis of various lipids and proteins required for rapid cellular growth. Both Akt and the nutrient sensor mTOR work together to control cell size and growth. PtdIns(3,4,5)P₃-activated Akt phosphorylates and inhibits the action of tuberin, which is in a complex with hamartin, known as the tuberous sclerosis complex (TSC) [46]. Once phosphorylated the complex is no longer able to suppress mTOR. Active mTOR binds to eukaryotic initiation factor 3 (eIF3) and phosphorylates its downstream targets, p70S6K and 4EBP1 (eIF4E binding protein) [47]. p70S6K in turn phosphorylates multiple effectors required for cell growth and protein synthesis including ribosomal protein S6, a protein essential for the regulation of cell size [48]. 4EBP1, upon phosphorylation, dissociates from its target eIF4E and increases the cap dependent translation [47]. In addition to the regulation of translation, the action of mTOR has also been linked to the control of the availability of endogenously produced amino acids required for biosynthesis, mitochondrial biogenesis, and *de novo* lipogenesis [49].

Intracellular vesicle trafficking, cytoskeletal rearrangement, and chemotaxis

It has been postulated that PI3K plays an important role in vesicle recruitment to the plasma membrane based upon the observation that both the PI3K inhibitor and dominant-negative PI3K block the insulin stimulated translocation of glucose transporter 4 (GLUT4) to the plasma membrane [50, 51]. Considerable evidence reports that a signaling pathway necessary for GLUT4 translocation is the one that proceeds from the insulin receptor to the protein kinase Akt via activation of the PI3K/PtdIns(3,4,5)P₃ signaling pathway [52].

Activation of Akt stimulates GLUT4 translocation by phosphorylation of its substrate protein AS160, which possesses a GAP (GTPase-activating protein) domain that catalyzes the inactivation of Rab protein [53]. Rab proteins are the small G-proteins that in their GTP-bound form participate in vesicle movement and fusion [54]. The GAP stimulates the GTPase activity of Rab by triggering GTP hydrolysis, thus generating the inactive GDP-bound form of the Rab. Phosphorylation of AS160 by Akt inhibits its GAP activity, subsequently elevating the GTP-bound form of the cognate Rab protein, and thus triggering GLUT4 translocation [55]. When Rab proteins are converted into their GDP-bound form on the acceptor membrane, they are removed from the membrane into the cytosol by GDI (guanine-nucleotide-dissociation inhibitors) [56]. Several Rab proteins that probably participate in insulin stimulated GLUT4 translocation have been identified, including Rab10 in adipocytes [57-59], and Rab8A and Rab14 in muscle cells [60]. Identification of the PtdIns(3,4,5)P₃-dependent effector molecule, GRP1, a nucleotide-exchange factor, led to report that PtdIns(3,4,5)P₃ mediated localization of GRP1 to the membrane stimulates the nucleotide exchange activity of GRP1 toward Arf1, which provides an explanation for PtdIns(3,4,5)P₃ induced regulation of the translocation of intracellular vesicles to the plasma membrane [61, 62]. PtdIns(3,4,5)P₃ dependent activation of VAV2 or other Rac exchange factors, followed by binding of Rac to GTP, explain how PI3K mediates the effect of growth factor and Ras-stimulated cytoskeleton arrangement, leading to cell migration [28]. Other PtdIns(3,4,5)P₃ dependent effector molecules, Akt and PDK, have also been implicated in the regulation of cell migration and motility. Akt1 has been found to inhibit cell migration via phosphorylation of the actin-binding factor paladin, as well as via the regulation of the nuclear factors of activated T-cells, ERK and TSC2 [63]. PDK^{-/-} cells also show defects in cell migration [64].

Estimation of PtdIns(3,4,5)P₃ concentration

The quantitative detection of PtdIns(3,4,5)P₃ is an important challenge in cell biology studies [65]. Cellular PtdIns(3,4,5)P₃ levels have been quantified by several methods. The classical method for quantitative detection of PtdIns(3,4,5)P₃ in cell extract entails labeling cells with [³H] myo-inositol or [³²P] phosphate followed by chloroform-methanol lipid extraction, paper chromatography, and autoradiography [66]. In the modern version of this method, radiolabelled lipid extracts are separated by thin-layer chromatography (TLC) and analyzed by reverse-phase high performance liquid chromatography (HPLC), or lipid products are first deacylated and then analyzed by anion exchange HPLC [67, 68]. Although these radioactive methods have the advantage of yielding quantitative results, one of the major problems is that radiotracer labelling of cells is often not very efficient and labelling to isotopic equilibrium can take days. In addition, these radioactive methods are not very practical for analysis in tissue samples.

To avoid this metabolic labeling issue, several non-radioactive detection methods for PtdIns(3,4,5)P₃ have been reported. One method is based upon the anion-exchange HPLC separation of the deacylated lipid products followed by conductivity detection [69]. This method measures the changes in resistance between two electrodes, which depends upon the concentration of the deacylated lipid products. Due to the lower sensitivity limit of this conductivity detection method compared with that of the radioactivity based method, another non-radioactive approach has been developed. This new non-radioactive method is based upon electrospray ionization mass spectrometry (ESI-MS) of total extracted lipid upon addition of triethyl ammonium acetate, which improves signal intensity [70-72]. This method can distinguish different PI depending upon their distinct masses; MS analysis also yields information about the type of acyl constituents of the PI. Although this method is very sensitive, quantitative, avoids radioactivity, and allows analysis of PI in tissue samples, it does not distinguish between different isomers and requires sophisticated as well as expensive instruments.

Recently, a protein–lipid overlay assay has been found to be the simplest and fastest way of measuring PtdIns(3,4,5)P₃ levels, which involves incubation of PtdIns(3,4,5)P₃-binding protein (e.g. GRP1 PH domain probe) with a nitrocellulose membrane that contains aliquots of lipid extract [68, 73]. Since readymade blots of all PI are commercially available, this type of analysis has become highly popular. In a variation of this method, spotting of lipid extract has also been carried out in the wells of microtiter plates instead of on nitrocellulose membranes for high-throughput screens. Although these spot assays are very useful, data from more specific methods are required to confirm results obtained with this method.

Implication of PtdIns(3,4,5)P₃ signaling in metabolic disorders and its associated complications

As discussed above, the PtdIns(3,4,5)P₃ mediated signaling cascade is a highly ordered and concerted pathway in a variety of cells and tissues. The relatively low abundance and precise control over the levels of PtdIns(3,4,5)P₃ ensure the deliberate activation of its downstream effector molecules, which contribute to a wide variety of cellular functions. For this reason, it is not surprising that a significant number of human diseases can be linked to impaired PtdIns(3,4,5)P₃ signaling. PtdIns(3,4,5)P₃ is a prime mediator of insulin signaling and glucose metabolism and alterations in this pathway are associated with various metabolic disorders including obesity and diabetes, and their associated complications, such as cardiovascular diseases, inflammation, etc.

PtdIns(3,4,5)P₃, insulin signaling, obesity, and type 2 diabetes

Obesity and diabetes have emerged as major public health problems throughout the world and are associated with significant, potentially life-threatening co-morbidities. Results from metabolic and epidemiological studies provide strong evidence that the increasing prevalence of obesity is closely associated with the increase in type 2 diabetes [74, 75]. Some experts call this dual epidemic diabetes [76]. Insulin stimulated glucose uptake and metabolism is one of the fundamental regulators of glucose homeostasis in the body. Impaired insulin action or insulin resistance is associated with obesity and type 2 diabetes (T2D). PI3K/PtdIns(3,4,5)P₃ signaling plays an important role in the insulin stimulated glucose metabolism pathway [41]. When insulin binds with insulin receptor tyrosine kinase, it increases the phosphorylation of insulin receptor substrates (mainly IRS1 and IRS2) [77]. Mice lacking the IRS2 gene have increased food intake and they develop obesity, fatty liver, and diabetes [78, 79]. Tyrosine phosphorylated IRS proteins bind to the SH2 domains of the p85 regulatory subunit of Class IA PI3K. The catalytic subunit p110 has also been found to be involved in IRS/PI3K metabolic signaling. Mice lacking either p110 α or p110 β die in early embryogenesis, whereas mice heterozygous for p110 α or p110 β display impaired glucose metabolism [80, 81]. Peripheral insulin resistance has been suggested to be the product of impaired PI3K signaling in the effector cells [77, 82]. Inhibition of PTEN expression using PTEN antisense oligonucleotides normalized blood glucose levels in ob/ob (obese) mice [83], while overexpression of PTEN resulted in inhibition of PtdIns(3,4,5)P₃ production and glucose uptake in 3T3L1 adipocytes [84]. Mice lacking PTEN expression in adipose, muscle, or liver tissue have been found to have an increase in insulin sensitivity and glucose tolerance [85, 86]. SHIP2 heterozygous knockout mice have also demonstrated an increase in insulin sensitivity [87]. Both PTEN and SHIP2 have been implicated as negative regulators of the insulin signaling pathway [88].

Insulin stimulated glucose uptake in adipocytes and muscle tissues plays an important role in the regulation of body glucose homeostasis. Insulin treatment of fat and muscle cells causes a rapid increase in glucose transport by controlling the amount of GLUT4 translocation from cytoplasm to plasma membrane. Activation of Akt upregulates the glucose uptake mediated by GLUT4 translocation from the intracellular pool to the plasma membrane via the phosphorylation of its substrate protein AS160 containing GAP domain for Rab which are the small G proteins required for membrane trafficking [52, 89]. Overexpression of

AS160-4p, a constitutively active form of AS160, inactivates cognate Rab and decreases GLUT4 translocation [55], suggesting it is a negative regulator that is itself inhibited by insulin through the activation of Akt. Activation of cognate Rab by RNAi mediated knockdown of AS160 increases the GLUT4 concentration on the adipocyte surface [90, 91]. However, knockdown of AS160 partially releases the intracellular pool of GLUT4 mobilized by insulin, and careful analysis has shown that other unknown Akt substrate proteins must play an important role in overall GLUT4 regulation by insulin [91-93]. Activation of Akt also promotes glycogen synthesis via activation of glycogen synthase through GSK3 inhibition [94], and transcription of several genes involved in insulin secretion and action mediated by the regulation of the FoxO transcription factor [95]. Mice lacking the protein kinase Akt show insulin resistance and a diabetes mellitus-like syndrome [96]. Our previous study showed reduced levels of PtdIns(3,4,5)P₃, downregulation of Akt phosphorylation, and an increase in GLUT2 protein expression in the liver tissues of type 1 and type 2 diabetic rats, but the PtdIns(4,5)P₂ levels were unchanged [97]. We also observed that exogenous PtdIns(3,4,5)P₃ supplementation increased both glucose uptake and glucose utilization and that the effect is mediated by the activation of the Akt/PKCζ/λ/GLUT4 signaling pathway [98]. Jiang et al. reported that insulin stimulated glucose transport in adipocytes was inhibited by the PI3K inhibitor, wortmannin, but that supplementation with exogenous diC8-PIP3/AM (dioctanoyl-PIP3-acetoxymethyl ester) was capable of overcoming the inhibitory effect of wortmannin in insulin stimulated glucose transport [99]. Genetic mutation of the PH domain of PDK1 inhibits Akt, leading to glucose and insulin intolerance, indicating the critical role played by PtdIns(3,4,5)P₃ in glucose homeostasis [22]. The protein kinase Akt has also been identified as an important regulator of renal hypertrophy and apoptosis induced by hyperglycemia [100]. Recent studies in the literature focused on the role of endocannabinoids (EC) in diabetic nephropathy [101]. EC are the endogenous agonists of cannabinoid receptors type 1 and type 2 (CB1 and CB2) and this system participates in the regulation of lipid and glucose metabolism at several levels [101]. The activity of CB2 has been found to be linked with the Akt/MAPK signaling pathway, indicating that the Akt/MAPK signaling pathway plays a role in diabetic nephropathy [100, 102, 103].

In addition to protein kinase B/Akt, impaired activity of atypical protein kinase C (aPKC, including ζ and λ/ι) has also been observed in obese as well as type 2 diabetic patients [104, 105]. The function of aPKC in glucose metabolism is well established [104, 105] and it has been reported that different lipid components, such as phosphatidylinositols, phosphatidic acid, arachidonic acid, and ceramide, can activate aPKC [106, 107]. PtdIns(3,4,5)P₃ plays a crucial role in the complete and stable activation of PKCζ [106, 107]. Metformin is a well-known insulin-sensitizing drug widely used in the treatment of type 2 diabetic patients. Farese and colleagues reported that the PtdIns(3,4,5)P₃-induced activation of PKCζ is impaired in the muscles of type 2 diabetic or obese humans [104, 105, 108, 109]. However, PtdIns(3,4,5)P₃ supplementation increased the PKCζ activity in the muscles of long-term metformin-treated type 2 diabetic subjects compared to that seen in the muscles of diabetic subjects who were off long-term metformin treatment [110]. The improved responsiveness of PKCζ to PtdIns(3,4,5)P₃ suggests the important role played by PtdIns(3,4,5)P₃ in improving insulin-stimulated aPKC activation during long-term metformin treatment. However, knowledge about the effect of metformin on PtdIns(3,4,5)P₃ levels in different tissues of obese as well as diabetic subjects is still lacking and needs to be investigated.

Sirtuins, the mammalian homologue of SIR2, are a group of NAD(+) dependent enzymes that are widely regarded as fuel sensing molecules [111, 112]. SIRT1, the most extensively studied sirtuin, has a profound effect on glucose homeostasis [113, 114]. Decreased SIRT1 expression or activity contributes to the pathogenesis of diseases related to insulin resistance [114]. Inversely, activators of SIRT1 improve insulin sensitivity and ameliorate insulin resistance [113, 115]. Activation of SIRT1 directly regulates the phosphorylation of IRS2 through deacetylation of this substrate, which is a key player in the PI3K/PtdIns(3,4,5)P₃ signaling pathway [116]. SIRT1 knockdown in 3T3L1 adipocytes also results in a decrease in tyrosine phosphorylation of IRS1, and serine phosphorylation of AKT (with a subsequent

increase in JNK phosphorylation as well as serine phosphorylation of IRS1), followed by inhibition of insulin-stimulated glucose uptake and GLUT4 translocation [117]. Increased in SIRT1 expression of has also been found to downregulate both protein and mRNA levels of PTP1B, which acts as a negative regulator of insulin signaling mainly via the downregulation of IR or IRS1 phosphorylation [118]. These studies demonstrate a positive role for SIRT1 in the regulation of glucose metabolism in an insulin independent manner. In addition to sirtuins, AMP-activated protein kinase (AMPK) is another well-known fuel sensing molecule activated by a decrease in the cell's energy state as reflected by an increase in the AMP/ATP ratio [119, 120]. Studies in humans showed a decrease in AMPK activity in both muscle and adipose tissues of obese, insulin resistant patients [121-123]. A decrease in AMPK activity has also been observed in multiple tissues of animals with some or all of the hallmarks of metabolic syndrome, including obesity and diabetes [124, 125]. A potential mechanism by which AMPK improves insulin sensitivity includes suppression of the inhibitory serine phosphorylation of IRS via regulation of the expression of JNK, IKK, and S6 kinase, an important factor contributing to the development of insulin resistance in obesity and diabetes [119, 126]. Both SIRT1 and AMPK play important roles in the regulation of insulin sensitivity and glucose metabolism via regulating PI3K/PtdIns(3,4,5)P₃ signaling mediated by the activation of growth hormone receptor substrate (IRS) [116, 126]. However, the direct effect of these two fuel sensing molecules on the PtdIns(3,4,5)P₃ metabolism is not known.

PtdIns(3,4,5)P₃ signaling and β -cell response to glucose intolerance. Insulin secreted by the pancreatic β -cells instructs insulin responsive tissues to absorb glucose. Elevated PtdIns(3,4,5)P₃ levels in β -cells cause an activation of Akt via PDK1 [127, 128]. Activation of Akt regulates β -cell mass by activating the cell cycle regulators, Cyclin D1, Cyclin D2, p21^{Clp1} and Cyclin-dependent kinase 4 [129] as well as via regulating various anti-apoptotic genes [130]. IRS-mediated activation of the MAPK signaling pathway through GRB2, a SH2/SH3 containing protein, also stimulates β -cell replication and inhibits apoptotic cell death [131]. Impaired insulin signaling in β -cells causes a decrease in cell proliferation and an increase in apoptosis. Although increased insulin secretion and expansion of the β -cell mass can compensate for the elevated demand for insulin during the initial stages of glucose intolerance in the pathological state of insulin resistance, studies in both diabetic patients and rodents suggest that diabetes results when β -cells no longer proliferate or secrete enough insulin to compensate for insulin resistance [132].

PtdIns(3,4,5)P₃ signaling in insulin responsive tissues. Increased insulin resistance in insulin responsive tissues such as liver, muscle, and adipocytes elevates plasma glucose levels. As in β -cells, insulin stimulated upregulation of PtdIns(3,4,5)P₃ in hepatocytes also activates Akt and aPKC through PDK1/2. Akt in turn phosphorylates the transcription factor FoxO1 (forkhead box protein O1) which causes its translocation from nucleus to cytoplasm, thus inhibiting its transcriptional activity [133, 134]. In the nucleus FoxO1 activates the promoters of the gluconeogenic enzymes G6Pase (glucose-6-phosphatase) [135] and PEPCK (phosphoenolpyruvate carboxykinase) [136]. Thus PtdIns(3,4,5)P₃ signaling normally inhibits gluconeogenesis but impaired insulin signaling causes aberrant hepatic glucose production and increased blood glucose levels. In addition to the impaired gluconeogenesis, increased fat storage in the liver is another contributing event in the development of T2D. The transcription factors SREBP (sterol regulatory element binding proteins) play an important role in cell metabolism by regulating synthesis of fatty acids, triglycerides, and cholesterol [137]. Activation of aPKC in hepatocytes activates the transcription of SREBP1c, while Akt stimulates the processing of SREBP1c, which is required for its activity [138, 139]. SREBP1c in turn transactivates FAS, promotes fat storage within the liver, and thus substantially reduces overall insulin sensitivity [140, 141]. The importance of oxidative stress in the pathogenesis of diabetes has become increasingly apparent over the past few years [142]. High levels of ROS can cause severe damage to DNA, proteins, and lipids, which could lead to cell death and organ dysfunction [143-146]. Using liver tissue from streptozotocin-induced type 1 diabetic rats, Martinovic et al. reported that activation of JNK and Akt/JNK signaling pathways determines the extent of DNA damage [147].

In muscle, PtdIns(3,4,5)P₃-mediated activation of Akt and aPKC stimulates the translocation of GLUT4 from cytoplasm to the plasma membrane, which allows increased glucose uptake [89] followed by elevated glycogenesis [94]. Impaired PtdIns(3,4,5)P₃ signaling results in decreased levels of GLUT4 translocation and reduced glucose uptake leading to diabetic pathophysiology [148]. PtdIns(3,4,5)P₃ signaling plays a profound role in adipogenesis. The effector molecule Akt plays an important role in adipocyte differentiation [149]. An RNAi-mediated decrease in Akt1 inhibited the differentiation of 3T3L1 cells into adipocytes [150], whereas constitutively active Akt can promote the differentiation of 3T3L1 cells, even in the absence of other inputs [151]. Furthermore, mouse embryonic fibroblasts (MEF) lacking Akt1 also displayed an inability to differentiate into adipocytes [152]. Activated Akt regulates the activities of several downstream target proteins involved in adipogenesis and lipogenesis [153-155]. Phosphorylation of the transcription factor GATA2 by Akt increases adipose tissue differentiation and reduces adipose tissue related inflammation in high-fat diet fed mice [156]. Constitutive activation of Akt promotes the nuclear localization of SREBP1, the expression of lipogenic genes, and the production of various classes of lipids (unsaturated and saturated fatty acids, phosphatidylcholine, and phosphatidylglycerol) [154]. Porstmann et al. reported that inhibition of mTOR1 by rapamycin blocks Akt-induced nuclear accumulation of SREBP1 and the expression of lipogenic genes [157].

In addition to the insulin responsive tissues, insulin resistance in brain is also associated with obesity and diabetes [158]. Neuron-specific knockout mice models of IR or its receptor shows obese, physically inactive, and insulin resistant phenotypes [159, 160]. Besides saturated free fatty acids, elevated levels of leptin plays an important role with regard to impaired insulin action [161]. Elevated leptin concentrations decrease insulin-mediated phosphorylation of IR and Akt in both human primary astrocytes and brain tissues of mice, which leads to a decline in locomotor activity [162]. Thus approaches that keep leptin levels in the brain within the physiological range may be beneficial in promoting physical activity and weight loss.

Role of PtdIns(3,4,5)P₃ in the inhibition of insulin signaling. In addition to the stimulation of insulin signaling [41], PtdIns(3,4,5)P₃ also initiates cellular events that can cause the inhibition of insulin signaling [163, 164]. PtdIns(3,4,5)P₃ mediated recruitment of O-linked-β-N-acetylglucosamine transferase (O-GlcNAc, OGT) from the nucleus to the plasma membrane activates OGT, which catalyzes the addition of GlcNAc to the insulin signaling intermediates downstream of PtdIns(3,4,5)P₃, leading to the inhibition of the insulin signaling pathways [163]. Mitochondrial protein prohibitin (PHB) has also been shown to inhibit insulin signaling via its interaction with PtdIns(3,4,5)P₃ [164]. Studies have shown that the central nervous system (CNS) can also influence insulin action and glucose homeostasis via regulation of feeding behavior and energy expenditure in response to hormones and various nutrients [165]. Leptin and insulin are two well-known fuel sensors that can inhibit food intake and stimulate energy expenditure via their hypothalamic receptors, which leads to the activation of the neurons that express proopiomelanocortin (POMC), a key hypothalamic neuronal subset for energy homeostasis [166]. The intracellular signaling mechanism of these two hormones converges at the PI3K pathway. Enhanced PtdIns(3,4,5)P₃ signaling in POMC neurons of POMC cell-restricted PTEN knockout mice caused hyperphagia and sexually dimorphic diet-sensitive obesity [167]. As discussed above, any mechanism related to decreased or increased PtdIns(3,4,5)P₃ production or expression can result in obesity and diabetic phenotypes.

PtdIns(3,4,5)P₃ signaling and cardiovascular disease

Metabolic disorder is one of the major contributors to the prevalence of cardiovascular disease [168, 169]. Excessive body weight, insulin resistance, and glucose intolerance have been found to be directly associated with a number of cardiovascular risk factors including hypertension, impaired glycemic control, dyslipidemia, and hemostatic and rheological factors [170]. These changes lead to an increased risk for coronary heart disease, stroke, and cardiovascular death. A significant increase in cardiovascular morbidity and mortality

has been observed among obese people compared to that in normal-weight subjects [171]. Dysregulated PtdIns(3,4,5)P₃ signaling plays an important role in the development of cardiac pathophysiology including cell survival, hypertrophy, contractility, and metabolism [172]. Both PI3K and PTEN are expressed throughout the cardiac tissue, which includes cardiomyocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells [173]. Class I_A PI3K isoforms (p110α and p110β) have been found to regulate physiologic growth of cardiac tissue via activation of RTK [173, 174], whereas class I_B PI3K isoform (p110γ) is required for the contractility function of myocardium [174]. Overactivation of PI3K or loss of the PTEN mediated increase in PtdIns(3,4,5)P₃ signaling induces various pathological disorders, including myocardial hypertrophy and decreased contractility, thus impairing normal cardiac function [173, 174]. In contrast, activation of PtdIns(3,4,5)P₃ signaling has been shown to mimic the cardio-protective nature of ischemic preconditioning [175, 176]. Enhanced PI3K (p110α) activity in response to exercise has been shown to delay or prevent the progression of heart disease [177].

The downstream targets of PtdIns(3,4,5)P₃ signaling, such as Akt, GSK3, etc., also play an important role in cardiac complications [178, 179]. All three Akt isoforms are expressed in cardiac tissue; among them, Akt1 and Akt2 are the most prevalent [180, 181]. Activation of Akt in myocardium is required for cell growth, metabolism, and inhibition of apoptosis [182]. Cardiac tissue specific knockdown of Akt1 reduced the number of cardiomyocytes and decreased the overall heart size, but no effect was observed on contractility [183]. In contrast, either overexpression or increased Akt activity can cause cardiac hypertrophy and abnormal contractility [183-185]. Both isoforms of GSK3 (α and β) are expressed in cardiac tissue [179]. GSK3β has been found to be a regulator of important transcription factors, particularly the nuclear factors of activated T-cells, CREB, and the Jun family of proteins [179]. Constitutively active GSK3β has been shown to act as a hypertrophic restraint in the heart [186]; induction of hypertrophy by increased PtdIns(3,4,5)P₃ signaling has been linked to the inhibition of GSK3β [179]. Additional cardiac pathologies, such as diabetic cardiomyopathy [187], adrinmycin-induced cardiomyopathy [188], chronic β-adrenergic receptor stimulation [189], or pressure overload induced hypertrophy [190], have also been shown to involve an alteration in PI3K/Akt signaling.

High glucose treatment increases endothelial cell apoptosis [191], permeability [192, 193], and monocyte-endothelial cell (EC) adhesion [193, 194]. PI3K/Akt signaling in endothelial cells regulates cell survival, proliferation, microvascular permeability, and angiogenesis [195-197]. Varma et al. reported that high glucose-induced inhibition of the PI3K/Akt pathway causes endothelial cell proliferative dysfunction [198]. Using human umbilical vein endothelial cells (HUVEC) and THP-1 monocytes, our study demonstrates that high glucose treatment decreased intracellular PtdIns(3,4,5)P₃ levels and increased the expression of adhesion molecules such as ICAM-1 (intercellular adhesion molecule 1) in endothelial cells and CD11a (a subunit of lymphocyte function-associated antigen 1, LFA-1) in monocytes, and adhesion of monocytes to endothelial cells (EC) [199]. Treatment with a specific inhibitor of PtdIns(3,4,5)P₃, PIT-1, also increased the expression of adhesion molecules and monocyte-EC adhesion. Exogenous PIP3 supplementation, however, restored the loss in intracellular PtdIns(3,4,5)P₃ levels and downregulated the expression of adhesion molecules as well as monocyte-EC adhesion in HG-treated cells, suggesting a causal role for PtdIns(3,4,5)P₃ in the regulation of adhesion molecules and the adhesion of monocytes to endothelial cells treated with high glucose. Overall, the PtdIns(3,4,5)P₃ signaling pathway has been shown to exert multiple effects on the regulation of cardiac pathophysiology.

PtdIns(3,4,5)P₃ signaling and inflammation

Both obesity and diabetes are associated with excessive production of the pro-inflammatory cytokines seen in chronic low-grade inflammation. Progression of insulin resistance exacerbates inflammation. Class I_A PI3Kα and PI3Kβ are expressed ubiquitously, whereas class I_A PI3Kδ and class I_B PI3Kγ are predominantly found in cells of hematopoietic lineage [200]. Because of their restricted expression, it has been suggested that these isoforms

are critical regulators in different populations of immune cells such as neutrophils, mast cells, macrophages, T-cells, B-cells, and NK cells [201]. PI3K δ and PI3K γ mediated production of PtdIns(3,4,5)P₃ has been found to be an important target in a range of inflammatory and autoimmune diseases as well as in transplantation and hematological malignancies [201, 202]. In response to various extracellular stimuli, different cellular receptors such as antigen, cytokine, or chemokine receptors activate PtdIns(3,4,5)P₃ signaling in the immune system [203]. PtdIns(3,4,5)P₃ then recruits and activates GEF for Rac and Arf GTPases, resulting in actin cytoskeletal rearrangement and directional cellular movement [204]. Several studies reported the role of PI3K γ as the primary isoform responsible for cellular migration in response to chemoattractants in the early steps of inflammation [205, 206]. Using a selective PI3K δ inhibitor, IC87114, Sadhu et al. also reported the essential role played by PI3K δ -mediated amplification of PtdIns(3,4,5)P₃ levels, which leads to neutrophil polarization and directional movement [207]. The role played by PTEN phosphorylation and stability has also been found to be essential for proper inflammatory cell migration, suggesting the importance of PtdIns(3,4,5)P₃ signaling [208]. The downstream effector molecules of PtdIns(3,4,5)P₃ signaling, including PDK1 and Akt, also regulate the production of reactive oxygen species at the site of inflammation via the regulation of various NADPH oxidase family proteins [209, 210]. Increased activation of neutrophils and macrophages has been associated with various diseases including atherosclerosis, lupus, and rheumatoid arthritis, suggesting that impaired PtdIns(3,4,5)P₃ signaling plays a role in the development of these inflammatory diseases [211, 212].

Allergy and inflammation have been found to involve both mast cells and macrophages, followed by invasion of the inflamed area by effector cells such as monocytes, neutrophils, and mast cell precursors [213]. Mast cells express a high affinity receptor for IgE on their surface and, in response to allergens, this IgE receptor is crosslinked followed by phosphorylation of immune-receptor tyrosine-based activation motifs (ITAM) on the receptor β and γ chains. Class I PI3K are then activated via binding to the phosphorylated ITAM. Production of PtdIns(3,4,5)P₃ initiates the activation of Btk and PLC γ resulting in the opening of plasma membrane Ca²⁺ channels and granule release [214]. Activation of PI3K δ regulates the initial degranulation and release of cytotoxic molecules [215], whereas PI3K γ regulates the subsequent degranulation [216]. PTEN deficiency in mast cells, both as the result of genetic deletion or shRNA-mediated knockdown of PTEN, induces mastocytosis and increases allergic responses in mice [217, 218]. Increased PtdIns(3,4,5)P₃ signaling in mast cells has been implicated in a variety of immune disorders including allergic diseases, asthma, autoimmunity, and mastocytosis, suggesting that PtdIns(3,4,5)P₃ regulation is a promising therapy for mast cell mediated diseases [219].

T-cells and B-cells play an important role in adaptive immune responses. PI3K δ and PI3K γ have been found to be essential for the development and function of both T-cells and B-cells [220]. It has been suggested that PI3K γ mediates chemotaxis in T-cells, whereas PI3K δ is responsible for the migration of B-cells [221]. Several studies reported that both increased and decreased PtdIns(3,4,5)P₃ signaling have been associated with the development of autoimmunity, suggesting that PtdIns(3,4,5)P₃ plays a role in the regulation of the functions of T-cells [203, 222, 223]. The evidence discussed above provides information supporting the importance of PtdIns(3,4,5)P₃ signaling in immune function and diseases. Targeting the appropriate PI3K isoform could be a therapeutic tool for the treatment of various inflammatory diseases; several isoform specific inhibitors are currently under clinical investigation [202]. Impaired wound healing is a serious complication of diabetes, which diminishes physical activity, and, in some cases, leads to limb amputation [224]. Wound healing is a complex multistage process that includes different phases: inflammation, formation of granulation tissue, production of new structures, and tissue remodeling [225]. These processes are all regulated by the secretion of various pro-inflammatory cytokines (IL-1, IL-6, IL-10, and TNF- α) [226-228], chemokines [229, 230], and growth factors [226] that, upon binding to their specific receptor, activate various signaling pathways and transcription factors, such as Akt, STAT (signal transducer and activator of transcription), and NF- κ B [231,

232]. Supplementation with undenatured whey protein, a cysteine rich protein, restored the activation of Akt, STAT3, and NF-κB and improved the closure of diabetic wounds in diabetic mice compared to those in control mice [233].

Conclusion

PtdIns(3,4,5)P₃ has been established as an important second messenger in a variety of cellular processes, which has implications for a wide range of diseases. Activation of various PI3K isoforms and expression of different phosphatases such as PTEN and SHIP2 have emerged as important regulators of intracellular PtdIns(3,4,5)P₃ levels. Following formation, PtdIns(3,4,5)P₃ recruits various effector protein molecules such as PDK1, Akt, PKCζ, Btk, etc. at the inner plasma membrane, where they are able to exert effects on a number of cellular functions including cell survival, proliferation, cytoskeletal rearrangement, intracellular vesicle trafficking, and cell metabolism. Impaired PtdIns(3,4,5)P₃ signaling has been found to be associated with various metabolic diseases such as diabetes and obesity, and their associated complications, including cardiovascular diseases and inflammation. Overall, the PtdIns(3,4,5)P₃ signaling pathway has multiple effects on the regulation and pathophysiology of various human diseases. Although significant progress has been made in understanding the role of PtdIns(3,4,5)P₃ signaling in human diseases, further studies need to be performed on the activities of PI3K isoforms and the expression of PtdIns(3,4,5)P₃ phosphatases in various tissues among different patient populations. The information gained from these studies will aid in the development of target specific inhibitors; particular care should be taken to avoid the any kind of toxicity following exposure to various inhibitors. Additionally, the expression as well as the regulation of PtdIns(3,4,5)P₃ effector molecules remains to be fully characterized under normal as well as disease conditions. Understanding the precise mechanism of PtdIns(3,4,5)P₃ signaling in organ pathophysiology will be helpful in the development of potential therapeutic targets for the treatment of associated human diseases.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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