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Scaffolding function of PI3Kgamma emerges from enzyme's shadow

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Abstract

Traditionally an enzyme is a protein that mediates biochemical action by binding to the substrate and catalyzing the reaction that translates external cues into biological responses. Sequential dissemination of information from one enzyme to another facilitates signal transduction in biological systems providing for feed-forward and feedback mechanisms. Given this view-point, an enzyme without its catalytic activity is generally considered to be an inert organizational protein without catalytic function and has classically been termed as pseudoenzymes. However, pseudoenzymes still have biological function albeit non-enzymatic like serving as a chaperone protein or an interactive platform between proteins. In this regard, majority of the studies have focused solely on the catalytic role of enzymes in biological function overlooking the potentially critical non-enzymatic roles. Increasing evidence from recent studies implicate that scaffolding function of enzymes could be as important in signal transduction as its catalytic activity an antithesis to the definition of enzymes. Recognition of non-enzymatic functions could be critical as these unappreciated roles may hold clues to the ineffectiveness of kinase-inhibitors in pathology, which is characteristically associated with increased enzyme expression. Using an established enzyme phosphoinositide 3-kinase γ (PI3K γ), we discuss the insights obtained from the scaffolding function and how this non-canonical role could contribute to/alter the outcomes in pathology like cancer and heart failure. Also we hope that with this review, we provide a forum and a starting point to discuss the idea that catalytic function alone may not account for all the actions observed with increased expression of the enzyme.

Graphical Abstract

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Introduction

Proteins are the building blocks of all living organisms that can be broadly categorized into two groups: structural proteins and biologically active proteins. Structural proteins are the key main constituents of our bodies that contribute to the shape and elasticity of a cell, organ or body. Biologically active proteins can be sub-divided into contractile, transport, regulatory, storage, protective, or enzymes. Enzymes catalyze biochemical reactions in the cells wherein a complex sum of their activity accounts for the response of cells/organs. Enzymes that can be further classified into several groups based on the biochemical reactions they perform. The importance of the enzymatic function is reflected by the fact the even the simplest of organisms have hundreds of enzymes in a living cell catalyzing key reactions that are critical for life. Current classification system is designed based on the general class of reaction the enzymes catalyze. The most common broad umbrella of enzyme classes can be categorized into oxidoreductases, ligases, hydrolases, lyases, isomerases and transferases.

The enzyme of interest for the present review and discussion belongs to the class of transferases. They are the most comprehensively studied enzyme class in biological systems as they are involved in acute regulation of signaling mechanisms manifesting in responses to external cues. Transferases are enzymes that catalyze the movement/transfer of a functional group/moiety from one molecule to another. In this process, they mediate activation/inhibition of the proteins that receive the functional group undergoing post-translational modification. These functional groups/moieties are very diverse including the most common studied moieties like phosphate, methyl, glycosyl or ubiquitin groups.

Among the large repertoire of transferases, kinases have gained significant prominence due to their role in acute signal transduction mechanisms. Importantly, kinase function gained importance due to their integral role in regulation of glycolytic pathways determining the energy generation. Specifically, kinases are involved in catalyzing the transfer of phosphate group moieties on to molecules leading to phosphorylation of the molecules. The effects of these kinases are wide-ranging as they can act on variety of molecules including lipids, proteins, carbohydrates and nucleotide. Such an event of phosphorylation as an example of priming a pathway for accelerated movement of information package from beginning of chain to the end like in metabolic pathway of glycolysis or signal transduction pathway of extracellular regulated kinase 1/2 (ERK 1/2) activation by the upstream mitogen activated protein kinase (MAPK) pathway. The kinases are a very diverse set of enzymes and there are

more than 500 different kinases that have been identified in humans. The most commonly studied and characterized kinases are lipid and protein kinases. In this regard, there has been intense interest and resources that have been invested in determining the underlying mechanisms of phosphorylation by protein kinases with protein kinase A (PKA) being one of earliest kinases to be studied[1, 2].

In contrast to the classical paradigm that enzymes are designed for catalytic kinase functions, studies have identified many proteins that have conserved structural components of enzymes and yet do not harbor catalytic activity. Such observations opened up the idea of “dead enzymes” or “pseudo-enzymes” which have characteristic feature of enzymes but in many cases lack key amino-acids involved in mediating catalysis[3]. The synopsis of protein kinases with non-catalytic functions have been elegantly reviewed previously[4]. One of the earliest identified pseudo-enzymes was α -lactalbumin that has significant homology to lysozyme but has no identifiable lysozyme activity[5, 6]. The expression of these pseudo-enzymes is intriguing given that these non-catalytic proteins are still retained in the genome despite significant evolutionary pressure[5] suggesting critical biological roles that are non-enzymatic. Consistent with this idea, pseudo-enzymes have been found to assist in folding of functional enzymes, provide platform for interactions and/or act as escort for proteins[7, 8].

As our understanding evolves with the use of state-of-art technology, so is the case with the classification of these enzymes. The classification of the enzymes into the pseudo-enzyme cohort is based on the current understanding and can change based on new discovery or structural information. For example, integrin linked kinase (ILK) was originally thought to be a kinase, however, the structural determination of a fragment of integrin-linked kinase showed that it is not a kinase but instead serves a structural role linking the cell's cytoskeleton to surface receptors[9, 10]. On the other hand, Ca^{2+} /calmodulin-activated Ser-Thr kinase (CASK) was initially classified as pseudo-enzyme. However once the structure for CASK was solved, it became apparent that it could use alternative amino acids in the kinase reaction and can be reclassified as a kinase[11, 12]. These observations indicate that retention of these pseudo-enzymes in the genome could have functions that we currently are unaware and increasing technology would allow us to better understand their roles.

Analysis of the enzyme classification based on their function highlights a key issue that has been overlooked in the context of catalytic enzymes as functional enzymes harbor all structural components that could serve the non-catalytic duties. Thus, the idea that catalytic enzymes can simultaneously in parallel execute both kinase as well as kinase-independent functions was appreciated with the discovery of scaffolding function for Pbs2P protein in yeast[13]. One of the classes of enzymes that has been gaining significant scientific appreciation on kinase-independent scaffolding functions in addition to its well established kinase-dependent functions are Phosphoinositide 3-kinases (PI3Ks)[14–16]. PI3Ks are a family of dual kinases that catalyze both lipid and protein phosphorylation[17, 18] and ongoing studies are still identifying new protein kinase substrates[19–21] (Fig. 1 & 2). However, a new layer of regulation that has come be appreciated in the recent years for PI3K has been its non-catalytic scaffolding function that plays a significant role in physiology as well as pathology[14–16, 22] (Fig. 3). We believe that discussion is pertinent at this juncture because PI3K family members have both beneficial as well as deleterious effects[23–25] that

are only being recognized in the recent past and adding the non-catalytic function only increases the complexity in understanding its role. Given these key non-catalytic roles, the current review brings-to-fore recent advances on the unappreciated scaffolding functions of PI3K and its relevance in the context of developing therapeutic strategies targeting PI3Ks.

Overview of Phosphoinositide 3-kinase (PI3K) classes

PI3Ks were identified as unique enzymes that could use lipid as substrates and transfer phosphate moieties on to the D3 position of the inositol ring of phosphatidylinositol's (PtdIns)[18, 26]. However immediately following these studies, findings showed that PI3Ks can function as protein kinases by phosphorylating protein and the earliest example was the phosphorylation of the p85 regulatory sub-unit by the catalytic p110 sub-unit[27–29]. Despite these intriguing observations, the lipid kinase activity of PI3K has gained lot more functional prominence than the protein kinase activity. PI3K enzymes are divided into three classes (Class I, II and III) based on the nature of the lipid substrate and phosphorylated products consistent the recognized role of its lipid kinase function[17, 26].

Class I PI3Ks are most well studied family of PI3Ks as they are acutely activated by cell membrane receptors, either receptor tyrosine kinases (RTKs) or G protein-coupled receptors (GPCRs). Their lipid kinase activity generates lipid second messenger phosphatidylinositol (3,4,5)-triphosphate (PIP3)[17, 18, 26]. The PIP3 lipid secondary messenger activates multiple downstream intracellular signaling pathways to regulate cell proliferation, survival and migration. These enzymes are heterodimers of catalytic p110 subunit and adaptor p85/p101/p84/p87 subunits coded by different genes. There are four identified PI3K isozymes based on catalytic subunit composition namely, PI3K α , β , γ , and δ isoforms, and are associated with an adaptor subunit of either p85 or p101/p84/p87 family. Class I PI3Ks can be further subdivided into class IA and IB, according to the identity of the regulatory subunit as well as the method of activation. Class IA PI3Ks, PI3K α , β , and δ , associate with p85 adaptors and are ordinarily triggered by RTKs, while class IB PI3K γ binds to either p101, p84 or p87 subunit and has long been considered to respond only to GPCR agonists[30–37]. However, this paradigm is slowly shifting given the finding that PI3K β can also signal downstream of GPCRs[38, 39] and PI3K γ can be engaged by RTKs in a Ras/p84-dependent way[40].

Different from Class I, Class II PI3Ks include PI3K-C2 α , PI3K-C2 β and PI3K-C2 γ . Less is known about the function of this class of PI3Ks. PI3K-C2 α and PI3K-C2 γ are the best characterized isoforms and the available data suggests that they play a role in vesicular trafficking by regulating the production of PtdIns(3)P[41] and PI(3,4)P2[42] pools. Conversely, class III includes only one member, Vps34, which is basically involved in the regulation of vesicular trafficking and autophagy through modulation PtdIns(3)P production[43].

Unlike PI3K α , PI3K γ is not ubiquitously expressed or if expressed is present at very low levels under physiological conditions. Its protein and lipid kinase activity are enriched in leucocytes and cells of the cardiovascular system[44–46]. However, PI3K γ protein levels as well as kinase activities are known to be upregulated in cardio-vascular pathologies

including atherosclerosis and heart failure[24, 25, 47, 48]. A vast number of studies have amply demonstrated that PI3K γ plays a critical role in regulation of cardiovascular function, inflammation and immunity. Incidentally, it was believed that PI3K γ either through its lipid kinase or protein kinase activity exerts these physiological effects as most of the results were obtained by using PI3K γ null (PI3K $\gamma^{-/-}$) mice. With the primary idea that the kinase function of PI3K γ is a key contributor to the pathological effects, inhibitors have been developed to target PI3K γ for treatment with an idea to ameliorate pathological effects of PI3K γ . However, use of knock-in mice expressing kinase-dead form of PI3K γ unsuspectingly showed phenotypes that could not be attributed only to the kinase function of PI3K γ suggesting a role for kinase-independent function for PI3K γ [15, 16]. The roles of both kinase functions and kinase-independent functions of PI3Ks in physiology and pathology have been reviewed earlier[44, 49–51]. However, this review focuses on recent advances in kinase-independent function of PI3K γ and perspectives for pharmacologically targeting PI3K γ keeping the scaffolding functions in mind.

Appreciation of kinase-independent scaffolding function of PI3K γ in cardiovascular system

In cardiomyocytes, PI3K γ counteracts the positive inotropic action by affecting β -adrenergic receptor (β AR) signaling, a key regulator of myocardial contractility, through both protein and lipid kinase functions[20, 52]. Furthermore, PI3K γ contributes to cardiac function through regulation of β AR desensitization and internalization by its kinase activities[19, 20, 24, 53]. Notably, PI3K γ activity and expression is negligible in physiological conditions, but results in dramatic upregulation under pathological stress[25, 48, 52]. In this context, PI3K γ expression is upregulated in response to stress contributing towards pathological progress of heart failure. PI3K γ indeed cooperates with G-protein coupled receptor kinase 2 (GRK2), a key regulatory molecule upregulated in human heart failure in mediating comprehensive β AR desensitization[54].

Although evidence points to a key role for kinase activity of PI3K γ in cardiac pathophysiology, kinase-independent scaffolding function of PI3K γ was identified to play a critical role in manifestation of the cardiac phenotype[16] [55]. PI3K γ acts as an A-kinase anchoring protein (AKAP) rather than a kinase, tethering major PDE isoforms near their activator PKA[22, 52]. PI3K γ -associated PKA phosphorylates and activates PDE3 and PDE4 initiating specific PKA-mediated signaling events downstream of β ARs. Conversely, in the absence of PI3K γ , PKA-dependent phosphorylation of key Ca^{2+} handling proteins is dramatically increased, a crucial event in the regulation of cardiac excitation-contraction coupling[56]. L-type calcium channel and phospholamban have higher phosphorylation in PI3K $\gamma^{-/-}$ (PI3K γ null) cardiomyocytes that ultimately leads to spontaneous Ca^{2+} release events and arrhythmic Ca^{2+} transients. However this PI3K γ -mediated protein complex is functionally impaired in heart failure, a condition associated with ventricular arrhythmia, which is a major cause of death[22, 52]. Thus, deregulation of PI3K γ scaffold function appears to be an important component of heart failure-related arrhythmias. The regulation of cAMP by kinase-independent mechanism of PI3K γ in the cardiomyocytes is also implicated in diet-induced thermogenesis. It has been shown that the kinase-independent pathway of

PI3K γ is a negative regulator of PKA-mediated hormone sensitive lipase activation in white adipose tissue[57].

The role of kinase-independent function of PI3K γ in adverse cardiac conditions became evident from the studies in kinase-dead PI3K γ knock-in (PI3K γ -KD) mice wherein expression of kinase dead PI3K γ afforded cardio-protection in response to myocardial ischemia and reperfusion[14]. This is in contrast to the PI3K γ null mice which undergo significant fibrosis with no cardiac protection. These studies show that cardioprotective function of PI3K γ is mediated by both kinase as well as kinase-independent mechanisms. Regulation by kinase-independent mechanisms become important given that PI3K γ is upregulated in human pathologies. Furthermore, we have reported that PI3K γ synergizes with PI3K α signaling in regulating physiological cardiac hypertrophy[15, 23]. Mechanistically, PI3K γ inhibits GSK-3 activation downstream of the insulin-PI3K α -Akt pathway, through a kinase-independent mechanism. This eventually results in enhanced PI3K α downstream signaling and cardiac hypertrophy induction[15]. Correspondingly, recent study demonstrated the role of kinase-independent PI3K γ in regulation of inflammation induced in response to myocardial depression. The results identify the suppressive effect of PI3K γ on cAMP and inducible nitric oxide synthase (iNOS) signaling via its scaffolding function[58]. Overall, these observations unveil a key role for cardiomyocyte PI3K γ in the development of heart failure and provide proof-of-concept that pharmacological targeting of PI3K γ represents a unique opportunity to treat cardiovascular diseases but yet would need to consider the role of kinase-independent functions.

PI3K γ as a scaffold in other systems

PI3K γ exerts kinase-independent functions not only in the myocardium, but also in other organ systems and cell types. Kinase-independent functions of PI3K γ have also been observed in other cell types including platelets[59], endothelial progenitors EPCs[60], microglia[61], and neurons[62] indicating their role in diverse functions.

In platelets, PI3K γ in coordination with PI3K β orchestrate the activation of adhesive function. Findings show that kinase-independent function of PI3K γ is required for efficient aggregation and thrombus formation, through affinity modulation of the $\alpha_{IIb}\beta_3$ integrin. PI3K β predominately regulates Gi-dependent $\alpha_{IIb}\beta_3$ -mediated platelet aggregation through a classical lipid kinase mechanism involving Rap1 and Akt activation; PI3K γ appears to regulate this process principally through a non-catalytic signaling mechanism. Indeed, the combination of the lack of PI3K γ and Wortmannin-mediated inhibition of all PI3K together leads to a much greater defect in platelet aggregation than inhibition of the PI3K catalytic function alone. These data illustrate that in platelets, a cooperative PI3K signaling mechanism exists that involves the catalytic function of PI3K β as well as the kinase-independent function of PI3K γ [59].

Bone marrow-derived EPCs complement the regenerative potential of resident vascular cells at the site of neovascularization[63]. PI3K γ is expressed by EPCs and appears to modulate several aspects of the reparative angiogenesis supported by these cells. Indeed, PI3K γ deficient EPCs show defects in proliferation, survival and migration. This is corroborated

with the study using PI3K $\gamma^{-/-}$ (PI3K γ null) mice, which show reduced capillary formation and arteriogenesis of hind limbs following ligation of the femoral artery. Furthermore, PI3K γ null mice showed impaired incorporation of EPCs in the reforming vessels. In contrast, PI3K γ kinase-dead (PI3K γ -KD) mice exhibit restoration of this reparative neovascularization process, and PI3K γ -KD EPCs do not show defects in proliferation, survival and migration. This observation strongly supports that concept that kinase-independent function of PI3K γ is required for correct vascular repair[60]. However, indepth mechanistic underpinnings of the kinase-independent PI3K γ function in EPCs as well as in other bone marrow-derived cells have not been determined.

It is also reported that PI3K γ plays an important role in microglial phagocytosis, which plays a key role in neuro-protective and neurodegenerative responses of the innate immune system in the brain. Genetic and pharmacological approaches reveal that PI3K γ is an essential mediator of microglial phagocytosis in vitro and in vivo. Interestingly, microglia expressing kinase-deficient mutant of PI3K γ exhibited similar phagocytosis as wild-type cells suggesting a role for kinase-independent function. It is believed that kinase-independent function of PI3K γ in forming the adaptor AKAP complex with phosphodiesterase would underlie phagocytosis[64].

The breakdown of the blood–brain barrier (BBB) is a key event in the development of sepsis-induced brain damage. BBB opening allows blood-born immune cells to enter the central nervous system (CNS) to provoke a neuro-inflammatory response. Abnormal expression and activation of matrix metalloproteinases (MMP) was shown to contribute to BBB opening. Recent studies using a combination of genetically altered mice in a model of LPS-induced systemic inflammation show that PI3K γ is a key mediator of BBB deterioration and is associated with concomitant generation of MMP by microglia primarily through kinase-independent mechanism. Thus, kinase-independent role of PI3K γ is a key mediator of microglial cell activation, MMP expression and subsequent BBB deterioration[61]. As clearance of cAMP by phosphodiesterase is regulated by scaffolding function of PI3K γ , it is quintessential to recognize the expression of PI3K γ in cells could determine the course of pathology. Given the importance of cAMP elevation in the protection from acute pancreatitis[65], it is tempting to speculate that PI3K γ through its scaffolding could regulate acute pancreatitis[66]. Indepth studies would be important as understanding the underlying mechanism could provide insights for novel therapeutics especially for acute pancreatitis.

The absence of PI3K γ in mice leads to impaired synaptic plasticity, suggestive of a role in behavioral flexibility. Several neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD), involve an impairment of behavioral flexibility. A recent study found that expression of PI3K γ occurs throughout the noradrenergic neurons of the locus coeruleus (LC) in the brainstem, which could serve as a mechanism that regulates the control on attention, locomotion and sociality. Specifically, the findings show an unprecedented phenotype in PI3K γ null mice resembling ADHD symptoms. In addition, PI3K γ null mice exhibit deficits in the attentive and mnemonic domains, typical hyperactivity, as well as social dysfunctions. Moreover, these studies show that the ADHD phenotype is regulated by kinase-independent function of PI3K γ through its interaction with

PDE4D. In this context, the findings show that ADHD phenotype is due to a dysregulation of CREB signaling exerted by a loss of PI3K γ in the noradrenergic neurons of the locus coeruleus uncovering novel mechanism and tools for therapeutic research in ADHD[62].

Accumulating evidence from multiple studies have identified that kinase-independent function of PI3K γ could contribute to cardiac hypertrophy and failure, platelet activation and immune response[67] and acute pancreatitis. Given these key observations, it is imperative to recognize that just targeting kinase function of PI3K γ may be insufficient to provide salutary effects in pathology. In that context, it is possible that there are chemical small molecules that can regulate the scaffolding function of PI3K γ . However, the domains of PI3K γ responsible for protein-protein interaction contributing to the kinase-independent function of PI3K γ are not very well established. Further investigations are needed to identify molecular and structural aspects of how scaffolding function of PI3K γ regulates the function of interacting proteins. Unlike small molecules targeting enzyme functions, the development of small molecules targeting the scaffolding function of a PI3K γ would be a complex process. A pre-requisite step in this process would involve mechanistic molecular understanding of how scaffolding function of PI3K γ regulates the function of interacting protein. Following this determination, modern computer based “*in silico*” screening methods can be used to generate test compounds. These compounds can be assessed using rapid cell based assay system with proper read outs to generate potential small molecules targeting scaffolding function of PI3K γ .

Human studies addressing mutations of PI3K γ and their effect on human diseases is currently still lacking. A recent study showed association of genetic mutations in PI3K γ gene affecting plasma cholesterol. However, the single nucleotide polymorphisms (SNPs) reported in this study are present in the intronic region or the SNP leads to synonymous mutations[68] with altering the primary amino-acid sequence. These observations suggest that more studies are needed to assess for SNPs in the genome related to the sequences associated with coding regions of the protein. In this regard, except for our previous studies[15], none of the other studies have identified the scaffolding/interacting domain of PI3K γ that is responsible for kinase-independent function. Our study identified the N-terminal 143 amino acids of PI3K γ interacts with PP2A mediating the kinase-independent function. Since the N-terminal region of PI3K γ interacts and sequesters PP2A, we searched the SNP database of NCBI for the presence of SNPs in this domain. Although, several SNPs are reported in the database, the studies on association of these SNPs with human diseases are lacking suggesting that more studies are needed to better understand the yet unappreciated kinase-independent role of PI3K γ .

The N-terminal 143 amino acid domain of PI3K γ is unique and has very little homology to any other human proteins including other isoforms of PI3K following alignment and blast homology analysis. This domain is known to be involved in binding to p101 adapter protein, and therefore generally characterized as adapter binding domain (ABD)[69]. However, the p101 binding site can also lie outside this region given other interacting partners[70]. Importantly, NCBI Blast analysis revealed that the N-terminal region of PI3K γ is well conserved only in mammals suggesting that the scaffolding function of PI3K γ may have evolved to provide fine tuning of signaling. Unfortunately, the crystal structure of PI3K γ

does not include this region making it difficult to understand the domain architecture's influence on scaffolding function. Therefore, a clear path for understanding the scaffolding function of this domain is to express and generate protein interactome patterns in various cells and tissues.

Summary and Future prospects

The human kinome contains more than 500 proteins that coordinate much of the biology of the cell[3]. Incidentally, about 10% of proteins included in the kinome are designated as pseudokinases, because they lack one or more conserved catalytic residues in the catalytic pocket[71]. Although functions of most of the pseudokinases are still unclear, several of them act as key scaffolding proteins [72]. It is thus not surprising to find that kinases like PI3K γ might exert part of their function that is independent from their catalytic activity through protein–protein interactions. Based on the current evidence, kinase-dependent functions of PI3K γ is generally deleterious, whereas kinase-independent functions could be protective though there are conflicting observations (Fig 4). The kinase-independent activity may operate in concert with the kinase function. This is exemplified by PI3K γ in regulation of β AR signaling, desensitization, and internalization, where protein kinase or lipid kinase activities are required[19, 20, 53]. The kinase-independent function can also act in complete autonomy from the catalytic function like accelerating cardiac insulin signaling in synergistic coordination with PI3K α signaling in cardiac hypertrophy or modulating PDE4-cAMP control in modulation of cardiac contractility[14–16, 52]. It is therefore conceivable that these studies of PI3K scaffolding activity could extend the common knowledge of targeting to other PI3K surfaces with an aim to modulate scaffolding activities. Thus, conventional inhibitor of PI3K γ would abrogate catalytic activity but likely, still leave its kinase-independent functions intact[73] thus only partially mitigating the overall pathological outcomes. Nevertheless, future challenges lie in the generation of selective small molecules that inhibit protein–protein interactions modulating kinase-independent functions of PI3K γ directed towards specific purposes. These small molecules could act together with classical kinase inhibitors to synergistically alter kinase-dependent and -independent functions and achieve a more thorough efficacy with low side effects. Therefore re-thinking the classical paradigm of using kinase inhibitors as method of targeting PI3K γ for pharmacological inhibition is necessary by considering the growing multifaceted roles of PI3K γ isoform in health and disease.

Critical overview

Increasing technology and novel tools has provided the band-width and depth to parse out the distinction between specificity and non-canonical activities of enzymes. Such capability has led to realization that some of pseudoenzymes may perhaps not use classical substrates for their functional activity as previously determined thus leading to reassessment in the classification of enzymes as pseudoenzymes. Nevertheless, the functions of most of the pseudokinases are still unclear and further studies using novel tools will hopefully unravel their role in biological consequences. In recognition of non-canonical function mediated by pseudokinases, it is logical to contemplate that enzymes in addition to mediating catalysis can still use their diverse exposed surfaces to interact with proteins. These surfaces could

provide the docking sites for interaction with various proteins, including substrates, inhibitors, regulatory proteins and other signaling molecules[72]. If we embrace this idea, it is not surprising to discover that kinases like PI3K γ might exert physiological/pathological affects in part, by functions independent of its catalytic activity using protein–protein interactions. However, more comprehensive studies are required to unequivocally strengthen this nuanced role for PI3K γ and such findings can be expanded to other enzymes providing insights to many observations that cannot be explained by the classical linear kinase-dependent function. These observations add another wrinkle to the regulation of signals wherein kinase-independent activity may operate in concert with kinase activity or in complete autonomy. Therefore, it becomes all the more important to recognize that changes in expression of enzymes and their contribution to a phenotype may not all be due to kinase activity of the enzyme but may involve non-canonical kinase-independent scaffolding function. We believe this area of investigation is in its infancy as currently majority of the studies in knock-out mice or knock-down in cells of enzymes attribute the phenotype to kinase activity. Such a view point may need reconsideration given the our discussion on using PI3K γ as an example suggesting that it may be time to recognize the potential roles for kinase-independent function of enzymes in physiology and pathology.

Perspectives

A key aspect of this review is to initiate discussion on the idea that enzymes are proteins containing unique tertiary structures that may be active interacting/docking site for many proteins that may not be their cognate substrate. We have used PI3K γ as an example to elucidate the role of kinase-independent functions that may or may not act in concert with its kinase activity in manifestation of a phenotype. In recognition of the observations the kinase-dependent and -independent functions play in pathology and physiology, it becomes imperative that this non-canonical scaffolding mechanisms are considered in the development therapeutics. Indeed, extensive investment has been made to develop inhibitors that conventionally abrogate catalytic activity of enzymes likely, leaving its kinase-independent functions intact [73] or leading to conformational changes altering kinase-independent function. Given this knowledge regarding enzymes, it becomes imperative to delineate whether key enzymes that are acutely regulated to stress have kinase-independent functions that could contribute to the responses. Nonetheless, a clear future challenge in the realm therapeutic development lies in the generation of small molecules that could inhibit protein–protein interactions regulating kinase-independent function. Such small molecules could act together with classical kinase inhibitors to synergistically alter kinase-dependent and -independent functions achieving precision oriented efficacies with low side effects. Therefore, we believe that this review would be a start to re-think the classical paradigm of using kinase inhibitors targeting catalytic sites of enzymes in health and disease that does not currently consider the multifaceted role of enzymes including scaffolding that may have beneficial/deleterious consequences.

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Scope

The review presents a discussion on the concept of kinase-independent function of enzymes and how this function could unsuspectingly provide non-canonical regulation of signals in physiology and pathology. Determining the mechanistic underpinnings of this non-canonical function may provide insights into the holistic role of these proteins/enzymes in phenotypic manifestation. Although understanding of the role of kinase-independent function/scaffolding by enzymes is at its infancy, this review could be the starting point to dissect how this function could synergize or antagonize the kinase function of enzymes. Such roles may have significant implications in therapeutics as majority of the inhibitors are currently designed to specifically target kinase domains.

Highlights

1. Enzymes harbor tertiary structures providing non-catalytic docking sites for proteins.
2. Docked proteins are kinase-independently regulated by enzymes.
3. Enzyme scaffolded proteins could be recruited to non-classical sites or sequestered.
4. Kinase-independent function could synergize or antagonize with kinase activity.
5. Kinase-inhibitors may need reconsideration in view of kinase-independent function.

Figure 1.

Lipid Kinase Activity: PI3Ks are classically well known for this lipid kinase activity. Cytosolic PI3K is recruited to the plasma membrane following an extracellular signal that could range from growth factors like insulin, epidermal growth factor (EGF) to hormones like epinephrine, norepinephrine which are G-protein coupled receptor agonists. Class I PI3Ks will use the phosphoinositide 4,5-bis-phosphate (PtdIns (4,5)P2 or PIP2), a component of inner leaflet of the plasma membrane as a substrate to generate D-3 phosphorylated PtdIns (3,4,5)P3 (PIP3) products. These D-3 phosphoinositides activate multiple downstream signaling pathways including the well known anti-apoptotic pathway of phosphoinositide dependent kinase 1 (PDK1) - protein kinase B (Akt) axis. PI3K-Phosphoinositide 3-kinase. PM - Plasma Membrane.

Figure 2.

Protein Kinase Activity: In addition to the classical lipid kinase activity, PI3K enzymes have also been identified to harbor protein kinase activity. Solved structure of PI3K γ shows conserved protein kinase domains similar to proteins kinase A within the catalytic site. One of the first substrates identified for the protein kinase function of PI3K was the p85 adaptor subunit of Class 1A PI3Ks. In this context, PI3Ks use protein as their substrate and transfers the phosphate group by its kinase a function generated phosphorylated products. Over a period of time new substrates for PI3Ks have been identified and are listed under the substrate. These are used as substrates by Class I PI3Ks to generated phospho-protein that have implications in PI3K activation and signaling (p85 regulatory subunit), insulin signaling (IRS, Insulin receptor substrate), actin remodeling (non-muscle tropomyosin), phosphatase inhibition (I2PP2A, inhibitor of protein phosphatase 2A) and in receptor transactivation (Src).

Figure 3.

Kinase-independent Function: In recent years it has come to be recognized that PI3K γ isoform has unique functions that are independent of its kinase activity. Enzymes are proteins characterized tertiary structures allowing by unique and diverse surfaces for interaction and docking of substrates, inhibitors, regulatory proteins and other signaling molecules that could be non-canonically regulated by scaffolding. Thus, the ability of PI3K γ to act as an adaptor for PDE thereby, altering the local concentration of cAMP or as a scaffolding protein that sequesters protein phosphatase 2A (PP2A) activator PP2A methyl transferase (LCMT1) has implications in physiology and pathology. More comprehensive studies are needed in general to elaborate the role of kinase-independent mechanisms for enzymes as the kinase-independent functions may synergize or antagonize the kinase functions. This may be critical as the current therapeutic strategies are all focused on targeting the catalytic sites of enzymes to inhibit kinase activity with no consideration for the non-canonical kinase-independent function of these enzymes in disease states.

Effect of protein upregulation in pathology

Figure 4.

Effect of upregulation: Under pathological stress PI3K γ protein levels are increased. The change in the abundance of PI3K γ protein levels lead to two pronged effects. The increase in lipid or protein kinase activity is generally considered deleterious. However, based on the evidence of PI3K γ knock-in mice, kinase activity of PI3K γ may contribute to beneficial effects that are currently under investigation. Even though the full effects of kinase-independent functions are yet to be realized, the current evidences point towards beneficial role.