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Phosphatidylinositol 4-Kinase Enzymes: Functional Roles and Potential for Drug Target

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Abstract: *The two types of phosphatidylinositol 4-kinases (PI-4Ks) synthesize phosphatidylinositol 4-phosphate (PI-4P), a member of the phosphoinositide family. Phosphoinositides (PIPs) are synthesized from phosphatidylinositol (PI), a lipid containing the myo-inositol head group. PI can be phosphorylated at positions 3, 4, and 5 of the inositol ring which allows for seven different PIPs. Indeed, all of these enzymes have been identified in the cell. For instance, one prominent function of PIPs is to serve as membrane markers typically in concert with organelle specific proteins. PI(4,5)P_2 is the main lipid determinant of the plasma membrane and PI3P and PI(3,5)P_2 of the early and late endosomes. PI-4P is the main lipid determinant of the Golgi and trans-Golgi network (TGN) but, additionally, helps to define the specific character of the plasma membrane. This article reviews the recent developments in research on these enzymes and their potential for drug target.*

I. Introduction

The Organelle-specific phosphoinositide distributions are maintained by the tight regulation of PI-kinases and PI-phosphatases. Four distinct PI-4kinases have been described in mammalian cells, including type II (PI4KIIα and PI4KIIβ) and type III (PI-4KIIIα and PI-4KIIIβ) kinases [1-3].The type II PI-4kinases are palmitoylated and thus are strongly associated with the membrane, particularly in the trans-Golgi apparatus, and to a smaller extent, in endosomes [4-5]. PI-4KIIIβ localizes primarily to the Golgi apparatus, coincident with Arf1, a small GTP-binding protein. Although the molecular process of how these enzymes are linked to Golgi-derived biosynthetic transport remain unknown, they have all been implicated in Golgi function and secretion . Deletion of the gene for PI-4KIIIα is embryonically lethal in mice, and its normal subcellular distribution is complex, with evidence for cytosolic, plasma membrane, and ER concentration. Recently, PI-4KIIIα has also been identified as a critical host factor for hepatitis C viral replication [6-7]. With regards to biosynthetic trafficking, the phospholipid PI-4P localized to the Golgi apparatus has been implicated in the delivery of cargo from the Golgi to the plasma membrane (PM). The membrane phospholipid PI-4P *in vivo* is primarily produced by the action of PI-4Ks, although it can be also produced by dephosphorylating a higher PIP. All eukaryotes have two families of PI4Ks conserved from yeast to man called type II and type III PI-4Ks [8-9] that have different domain organization. The missing type I PI-4Ks is a historical artifact as it was later found out that the type I PI-4Ks were actually PI3Kinases. Type III kinases are known as typical PI-4Ks due to their similarity to PI3Ks and type II are known as atypical due to their dissimilarity to any other lipid kinases. Phosphatidylinositol phosphates (PIPs) are universal markers of intracellular membranes. Phosphatidylinositol 4-phosphate (PI-4P) is the single most abundant mono phosphoinositide that defines the membranes of the Golgi and TGN. It has been reported to play a key role in membrane biogenesis, vesicular transport, lipid dynamics, and protein and lipid sorting in the TGN. PI-4P is synthesized by phosphatidylinositol kinases (PI-4Ks) [10]. Humans have two type II PI-4Ks (alpha and beta) and two type III PI-4Ks (alpha and beta). On the other hand, the type III PI4Ks are carried by positive strand RNA (+RNA) viruses such as the Hepatitis C virus, poliovirus or SARS to create so-called membranous web, an extensively phosphorylated and modified membrane system dedicated to their replication [11].However, PI-4P produced by type II PI4Ks is used by intracellular bacteria (e.g. Legionella pneumophila, Chlamydia trachomatison). Thus, PI4Ks are a potential pharmacological target.Recent work has also established that different PI 4-kinase isoform permutations are required during receptor-activated PLC signalling and in Golgi-endosomal trafficking and all of which suggests that there is still much still to be discovered about this, the least well studied mammalian PI kinase family.

Phosphatidylinositol 4 kinase and cancer biology:

Uptil now increased expression of PI-4KIIα and PI-4KIIβ has been reported for a range of specific cancers with increased PI4KIIα levels associated with augmented activation of the HER2 receptor kinase pathway, HIF production and angiogenesis [12]. In a interesting study, a potential anti-metastatic role for PI-4KIIβ has emerged which involves this isozyme promoting the trafficking of CD81 tetraspanin proteins away from the plasma membrane into a population of intracellular trafficking vesicles that also contain actinin thereby

inducing anti-migratory remodelling of the actin cytoskeleton [13]. Meanwhile PI-4KIII has been identified in non-biased screens as contributing towards a more aggressive metastatic phenotype of pancreatic ductal carcinoma cells and was identified as one of several proteins that mediate resistance to the chemotherapeutic agents gemcitabine [14-15] and cisplatin. However, unlike the well established case of constitutively active phosphoinositide 3-kinase mutations as found in PI-3KCA or phosphatase PTEN deletions which can both elevate $PI(3,4,5)P_3$ levels and drive oncogenic signaling and [16], it is significant that neither activating mutations of the PI 4-kinases nor deletions of PI4P phosphatases have yet been discovered in cancer.

Estimation of PI4**Kinase activity:**

The activity of PI4KIIα can be determined by measuring ADP generation using an ADP-Glo kinase kit (Promega, USA) as previously described [17]. Briefly, 1 µg protein in a kinase buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 0.2% Triton X-100 (or 0.1% Anzergent), 1 mM EDTA and 20 mM $MgCl_2$ was used in this assay. PI dissolved in chloroform was dried using nitrogen, resuspended to 7 mM in the above kinase buffer and then sonicated for 30 min before use. Protein 1 µl (1 mg ml^{-1}) was mixed with 7 µl kinase buffer and 10 µl PI micelles. The kinase reaction was initiated by adding 2 µl ATP (10 mM) and carried out at room temperature (RT). All reactions were repeated three times and were controlled with blank reactions that lacked PI. Reactions were stopped by addition of 20 µl ADP-Glo reagent (Promega, USA). After 70 min incubation at RT, 40 µl of Kinase Detection Reagent was added and the mixture was incubated for another 60 min at RT. The fluorescence signal of the mixture was recorded on a Luminescence Spectrometer which was set to a sensitivity of 30%.

Technique to determine the Crystal structure of PI4Kinases:

Purified PI4KIIα (~30 mg ml^{-1}) is diluted with a buffer containing 25 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM DTT and 20 mM $MgCl_2$ to a final concentration of 4 mg ml^{-1} [18].The detergent hexaethylene glycol monooctyl ether was added to a final concentration of 20 mM. After incubation on ice for 40 min, the protein solution was centrifuged at 15000 *g* for 30 min. The pellet was discarded and the supernatant was collected for crystallization. Crystals of PI4KIIα were grown by the hanging-drop vapour diffusion method at 293 K by mixing 1 µl of protein solution with an equal volume of reservoir solution containing 0.1 M citric acid, pH 5.8, 150 mM NaCl and 18% PEG400. The crystal structure of the PI4K II alpha has been solved. The structure revealed an unusual kinase fold that could be divided between N- and C- lobes. The catalytically important ATP molecule is localized between the N- and C- lobes but surprisingly we found a second ATP molecule bound to a lateral pocket of the C-lobe. The adenine ring of the ATP is held inside the pocket mainly by hydrophobic interactions. We were able to show that the ATP is not the biological ligand for the lateral hydrophobic pocket but rather a suitable ligand present at or within the lipid bilayer is and the lateral pocket fine tunes the enzyme's activity *in vivo.*

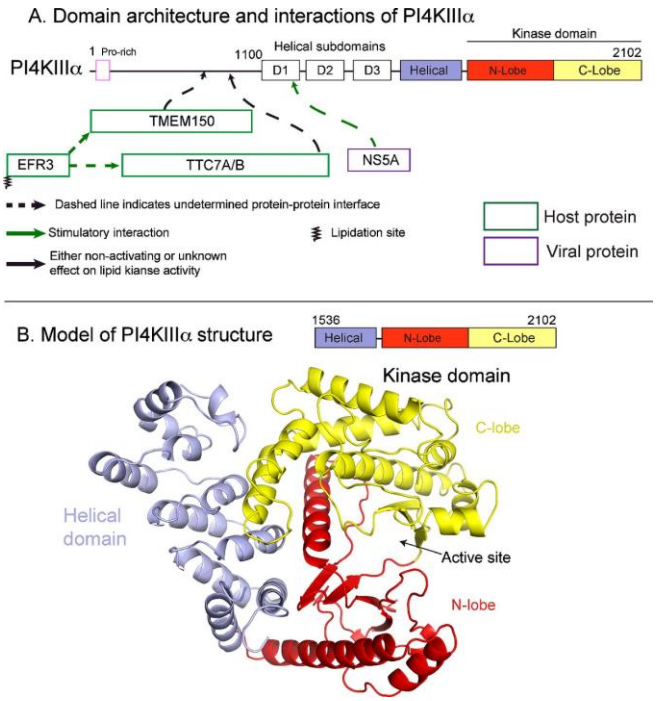

Fig.1: Structure of PI-4KIII (Courtesy: Gillian L. Dornan, Jacob A. McPhail, John E. Burke, Biochemical Society Transactions Feb 09, 2016, 44 (1) 260-266

Functional roles of PI4KII enzymes: It plays a important role in the development of a migratory phenotype in HCC but there is limited evidence for the direct involvement of mammalian PI-4KII isozymes in actin regulation. In contrast, several studies have described PI-4KII functions in post-TGN traffic through PI(4)P biosynthesis and direct binding to clathrin adaptors AP-1 and AP-3 [19], both pathways that contribute to endo-lysosomal traffic (Refer Fig.1). PI-4KIIα controls LE traffic of the EGFR [20], endolysosomal traffic mediated by AP-3 and of lysosomal membrane proteins and hydrolases such as the enzyme β-glucocerebosidase [21].This explains the similar effects of PI-4KIIα and PI-4KIIβ depletion on EGFR degradation and LE traffic. Keeping in mind that most PI4KIIα and a substantial portion of PI-4KIIβ exist as membrane proteins in the cytoplasm owing to the palmitoylated cysteine residues, it appears inevitable that the nuclear PI4KIIα and IIβ also exist as membrane proteins in the nucleus. Moreover, in view of the fact that the several small Ins(1,4,5)P_3-sensitive nucleoplasmic Ca^{2+} store vesicles are the only known nucleoplasmic entity with membranes, the localization of PI4KIIα and IIβ in the nucleoplasmic vesicles is in accordance with their biochemical properties [22]. In addition to this nucleoplasmic Ca^{2+} store vesicles not only contain the high capacity, low affinity Ca^{2+} storage proteins chromogranin B and secretogranin II but also are loaded with the Ins(1,4,5) P_3R/Ca^{2+} channels [23].

Therapeutic potential of targeting the PI4-kinase enzyme: A further challenge in targeting the PI-4-kinases has emerged from more recent studies which have revealed differential cell and tissue dependencies on the PI 4-kinase isoforms that are not always absolutely predictable. Srivastava *et al* have shown that in T cell leukemia cells the PI-4 kinase associate with TCR- CD cells and this can be a potential for drug target and lead to curative prospects in blood cancers [24]. Another example is pertaining to the animal studies have shown that genetic knockdown of PI-4KIIIα leads to severe changes in the gastrointestinal mucosal epithelium whereas loss of PI4KIIα leads to selective loss of specific neuronal cell populations such as cerebellar Purkinje cells [25]. Therefore, it is not yet clear if inhibiting individual PI 4-kinase isoforms in a therapeutic setting represents a feasible strategy [26]. Imidazopyrazines demonstrate potent preventive, therapeutic, and transmission-blocking activity in rodent malaria models, are active against blood-stage field isolates of the major human pathogens P. falciparum and P. vivax, and inhibit liver-stage hypnozoites in the simian parasite P. cynomolgi. It has been shown that imidazopyrazines exert their effect through inhibitory interaction with the ATP-binding pocket of PI(4)K, altering the intracellular distribution of phosphatidylinositol-4-phosphate [27-28]. Collectively, these data define PI(4)K as a key Plasmodium vulnerability, opening up new avenues of target-based discovery to identify drugs with an ideal activity profile for the prevention, treatment and elimination of malaria. However, a more comprehensive knowledge of the roles of different PI 4-kinase permutations in modifying lipid metabolism, signalling and trafficking, may reveal which PI4P pathways to target in human diseases [29-31] . An excellent example of this is the Gaucher disease which is a lysosomal storage disorder caused by a defect in the degradation of glucosylceramide catalyzed by the lysosomal enzyme β-glucocerebrosidase (GBA). GBA reaches lysosomes via association with its receptor, lysosomal integral membrane protein type 2 (LIMP-2). It was found that distinct phosphatidylinositol 4-kinases (PI4Ks) play important roles at multiple steps in the trafficking pathway of the LIMP-2/GBA complex. Acute depletion of phosphatidylinositol 4-phosphate in the Golgi caused accumulation of LIMP-2 in this compartment, and PI4KIIIβ was found to be responsible for controlling the exit of LIMP-2 from the Golgi. In contrast, depletion of PI4KIIα blocked trafficking at a post-Golgi compartment, leading to accumulation of LIMP-2 in the endosomal vesicles. PI4KIIα depletion also caused secretion of missorted GBA into the medium, which was attenuated by limiting LIMP-2/GBA exit from the Golgi by PI4KIIIβ inhibitors [32]. These studies identified PI4KIIIβ and PI4KIIα as important regulators of lysosomal delivery of GBA, revealing a new element of control to sphingolipid homeostasis by phosphoinositides. The further goal is to get atomic insight in the PI4P biosynthesis that takes place at intracellular membranes of all eukaryotic organisms and to use the structural information to design specific inhibitors targeting PI-4Ks.

II. Conclusions

Future work to visualize and selectively deplete PI4P either in the ER or the Golgi apparatus may lead to better understanding of the roles of the pools of PI4P in each of these organelles that are important for biosynthetic trafficking. The Sac1 phosphatase cycles between the ER and Golgi apparatus and is a key regulator of PI4P in mammalian cells. While the localization of PI4P to the Golgi has been extensively reported, new tools to visualize PI4P pools in cells have revealed pools of this lipid associated with the PM and late endosomes/lysosomes. It will be interesting and informative to test whether these probes are capable of recognizing an ER-associated pool of PI4P in this cell system. Alternatively, further support for the necessity of PI4KIIIα activity, as implicated by our pharmacological approach, could be provided with the use of siRNA to genetically silence this group of enzymes.

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