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Review Article

What we have learnt about PIKE from the knockout mice

Chi Bun Chan, Keqiang Ye

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA.

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Abstract: Phosphoinositide 3-kinase enhancer (PIKE) is a group of GTPase that belongs to the centaurin superfamily. These proteins have been discovered for more than a decade but our understandings on their functions are still limited. Studies from our research group and others have revealed some of their functions in a cellular context but their roles in organ development or systemic homeostasis just begin to unveil. The generation of PIKE knockout mice thus provides the valuable model to delineate the physiological roles of PIKE. In addition to being a PI3K/Akt enhancer, phenotypic characterization of the PIKE knockout mice demonstrates that the proteins are involved in multiple signaling cascades including Janus kinase (JAK)/ Signal Transducer and Activator of Transcription (STAT), AMP-activated protein kinase (AMPK)/Acetyl-CoA carboxylase (ACC) and insulin receptor (IR)/Akt. In this article, we will review the current findings from the PIKE knockout mice studies and will discuss how these *in vivo* observations lead to the identifications of novel signaling cascades regulated by PIKE.

Keywords: Adipocyte, BDNF, GluR2, Insulin receptor, Liver, Mammary, Neuron, Obesity, PIKE

Introduction

Phosphoinositide 3-kinase enhancers (PIKE) are GTPases that belong to the gamma 1 subgroup of the centaurin superfamily [1]. There are three members in PIKE family, which are designated as PIKE-S, PIKE-L and PIKE-A (also known as AGAP2 or GGAP2). PIKE-S is a nuclear protein that responses to NGF to activate nuclear PI3K [2]. Using cDNA library screening, we have isolated a long isoform of PIKE-S, PIKE-L, from the fetal brain library [3]. This isoform differs from PIKE-S in that it has a C-terminal extension containing an Arf-GAP domain. The identification of the third PIKE isoform, PIKE-A, was first reported on 1996 by Nagase *et al.* [4], a time well earlier than PIKE-S was found. However, the authors only reported PIKE-A as a novel sequence with no discussion on its structural similarity to any protein family. On 2003, Xia *et al*. reported the cloning of PIKE-A from human heart cDNA library [5]. The authors did not realize the sequence homology between GGAP2 and PIKE-S, thus they assigned their protein as GGAP2, which is an isoform of another novel GTPase GGAP1. During our investigation on the mechanism of glioblastoma multiform formation, we found that the loci of *GGAP2* (or *CENTG1*) are highly amplified in glioblastomas [6, 7]. After sequence alignment analysis, we clarified that GGAP2 is a new isoform of PIKE-S and PIKE-L. Structure comparison shows that PIKE-A contains the identical structure of PIKE-L C-terminus that they both possess a GTPase, a PH domain and a ArfGAP domain [7]. However, PIKE-A lacks the proline rich N-terminal of PIKE-L but comprises a unique sequence from other PIKE members [8].

The expression profile of PIKE has not been systematically studied. Neither a detailed tissue distribution of PIKE in any animal model nor a comparative study on their expressions among different species has been performed. Today, only fragmented information on their tissue expression levels has been reported. Most of these studies focus on PIKE-A. Nagase *et al.* [4] and Elkahloun *et al.* [9] showed that PIKE-A is expressed in human brain, lung, liver, skeletal muscle, spleen, thyrus, small intestine and leukocytes. Among these tissues, PIKE-A has the highest expression in the central nervous (CNS) system. Within the CNS, its expression can be readily detected in cerebellum, cerebral cortex, occipital pole, frontal lobe, temporal lobe and putamen [5]. Xia *et al*. further demonstrated

that PIKE-A mainly expresseed in the neural tube, forebrain and midbrain during mouse embryogenesis [5]. We have made a similar observation for both PIKE-S and PIKE-L that they were neuronal specific [2, 3]. Recently, we found that PIKE-A was also expressed in white adipose tissue (WAT), brown adipose tissues (BAT) and mammary epithelials [10].

The ubiquitous expression profile of PIKE proteins, especially PIKE-A, suggests that they have a broad spectrum of undefined function. Because of the lack of suitable animal models, previous researches on PIKE were restricted to *in vitro* studies. To identify the functions of PIKE in a systemic context, we generated the whole body PIKE knockout (*PIKE -/-*) mice using the loxP/Cre recombination [11] that the exons 3 to 6 of the *CENTG1* were removed, thus introducing a shift to the original reading frame and producing a truncation in the GTPase domain of all PIKE isoforms. The mutant animals are viable, fertile and indistinguishable from their wild-type littermates on morphological assessment. However, they show some peculiar phenotypes that are obvious under specific examinations (**Table 1**). In this review, we will discuss how we used the *PIKE -/-* mice to verify the previously reported functions of PIKE as well as the identifications of novel signaling pathways modulated by PIKE in these animals.

Role of PIKE in brain development, survival and memory formation

Controlling cell death during development or pathological conditions is critical for preserving the functional activity of the brain. During brain development, apoptosis is induced in those differentiated neurons projected to an inappropriate target [12]. On the other hand, apoptosis is inhibited in neurons challenged with neurotoxical insults to impede the number of neurons affected. The initiation of the decision between "death or survival" is controlled by an orchestration of various signaling pathways, but the PI3K/Akt cascade is on the central part of this network [13]. During excitotoxic stimulations like stroke or glutamate receptor overactivation, the surges of intracellular Ca^{2+} activates the apoptotic machineries leading to caspases activation, DNA damage and mitochondrial membrane potential change. A great deal of evidence suggests that an intact PI3K/Akt pathway is critical for buffering these processes

Table 1. Phenotypes of PIKE knockout mice

Tissues	Phenotypes
Brain	Smaller brain Thinner cortex Fewer cortical neurons Diminished dendritic arborization More cell death under excitotoxic insults
Mammary gland	Enhanced apoptosis during pregnancy Impaired postpartum development Reduced milk production
Liver	Insensitive to insulin Enhanced neoglucogenesis Reduced lipid content
Muscle	Hypersensitive to insulin Enhanced lipolysis
Adipose tissue	Hypersensitive to insulin Enhanced lipolysis Reduced fat mass Defective adipogenesis

[14]. Therefore, proteins that modify the activity of PI3K/Akt are also crucial for maintaining the neuronal survival. Our *in vitro* studies in hippocampal neurons suggest that PIKE-L prevents neurotoxic insults by linking various survival clues to PI3K activation. It was found that PIKE-L complexed with mGluRI through the adaptor proteins Homer 1 when the receptor was activated by its ligand [3]. In this complex, PIKE-L serves as a linkage that couples activated mGluRI to PI3K, thus preventing the cells from staurosporine-induced cell death. Similarly, we found that PIKE-L interacted with netrin receptor (UNC5B) in a ligand-dependent manner [15]. Since UNC5B is a dependence receptor, which induces apoptosis in the absence of the ligands, netrins [16], the binding of PIKE-L to UNC5B after netrin stimulation leads to PI3K activation and thus prevents the neurons from committing apoptosis. We also found that PIKE-L protected the DNase inhibitor SET from proteolytic degradation by asaparagine endopeptidase (AEP) during neuroexcitotoxic insults [17]. The cleavage of SET by AEP is a critical step in acidosis-triggered neuronal apoptosis after stroke or kainic acid stimulation. Under acidic conditions, AEP is activated and cleaves SET into non-functional fragments. However, direct association of PIKE-L to SET protects it from the degradation possibly by inhibiting its accession to AEP.

All the above studies point to the conclusion that PIKE-L is a protector that prevents neurons from apoptosis *in vitro*. We have verified this conclusion *in vivo* using the *PIKE -/-* mice. When the knockout animals were subjected to middle cerebral artery occlusion (MCAO), they developed larger stroke area with higher number of cells perusing apoptosis [18]. Similar observation was found in *PIKE -/-* mice injected with KA that elevated caspase 3 activity and PARP cleavage were detected in the hippocampus (unpublished observation). Interestingly, KA stimulation induces more Ca^{2+} influx into *PIKE -/-* neurons, which results in a lower threshold towards KA-induced seizure [19]. *In vitro* culture of *PIKE -/-* neurons also revealed a high vulnerability under glutamate stimulation (unpublished observation), suggesting that PIKE -L is necessary for cell protection during stroke and glutamate challenges both *in vitro* and *in vivo*.

PIKE are not only critical for protecting the neurons from pathological damage, they are also necessary for normal brain development. During embryonic growth, markedly enhanced apoptosis is observed in the nestin-positive progenitor cells in the ventricular zone of *PIKE -/-* neocortex at E12.5 [18]. However, A comparable density of proliferating cells (as determined by BrdU chasing and ki67 staining) is detected between wild-type and *PIKE -/-* cortex at the same developmental stage, indicating neuronal survival but not proliferation is regulated by PIKE. The apoptosis in cortical neurons is sustained beyond the developmental stages as we observed positive caspase-3 staining in the somatosensory cortex of adult *PIKE -/-* mice but not wild-type controls [18]. As a result, the number of neurons in the neocortex is reduced in PIKE-null animals, leading to a thinner cortical layers and reduced brain mass.

In addition to the lower neuronal number in brain, we also found that *PIKE -/-* neurons have decreased dendritic length and complexity [18]. When the PI3K/Akt pathway is augmented in *PIKE -/-* neurons by overexpressing constitutively active Akt or p110 subunit of PI3K, the arborization pattern was rescued [18]. We extended the studies by generating the *PIKE-/-PTEN-/-* double mutant animals to mend the defective Akt signaling *in vivo*. PTEN is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase that negatively regulates the PI3K/Akt pathway [20]. In *PTEN* knockout mice, the brain mass and neuronal size is enlarged [21, 22]. Therefore, *PTEN* ablation in *PIKE -/-* neurons should restore the PI3K/Akt function, thus rescuing the defective arborization. Indeed, the dendritic patterns as well as the total dendritic length were increased in the double mutant neurons both *in vitro* and *in vivo* [18]. These observations are in good agreement with the *in vitro* reports that the integrity of PI3K/Akt is necessary for proper dendritic arborization [23, 24]. We have also performed studies to search for the upstream factors responsible for initiating the PIKE/PI3K/Akt pathway during neuronal development. It has been reported that dendritogenesis is regulated by an intricate signaling network involving temporal and spatial activation of the particular cascades. Extracellular factors like brain-derived neurotrophic factor (BDNF), agrin, semaphorin, reelin, etc, are inducers for dendritogenesis [25]. We are particular interested in BDNF signaling because it is a renowned factor to exert its biological functions through PI3K/Akt. *PIKE -/-* neurons are "partially" resistance to BDNF stimulation as PI3K/Akt cascade, but not Ras/ERK, is impaired when treated with BDNF [18]. As a result, the BDNF-induced dendritic arborization, dendritic outgrowth and formation of new dendrites are diminished in *PIKE -/-* neurons. Therefore, the BDNF inactivation in *PIKE -/-* neurons represents one of the mechanisms leading to the defective neuronal arborization.

The development of a highly branched dendritic tree is essential for establishing the functional connections between neurons. Several researches have shown that dendritic patterning of pyramidal cells in the cerebral cortex is escalated after learning [26-28]. Accumulating evidence also shows that learning behaviors induce the expression of BDNF in neurons [29, 30], which may trigger dendritic development to facilitate memory formation. In addition, PI3K/Akt is involved in the memory formation and retrieval [31, 32]. Conceivably, it is reasonable to infer that *PIKE -/-* mice should display an impaired memory. To test this hypothesis, we have performed two classical behavioral tests, Morris water maze and Y-maze [33], on *PIKE -/-* mice to examine the effect of *PIKE* ablation on memory formation. As anticipated, *PIKE -/-* mice displayed a poor performance in both tests [18], which is a logical consequence of BDNF resistance in *PIKE -/-* neuron. Presumably, wiring of

the neuronal circuit in *PIKE -/-* brains is impaired, causing a reduced ability in performing memory task.

We also found that the *PIKE -/-* hippocampal neurons are unable to substantiate the long-term potentiation (LTP) induced by high-frequency or glycine stimulation [34, 35]. Moreover, the number of α-amino-3-hydroxy-5-methyl -isoxazole-4-propionic acid receptor (AMPAR) on cell surface of both cortical and hippocampal neurons are reduced in *PIKE -/-* brain [36]. Ionotropic glutamate receptors like AMPAR and N-Methyl-D-aspartic acid receptor (NMDAR) are critical ion channels in modulating the synaptic plasticity [37, 38]. In particular, AMPAR mediates the majority of the fast excitatory synaptic transmission and its postsynaptic surface expression is necessary for LTP expression [38]. The down-regulated surface expression of AMPAR in *PIKE -/-* neurons thus implies that PIKE is involved in controlling the membrane retention of AMPAR. It is noteworthy that BDNF can induce LTP and AMPAR trafficking by increasing intracellular Ca^{2+} concentrations via phospholipase C-γ1 (PLC-γ1) and ERK activations [40-43]. Since PLC-γ1 is the guanine exchange factor of PIKE [44], it is possible that PIKE-L serves as the linkage between BDNF and AMPAR signaling to mediate memory formation: on one hand, it is a downstream effector of BDNF to induce neurite outgrowth to form the correct networking in the cortex; on the other the hand, it enhances the AMPAR surface expression during LTP in the hippocampus (**Figure 1**).

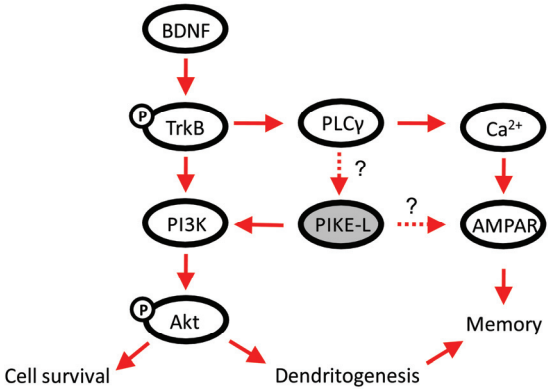

Figure 1. Role of PIKE-L in brain mass maintenance and memory formation.

PIKE in mammary gland development

During the breeding of *PIKE -/-* mice, we found that the survival rate of new born pups from *PIKE -/-* dams was low and autopsy result indicated a dehydration and lack of milk in the stomach of the dead pups [45]. However, we did not find deceased *PIKE -/-* pups raised by heterozygous dams, indicating the high infant mortality rate is caused by insufficient milk production from the knockout mother but not any defective sucking behavior of the pups. Mammary gland is an excellent tissue for studying physiological apoptosis, since it switches constantly between cell proliferation and apoptosis. It is a complex tissue that proliferates and differentiates under the control of systemic hormone during puberty, pregnancy and lactation [46]. During pregnancy, the mammary gland epithelial cells performed extensive mitosis to form a highly branched ductal structure over the fat pad for milk storage. The end of these newly formed ducts will further differentiate into specific structures called alveoli that are major site for milk production [47]. On the other hand, the mammary epithelials undergo apoptosis and tissue remodeling to reduce the cell number at weaning, when milk production is not necessary for nourishing the pups [48]. In fact, we detected a massive apoptosis in the *PIKE -/-* mammary epithelial cells with a reduction of Akt phosphorylation in mid and late gestation. In parallel with this observation, *PIKE -/-* epithelials fail to form mature lobuloalveolar structure during the parturition. These observations imply that *PIKE -/-* mammary gland is defective in proliferation and differentiation during late pregnancy [48].

Although Akt is important for cell growth in a variety of tissues as Akt knockout mice showed reduced body growth, however, they are dispensable for mammogenesis during pregnancy and lactation {49, 50]. Thus, the down-regulated Akt activity is not the major reason for the defective mammary structure observed in *PIKE -/-* mice. In search for the potential signaling cascades for PIKE-A to regulate the mammary gland development, special attentions were given to the prolactin (PRL)/Janus kinase 2 (JAK2)/ signal transducer and activator of transcription 5 (STAT5) pathway as PRL has long been recognized as the crucial factor in triggering the development and milk secretion [51]. There are several observations that lead to the hypothesis of PIKE-A/PRL crosstalk: first, defective mammary development is rescued in *PIKE -/-* mice after multiple gestations, which resembles the

unique situation reported in prolactin receptor (PRLR) knockout mice [52]; second, the mammary gland development before mid pregnancy is normal in *PIKE -/-* mice, which fits with the idea that intact PRL signaling is only necessary for mid to late gestation; third, gene expressions of milk proteins (e.g. β-casein) and proliferation regulators (e.g. cyclin D1) are downregulated in *PIKE -/-* mammary gland, which are the targets of the transcription factor STAT5a [53]. *In vitro* binding assays confirm our hypothesis that PIKE -A specifically interacts with STAT5a but not other STAT isoforms. Moreover, it complexes with the PRLR during PRL stimulation in mammary epithelial cell line HC11. Depletion of PIKE -A in HC11 cells diminishes PRL-stimulated STAT5a phosphorylation and transcriptional activity on cyclin D1 promoter. The STAT5a phosphorylation is also curtailed in *PIKE -/-* mammary tissues. Moreover, the coupling of STAT5a to PRLR is reduced when PIKE-A is depleted in HC11 cells or in *PIKE -/-* mammary tissues. Thus, PIKE-A serves as an adaptor that mediates the interaction between STAT5a and PRLR during PRL stimulation. Depletion of PIKE-A abolishes STAT5a phosphorylation by JAK2. As a result, the expression of cyclin D1 is downregulated, causing a reduced epithelial cell proliferation (**Figure 2**).

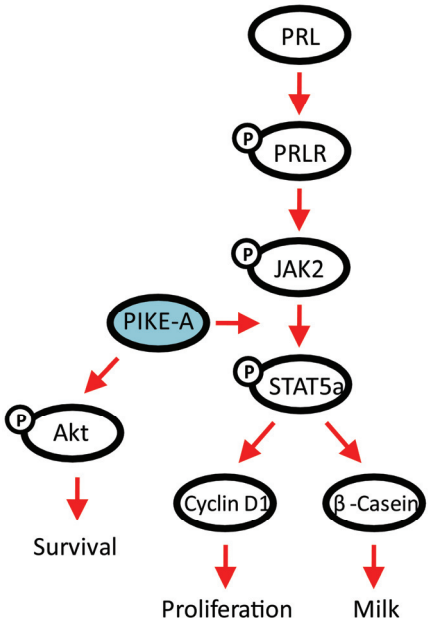

Figure 2. Role of PIKE-A in mammary gland development.

The finding of PIKE-A is a novel component of PRL/JAK/STAT5 pathway not only updates the paradigm of PRL signaling but also proposes a mechanism for the isoform-specific STAT5 activation by PRL, as PIKE-A association is highly specific towards STAT5a but not STAT5b. Most important, it provides a new research direction on PIKE signaling as PRL is involved in more than 300 functions [54]. In particular, our previous studies show that PIKE-A is overexpressed in breast cancers, and dysfunction of STAT5 proteins is implicated in mediating the tumor malignancy [55]. Therefore, further research on the roles of PIKE-A/STAT5a association in these tumors might provide a potential therapeutic target for treating breast cancers.

PIKE in obesity development and energy metabolism

In addition to the reduced brain mass, we found that the WAT weight is also diminished in *PIKE -/-* mice [10]. Adipose tissue is the major energy depot to store extra nutrients in form of lipid during energy surplus. New adipocytes are not generated by mitosis but through the differentiation of mesenchymal stem cells-derived preadipocytes via a series of morphological and biochemical changes called adipogenesis. During the process, the preadipocytes have to go through the determination (the change of stem cells to adipogenic linage), clonal expansion (limited mitosis of pre-adipocyte) and terminal differentiation (the formation of cellular equipments to store lipids) [56]. The reduced adipose mass in *PIKE -/-* mice is a result of defective adipogenesis as *PIKE -/-* mouse embryonic fibroblasts (MEF) were unable to differentiate into mature adipocytes under dexamethasone/isobutylmethylxanthine/insulin (MDI)-induction. This conclusion is further supported by the observations that mature adipocyte markers like aP_2 and PPARγ, but not the preadipocyte marker Pref-1, were reduced in *PIKE -/-* adipocytes [10].

Since the increase in body weight during obesity is caused primarily by the expansion of adipose tissue; presumably, the defective adipogenesis in *PIKE -/-* adipocytes will protect the mice from obesity development. When *PIKE -/-* animals were fed with high fat diet (HFD) for 20 weeks, the body weight and adipose tissue mass increases were significantly smaller than the wild-type control [10]. Lipoatrophy should result in severe hyperlipidemia and ectopic lipid storage in peripheral tissues [57], but less liver steatosis was detected in *PIKE -/-* mice. Together,

these findings suggest that the excessively absorbed lipid may be metabolized rather than deposited as storage in the mutant animals [10]. Indeed, we found that phosphorylations of AMP-activated protein kinase (AMPK) and its downstream target acetyl Co-A carboxylase (ACC) are upregulated in *PIKE -/-* fat and muscle. AMPK is a serine/threonine kinase that functions as an energy sensor for glucose and lipid metabolism in cell. Once activated, it inhibits the energy-consuming biosynthetic pathways and activates the ATP-production reactions like fatty acid oxidation [58]. Therefore, *PIKE -/-* tissues have enhanced β-oxidation as revealed by the higher *in vitro* lipolysis and reduced respiratory exchange ratio (RER) in the metabolic cage measurement [10]. The reduced obesity in *PIKE -/-* mice also protects it from diet-induced diabetes as obesity a major cause of insulin resistance [59].

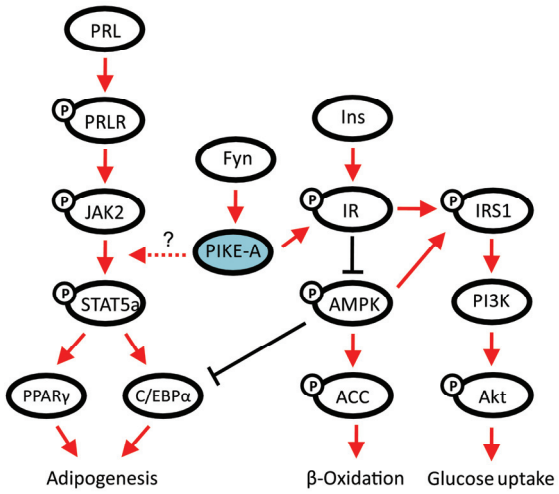

Figure 3. Role of PIKE-A in adipogenesis and energy metabolism.

Interestingly, we also found that *PIKE -/-* fat and muscle are hypersensitive to insulin stimulation under chow diet feeding. Several lines of evidence support the enhanced insulin responsiveness in *PIKE -/-* mice. First, the concentration of circulating insulin is lower in *PIKE -/-* mice, suggesting less insulin is sufficient to maintain normoglycemia; second, glucose infusion rate is higher in *PIKE -/-* during the hyperinsulinemia-euglycemia clamp assay; third, insulin stimulation triggers higher glucose uptake in the isolated soleus muscle and adipocytes from *PIKE -/-* mice [10]. We also detected a higher PI3K/Akt activation in *PIKE -/-* muscle and fat after insulin stimulation *in vivo*. These results contrast with the PI3K/Akt enhancing role of PIKE, as depletion of PIKE should result in reduced Akt activation and the development of insulin resistance. However, it is reasonable to observe the phenotypes if we consider the critical role of AMPK in glucose metabolism. AMPK cross-talks with PI3K/Akt pathway by increasing the activity of IRS1 [60]. Moreover, agonist-induced AMPK activation increases glucose uptake in muscle and fat [61, 62]. Long-term activation of AMPK augments the systemic insulin sensitivity and protects animals from obesity-induced diabetes, which concurs with our findings in *PIKE -/-* mice [63]. The upregulation of AMPK activity in *PIKE -/-* adipocytes may also provide a possible mechanism for the defective adipogenesis, as activation of AMPK inhibits 3T3-L1 cells from differentiated into adipocytes [64]. Interestingly, the PRL/JAK2/STAT5a pathway is reported as a crucial factor for adipocyte differentiation [65-67]. Given the important role of PIKE-A in mediating STAT5a activation [45], we could not exclude the possibility that the PRL-induced signaling is also defective in *PIKE -/-* adipocytes (**Figure 3**).

Although the negative role of PIKE-A in AMPK activation is evident, the molecular mechanism of how PIKE-A suppresses AMPK phosphorylation remains unknown. Our *in vitro* studies suggested that PIKE-A interacts with insulin receptor (IR) in muscle, which is necessary for insulin to suppress AMPK phosphorylation [10]. The PIKE-A/IR association is mediated by Fyn tyrosine kinase, as Fyn-phosphorylation sites mutated PIKE-A is unable to associate with IR or trigger the insulin-suppressed AMPK phosphorylation [68]. Bastie et al also reported that Fyn kinase is a negative regulator of AMPK [69]. Although the authors have also elucidated that Fyn negatively regulated the AMPK activity by controlling the cellular localization of the AMPK kinase, LKB1 [70], our observation of PIKE-A/IR/Fyn/AMPK interplay may provide an additional mechanism for Fyn to control energy homeostasis.

PIKE in insulin resistance

While *PIKE -/-* muscle and fat are hypersensitive towards insulin stimulation, it is interesting to note that *PIKE -/-* liver is insulin resistant. In contrast to the observations in muscle and fat,

activation PI3K/Akt pathway in liver is significantly reduced in *PIKE -/-* mice after insulin injection [71] . However, we did not observe any change in systemic glucose tolerance in the knockout animals because the upregulation of insulin responsiveness in muscle and fat of *PIKE -/-* mice compensated for the hepatic insulin resistance [10]. To further study the physiological consequence of hepatic PIKE-A inactivation and overcome the interferences of *PIKE* ablation from other tissues, we thus generated liver-specific *PIKE* knockout (LPKO) mice [71]. Similar to the observations in *PIKE -/-* mice, hepatic insulin signaling in LPKO mice is aberrant that *in vivo* insulin injection stimulates less PI3K/Akt activation. The major function of insulin is to suppress glucose production in liver. It suppresses hepatic glucose output by inhibiting the expressions of enzymes involved in the gluconeogenesis like glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxylase (PEPCK) [72]. Using RT-PCR analysis, we found that both G6Pase and PEPCK expressions are augmented in LPKO liver, indicating the glucose output from liver is increased in PIKE-null hepatocytes. Indeed, glucose production is enhanced in cultured *PIKE -/-* hepatocytes, which could not be inhibited by insulin stimulation. Consequently, LPKO mice display symptoms resembling prediabetes patients including fasting hyperglycemia, hyperinsulinemia, impaired glucose tolerance and defective systemic insulin tolerance [73].

Another function of insulin is to promote lipid synthesis in liver by increasing the expression of lipogenic enzymes [74]. A defective hepatic insulin signaling pathway should thus reduce the hepatic lipid content. As expected, triglyceride, free fatty acid and cholesterol contents were lower in the LPKO liver [71]. The low lipid concentrations fit with the reduced hepatic expressions of fatty acid synthase (FAS) and acetyl Co-A (ACC), two key enzymes in lipid synthesis that are regulated by insulin [75, 76], in LPKO mice. Because excessive lipid accumulation in liver is a major denominator for hepatic insulin resistance [77], the low lipid contents in LPKO liver thus exert a protective role towards obesity induced hepatic steatosis and hyperglycemia. However, we did not observe any significant improvement of obesity-induced diabetes or liver steatosis in LPKO mice after HFD feeding. Presumably, most of the lipids found in the liver of these animals are coming from ectopic lipid

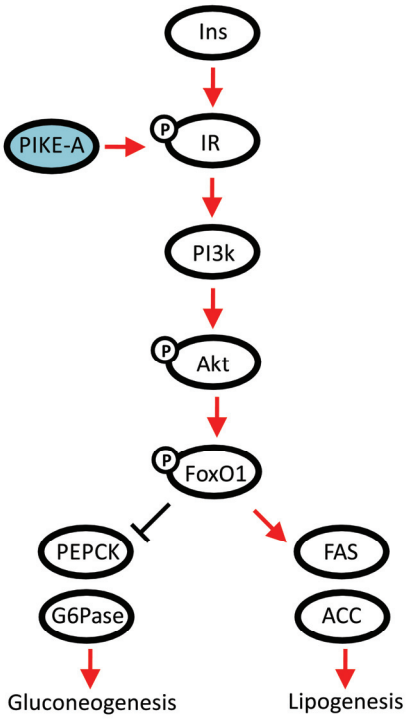

Figure 4. Role of PIKE-A in insulin receptor signaling.

deposition spilled from the WAT, which overwhelmed the low *de novo* lipogenesis in LPKO liver during HFD feeding [68].

Another interesting finding on the role of PIKE-A in liver insulin signaling is its association with IR. In both *PIKE -/-* and LPKO liver, we found that insulin provoked less IR phosphorylation, indicating PIKE-A might regulate the receptor autophosphorylation by an unknown process [71]. IR is a transmembrane receptor consists of two disulfide-bonded extracellular α- and two transmembrane β-subunits with tyrosine kinase activity [78]. When insulin binds to the IR, it causes a conformational change that enhances the transphosphorylation between the β-subunits of IR to activate its kinase activity [76], which in turns phosphorylates various cellular substrates including IRS-1, Shc and Cbl to regulate glucose metabolism [80]. We found that IR activation is facilitated by PIKE-A and insulin stimulation enhances the direct interaction between PIKE-A and IR, which is critical for IR autophosphorylation. In HEK293 cells, overexpression of PIKE-A enhances the IR autophosphorylation and its kinase activity. On the other hand, depletion of PIKE-A in *PIKE -/-* MEF dimin-

ishes insulin-induced IR autophosphorylation and its kinase activity. Interesting, PIKE-A is a substrate of IR which can phosphorylate the N-terminal of PIKE-A. However, it remains to be determined if such phosphorylation causes any activity changes on PIKE-A (**Figure 4**).

Our *in vivo* studies using *PIKE -/-* and LPKO mice show that Akt might not be the major target of peripheral PIKE-A under a physiological context though it is identified as an Akt enhancer in glioblastoma [6]. In muscle and fat, PIKE-A ablation results in AMPK activation, leading to insulin hypersensitivity [10]. Therefore, insulin-provoked Akt activation is enhanced in *PIKE -/-* mice, which is a secondary effect of elevated IRS-1/PI3K activation induced by AMPK [81]. In liver, insulin-induced Akt activation is reduced in LPKO/*PIKE -/-* mice, which is a result of diminished IR activation, suggesting the preferential role of PIKE-A in maintaining IRTK activity over the kinase activity of Akt. Importantly, we did not observe any changes of basal hepatic Akt activity neither in fed nor in fasting status. Presumably, the Akt enhancer role of PIKE-A is more prominent in cancer progression but not under physiological conditions.

Perspective

In all, the availability of PIKE knockout animals provides the essential tool to study the authentic functions of PIKE under physiological or pathological conditions. However, some of the well-documented functions of PIKE have not been fully evaluated, although we have successfully testified the protective activities of PIKE in neurons and identified several new activities of PIKE from characterizing the *PIKE -/-* mice. In particular, the role of PIKE-A in tumor formation has not been validated in the knockout animals. We and others have reported that PIKE-A is a proto-oncogene, which has an elevated expression in glioblastoma, breast, prostate and liver cancers [6, 82-84]. Thus, the *PIKE -/-* mice may be resistant to the spontaneous or chemical-induced formation of cancers. However, experiments performed in *PIKE -/-* mice could not address the question convincingly as it is unknown if the high PIKE-A expression is the cause or consequence of cellular transformation. In this regard, a parallel carcinogenic research in PIKE-A transgenic and *PIKE -/-* mice will provide a more definitive answer.

PIKE -/- animals also cannot be used to distinguish the isoform-specific role of PIKE in a particular phenotype. Since all PIKE isoforms are produced from the *CENTG1* gene, traditional knockout technology by removing the exons inevitably results in the loss of all PIKE proteins, and the phenotypes observed in *PIKE -/-* animals may be a combined consequence of the depletion of all PIKE proteins. For example, we have recently found that both PIKE-L and -A interacts with UNC5B that PIKE-A also has a functional contribution to prevent neuronal cell death via inhibiting the apoptotic activity of UNC5B [85]. Generation of transgenic animals overexpressing shRNA against specific PIKE isoforms may provide a feasible solution to the problem [86]. Nevertheless, more studies should be devoted on the transcriptional regulation of different PIKE family members, which is an unexplored area today, so that a more specific strategy could be applied to design a better construct in making the isoform-specific PIKE -/- animals.

Tissue specificity is another problem when analyzing the role of PIKE proteins in a particular tissue. The use of tissue specific knockout mice together with *in vitro* culture assays may exclude this possibility. For instance, hepatic glucose production can be regulated by central nervous system through the hepatic branch of vagus nerve [87]. It is thus skeptical that the defective hepatic glucose output in *PIKE -/-* mice might be a secondary effect of neuronal defect. Indeed, our finding that ablation of PIKE-A in LPKO mice is sufficient to trigger insulin insensitivity and hyperglycemia with the liver-brain circuit remains intact. Hence, this finding supports the cell autonomous role of hepatic PIKE-A in regulating hepatic glucose production. Similarly, it remains unknown if the defective adipogenesis in adipose tissue or the enhanced lipolysis in muscle is the primary factor in protecting the mice from diet-induced obesity. Future research on muscle-specific and fat-specific PIKE -/- mice will be useful in providing the answer.

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Address correspondence to: Dr. Keqiang Ye, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, 615 Michael Street, Atlanta, GA 30322, Tel: 404-712-2814; Fax: 404-712-2979, E-mail: kye@emory.edu

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