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Review

The roles of PIKE in tumorigenesis

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Tumorigenesis is the process by which normal cells evolve the capacity to evade and overcome the constraints usually placed upon their growth and survival. To ensure the integrity of organs and tissues, the balance of cell proliferation and cell death is tightly maintained. The proteins controlling this balance are either considered oncogenes, which promote tumorigenesis, or tumor suppressors, which prevent tumorigenesis. Phosphoinositide 3-kinase enhancer (PIKE) is a family of GTP-binding proteins that possess anti-apoptotic functions and play an important role in the central nervous system. Notably, accumulating evidence suggests that PIKE is a proto-oncogene involved in tumor progression. The PIKE gene (CENTG1) is amplified in a variety of human cancers, leading to the resistance against apoptosis and the enhancement of invasion. In this review, we will summarize the functions of PIKE proteins in tumorigenesis and discuss their potential implications in cancer therapy.

Keywords: PIKE; tumorigenesis; cancer; Akt; phosphoinositide 3-kinase

Introduction

Phosphoinositide 3-kinase enhancer (PIKE) is a group of GTP-binding proteins that belong to the α1 subgroup of centaurin GTPase family[1]. The PIKE family includes three members, PIKE-L, PIKE-S and PIKE-A, which are originated from a single gene (CENTG1) through alternative splicing or differential transcription initiation[2, 3]. PIKE-L is the longest isoform among the three family members, containing three proline-rich domains (PRD) at the N-terminus, a central GTP-binding domain, a pleckstrin homology (PH) domain, an Arf-GAP-like sequence, and two ankyrin repeats (ANK) in the C-terminus[3]. Compared to PIKE-L, PIKE-S lacks the Arf-GAP-like domain and ankyrin repeats[4]. PIKE-A differs from PIKE-L in that the N-terminal proline-rich domain is replaced with a unique short peptide of 72 amino acids[2, 5] (Figure 1). Both PIKE-S and PIKE-L bind to PI3K and enhance its activity. However, PIKE-A does not interplay with PI3K. Instead, it interacts with the downstream effector Akt and promotes its activity. These actions are mediated by their GTPase activity. Both PIKE-S and PIKE-L are prominently distributed in brain[3, 4], while PIKE-A shows a distinct tissue distribution pattern from other PIKE isoforms. Northern blot analysis reveals a high level of PIKE-A mRNA is expressed in brain and a small amount is also detected in liver, lung, skeletal muscle, spleen thymus, small intestine and periphery blood leukocytes[6–8]. For the cel-
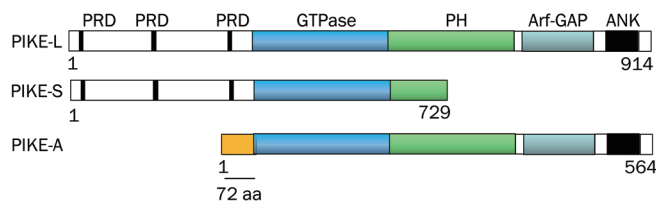
Figure 1. Schematic representation of PIKE proteins. PRD, proline-rich domain; GTPase, GTP-binding motif; PH, pleckstrin homology domain; Arf-GAP, a domain showing sequence homology to ARF/GAP protein; ANK, ankyrin repeat.

lular localization, PIKE-L and PIKE-A are present in both the nucleus and the cytoplasm, while PIKE-S resides exclusively in the nucleus[9].

In the last decade, our research group endeavored to delineate the physiological roles of PIKE proteins. With the availability of the whole body PIKE knockout (PIKE−/−) mice, the roles of PIKE in neuronal survival[3, 10–12], brain development[13], mammary gland development[14], obesity development[15], insulin resistance[16], and cell transformation[17] have been characterized. Remarkably, PIKE proteins are involved in multiple signaling pathways in addition to the PI3K/Akt cascade. PIKEs are implicated in regulating the activity of signal transducer and activator of transcription 5A (STAT5) via prolactin (PRL)-stimulated JAK2 (Janus kinase 2) phosphorylation[14]. In addition, PIKE-L strongly binds SET, a DNase inhibitor, and prevented its degradation by asparaginyl endopeptidase

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(AEP), leading to resistance of neuronal cell death provoked by neuroexcitotoxic insults or ischemia[10].

PIKE is a proto-oncogene

The accumulation of genetic damage in the forms of activated proto-oncogenes and inactivated tumor-suppressor genes is the driving force in the evolution of a normal cell to a malignant cell. Proto-oncogenes are a group of genes that cause normal cells to become cancerous when they are mutated or highly expressed[18, 19]. Examples of proto-oncogenes include RAS, WNT, MYC, ERK, and TRK[20], and they are considered as potential targets for molecular cancer therapy[21, 22].

The most crucial discovery of PIKE's activity in disease onset is its role in neuro-oncology. During our investigation on the mechanism of glioblastoma multiform formation, we found that the loci of GGAP2 (PIKE-A) are highly amplified in glioblastoma[5]. After sequence alignment analysis, we clarified that the resultant protein of GGAP2 is an isoform of PIKE, PIKE-A, which was originally identified in the human genome sequencing effort as KIAA0167[7]. Indeed, PIKE-A displays an elevated expression in glioblastoma and astrocytoma, which result from gene amplification[2]. In addition, it is highly expressed in various cancers that originate from breast, prostate, skin, uterus, colon, ovary, liver, stomach, lung, cervix, rectum, testis, and kidney[5, 17, 23, 24]. It is also demonstrated that PIKE-A expression is increased in 93% of brain tumors without CENTG1 amplification. Most recently, Xie et al found that PIKE-S is highly expressed in malignant human keratinocytes (SCC4 and SCC12B2) but had low expression in normal human keratinocytes[25]. EGF-induced squamous cell carcinoma (SCC) proliferation requires SH3 domain of Phospholipase C-γ1 (PLC-γ1), which is a guanine nucleotide exchange factor (GEF) for PIKE. Knockdown of PLC-γ1 or PIKE blocks EGF-induced SCC cell proliferation. These findings support the notion that PIKE plays a critical role in EGF-induced SCC cell proliferation and functions as a proto-oncogene in SCC.

Amplification of chromosome 12q13-q15, where CENTG1 is located, is frequently observed in numerous human cancers[26-29]. In 1994, Reifenberger et al revealed that CENTG1 is frequently co-amplified with cyclin-dependent kinase 4 (CDK4), which is a well-known proliferation activator that promotes E2F- and CDK2-dependent cell cycle progression in tumors[28], it would be logical to surmise that PIKE-A amplification or overexpression coordinately acts with CDK4 amplification or overexpression to drive tumorigenesis. Cancer cells with this amplicon are more resistant to apoptotic stimuli compared with cells that express a normal CENTG1 copy number[5]. Indeed, from an automated network analysis on the core pathway of glioma formation, PIKE-A has recently been confirmed as a driver gene of glioblastoma[30]. These data suggest a strong correlation between PIKE-A expression and tumor formation. As a matter of act, PIKE-A overexpression is sufficient to transform NIH3T3 cells and enhance the proliferation and invasion of U87MG, a glioblastoma cell line without CDK4 amplicon and with modest PIKE-A expression[17]. Therefore, PIKE satisfies the criterion of a proto-oncogene,

which implies its potential role in tumorigenesis.

Functions of PIKE in tumorigenesis

Three members (PIKE-L, PIKE-S, and PIKE-A) have been identified in the PIKE family so far, and accumulating evidence indicates that functions of PIKE are characterized by different isoforms at different subcellular compartments. PIKE-L and PIKE-A reside in multiple intracellular compartments, while PIKE-S localizes exclusively in the nucleus[9]. To understand the functions of PIKE in tumorigenesis, we will discuss the role of PIKE based on its cellular localization.

The functions of PIKE in the cell membrane

Cells transmit extracellular signals via membrane receptors. PIKE-L has been identified as a component of the netrin-1 signaling pathway, which protects neurons from apoptosis[11]. Traditionally, netrin-1 is a chemotropic cue for axon migration and arborization during neural development[31]. The most important receptors of netrin-1 are deleted in colorectal cancer (DCC) and the UNC5 family[32]. Recently, the roles of netrin-1 and its receptors in tumorigenesis have been broadly studied[33] and DCC and UNC5 proteins are considered dependence receptors that regulate apoptosis depending on the interaction with their ligands, netrins[34]. They are also considered to be tumor suppressors, since they suppress tumor progression in the absence of netrin-1[35, 36]. PIKE-L/UNC5B association enhance cell survival via PI3K signaling[11], which is controlled by a protein kinase Fyn. Fyn phosphorylation on both the receptor and PIKE-L is necessary for their interaction[11, 37]. As Fyn is constitutively associated with DCC, presumably, PIKE-L may not interact with UNC5B but it may also associate with DCC[38]. Indeed, PIKE-L and DCC have been co-immunoprecipitated from rat brain lysates, which further supports this hypothesis[11].

It has also demonstrated that PIKE-A associates with UNC5B in glioblastoma cell lines[39]. The PIKE-A/UNC5B binding is tightly regulated by Akt, in which Akt-induced phosphorylation of PIKE-A on Ser-472 promotes its interaction with UNC5B. PIKE-A suppresses UNC5B transcription by down-regulating p53, which is a transcriptive regulator of UNC5B[40, 41]. As such, netrin-1 might stimulate Akt activation, which subsequently phosphorylates PIKE-A, escalating its binding to UNC5B, and thereby inhibiting the receptor cleavage and apoptosis-inducing activity. In addition, PIKE-A (S472) phosphorylation feed backs positively to further elevate the Akt activity and leads to p53 degradation through the Akt-MDM2 pathway, resulting in down-regulation of UNC5B[39]. Since PIKE-L associates with DCC in the brain, conceivably, other PIKE isoforms may interact with DCC and demonstrate a potential function in tumorigenesis.

Recently, we have found that PIKE-A regulates insulin receptor tyrosine kinase (IRTK) activity, leading to suppression of AMP-activated protein kinase (AMPK) activation. The association of PIKE-A with the insulin receptor is important for insulin to fully initiate the hepatic IRTK[16]. As IRTK also plays an important role in cancer progression[42, 43], it is reason-

able to infer the IRTK-PIKE-A crosstalk may play a role in the regulation of PIKE-A in tumorigenesis.

The functions of PIKE in the cytoplasm

Cytoplasmic PI3-kinase regulates the membrane translocation and activation of many signaling molecules. As a PI 3-kinase enhancer, PIKE also functions through cytoplasmic molecular interactions. Our previous works demonstrate that PIKE-L reduces the anti-proliferation activity induced by merlin (schwannomin, a tumor suppressor), which is encoded by the NF2 gene. It is now recognized that mutation of the NF2 gene is not restricted to schwannomas but also extends to thyroid carcinomas, hepatocellular carcinomas, and perineurial tumors[44-47]. The N-terminus of PIKE-L specifically binds to the N-terminal domain of merlin[48], which assists merlin's inhibition of PI3-kinase activity[48]. Notably, PIKEs are also implicated in regulating the activity of transcription factors such as STAT5A, which can be activated by PRL[14], one important ligand of JAK2[49]. As the JAK2/STAT5 cascade plays an important role in cancer initiation and progression[50, 51], it is logical to infer the roles PIKE in tumorigenesis via JAK2/STAT5 signaling regulation. PIKE-A is amplified in human cancer cells and Akt activity correlates with PIKE-A amplification[5]. It has been demonstrated that PIKE-A directly binds to activated Akt but not PI3-kinase in a guanine nucleotide-dependent fashion and stimulates the kinase activity of Akt, promoting cell survival, migration, and invasion[52]. Overexpression of PIKE-A enhances Akt activity and promotes cancer cell invasion, whereas dominant-negative PIKE-A and PIKE-A knockdown markedly inhibit these processes[2]. Further studies indicate that PIKA-A binds active Akt and enhances its activity through maintaining Akt active conformation or initiating its activation[2]. Most recently, the interaction of PIKE-A and Akt has been validated using fluorescence-based protein complementation assay in live cells. Disrupting the PIKE-A/Akt association, using a small peptide derived from the binding fragment on PIKE-A or Akt, inhibits glioblastoma cell proliferation, migration, and invasion, re-confirming the essential role of PIKE-A in Akt activation[53].

Focal adhesion kinase (FAK) is a key player in tumor formation and progression[54-56]. Recently, Zhu et al showed that PIKE-A is a novel binding partner of FAK, and PIKE-A enhances its kinase activity in response to growth factor stimulation[57]. It is also revealed that PIKE-A functions as a FAK enhancer through two mechanisms: activating FAK to disassemble focal adhesions and control the trafficking of regulators to or from the focal adhesion, when the focal adhesion is being remodeled, which are key processes for cell motility and migration[58, 59]. Moreover, phosphorylation of FAK by cyclin-dependent kinase 5 (CDK5) is critical for FAK-mediated microtubule fork formation and cell migration[60] and phosphorylation of FAK by CDK5 regulates FAK's function in mediating cell migration[61]. Interestingly, PIKE-A has also been demonstrated as a physiological substrate of CDK5. In glioblastoma cells, CDK5 directly phosphorylates PIKE-A at Ser-279 in its GTPase domain, which stimulates PIKE-A

GTPase activity and the activity of its downstream effector Akt[52]. Moreover, phosphorylation of PIKE-A by CDK5 mediates growth factor-induced migration and invasion of human glioblastoma cells[52]. Collectively, these data indicate that PIKE-A, FAK, and CDK5 are involved in cross-talk to regulate cell migration and invasion in cancer cells.

The functions of PIKE in the nucleus

While most PI3K signaling studies have focused on its activities at the plasma membrane, it is now clear that the nucleus has a distinct set of PI3K signaling machinery and effectors with different regulatory mechanisms[62, 63]. Nuclear phosphoinositide lipids appear to regulate cell proliferation and differentiation as well[64, 65]. PLC-γ1 is a tyrosine kinase substrate for many RTKs and non-RTKs and is essential for cell proliferation and differentiation[66]. It has been detected in the nucleus and is localized to the nucleus in highly transformed and proliferating cell lines[67, 68]. It has been demonstrated that PLC-γ1 functions as a GEF (guanine nucleotide exchange factor) for PIKE-S and thereby activates PI3-kinase activity[69]. Moreover, Nerve growth factor(NGF) treatment elicits the membrane-associated protein, 4.1N, to translocate to the nucleus and bind PIKE, which prevents the interaction between protein 4.1N and nuclear PI3-kinase, leading to diminish the activation of PI3-kinase[4].

A recent report reveals that hsa-miR26a, CDK4, and PIKE-A comprise a functional integrated oncomir/oncogene DNA cluster, which promotes the aggressiveness in glioblastoma[70]. Cai et al demonstrated that PIKE-A promotes tumor progression by increasing the transcriptional activity of nuclear factor κB by directly interacting with its p50 subunit[23]. Most recently, using a SCC system, PIKE was found to mediate epidermal growth factor receptor (EGFR)-dependent SCC cell proliferation[25]. In the SCC cells, PIKE is abundantly expressed in vitro and in vivo. Previous studies have indicated that PLC-γ1 is required for EGF-induced SCC cell proliferation[71], and the proliferative role of PLC-γ1 relies on its SH3 domain but is independent of its lipase activity[72]. Conceivably, PIKE plays a role in SCC cell proliferation because of the interaction between PIKE and PLC-γ1 in the nucleus[73]. These data provide evidence of PIKE-EGFR signaling crosstalk; further validate the function of PIKE in tumorigenesis.

Targeting PIKE in cancer therapy

The role of a proto-oncogene has been clearly defined and targeting proto-oncogene is a strategy for cancer therapy[20, 21]. PIKE, as a new member of the proto-oncogene family, is a potential target for cancer therapy. Similar with many known proto-oncogenes, the functions of PIKE rely on different kinds of signaling pathways associated with many binding partners (Table 1). From these molecules, we can clearly sort out the potential mechanisms for targeting PIKE in cancer therapy. Although how to manipulate PIKE activities to contribute cancer treatment is still not well clarified yet, we can speculate the regulation of PIKE in three directions.

Table 1. PIKE interaction partners summary.

PIKE isoforms	Interacting proteins	Functions
PIKE-S	PI3K	Enhancing kinase activity of nuclear PI3K
	PLCγ1	Enhancing PIKE-S GTPase activity
	4.1N	Abolishing PIKE-S/PI3K association
	BRAG2	Accumulating BRAG2 in nucleoli
PIKE-L	PI3K	Enhancing kinase activity of PI3K
	Homer 1c	Activating PI3K by quisqualate
	Merlin	Inhibiting merlin-induced PI3K activity
	UNC5B	Activating PI3K by netrin-1
	SET	Preventing SET from proteolytic cleavage
PIKE-A	Akt	Enhancing Akt kinase activity
	Arf1	Enhancing GTPase activity of Arf1
	AP1	Inhibiting GAP activity of PIKE-A;
		Altering the intracellular localization of AP1
	Fyn	Preventing PIKE-A degradation
	AMPK	Regulating AMPK activity
	CDK5	Enhancing the GTPase of PIKE-A
	FAK	Enhancing the kinase activity of FAK
	UNC5B	Regulating UNC5B apoptosis-inducing effect
	Insulin receptor	Inhibiting AMPK phosphorylation
	STAT5a	Enhancing STAT5a phosphorylation

Regulating PIKE GTPase activity

In response to stimulatory signals, GTPase activities are controlled by the GTP/GDP ratio and subcellular distribution in the cell through the joint action of multiple regulatory molecules: GEFs, which activate GTPases by promoting GDP-to-GTP exchange, and GAPs, which inactivate the GTPases by enhancing intrinsic GTP hydrolysis activity[74]. It has been reported that Dynamin 2 (a potent activator of metastatic migration) enhances invasive migration of pancreatic tumor cells through stabilization of the Rac1 GEF Vav1[75]. The anticancer agent cucurbitacin I, a Jak2 inhibitor, reduces the activation of Rac1 in response to the ErbB3 ligand heregulin in breast cancer cells[76]. As a GTPase, PIKE can be activated by intrinsic or extrinsic cues, setting off a signaling cascade. Therefore, regulating the GEFs and GAPs of PIKE is a potential way to interfere with the functions of PIKE. So far, we have characterized that PLC-γ1 is a GEF of PIKE[73], accordingly, functions of PIKE may be able to be regulated by manipulating PLC-γ1 activity. Discovering other GEFs and GAPs of PIKE in the future may also contribute to the regulation of PIKE functions.

The PH domain is a structural motif that binds phosphatidylinositol lipids to biological membranes[77]. The PH domain of PIKE plays a regulatory role in regard to the cellular localization and GTPase activity of the protein[78]. Binding of phospholipids to the PIKE PH domain increases the GTPase activity. It is suggested that the phospholipid/PIKE interaction causes a conformational change in the protein, rendering the C-terminal Arf-GAP domain accessible to its N-terminal domain, thus inducing the GTPase activity. This hypothesis is supported by the observation that the enzymatic activ-

ity of PIKE-A is low when the Arf-GAP domain is deleted[8]. Moreover, a recent structural study of the PIKE PH domain also suggests that the motif is responsible for binding the head groups of phosphoinositides in the cell membrane[79]. Together, these results indicate that PIKE cellular localization and activity are controlled by the interaction between phospholipids and the protein's PH domain.

Cytoplasm-nucleus shuttling of PIKE is known to correlate with its GTPase activity and cellular functions. Based on the structure of its PH domain, PIKE has been considered as one of the split PH domain containing proteins[79]. The split PH domain mediates the cytoplasmic-nuclear localization of PIKE via a positively charged nuclear localization sequence (NLS). Lipid membrane binding of the PH domain is further enhanced by the NLS[79]. Therefore, based on this model, it is conceivable that regulation of phosphatidylinositol phosphate (PIP) concentration at cytoplasmic membranes could possibly control the localization of PIKE, leading to alterations in its activity and cellular functions.

Modulating PIKE phosphorylation

It has been demonstrated that Akt1/PKBα regulates invasion of inflammatory breast cancer cells through Akt1 phosphorylation of Rho C GTPase[80]. Calmodulin binds to K-Ras B and inhibits phosphorylation of K-Ras B at Ser1-81, near to the membrane anchoring domain, modulating the signaling of oncogenic K-Ras B, which may be relevant to normal cell physiology, and opens new therapeutic perspectives for the inhibition of oncogenic K-Ras B signaling in tumors. PIKE can also be regulated by protein phosphorylation. It has been reported that PIKE-A can be phosphorylated by CDK5, Akt, and Fyn on Ser-279[52], Ser-629[23]/Ser-472[39], and Tyr-682/Tyr-772[81], respectively. In addition, it has been shown that PIKE-A can also be phosphorylated upon growth factor stimulation[82].

Phosphorylation of PIKE plays an important role in dictating its biological functions. To regulate the functions of PIKE through altering the levels of PIKE phosphorylation is rational. As described above, serine/threonine and tyrosine kinases are involved in the phosphorylation of PIKE; these kinases contribute to cancer progression and their cross-talk also plays a role in tumorigenesis[83]. Since PIKE-A acts as a proto-oncogene and contributes to tumorigenesis, it is reasonable to assume that suppressing activity of the kinases with inhibitors is a good approach for targeting PIKE in cancer therapy. It should be noted that there are many other putative phosphorylation sites on PIKE, which suggests that there are additional potential roles of PIKE in tumorigenesis, as well as more ways to impinge on PIKE function.

Disrupting protein-protein interactions

Protein-protein interactions (PPI) play an important role in tumorigenesis and disrupting PPI is considered a novel therapy for cancer treatment[84]. It has been reported that the ERK1/2-binding IQGAP1 (IQ motif-containing GTPase activating protein 1) WW domain peptide disrupts IQGAP1-

ERK1/2 interactions and inhibits RAS- and RAF-driven tumorigenesis[85]. EHop-016, a novel small molecule, inhibits Rac GTPase activity by disrupting the interaction of Rac with its direct guanine nucleotide exchange factors[86]. It is now clear that PIKE interacts with numerous molecules to trigger multiple physiological functions potentially involved in tumorigenesis (Table 1). As cancer is a complex disease, the representation of a malignant cell as a protein-protein interaction network (PPIN) and its subsequent analysis can provide insight into the behavior of cancer cells and lead to the discovery of new biomarkers[84]. The feasibility of targeting PPIs for functional studies has been well established[87] and targeting PPI is now considered a potential strategy for cancer therapy[88]. Therefore, disrupting the PIKE binding networks may provide an efficient strategy for regulating PIKE activity in tumorigenesis.

As such, we have demonstrated that the disruption of PIKE-A/Akt interaction leads to reduction of glioblastoma cell proliferation, survival, migration and invasion[53]. Moreover, peptides derived from the binding fragments of PIKE-A/Akt are able to inhibit this protein complex and sensitize glioblastoma cells to clinical anti-gloom chemotherapeutics[53]. Since PIKE-A/Akt association also plays an important role in prostate cancer progression[23], it is reasonable to consider targeting this PPI for prostate cancer treatment. Among the binding partners of PIKE (Table 1), most of them are involved in tumorigenesis, such as UNC5B[89], AMPK[90], and Fyn[37]. Therefore, regulating PIKE-related PPI may serve as a good strategy for targeting PIKE in tumorigenesis.

Future perspectives

The role of PIKE GTPase in tumorigenesis is evident based on the studies performed in the past 10 years, yet we believe that the functional activity of PIKE in tumorigenesis has not been fully elucidated. With the availability of PIKE knockout animals, we will further delineate the authentic functions of PIKE in tumorigenesis. We and others have established that PIKE acts as a proto-oncogene. Thus, knockout mice may be resistant to the spontaneous or chemical-induced formation of cancers. However, experiments performed in PIKE knockout mice could not address the question convincingly as it is unknown if the high PIKE expression is the cause or consequence of cellular transformation. Another fundamental question regarding PIKE signaling is whether PIKE activation is growth factor specific, *ie* its activity can be triggered by NGF/EGF. In this regard, a parallel carcinogenic research study in PIKE transgenic and PIKE knockout mice may provide a more definitive answer. Additionally, the role of PIKE as an initiator or promoter of tumorigenesis still needs to be determined. Further study of PIKE signaling in tumorigenesis will not only enhance our knowledge about its physiological activities, but may also provide significant implications for cancer prevention and treatments.

Acknowledgements

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