PDK4 Protein Promotes Tumorigenesis through Activation of cAMP-response Element-binding Protein (CREB)-Ras Homolog Enriched in Brain (RHEB)-mTORC1 Signaling Cascade**

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The mechanistic target of rapamycin (mTOR)2 is an evolutionarily conserved serine/threonine kinase that integrates various intracellular and extracellular signals to regulate cell survival, growth, proliferation, differentiation, and metabolism (1–4). The receptor tyrosine kinase/P13K/AKT pathway relays signaling from growth factors to mTOR by first suppressing the tuberous sclerosis complex 1 and 2 (TSC1 and TSC2) protein complex. The TSC1/2 protein complex is a negative regulator of the small GTPase enrichment in brain (RHEB) as TSC2 exerts its GTPase-activating activity toward RHEB. The unleashed RHEB then activates mTOR by binding to its catalytic domain and stimulating mTOR phosphorylation (5–6). mTOR exists in rapamycin-sensitive mTOR complex 1 (mTORC1) and rapamycin-insensitive mTOR complex 2 (mTORC2). Activated mTORC1 exerts its cellular functions by modulating downstream effectors such as S6 kinase and 4E-binding protein 1 (4E-BP1), whereas mTORC2 phosphorylates AKT at Ser-473 (4). Conversely, hyperactive mTORC1 also induces feedback inhibition of AKT through up-regulation of S6 kinase, growth factor receptor-bound protein 10 (GRB10), and store-operated Ca2+ entry and down-regulation of platelet-derived growth factor receptors and insulin receptor substrate (5–8). Genetic mutations in human cancers that cause gain of function for proto-oncogenes or loss of function for tumor suppressors in this pathway lead to hyperactivation of mTORC1. Mutations of tumor suppressor TSC1 or TSC2 cause TSC, a multiorgan tumor syndrome, through activation of mTOR (9–12). mTOR is also a sensor of energy status downstream of the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK)-TSC1/2 signaling pathway. Deletion of either LKB1 or AMPK also activates mTOR (13). An important role of mTOR has been established in the regulation of cellular pro-
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cesses such as metabolism, autophagy, and protein synthesis (14–17). Hyperactive mTOR augments angiogenesis, aerobic glycolysis (Warburg effect), and cell proliferation in tumor development (18–20). Therefore, the regulation of mTOR activity is of significance in normal cellular processes as well as tumor development.

Pyruvate is an intermediate pivot in glucose metabolism and can be decarboxylated to acetyl-CoA by pyruvate dehydrogenase (PDH) (21). PDH links glycolysis to the citric acid cycle and releases energy via NADH. Pyruvate dehydrogenase kinases (PDKs) inactivate PDH through phosphorylation of its E1a subunit. PDKs are mitochondrial proteins encoded by genomic sequences for human PDK4 was 5'-GGATAGTGTA-3'. The role of PDK4 in tumorigenesis is unclear. As an inhibitor of PDK4 and its isoforms, dichloroacetic acid (DCA) has been used to treat some mitochondrial diseases (23). DCA has antiproliferative and pro-apoptotic effects on cancer cells due to unknown mechanisms. Furthermore, DCA can suppress hypoxia-inducible factor 1 (HIF-1α) (24), a downstream effector of mTOR signaling (25).

Here, we show that PDK4 activates mTORC1 through up-regulation of the CREB-RHEB cascade. Overexpression of PDK4 sensitizes cells to mTOR inhibition, and suppression of PDK4 blunts tumorigenesis. Therefore, PDK4 may be targeted for the treatment of certain cancers.

EXPERIMENTAL PROCEDURES

Reagents—Rapamycin and DCA were obtained from Sigma. MG-132 was obtained from Beyotime (Shanghai, China). Cycloheximide (CHX) was obtained from Genview (Galveston, TX). Torin-1 was obtained from Selleckchem (Houston, TX). Fetal bovine serum, Dulbecco’s modified Eagle’s medium, Lipofectamine 2000, and 4–12% BisTris NuPAGE gels were purchased from Invitrogen. Restriction enzymes were from Takara (Otsu Shiga, Japan).

Antibodies—The antibodies against total S6 and phospho-S6 (Ser-235/236) have been previously described (6). PDK1, PDK2, PDK3, PDK4, and phospho-PDH (Ser-293) antibodies were from Abgent (San Diego). Antibodies for α-tubulin, β-actin, ERK, phospho-ERK (Tyr-204), GRB10, AKT, normal IgG antibody, and all HRP-labeled secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). TSC2, PTEN, phospho-AKT (Ser-473), RHEB, CREB, and phospho-CREB (Ser-133) antibodies were from Cell Signaling Technology (Beverly, MA). Phospho-AMPK (Thr-183/172) antibody was from Bioworld (Atlanta, GA), and phospho-GRB10 (Ser-501/503) antibody was from Millipore (Bedford, MA). PDH antibody was from Invitrogen.

Preparation of Plasmids and Viruses—The pLL3.7-shRNA vector was used for suppression of PDK4 expression. The target sequence for human PDK4 was 5'-GGACGTAAAGAATTCTCAT-3'. The target of mouse Pdk4 was 5'-GGATTTGGTGGAGTTCCAT-3'. Lentiviruses were generated by co-transfecting pL3.7 and the packaging vectors (VSVG, REV, and pMDL) into HEK293T cells. After 48 h of transfection, viruses were collected by filtering through a 0.45-μm filter. PC3 cells were infected by incubation with viruses for 2 days, and the expression of GFP protein was used to evaluate the infection rate.

To generate a Pdk4 overexpression vector, Pdk4 was amplified from cDNA of wild-type mouse embryonic fibroblasts (MEFs) as a template using the following primers: 5'-TAACGCGGCCGACCATGAAAGGCGCCGCTTT-3' and 5'-CCCCAGGATCTCTACACTGCGAGTCTTCTCCTG-3'. The PCR fragment was then cloned into the pQCXIP-puro vector at NotI/BamHI sites to generate pQCXIP-puro-PDK4.

Cell Culture—Pten<sup>−/−</sup>, Tsc2<sup>−/−</sup>, myr-Akt, Ampk<sup>−/−</sup> MEF cells and ELT-3 cells have been described previously (2, 7, 26, 27). A549, PC3, MDA-MB-468, PANC-1, MCF7, NIH/3T3, and HEK293T were obtained from ATCC. ELT-3 cells were cultured in Dulbecco’s modified Eagle’s medium/F-12 (1:1) with 10% fetal bovine serum and 1% antibiotics in 5% CO₂ at 37 °C. The other cell lines used in this study were maintained and propagated in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 1% antibiotics in 5% CO₂ at 37 °C. The dosage of DCA treatment of cells was chosen according to McFate et al. (28).

Immunoblotting—Immunoblotting was conducted as described previously (2, 9). Cells were lysed in lysis/loading buffer (10 mM Tris, pH 6.8, 10% glycerol, 2% SDS, and 100 mM DTT) and boiled for 10 min. The cell lysates were then subjected to immunoblotting.

Cell Proliferation Assay (MTT)—Cell proliferation was measured using an MTT assay kit (BioDev-Tech, Beijing, China). Cells were plated at 4 × 10⁴ cells per well, seeded in quintuplet in 96-well plates for 24 h, and then treated with rapamycin in various concentrations. Cells were incubated with 200 μl of medium containing 20 μl of MTT reagent at 37 °C. After 2 h, the supernatant was removed, and 150 μl of dimethyl sulfoxide was added. The plates were shaken under protection from light for 10 min, and the spectrometric absorbance at 490 nm was then recorded. Cell proliferation assays were replicated in at least two independent experiments.

RNA Interference—A total of 5–8 × 10⁴ cells were seeded in a 12-well plate and transfected with synthesized siRNA by Lipofectamine 2000 following the manufacturer’s instructions. Cell lysates were harvested for immunoblotting after 48 h of transfection. The target sequences for RNAi were as follows: pdk4-target 1, 5'-GGACGTAAAGAATTCTCAT-3'; pdk4-target 2, 5'-GGATTTGGTGGAGTTCCAT-3'; rbad-target 1, 5'-GGACGTCTCACAAGGAA-3'; rbad-target 2, 5'-GGAGTCTGTTGGAAGTTCTACAT-3'; rbad-target 3, 5'-GAACGTCTTACAAGGAA-3'; rbad-target 4, 5'-GGACGTCTCACAAGGAA-3'; and negative control, 5'-TTTCCCGAACGGTGTCAGT-3'.

Reverse Transcription of mRNA and Quantitative Real-Time PCR (qRT-PCR)—A total of 1 μg of RNA was reverse-transcribed using the PrimeScript<sup>™</sup> RT reagent kit (Takara). cDNA was used as template using TransStart Green quantitative PCR SuperMix (TransGen Biotech, Beijing, China) in a 20-μl qRT-PCR. Amplifications were performed for 40 cycles using CFX on an iCycler (Bio-Rad). β-Actin served as the inter-
nal control. The number of samples from an individual experiment is three or 5, and the qRT-PCR assays data were replicated in at least two independent experiments. The qRT-PCR primer sequences were as follows: BACT forward, AGAGGAAAATCG- TGGCTGAC and reverse, CAATGCTGACCTTGGCCGT; pdk1 forward, AGATCGAAGACCACGCAAT, and reverse, GTGCTGTTGAGTACCTTCA; pdk2 forward, AACCTGGTTCCTGACCCGAG; and reverse, GAACTGGC- TTAGGCTCCGCTG; pdk3 forward, CCAGAAGACCCAGCA- GTTTTG, and reverse, GGCCATTGTAGAACAACAACA- TCA; pdk4 forward, CCGGTTAGAACAACCTTTC, and reverse, TCTACAACCTGACAGGGCTTT; rhp forward, AAGTCGGGAAAGATCGCA, and reverse GGTGGAT- GTGGAAATCAACCA; c-fos forward, ATCGGCAAGACGCGGAAGATGTAGG, and reverse, GCAACGGCAACTCTCA-TCTCAAG; sst-1 forward, CCACCGGAAAACAGGAATCTG, and reverse, TTGTGCGGTTCAGGTGAGGCC; nuru forward, GTGTTACGGGCAGATGG, and reverse, TGCGCTCCACCCTT, and PBR reverse, TCCCACCTACTTCC-

**DCA, a PDK inhibitor, was pre-**

**RESULTS**

**PDK4 Activation of mTORC1**—DCA, a PDK inhibitor, was previously shown to suppress HIF-1α expression. Because mTORC1 is a critical regulator of HIF-1α, we treated cells with DCA and examined mTOR activity. As a substrate of PDKs, phosphorylated PDH (p-PDH) was used to indicate the action of DCA. DCA reduced the phosphorylation of S6, 4EBP1, and PDH. To investigate the specificity of PDK4 regulation on mTORC1, we knocked down pdk4 and the other three pdk isozymes in NIH/3T3 cells. Knockdown of pdk4, but not pdk1,
pdk2, or pdk3, reduced mTORC1 activity (Fig. 1, d and e). Taken together, our data demonstrate that DCA suppresses mTORC1 specifically through Pdk4 and independent of Pdh.

To further investigate PDK4 regulation of mTOR, we overexpressed PDK4 in NIH/3T3 cells. p-S6 and p-4EBP1 were elevated, and p-Akt was decreased in response to Pdk4 expression in a dose-dependent manner (Fig. 1f). In contrast, Pdk4 overexpression had no impact on p-Erk, a marker of the MAPK signaling pathway (Fig. 1f). Similarly, overexpression of Pdk4 increased phosphorylation of S6, 4EBP1, and Grb10 but not Erk upon serum stimulation after 24 h of starvation of NIH/3T3 cells (Fig. 1g), with a decrease of p-Akt. Consistently, inhibition of Pdk4 did not affect p-Erk (Fig. 1b). We also observed an increase of p-Akt in DCA-treated cells in a dose-dependent manner (Fig. 1b) and an increase of p-Akt in PDK4 knocked down cells (Fig. 1d), which attributed to the feedback inhibition of mTORC1.

**DCA-induced Inactivation of mTORC1 is Independent of AMPK and PTEN-AKT-TSC2**—After knocking down PDK4, suppression of mTOR activity by DCA was compromised (Fig. 2a), consistent with PDK4 regulation on mTOR. Both LKB1-AMPK and PTEN/AKT/TSC1/2 pathways are major mTOR regulatory networks. To examine the potential involvement of AMPK in PDK4 regulation of mTOR, AMPK α1/α2 double knock-out MEFs were used. DCA-mediated p-S6 and p-4EBP1 reductions were not affected in Ampk−/− MEFs compared with wild-type MEFs. Furthermore, DCA did not alter p-Ampk levels in wild-type MEFs (Fig. 2b). Finally, overexpression of pdk4 had no effect on p-Ampk levels in NIH/3T3 cells (Fig. 2c). Taken together, these data indicate that PDK4 is unlikely upstream of AMPK.

DCA down-regulated S6 and 4EBP1 phosphorylation in both wild-type and Pten−/− MEFs (Fig. 2d). Neither constitutive AKT1 activation (myr-AKT1) (Fig. 2e) nor TSC2 deletion (Fig. 2f) compromised DCA-induced mTORC1 suppression. DCA also decreased p-S6 in ELT-3 cells, a Tsc2-null cell line derived from an Eker rat uterine leiomyoma (Fig. 2g). Collectively, these data demonstrate that PDK4 regulation of mTORC1 is in parallel with PTEN/AKT/TSC1/2 and LKB1/AMPK/TSC1/2 pathways.

**PDK4 Up-regulates RHEB Transcription**—Through its GTPase-activating protein (GAP) activity, TSC2 negatively regulates the small GTPase RHEB, which functions as an activator of mTOR (30). Because PDK4 up-regulated mTOR independently of TSC2, we explored the possibility of RHEB serving as a link between PDK4 and mTOR. We knocked down RHEB in both Tsc2+/− and Tsc2−/− MEFs and then treated these cells with DCA. DCA-induced depression of p-S6, p-4EBP1, and p-Grb10 was abolished in rheb knockdown cells (Fig. 3a). Furthermore, overexpressed Pdk4 stimulated Rheb expression in NIH/3T3 cells (Fig. 3b). Taken together, RHEB is involved in PDK4 regulation of mTORC1.

In addition to TSC2 suppression of RHEB, there are other distinct mechanisms that regulate RHEB (27, 29, 31). pdk4 knockout reduced both mRNA (Fig. 3c) and protein levels (Fig. 3d) of Rheb, suggesting that Pdk4 regulated Rheb at the
transcription level. Knockdown of pdk1, pdk2, or pdk3 had no effect on rheb mRNA levels (Fig. 3e).

**PDK4 Stimulates RHEB Expression through CREB**—To identify the link between PDK4 and RHEB, we analyzed the promoter region of rheb for potential protein-binding sites and identified binding sites for the transcription factor CREB (Fig. 4a). CREB is a cellular transcription factor. After phosphorylation at Ser-133, CREB binds to certain DNA sequences and increases or decreases the transcription of targeted genes (32, 33). Chromatin immunoprecipitation assay was performed...
with cross-linked chromatin from NIH/3T3 cells treated with p-CREB antibody or control IgG. CREB indeed bound to the predicted CREB-binding regions of the RHEB gene (Fig. 4b).

To provide more direct evidence of CREB involvement in PDK4-regulated mTORC1 activity, we treated CREB-knocked down NIH/3T3 cells with DCA. DCA-induced depression of mTOR activity was abolished in rheb knockdown cells (Fig. 4c, right panel), which indicates that CREB is involved in PDK4 regulation of mTOR. A marked reduction of Rheb was observed in CREB knockdown cells compared with control cells (Fig. 4d), implicating PDK4 in the upstream of CREB and CREB in the stimulation of RHEB expression. Furthermore, Creb downstream target genes, such as c-fos, nurx-1, sst1, were enhanced in pdk4 overexpression cells (Fig. 4e, left and middle panels) and suppressed in pdk4
knockdown cells (Fig. 4e, right panel). The interaction between CREB protein and the RHEB promoter was significantly higher in pdk4-overexpressing cells than in control cells (Fig. 4f, left panel) and was suppressed in NIH/3T3 cells treated with DCA (Fig. 4f, right panel), indicating that CREB is the potential bridge between PDK4 and RHEB.

Next, we examined the regulatory mechanism of PDK4 on CREB. PDK4 overexpression increased both total Creb and p-Creb protein levels in NIH/3T3 cells (Fig. 4g). However, PDK4 did not affect the mRNA levels of Creb (Fig. 4h), suggesting that PDK4 regulates CREB post-transcriptionally. To test whether PDK4 stabilizes CREB, we established pdk4 knockdown NIH/3T3 cells (Fig. 4i, left panel). The synthesis rate of Creb protein, measured by inhibiting degradation with the proteasome inhibitor MG-132, was similar between shPDK4 NIH/3T3 cells and shScr control cells (Fig. 4i, right panel). To test whether Creb degradation was inhibited in pdk4-overexpressing cells (Fig. 4j, left panel), we treated cells with protein biosynthesis inhibitor cycloheximide (CHX). We found apparent suppression of Creb degradation in PDK4-overexpressing cells compared with control cells (Fig. 4j, right panel). In addition, endogenous PDK4 co-immunoprecipitated CREB (Fig. 4k), indicating that PDK4 physically interacts with CREB. Taken together, we speculated that PDK4 interacts with CREB and prevents CREB degradation. Therefore, PDK4 enhances the transactivation of CREB on RHEB.

**PDK4 Potentiates HIF-1α and Its Target Pyruvate Kinase Isozymes M2 and Promotes Aerobic Glycolysis**—Among the mTOR effectors, HIF-1α is one of the critical regulators in tumorigenesis. Elevated/attenuated HIF-1α and its downstream target pyruvate kinase isozymes M2 (PKM2) were observed in PDK4-overexpressing/knockdown cells (Fig. 5a). Increased glucose consumption and lactate production are manifestations of aerobic glycolysis in mTOR-hyperactivated cells (19). The potentiated Warburg effect was reversed by the mTOR inhibitor rapamycin in PDK4-overexpressing NIH/3T3 cells (Fig. 5b and c). Glucose consumption (Fig. 5e) and lactate production (Fig. 5f) were reduced by PDK4 depletion in three human cancer cell lines, PC3, A549, and MCF7 (Fig. 5d). To clarify PDK4-induced metabolic changes whether through mTORC1 or PDH, we knocked down PDH in shPDK4 and control cells. Glucose consumption and lactate production showed a slight
increase in both cells (Fig. 5g). On the contrary, after knocking down PDK4, the inhibition of the Warburg effect by mTOR inhibitor rapamycin was significantly compromised (Fig. 5g). Thus mTOR indeed plays an important role in PDK4-related metabolic changes. These data also add additional lines of evidence to the PDK4 activation of mTORC1.

Inhibition of PDK4 Suppresses Cell Proliferation and Tumorigenesis—To investigate the role of PDK4 in tumor development of mTOR-activated cancer cells, we injected PTEN-mutated PC3 cells expressing control shRNA (shScr) or shPDK4 subcutaneously into nude mice. Reduction of PDK4 in PC3 cells dramatically retarded tumor initiation and development and extended the survival of tumor-bearing mice (Fig. 6, a and b). Additionally, the attenuated PDK4 expression with the marked decrease of RHEB expression and lower mTOR activity were detected in tumor tissues dissected from nude mice (Fig. 6c). Reduced Ki67 staining and microvessel density (CD31 staining) illustrated suppressed proliferation and angiogenesis, manifestations of blunted mTOR activity, in PC3 shPDK4 tumors (Fig. 6d). In addition, PDK4 highly expressing cells displayed greater sensitivity to rapamycin treatment (Fig. 7a). Consistently, knockdown of PDK4 rendered NIH/3T3 cells less sensitive to rapamycin (Fig. 7b) Furthermore, enhanced inhibition of proliferation was observed in PC3 cells treated with DCA and rapamycin (Fig. 7c). Consistently, the catalytic mTOR inhibitor Torin-1 also enhanced the inhibition of PC3 cell proliferation by DCA (Fig. 7d). DCA induced cleavage of caspase3 (Fig. 7e), a manifestation of cellular apoptosis. The MTT readout correlated with caspase activation, indicating a connection between MTT and viability.

DISCUSSION

mTOR is a central controller of multiple cellular processes. Here, we have identified PDK4 as a positive regulator of mTORC1 and demonstrated that PDK4 activates mTORC1 through CREB-mediated transcriptional regulation of RHEB (Fig. 7f). PDK4-CREB-RHEB-mTOR signaling cascade is critical for tumorigenesis.

mTOR integrates intracellular and extracellular signals and modulates cell growth and proliferation. Environmental cues that affect mTOR include growth factors, stress, energy status, and amino acids (14). By coordinating these upstream inputs, mTORC1 controls a variety of cellular processes. Gain of function mutations of proto-oncogenes or loss of function mutations of tumor suppressors can augment mTORC1 signaling and result in tumorigenesis. Therefore, the regulation on mTOR is of considerable importance both in physiological and pathological states. Guo et al. (34) observed increased PDK4 and reduced mTOR signaling in H2O2-induced senescent cells in comparison with control cells. The authors proposed that PDK4 negatively regulated mTOR. In contrast, our findings from MEFs, human cancer cells, and a xenograft tumor model demonstrated that PDK4 is an activator of mTORC1 in various tissues (Figs. 1 and 6).

Both AMPK and TSC2 are negative regulators of mTOR (13, 35–37). Our data suggest that PDK4 regulation of mTOR is independent of AMPK or TSC2 (Fig. 2). We identified RHEB, an immediate activator of mTOR, as the connector between PDK4 and mTOR. TSC1/2, Bnip3, or p38-PRAK cascade inactivates mTORC1 through suppression of RHEB (27, 29, 38, 39). The transcriptional regulation of Rheb was discovered in ATF6-Rheb-mTOR signaling (31). We found that the tran-
scriptional activity of RHEB is significantly higher in PDK4-overexpressing cells than in control cells, demonstrating that PDK4 activation of mTORC1 occurs through up-regulation of RHEB transcription. We then demonstrated that CREB serves as the transcriptional activator of RHEB. Furthermore, PDK4 post-transcriptionally regulates CREB by enhancing CREB accumulation (Fig. 4). Previous studies have shown that CREB is degraded by phosphatases or the ubiquitin-proteasome pathway (40, 41). Because PDK4 physically interacts with CREB, PDK4 may bind to CREB and prevent its degradation. Taken together, we speculate that PDK4 stabilizes CREB and potentiates CREB activation of RHEB transcription.

Hyperactive mTOR promotes angiogenesis, potentiates aerobic glycolysis, and accelerates tumor growth (19, 20). In this study, we identified PDK4-CREB-RHEB as the activating axis upstream of mTOR. Furthermore, PDK4 post-transcriptionally regulates CREB by enhancing CREB accumulation (Fig. 4). Previous studies have shown that CREB is degraded by phosphatases or the ubiquitin-proteasome pathway (40, 41). Because PDK4 physically interacts with CREB, PDK4 may bind to CREB and prevent its degradation. Taken together, we speculate that PDK4 stabilizes CREB and potentiates CREB activation of RHEB transcription.

As PDK4-CREB-RHEB signaling cascade activates mTORC1 and promotes tumorigenesis, components in this cascade may be potential targets for cancer therapy. Because PDK4-overexpressed cells displayed higher sensitivity to rapamycin, the level of PDK4 may be a sensitivity biomarker of cancer cells to mTOR inhibitors. In addition, combination of mTOR inhibitors and the PDK4 inhibitor exerted synergistic suppression on the proliferation of cancer cells with activated mTOR signaling (Fig. 7). This result is a proof of principle that combination therapy may be a superior approach for the management of cancers caused by the hyperactivated mTOR signaling cascade with fewer side effects. As the pan-inhibitor of PDKs, DCA has antiproliferative and pro-apoptotic effects on cancer cells, possibly through depolarization of mitochondria and induction of apoptosis of cancer cells (24). However, its potential to be translated into clinical treatment is debatable because of its broad
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side effects (42, 43). Our data indicate that DCA inhibits mTOR activity, and this effect is specifically through PDK4, independent of PDH and the other three PDK isoforms (Fig. 1). Our study reveals an additional mechanism of DCA inhibition of cancer. To avoid the toxicity of DCA, it will be worthwhile to screen specific inhibitors of PDK4.

In summary, we have identified PDK4 regulation of mTOR through a newly identified PDK4-CREB-RHEB-mTOR signaling cascade. Activated PDK4 may potentiate mTOR and promote cell growth and tumorigenesis. Our study uncovers a novel therapeutic target that controls mTOR signaling, and the development of PDK4 inhibitors is thus warranted. In addition, the interplay between PDK4 and mTOR may display wider significance in physiological and pathological states, such as metabolic disorders, beyond the potential connection in cancers.

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