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PERSPECTIVE

Striking the balance between PTEN and PDK1: it all depends on the cell context

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The phosphatidylinositol-3 kinase (PI3K) signaling pathway is critical for normal brain development and function and is commonly hyperactivated in brain cancer. The PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor protein and phosphate-dependent kinase 1 (PDK-1) are critical regulators of this pathway. In the July 15, 2009, issue of *Genes & Development*, Chalhoub and colleagues (pp. 1619–1624) demonstrate PDK1-dependent and PDK1-independent effects of conditional PTEN deletion in the brain, and they identify cell type-specific differences in feedback regulation of the PI3K pathway. These studies provide important insights as to how neurons and glia may differentially regulate PI3K signaling, yielding intriguing clues about targeting PTEN-deficient brain cancers.

Phosphatidylinositol-3 kinases (PI3Ks) are a family of highly conserved intracellular lipid kinases that regulate cellular proliferation, differentiation, metabolism, and survival (Engelman et al. 2006). Akt/PKB (henceforth referred to as Akt) and mammalian target of rapamycin (mTOR) are enzymatic effectors of PI3K signaling (Engelman et al. 2006). PI3K signaling is critical for normal development and function, and defects in its regulation result in a variety of neurological and developmental diseases and cancer (Vivanco and Sawyers 2002; Engelman et al. 2006; Endersby and Baker 2008).

Class IA PI3Ks are activated by growth factor receptor tyrosine kinases (RTKs) either directly or through interaction with the insulin receptor substrate family of adaptor molecules (Engelman et al. 2006). The activity of PI3K results in the production of phosphatidylinositol-3,4,5-trisphosphate (PIP₃), a critical regulator of the serine/threonine kinase Akt to link growth factor signaling with cellular growth, proliferation, metabolism, and survival (Engelman et al. 2006; Chalhoub and Baker 2009). Akt is recruited to the membrane through interaction of its pleckstrin homol-

ogy (PH) domain with PIP₃, thus exposing its activation loop and enabling phosphorylation at Thr308 by the constitutively active phosphate-dependent kinase (PDK1) (Alessi et al. 1997; Calleja et al. 2007, 2009). For maximal activation, Akt is also phosphorylated on Ser473 of its C-terminal hydrophobic motif by mTOR, as described below.

mTOR is both an effector and an activator of Akt signaling, thus linking growth factor signaling through PI3K with protein translation, cell growth, proliferation, metabolism, and survival. mTOR exists in two multiprotein complexes, each characterized by different binding partners with distinct activities. mTOR resides in a complex with PRAS40, raptor, and mLST8/GβL as part of mTORC1 complex 1 (mTORC1). mTORC2 is composed of mTOR in complex with rictor, mSIN1, proTOR, and mLST8 (Guertin and Sabatini 2009). The recently identified mTOR inhibitor DEPTOR associates with both complexes, as will be described below (Peterson et al. 2009). Akt activates mTORC1 by inhibitory phosphorylation of TSC2, which, along with TSC1, negatively regulates mTORC1 by inhibiting the Rheb GTPase, a positive regulator of mTORC1. Rather surprisingly, a PKC α -dependent, Akt-independent route linking PI3K with mTORC1 has been described recently, suggesting that there may be multiple routes between PI3K and mTORC1 (Fan et al. 2009). mTORC1 has two well-defined substrates—p70S6K (referred to hereafter as S6K1) and 4E-BP1—both of which critically regulate protein synthesis (Guertin and Sabatini 2009). Unlike mTORC1, which is regulated by Akt, mTORC2 is an Akt activator. PI3K signaling activates mTORC2, which in turn activates a number of kinases in addition to Akt, including serum glucocorticoid-induced protein kinase (SGK1) and PKC α , all of which may also play important roles in regulating cellular proliferation and growth.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is the critical negative regulator of PI3K signaling: role in neurological disease and cancer

Unrestrained signaling through the PI3K pathway has disastrous consequences for the cell and the organism,

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including the development of cancer. The PTEN tumor suppressor protein is the major negative regulator of the PI3K pathway. PTEN antagonizes PI3K signaling by dephosphorylating PIP3. Unlike other pathway components, there are no other PTEN-like proteins that can compensate for its loss of function, so PTEN loss of function has a substantial impact in cell development and tumorigenesis (Furnari et al. 2007; Endersby and Baker 2008).

Germline mutations in *PTEN* are associated with a number of developmental, neurological, and neoplastic diseases, including Cowden's syndrome (85%), Bannayan-Riley-Ruvalcaba syndrome (65%), and *Proteus* syndrome (20%) (for review, see Orloff and Eng 2008). These diseases are characterized by hamartomatous growths, abnormal brain development including cerebellar hamartomas, Lhermitte-Duclos syndrome (LHD), and cancer predisposition (Chalhoub and Baker 2009). Homozygous *PTEN* deletion is embryonic lethal. Therefore, mouse models to assess the biology of PTEN have benefitted greatly from *Cre-loxP*-mediated conditional deletion approaches. Due to the preponderance of brain-associated phenotypes in patients with *PTEN* germline mutations, significant attention has been given to the development of models in which *PTEN* is conditionally deleted in the brain. Backman et al. (2001) and Kwon et al. (2001) crossed mice in which PTEN was floxed with transgenic mice expressing Cre-recombinase under a *GFAP* promoter. This resulted in *PTEN* deletion primarily in cerebellar granule cells and granule cells of the dentate gyrus of the hippocampus. Both models demonstrated markedly enlarged brains with nearly a doubling in neuronal cell size and abnormal neuronal organization—phenotypes similar to Lhermitte-Duclos disease (Backman et al. 2001; Kwon et al. 2001). Subsequent work from the Baker group (Kwon et al. 2003; Chalhoub et al. 2006) demonstrated that the neuronal hypertrophy was dependent on mTOR, but not S6K1.

Taking a different approach, Groszer et al. (2001) crossed *PTEN*-floxed mice with transgenic mice expressing Cre-recombinase under the control of a *nestin* promoter (a gene whose expression is enriched in neural precursor cells). These mice similarly showed massive brain enlargement with increased neuronal cell size and abnormal neuronal organization (Groszer et al. 2001). This group subsequently demonstrated that *PTEN* deletion in neural stem/progenitor cells enhances constitutive neurogenesis (Gregorian et al. 2009). Taken together, these models demonstrate representative features of PTEN-associated human disease, and demonstrate a critical role for PTEN in regulating neuronal size, proliferation, and organization. Additional mouse models have demonstrated a role for PTEN in regulating neural organization, migration, neuronal and glial differentiation, synaptogenesis, and axonal regeneration (Jaworski et al. 2005; Kwon et al. 2006; Otaegi et al. 2006; Park et al. 2008; for review, see Endersby and Baker 2008). In addition, effects of *PTEN* deletion have been observed on learning and social behavior (Kwon et al. 2006). Thus, the effects of *PTEN* deletion on brain architecture and

function appear to be highly dependent on the cell type and stage at which *PTEN* deletion occurs. It is interesting to note that none of these models developed gliomas. Thus, *PTEN* loss alone is not sufficient for glioma development, a finding consistent with the requirement for multiple pathway coactivations in mouse models of glioblastomagenesis (Endersby and Baker 2008), as well as the evidence for *PTEN* alterations in association with other mutations in human glioblastoma (The Cancer Genome Atlas Research Network 2008).

PDK1 in balance with PTEN?

If PTEN can be considered to be the “brakes” on the PI3K pathway, PDK1 potentially could be considered the “gas pedal.” PDK1 was first purified from tissue extracts as an enzyme that phosphorylates the T-loop of Akt (Thr308) in the presence of PIP3 (Alessi et al. 1997; Stokoe et al. 1997). PDK1 is known as a master kinase, because in addition to Akt, it also phosphorylates the conserved activation loop residue of many members of other protein kinase families (equivalent to Thr308 of Akt) in different cell types, including S6K, SGK, and PKC kinases (Mora et al. 2004; Bayascas 2008). Similar to *PTEN* deletion, homozygous deletion of *PDK1* is also embryonic lethal, requiring the development of mouse models in which *PDK1* is deleted conditionally or in which a hypomorphic allele is expressed. *PDK1* hypomorphic mutant mice are only half the size of their wild-type littermates, with markedly diminished somas of many cell types (Lawlor et al. 2002). PDK1 has been postulated recently to play a role in brain development, as it has been shown to be indispensable for neuronal survival during development (Kharebava et al. 2008) and to selectively induce the differentiation of GABAergic neurons through activation of Mash1 (Oishi et al. 2009). Because of its role in promoting PI3K signaling as well as regulating a series of signal transducers involved in promoting cancer growth, proliferation, and survival (Akt, S6K, SGK1, RAK, and PKC), PDK1 has been postulated to be a promising anti-cancer drug target (Pullen et al. 1998; Peifer and Alessi 2008). In particular, PDK1 may have heightened importance in PTEN-null cancers. An epistatic relationship between *Pten* and PDK1 has been reported in *Drosophila*, in which *PDK1* deficiency rescued the lethality of dPten-deficient flies (Rintelen et al. 2001). Reducing PDK1 expression in cancer-prone heterozygous *PTEN*^{+/-} mice has been shown to greatly reduce the development of a wide range of tumors (Bayascas et al. 2005). However, the contribution of PDK1 in balancing PTEN signaling, particularly in the nervous system, has, up until now, not been elucidated.

In the July 15, 2009, issue of *Genes & Development*, Chalhoub et al. (2009) use an elegant mouse genetic approach to determine whether conditional deletion of *PDK1* can rescue the effects of *PTEN* deletion in the brain. They generate strategic conditional knockout models using the *Cre-loxP* system. They cross a *GFAP-cre* transgenic line directing Cre-mediated activity throughout the brain in both neurons and glia with nearly complete

activity in the hippocampus ("*hp-cre*") (Fraser et al. 2004) with *Pten*; *Pdk1*-floxed mice to generate four genotypes used in the study: (1) *Pten*^{loxP/loxP}; *Pdk1*^{loxP/+}*cre* (*Pten* cKO); (2) *Pten*^{loxP/+}; *Pdk1*^{loxP/loxP}; *cre* (*Pdk1* cKO); (3) *Pten*^{loxP/loxP}; *Pdk1*^{loxP/loxP}; *cre* (*Pten*; *Pdk1* dKO); and (4) *No-cre* mice (controls). As expected, conditional *Pten* deletion resulted in macrocephaly, increased neuronal cell size, and abnormal cerebellar neuron migration and layering (Fig. 1A,B). In contrast, *Pdk1* cKO mice showed microcephaly (Fig. 1C). Combined *Pten*; *Pdk1* dKO mice showed normal brain size and normal neuronal cell size; however, the migration defect remained (Fig. 1D). Taken together, these results demonstrate PDK1-dependent effects of PTEN loss on brain size and neuronal cell size. They also identify PDK1-independent effects on neuronal migration that were most prominently identified in the cerebellum. A significant reduction of neurons was detected in the cerebella of *Pten*; *Pdk1* dKO mice, consistent with the requirement for PI3K signaling in the granule cell progenitor and survival in the cerebellum (Dudek et al. 1997). Therefore, Chalhoub et al. (2009) used a second *GFAP-cre* transgenic line with later-onset Cre activity in postnatal cerebellar granule cells ("*cb-cre*") (Kwon et al. 2001). This strategy allowed them to bypass the early cerebellar requirement for PDK1 for survival and allowed a controlled comparison of cerebella. In this model, *Pdk1* deletion rescued the *Pten*-mediated effects on cerebellar size and neuronal cell size, but it did not rescue the migration defect. This elegant use of models identified both PDK1-dependent and PDK1-independent effects of PTEN loss in the brain.

In addition to transducing PI3K–Akt-mediated signals toward protein synthesis, mTORC1 also acts as a negative regulator of PI3K pathway activation (Sabatini 2006). Thus, deletion of *PDK1* could potentially derepress this negative regulator to activate PI3K signaling through Akt, as can be determined by biochemical analysis of Akt Ser473. Unexpectedly, Western blotting analyses of cerebella and hippocampi in the various models revealed up-regulation of Akt (Ser473) phosphorylation in *Pdk1*-deleted glia, but not in *Pdk1*-deleted neurons, which was confirmed by immunohistochemical analyses of phospho-Akt Ser473 (Fig. 1C). This was shown to be mediated through mTOR/PI3K, as the dual PI3K/mTOR inhibitor BEZ235 abrogated this response. Taken together, these results demonstrate that deletion of *Pdk1* differentially activates PI3K signaling in glia relative to neurons, raising the concept that the signaling feedback pathways are wired differently in different CNS cells types.

Implications of these findings

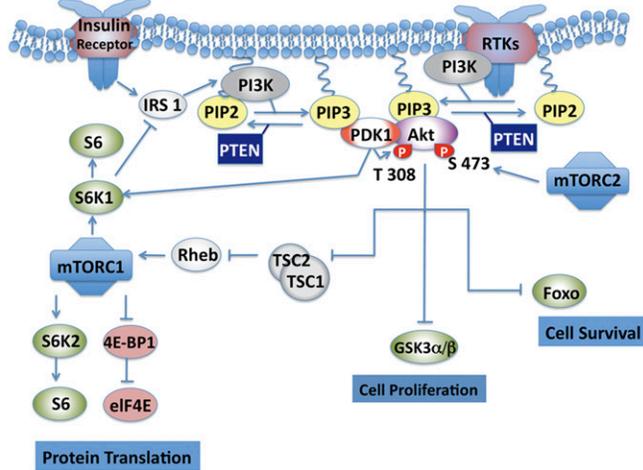
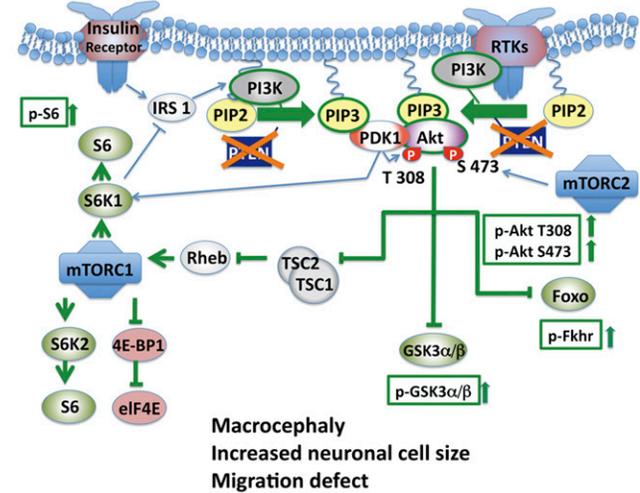
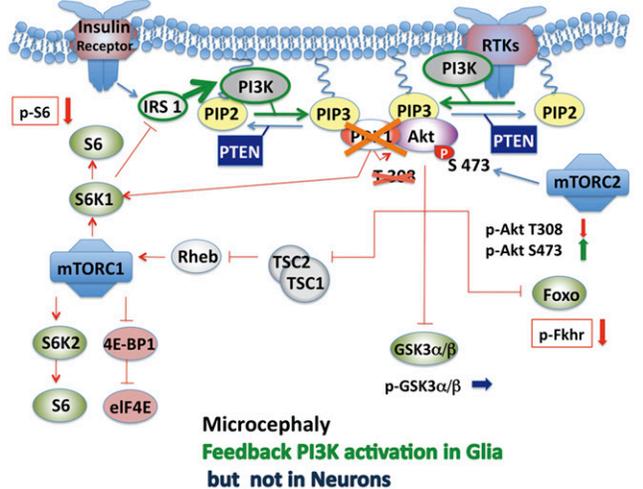
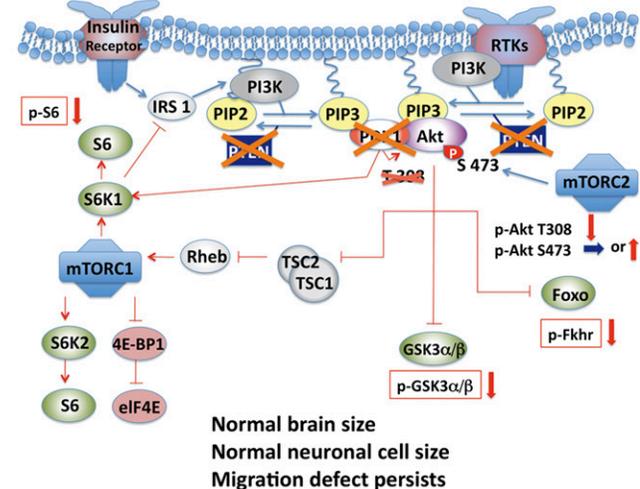
These results have important implications for cancer therapy. PTEN loss is common in many cancers, including glioblastoma. PTEN-null tumors are proving to be particularly difficult to treat, and PTEN loss renders EGFR-activated glioblastomas resistant to EGFR kinase inhibitors (Mellinghoff et al. 2005). Is PDK1 a good target in PTEN-deficient cancer, either alone or in combination with other targeted agents? The results from the study by

Chalhoub et al. (2009) suggest that targeting PDK1 may not fully inhibit the spectrum of pathology induced by PTEN loss, as PDK1 did not prevent abnormal cellular migration induced by PTEN deficiency. It is not clear whether these PTEN-mediated effects on migration are PI3K-dependent or PDK1-independent, or whether they are mediated by PI3K-independent signaling. PTEN contains both a lipid phosphatase that is required for antagonizing PI3K signaling and a protein phosphatase domain that has been implicated in regulating other cancer-promoting signaling pathways, such as Src family kinases (Dey et al. 2008). Future studies will be needed to determine whether these effects are mediated through, or are independent of, PI3K signaling, and to assess their downstream effectors. However, before PDK1 inhibitors are discounted in PTEN-null cancers, it needs to be recognized that in addition to Akt signaling, PDK1 also regulates a number of other enzymes such as SGK1, PKC α , and S6K, all of which are up-regulated in many cancers. Thus, PDK1 inhibitors could have significant anti-growth effects on tumors independent of their effect on PI3K signaling.

The most intriguing and unexpected finding in the study by Chalhoub et al. (2009) is the demonstration that feedback signaling to PI3K occurs in glia but not neurons. mTORC1 links growth factor signals with cellular proliferation and protein translation, but it also can serve as a negative regulator of PI3K pathway activation. mTORC1 impairs PI3K activation in growth factor-stimulated cells by down-regulating IRS1, IRS2, and PDGFR (Sarbasov et al. 2005; Sabatini 2006; Zhang et al. 2007), presumably to keep PI3K pathway signaling in check. In fact, derepression of mTORC1-mediated feedback by treatment with rapamycin has been shown to be associated with more rapid clinical progression in glioblastoma patients (Cloughesy et al. 2008). Thus, understanding how feedback is regulated is critical for cancer therapy. These studies suggest that feedback activation is cell context-dependent, and demonstrate that feedback signaling cannot be studied adequately outside relevant cell contexts.

These studies also raise the need to identify interacting proteins that differentially regulate feedback activation of PI3K signaling in glia versus neurons. Chalhoub et al. (2009) point out that post-mitotic neurons do not require the exquisite level of pathway regulation required by astrocytes and oligodendrocytes, which maintain the ability to proliferate. What are the molecular mechanisms underlying this phenomenon? The fact that the dual PI3K/mTOR inhibitor BEZ235 inhibits feedback activation in *Pdk1*-deleted glia (Supplemental Fig. S6B of Chalhoub et al. 2009), indicates that the feedback loop is mTOR-mediated and/or PI3K-mediated. However, is the feedback activation mTORC2-dependent, as suggested by Akt473 phosphorylation? If so, is it amenable to inhibition by mTOR kinase inhibitors? The mTOR-interacting protein DEPTOR has been identified recently as a key determinant of mTORC1-mediated inhibition of PI3K signaling. DEPTOR is both a negative regulator of and negatively regulated by mTORC1 and mTORC2, and

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A. Normal PI3K/Akt/mTOR pathway**B. *Pten* conditional KO mice****C. *Pdk1* conditional KO mice****D. *Pten/Pdk1* double KO mice**

— Normal
 — Upregulation
 — Downregulation

Figure 1. Summary of consequences on signal transduction and brain architecture of conditional *Pten* or *Pdk1* deletion, alone or in combination. (A) Under normal levels of PI3K/Akt/mTOR pathway activation, PTEN and PDK1 activities are in balance. (B) Conditional deletion of *Pten* with nearly complete activity in the hippocampus “*hp-cre*” results in hyperactivation of Akt/mTOR signaling with macrocephaly, increased neuronal cell size, and cerebellar neuronal migration defect. (C) Conditional deletion of PDK1 suppresses Akt/mTOR signaling and causes microcephaly. Up-regulation of PI3K via derepression of mTORC1 is detected in glia but not in neurons. (D) *Pdk1* deletion rescues *Pten* deletion-mediated macrocephaly and prevents *Pten* deletion-mediated increase in neuronal cell size. However, *Pdk1* deletion cannot rescue the effect on abnormal neuronal migration.

its overexpression can activate PI3K by relieving mTORC1-mediated inhibitory feedback (Peterson et al. 2009). It remains to be determined whether DEPTOR plays a role in the differential PI3K pathway activation observed upon PDK1 loss in neurons versus glia. Furthermore, DEPTOR has also been shown to be down-regulated in many cancers, including malignant gliomas in which its expression is greatly reduced relative to normal brains (Peterson et al. 2009). Does DEPTOR loss in these cancers

imply a loss of control of the mTOR regulation above and beyond that promoted by unrestrained PI3K activation and, if so, what are the cellular consequences of unrestrained mTORC2 signaling?

Lastly, the study by Chalhoub et al. (2009) suggests that biologically important signaling systems are not simple linear pathways, but rather complex dynamic networks in which feedback and cross-talk are critical for determining cellular outcome. These studies raise the question

of whether magnitude of flux through the PI3k/Akt/mTOR signaling pathway is important for determining its wiring. In an elegant study published in the March 15, 2007, issue of *Genes & Development*, Hietakangas and Cohen (2007) demonstrated through genetic disruption of the TORC2 complex proteins rictor and sin1 and/or genetic disruption of the Akt hydrophobic motif containing Ser473 in *Drosophila* that failure of TORC2-mediated phosphorylation of Akt results in only minor growth impairment at normal levels of PI3K pathway activation. However, in PTEN-deficient flies, loss of TORC2 activity significantly inhibited hyperplasia (Hietakangas and Cohen 2007). Does mTORC2 signaling have enhanced importance in the context of increased signal flux through the PI3K pathway? What about in the context of PI3K pathway hyperactivation, as occurs in many cancers, including malignant gliomas (The Cancer Genome Atlas Research Network 2008)? *EGFR* amplification in combination with *PTEN* loss is extremely common in glioblastomas (The Cancer Genome Atlas Research Network 2008), as are other PI3K coactivating alterations (Stommel et al. 2007). Constitutive coactivation of *EGFR*-*Ras*-*Akt* pathways in *Drosophila* glia and glial precursors gave rise to invasive gliomas in which TORC2 complex members (*Sin1* and *rictor*) were required for glial proliferation, but not for normal development (Read et al. 2009). These studies suggested that in the context of PI3K pathway hyperactivation, mTORC2 signaling may take on heightened importance. In summary, the work of Chalhoub et al. (2009) has provided a novel and important demonstration of cell context-specific differences in feedback regulation that hint at crucial differences in the wiring of the PI3K/Akt/mTOR signaling pathway between neurons and glia and suggest even further alterations in glial tumors. Future work, particularly studies that tease out this circuitry in well-defined cell contexts, is needed.

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