gions. Surface wave microseisms from the deep ocean are generally not observed on land (7). However, microseismic body waves penetrate the deep Earth structure and can be observed at distant land seismic stations, with compressional P waves predominant (8). The recent observation of microseismic S waves by Nishida and Takagi and by Liu et al. (9) gives seismologists a new tool with which to study Earth's deeper structure.

Seismic arrays provide an opportunity to study microseism source locations and propagation characteristics. For surface waves, only the back-azimuth to their generation region can be determined. However, for seismic body waves, both back azimuth and range to the generation region can be obtained, giving additional information on propagation paths and crustal structure between the source and the receivers. These attributes of P wave microseisms allowed estimates of cyclone locations by using the Large Aperture Seismic Array in Montana installed in the 1960s for nuclear monitoring (8). In the 2000s, the transformative USArray has enabled more observations of microseism P wave body waves (10). The observed P waves can be linked to specific storms, such as waves from Hurricane Katrina hitting the coast near New Orleans in 2005 (11), and allowed the tracking of Super Typhoon Ioke across the Pacific (12) (see the figure) and Hurricane Sandy along the New England coast in 2012 (13).

By cross-correlating microseismic P waves, it is possible to determine the fine layer structure of the mantle-layer transition zone (14). Because S waves have shorter wavelengths than those of P waves and are more sensitive to state changes related to temperature and Earth composition, array processing of microseismic S waves (2, 9) has the potential to add to our understanding of the deeper crust and upper mantle structure. ∎

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Proline hydroxylation linked to Akt activation

Oxygen-sensing enzymes regulate the Akt kinase

By Michael Voulgarelis and Philip N. Tsichlis

Prolyl hydroxylases are oxygen-sensing enzymes that regulate the abundance of hypoxia-inducible factors (HIF)1α and HIF2α, which control the cellular response to oxygen deprivation (hypoxia). Three prolyl hydroxylases target the HIFα molecules, EglN1 (PHD2), EglN2 (PHD1), and EglN3 (PHD3) (1). All three are widely expressed iron (Fe^{++}) and α-ketoglutarate–dependent dioxygenases. When the oxygen tension is normal, they hydroxylate HIF1α and HIF2α. Hydroxylated HIFα molecules are recognized and ubiquitinated by an E3 ubiquitin ligase complex containing the von Hippel-Lindau tumor–suppressor protein pVHL (1), leading to their proteasomal degradation. Inactivation of prolyl hydroxylases during hypoxia results in the stabilization of HIFα molecules. On page 929 of this issue, Guo et al. present evidence that a prolyl hydroxylation–dependent pathway also plays an important role in the regula-

> "...inhibition of Akt may be therapeutically beneficial in pVHL-null and other tumors with mutations interfering with Akt hydroxylation."

tion of the Akt kinase, which in turn is a key player in oncogenesis (2).

Akt, also known as protein kinase B (PKB), is a member of the AGC family of serine-threonine protein kinases (3). There are three closely related Akt isoforms (Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ). Activation of Akt, which in the absence of activation signals is catalytically-inactive, is phosphoinositide 3-kinase (PI3K)–dependent (4). PI3K generates D3-phosphorylated phosphoinositides at the cell membrane. Binding to these phosphoinositides results in the recruitment

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of Akt to the cell membrane, where it is phosphorylated at multiple sites by other membrane-associated kinases (4, 5). Phosphorylation plays a critical role in Akt activation. One of the two main sites of Akt phosphorylation, Thr^{308}, is dephosphorylated by protein phosphatase 2A (PP2A) (6). PP2A is a multimeric phosphatase composed of a scaffold A subunit, a catalytic C subunit, and one of 26 regulatory/targeting B subunits. Access of PP2A to the phosphorylated Thr^{308} site is modulated by intramolecular interactions in the Akt catalytic cleft and by phosphorylation of the B55 subunit (7). It can also be modulated by Akt-interacting proteins. It is not known whether these interacting proteins simply join phosphorylated Akt with the phosphatase, or also modulate the intramolecular interactions that control accessibility of the phosphatase to phosphorylated Thr^{308}. Other modifications contributing to the activation of Akt include acetylation, glycosylation, oxidation, ubiquitination, and SUMOylation (5). Guo et al. now add proline hydroxylation to this list.

There are two types of mutations in the tumor-suppressor gene pVHL that are associated with cancer (8). Type 1 mutations result in deletion or truncation, and when truncated, the pVHL protein becomes misfolded and inactive. The loss of pVHL function results in the up-regulation of HIFα, high risk of renal cell carcinoma, and low risk of pheochromocytoma. Earlier studies had shown that, in addition to upregulating HIFα, type 1 mutations also activate Akt (9). Guo et al. confirmed this finding and also showed that HIFα induction is not required for the activation of Akt in pVHL-null tumors. Because pVHL is recruited to its targets by proline hydroxylation, the authors proceeded to show that EglN1, one of the three prolyl hydroxylases that target HIFα molecules, also binds and hydroxylates phosphorylated Akt (Akt1 and Akt2, but not Akt3) at four proline sites. Hydroxylation of two of these sites (Pro^{125} and Pro^{313}) promotes Akt binding to pVHL. The latter molecule also interacts with PP2A, which dephosphorylates the Thr^{308} site, resulting in Akt inactivation. Consistent with this, genetic or pharmacological inhibition of EglN1, and type 1 pVHL mutations, re-

Shutting down Akt

Upon growth factor stimulation, Akt is recruited to the cell membrane by the actions of PI3K, where it is activated by phosphorylation (P) by other membrane-associated kinases such as 3-phosphoinositide–dependent protein kinase-1 (PDK1). Phosphorylated Akt1 and Akt2, but not Akt3, undergo proline hydroxylation (OH) by the prolyl hydroxylase EglN1 under conditions of normal oxygen tension. Hydroxylated Akt interacts with, and is dephosphorylated by, pVHL-associated PP2A, leading to Akt inactivation. Ptdins(4,5), phosphatidylinositol 4,5-bisphosphate; Ptdins(3,4,5), phosphatidylinositol 3,4,5-triphosphate; mTORC2, mTOR complex 2.
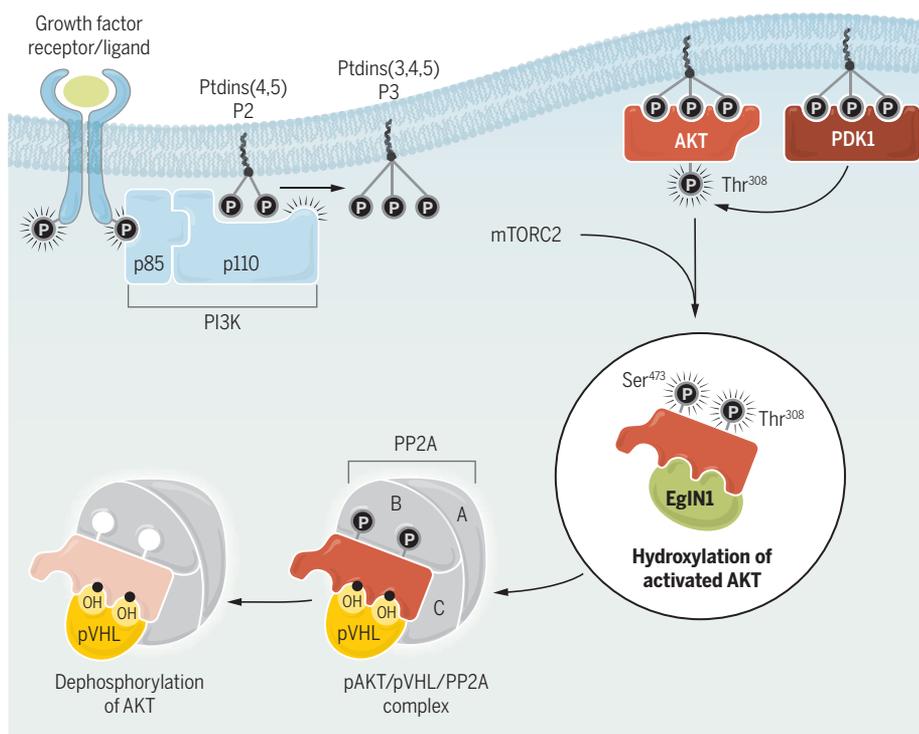
sult in Akt activation by interfering with Akt dephosphorylation (see the figure).

The pathway of Akt deregulation described above may be activated in human cancer by several mechanisms. Although rare, mutations that alter the Akt hydroxylation sites (G311D in Akt1 and P127N in Akt2) do occur and are associated with Akt activation. It remains to be determined whether other Akt mutations also interfere with the hydroxylation and subsequent dephosphorylation of Akt. In addition, hypoxia, which is common in human cancer, results in the inactivation of EglN1, and this should interfere with proline hydroxylation and dephosphorylation of Akt. Mutations in pVHL, which are common in certain forms of human cancer, may also activate Akt by preventing its dephosphorylation. Other cancer-associated loss-of-function mutations targeting genes encoding enzymes involved in the Krebs cycle, such as succinate dehydrogenase and fumarate hydratase, result in the accumulation of succinate and fumarate, which inhibit the activity of EglN1 by competing with α-ketoglutarate (1). Also, regulation of Akt via proline hydroxylation may be under the control of additional signals that control the activity of EglN1 and the hydroxylation of Akt, or the expression of pVHL and its interaction with hydroxylated Akt.

EglN1 and other prolyl hydroxylases are activated by the metabolite D-2-hydroxyglutarate. An increase in D-2-hydroxyglutarate levels is caused by mutations in isocitrate dehydrogenase 1 (IDH1), which are commonly observed in diffuse gliomas and glioblastomas (10). According to the results of Guo et al., this would inhibit Akt by activating EglN1, which is consistent with clinical observations showing that patients with IDH1 mutations tend to harbor lower-grade tumors and have a better prognosis (11). If the better prognosis of these patients is due to the inactivation of Akt, one would expect that the beneficial effect of the mutation would be more pronounced in patients with Akt1 and Akt2 rather than Akt3-expressing tumors. The observations in glial tumors are in sharp contrast to observations in acute myelogenous leukemia (AML) with normal cytogenetics and mutations in IDH1/IDH2, which also produce D-2-hydroxyglutarate. Despite the fact that D-2-hydroxyglutarate should inhibit Akt, the IDH2 mutations in AML are associated with bad prognosis, and the inhibition of the mutated IDH2 is therapeutically beneficial (12). It is not known why these mutations exert opposite effects in glial tumors and AML. Perhaps the pathway of Akt regulation by hydroxylation is active in glial but not in hematopoietic cells. Alternatively, Akt may be activated in AML by other pathways that supersede its inhibition by D-2-hydroxyglutarate.

Although not the focus of Guo et al., hydroxylation of Akt should play an important role in the activation of Akt1 and Akt2 during hypoxia. Other mechanisms contributing to Akt activation in hypoxia include the down-regulation of phosphatase and tensin homolog (PTEN) by miR-21, which is induced by Akt2 and the inactivation of PTEN by hypoxia-induced reactive oxygen species (13, 14). In addition, Akt may be activated via a feedback loop that is induced by inactivation of mechanistic target of rapamycin (mTOR) via HIF-induced expression of regulated in development and DNA damage responses 1 (REDD1) (15). The relative contribution of these pathways to Akt activation during hypoxia in different cell types remains to be determined.

The data of Guo et al. suggest that inhibition of Akt may be therapeutically beneficial in pVHL-null and other tumors with mutations interfering with Akt hydroxylation. It will be interesting to determine how efficiently currently available inhibitors interfere with Akt activation via this pathway. ∎

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