goblet, or enteroendocrine. Without *Math1*, cells remain in the progenitor stem cell pool and can only become enterocytes.

To fully understand epithelial cell lineage determination in the gut, more questions need to be answered. For example, *Math1* is not expressed in the stomach or pancreas, locations where Hes1-deficient mice show considerable enteroendocrine cell loss (*7*). This implies that there might be other factors similar to Math1 at these sites. The hedgehog family of morphogens are candidate Math1-like factors. The hedgehog signaling pathway is critical in gastric (*8*), pancreatic (*9*), and intestinal (*10*) epithelial cell differentiation. Although interactions between the Notch and hedgehog pathways have been described in other systems (*11*, *12*), there is much to be learned about how these pathways interact in gut epithelium.

Yang *et al.*'s findings do not solve the puzzle of how epithelial cell differentiation is influenced by the cell's position along the crypt-to-villus axis. Cell fate decisions modulated by the crypt-to-villus axis are likely to involve morphogens expressed in the mesoderm and endoderm. A morphogen gradient of Bmp4, for example, may extrinsically regulate the outcome of Notch signaling in progenitor stem cells. Bmp4, a secreted morphogen expressed by the mesoderm (*13*), may be important in development of the intestinal epithelium (*14*) and is known to affect Math1-regulated cell fate decisions in other systems (*15*, *16*). Elucidating different molecular regulators of epithelial cell differentiation along the crypt-to-villus axis will be the focus of future work.

With the Yang *et al.* results, we can now begin to understand the signals needed for intestinal epithelial cell fate decisions. It is interesting that one molecule, Math1, provides the signal for cells to pick one of three fate choices, leaving the enterocyte as the default state. Whether a different signal would be required for gut epithelial precursor cells to become enterocytes in Math1-deficient animals has yet to be investigated. Doubtless, many interesting discoveries about intestinal cell fate decisions will be revealed in the near future.

References

1. Q. Yang, N. A. Bermingham, M. J. Finegold, H. Y. Zoghbi, *Science* **294**, 2155 (2001).
2. J. P. Clatworthy, V. Subramanian, *Mech. Dev.* **101**, 3 (2001).
3. M. L. Hermiston, R. P. Green, J. I. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8866 (1993).
4. J. Lewis, *Semin. Cell Dev. Biol.* **9**, 583 (1998).
5. R. Kageyama, T. Ohtsuka, K. Tomita, *Mol. Cells* **10**, 1 (2000).
6. M. Skipper, J. Lewis, *Nature Genet.* **24**, 3 (2000).
7. J. Jensen *et al.*, *Nature Genet.* **24**, 36 (2000).
8. G. R. van den Brink *et al.*, *Gastroenterology* **121**, 317 (2001).
9. J. Zhang, A. Rosenthal, F. J. de Sauvage, R. A. Shivdasani, *Dev. Biol.* **229**, 188 (2001).
10. M. Ramalho-Santos, D. A. Melton, A. P. McMahon, *Development* **127**, 2763 (2000).
11. N. E. Baker, S. Y. Yu, *Curr. Biol.* **7**, 122 (1997).
12. C. Alexandre, M. Lecourtois, J. Vincent, *Development* **126**, 5689 (1999).
13. M. J. Bitgood, A. P. McMahon, *Dev. Biol.* **172**, 126 (1995).
14. P. de Santa Barbara, G. R. van den Brink, D. J. Roberts, *Semin. Med. Genet.*, in press.
15. K. J. Lee, M. Mendelsohn, T. M. Jessell, *Genes Dev.* **12**, 3394 (1998).
16. N. A. Bermingham *et al.*, *Neuron* **30**, 411 (2001).

PERSPECTIVES: STEM CELLS

PTEN—Coupling Tumor Suppression to Stem Cells?

Josef M. Penninger and James Woodgett

In medieval times people dreamed of finding the Holy Grail, which promised them eternal youth and renewal of life. Stem cells have arguably achieved similar mythological status in our world with their potential to self-renew and to regenerate heart muscle, neurons, and blood cells. However, to realize the full potential of both embryonic and adult stem cells, we need to fully understand their biology. Taking a big step in this direction, Groszer and co-workers (*1*) report on page 2186 of this issue that a tumor suppressor protein called PTEN controls the proliferation and possibly also the self-renewal of neuronal stem cells.

PTEN (phosphatase and tensin homolog deleted from chromosome 10)—also known as MMAC1 (mutated in multiple advanced cancers) or TEP1 (TGF-regulated and epithelial cell–enriched phosphatase)—was identified as a candidate tumor suppressor on chromosome 10q22-24 (*2–4*). Loss of heterozygosity (inactivation of the second *PTEN* allele) at this locus is observed in several spontaneous human malignancies including glioblastomas, endometrial tumors, and breast as well as prostate cancers. Germ line PTEN mutations have been detected in more than 80% of patients with Bannayan-Zonana syndrome, Cowden disease, and Lhermitte-Duclos disease (*5*). Although these autosomal dominant disorders have distinctive phenotypic features (visible characteristics), they also share common clinical features such as benign growths and a high incidence of systemic malignancies. Identical PTEN mutations have been found in these three diseases, indicating that the same PTEN mutation induces distinct clinical manifestations depending on the type of tissue and the involvement of additional gene loci.

Mice carrying germ line mutations of the *PTEN* gene show that PTEN is indeed a critical tumor suppressor protein. Mouse embryos homozygous for the *PTEN*-inactivating mutation die between day 6.5 and 9.5 of development. They show abnormal patterning of ectodermal and mesodermal germ layers and overgrowth of the cephalic and caudal regions, probably due to enhanced proliferation of the neuroepithelium (*6*, *7*). Mice heterozygous for the *PTEN* mutation are viable but display hyperplastic-dysplastic changes in the prostate, skin, and colon and a high incidence of spontaneous tumors of various histological origins (*6*, *8*). In general, tumors from heterozygous *PTEN*-mutant mice display loss of heterozygosity at the second allele, demonstrating that loss of PTEN contributes to tumor formation (*9*).

The PTEN tumor suppressor is a lipid phosphatase that dephosphorylates the D3 position of phosphatidylinositol 3,4,5-trisphosphate (PIP_3), a product of phosphatidylinositol 3-kinase (PI3K) (*10*). Thus, PTEN lowers the amount of the PI3K product, PIP_3, within cells and antagonizes PI3K-mediated cellular signaling pathways (see the figure, top of the next page). There also may be additional PTEN substrates because PTEN can also dephosphorylate (remove phosphate groups from) both phosphotyrosine- and phosphoserine/threonine-containing substrates in vitro (*11*). However, negative regulation of PIP_3 is the critical determinant for control of tumor growth (*12*). PI3K and its product PIP_3 regulate many cellular processes including proliferation, transcriptional regulation, glucose metabolism, cell migration, and protein synthesis, and they also protect against apoptosis. Groszer *et al.* (*1*) now add another important cellular process to this list: the negative regulation of neural stem cell proliferation.

Given that inactivation of PTEN in all cells results in death of the embryo, Groszer and colleagues (*1*), in parallel

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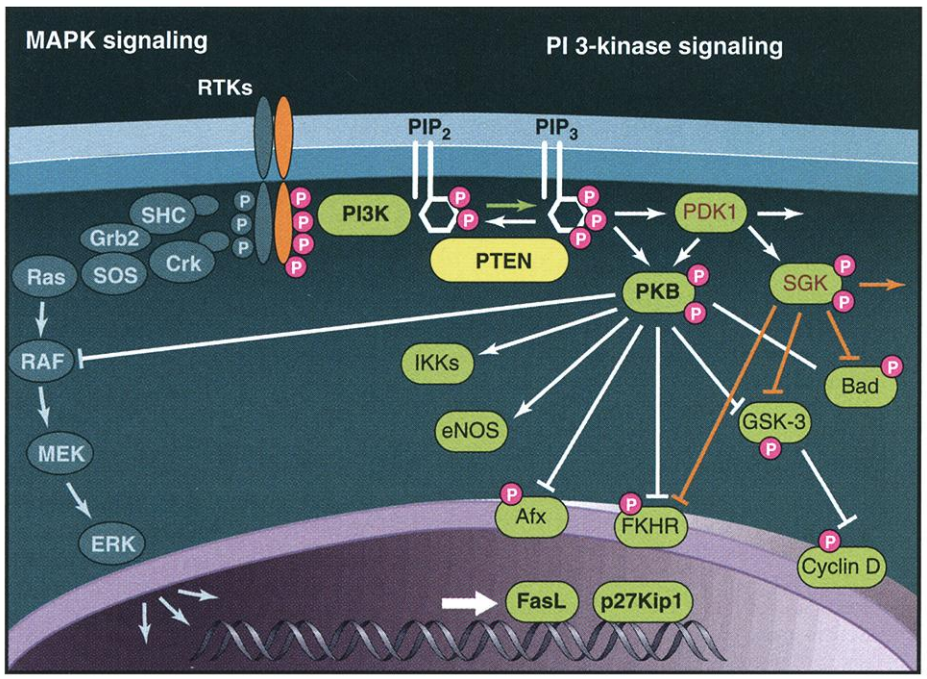


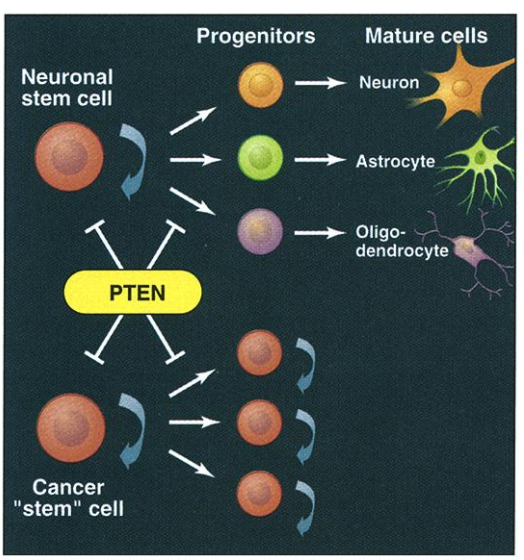
Out of control. Regulation of PI3K signaling by the tumor suppressor PTEN. Inactivation of PTEN increases the intracellular concentration of 3′-phosphorylated phosphatidylinositols. This mimics the effect of constitutive PI3K activation and induces various protein kinase cascades that impact other important signaling pathways and gene transcription. PIP_3 production is critical for the activation of PKB/Akt, PDK1, or SGK. Several substrates for PKB/AKT and SGK are shown, including the death-promoting molecule Bad and the Afx and FKHR transcription factors, which induce expression of genes such as FasL and p27Kip1. PKB/AKT suppresses these transcription factors by promoting their nuclear export. Affected proteins include regulators of apoptosis (Bad, Forkhead, GSK-3), the cell cycle (p27, cyclin D), and metabolism (GSK-3, eNOS).

with two other groups (*13*, *14*), mutated PTEN only in embryonic neuronal tissues using a conditional gene targeting approach. Depending on the mutant PTEN mouse lines, these conditional PTEN mutant mice either died within 12 hours after birth (*1*), or survived with seizures and ataxia of variable severity (*13*, *14*). In all three mutant mouse lines, the animals exhibited enlarged brains with severely disturbed patterning of brainstem nuclei, hippocampus, cortex, and cerebellum. This macrocephaly appeared to result from a combination of increased cell numbers, decreased cell death, and in particular enlarged neuronal cell size. Thus, PTEN controls neuronal cell size in vivo. The histoarchitecture of these brains resembles that of Lhermitte-Duclos disease (*15*). This intriguing finding confirms that Lhermitte-Duclos disease is a neurological manifestation of Cowden disease, and so the conditional PTEN-mutant mouse may serve as a model for this disorder.

To determine the pluripotential nature of neuronal stem cells in the PTEN-mutant mice, Groszer and co-workers isolated neurospheres (aggregates of neuronal progenitors) from the brains of these animals. They found that the neurospheres contained more cells per sphere, and that the individual cells were larger and displayed an increased number of cell divisions. Loss of PTEN in the brain altered the cell fate commitment of neuronal stem cells, which would normally differentiate into neurons, astrocytes, and oligodendrocytes (see the figure, bottom). When the neurospheres were propagated to test for self-renewal capacity, PTEN-null neural stem cells showed more self-renewal capacity than their normal counterparts. The authors conclude that PTEN deficiency results in increased proliferation and self-renewal of neural stem cells.

The brain-specific phenotype of the PTEN-mutant mice could also be explained by other factors (*1*, *13*, *14*). For example, because PTEN inhibits cell migration, integrin-mediated cell spreading, and formation of focal adhesions by focal adhesion kinase and c-Src in cultured cells (*16*), the observed defects in brain patterning could be due to alterations in cell adhesion and migration. The proliferative phenotype observed in the mutant mice could be a secondary effect caused by a block in neuronal stem cell differentiation or apoptosis. Genetic inactivation of a lipid phosphatase called SHIP1—which dephosphorylates the D5 position of PIP_3 and thus (in the same way as PTEN) negatively regulates PI3K signaling—results in enhanced proliferation and differentiation of hematopoietic stem cells in response to growth factors, and a reduction in apoptosis of myeloid blood cells (*17*, *18*). In contrast to PTEN, however, there is no evidence that inactivation of SHIP1 or other D5 lipid phosphatases causes malignancies. Rather, inactivation of SHIP1 results in progressive myeloid hyperplasia (*17*, *18*), and inactivation of its cousin, SHIP2, leads to early neonatal death due to increased insulin receptor signaling and hypoglycemia (*19*). Nonetheless, the Groszer *et al.* work raises intriguing possibilities for neuronal stem cell research and suggests how loss of PTEN in tumors might rewire differentiated cells so that they regain their pluripotentiality.

The discovery of neuronal stem cells in the embryo has opened new avenues for the repair and regeneration of neuronal tissues damaged through disease. Neuronal stem cells have been isolated from embryos, the adult brain, and even the skin of adult animals and humans (*20*, *21*). Multiple growth and differentiation factors have been identified for committed hematopoietic stem cells and are being used clinically to force the maturation of leukemic cells stuck at one stage of differentiation. This strategy to differentiate and treat tumor cells with growth factors may also work for



Not just a tumor suppressor. The PTEN tumor suppressor may affect the self-renewal and proliferation of neuronal stem cells. Mutant PTEN is involved in tumor proliferation and may enable tumor cells to regain pluripotentiality. Mutant PTEN has also been implicated in tumor invasion and metastasis and in the survival of metastatic tumor cells in the "wrong" environment.

nonhematopoietic tumors although we first need to identify the specific growth and differentiation factors for such tissues. Furthermore, we know very little about the signals that control self-renewal and proliferation of pluripotent stem cells (*22*, *23*). If, indeed, PTEN controls self-renewal and proliferation of neuronal stem cells, then this protein could be used to harvest increased numbers of these cells for research. The Groszer *et al.* findings also provide insight into how neuronal stem cells remain pluripotent. Perhaps stem cell pluripotentiality could be maintained by expressing activated PKB/AKT or other components of signaling pathways that are suppressed by PTEN. PTEN may also be important for maintaining the pluripotentiality of other types of stem cells.

One striking feature of stem cells is their ability to self-renew, a property that also defines cancer cells. Tumors often originate through the transformation of stem cells, and it has been postulated that stem cell transformation, self-renewal, and proliferation may be controlled by the same signaling pathways (*22*). The notion that the tumor suppressor PTEN, which is mutated in many different human tumors, may regulate neuronal stem cell renewal and proliferation is very exciting. One could speculate that loss of PTEN in tumors would help them to become pluripotent, although PTEN loss is often a late event in tumor formation (*21*). The conditional PTEN-mutant mice develop macrocephaly and perturbed neuronal patterning, so loss of PTEN alone is insufficient to drive transformation and there must be an additional mutational event for brain tumors to develop. If this hypothesis is correct, then D5 lipid phosphatases such as SHIP1, which control proliferation and differentiation of hematopoietic progenitor cells, must also have tumor suppressor activity.

From a therapeutic standpoint, transient inactivation of PTEN could provide a booster shot for a rare stem cell population needed to treat certain neurodegenerative diseases. The caveat is that such an approach would have to circumvent the pro-cancer consequences of PTEN inactivation in other cell types. If we have indeed found our modern Holy Grail, then we must be sure that it does not harbor poison.

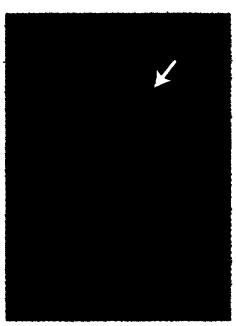
References

1. M. Groszer *et al.*, *Science* **294**, 2186 (2001).
2. D. M. Li, H. Sun, *Cancer Res.* **57**, 2124 (1997).
3. J. Li *et al.*, *Science* **275**, 1943 (1997).
4. P. A. Steck *et al.*, *Nature Genet.* **15**, 356 (1997).
5. A. Di Cristofano, P. P. Pandolfi, *Cell* **100**, 387 (2000).
6. A. Di Cristofano *et al.*, *Nature Genet.* **19**, 348 (1998).
7. A. Suzuki *et al.*, *Curr. Biol.* **8**, 1169 (1998).
8. V. Stambolic *et al.*, *Cell* **95**, 29 (1998).
9. V. Stambolic *et al.*, *Cancer Res.* **60**, 3605 (2000).
10. T. Maehama, J. E. Dixon, *J. Biol. Chem.* **273**, 13375 (1998).
11. N. K. Tonks, M. P. Myers, *Science* **286**, 2096 (1999).
12. M. P. Myers *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13513 (1998).
13. S. A. Backman *et al.*, *Nature Genet.*, 19 November 2001 (10.1038/ng782).
14. C.-H. Kwon *et al.*, *Nature Genet.*, 19 November 2001 (10.1038/ng781).
15. N. Vantomme *et al.*, *Surg. Neurol.* **56**, 201 (2001).
16. M. Tamura *et al.*, *Science* **280**, 1614 (1998).
17. C. D. Helgason *et al.*, *Genes Dev.* **12**, 1610 (1998).
18. Q. Liu *et al.*, *Genes Dev.* **13**, 786 (1999).
19. S. Clement *et al.*, *Nature* **409**, 92 (2001).
20. B. A. Reynolds, S. Weiss, *Science* **255**, 1707 (1992).
21. J. G. Toma *et al.*, *Nature Cell Biol.* **3**, 778 (2001).
22. T. Reya *et al.*, *Nature* **414**, 105 (2001).
23. S. Temple, *Nature* **414**, 112 (2001).

NOTA BENE: BIOMEDICINE

In the Nic of Time

Alzheimer's disease (AD), the most common neurodegenerative disease of old age, is characterized by two types of deposits in the brain: amyloid plaques and tau tangles. Amyloid plaques are composed of short, sticky β-amyloid peptides formed by aberrant cleavage of an amyloid precursor protein (APP) at a site in its transmembrane domain. The enzyme responsible for this aberrant cleavage is a mysterious γ-secretase that has yet to be fully characterized. The favored candidate is a transmembrane protein called presenilin that is mutated in some patients with an early-onset form of AD. But there are also other contenders, including nicastrin, a transmembrane protein that associates with presenilin in a giant, multisubunit complex that has γ-secretase activity. A trio of recent papers (*1*–*3*) confirms that nicastrin is indeed essential for activity of the γ-secretase complex, but the reports differ when it comes to speculating about what nicastrin actually does in the cell.



The humble fruit fly has proved to be a boon for those interested in AD because a membrane-tethered signaling protein called Notch, which is essential for normal fly development, must be cleaved by γ-secretase before it can transduce signals. Cleavage releases an intracellular fragment that moves to the nucleus and alters the transcription of target genes required for development. Using mutant flies deficient in nicastrin (*nic*), the three groups examined the effects of a lack of nicastrin on the cleavage of Notch by γ-secretase. They found that *nic* mutant flies could not cleave Notch (or other substrates including APP). This resulted in a series of developmental abnormalities—such as large notches and thickened veins in the wings, and altered segregation of neuroblasts—that were indistinguishable from the abnormalities seen in flies deficient in either Notch or presenilin.

The finding that nicastrin is essential for γ-secretase activity does not prove that it is proteolytically active. So, what does nicastrin do? Chung and Struhl (*1*) discovered that in the absence of nicastrin, presenilin could not move to the apical plasma membrane of the cell, the location where γ-secretase cleavage of Notch usually takes place (see the figure). They suggest that nicastrin may be required for the subcellular trafficking of presenilin (or another γ-secretase component) or for assembly of the complex itself. Hu *et al.* (*2*), on the other hand, propose that nicastrin (either on its own or bound to substrate) may be important for the stabilization or maturation of presenilin. Using small RNAs to interfere with nicastrin activity in cultured fly cells, this group showed that loss of nicastrin activity was accompanied by decreased accumulation of the mature form of presenilin.

Supporting the Hu *et al.* proposal is the work of López-Schier and St. Johnston (*3*), which hints that nicastrin may be important for the long-term stability of presenilin. But perhaps even more intriguing is the claim by the third group that nicastrin is necessary for maintaining the integrity of the cell's spectrin cytoskeleton. In both nicastrin-deficient and presenilin-deficient fly cells, the normal distribution of α-spectrin and β-spectrin was disrupted. The former increased in apical regions of the cell, whereas the latter disappeared from these regions altogether. Through control experiments, the investigators showed that the disruption in spectrin organization was independent of Notch signaling, suggesting that both nicastrin and presenilin do something else besides helping γ-secretase to cleave Notch and APP. That two central components of the γ-secretase complex seem to be important for another cellular process hints that γ-secretase inhibitors under development for the treatment of AD may have harmful side effects.

—ORLA SMITH

References and Notes

1. H.-M. Chung, G. Struhl, *Nature Cell Biol.* **3**, 1129 (2001).
2. Y. Hu *et al.*, *Dev. Cell*; published online 30 November 2001 (10.1016/S15345807(01)001058).
3. H. López-Schier, D. St. Johnston, *Dev. Cell*; published online 30 November 2001 (10.1016/S15345807(01)00106X).

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