

Myfibroblast Differentiation by PTEN with

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What is known about PTEN?

Eric S. White: PTEN (phosphatase and tensin homologue on chromosome 10) is a tumor suppressor phosphatase capable of dephosphorylating lipid and protein substrates (1,2). Also known as MMAC-1 (mutated in multiple advanced cancers-1) and TEP-1 (transforming growth factor β -regulated, epithelial cell enriched phosphatase-1), PTEN is considered a tumor suppressor based on a number of lines of evidence: first, the PTEN gene localizes to chromosome 10q23, a locus commonly mutated in a wide variety of cancers (3); second, loss of PTEN is observed in 17-66% of cancers, depending on cell type (4); third, reconstitution of PTEN into malignant epithelial cells reverses tumorigenicity (5-8); and heterozygous deletion of PTEN in mice enhances cancer susceptibility (homozygous deletion is embryonic lethal) (9).

The primary physiologic function of PTEN is to inhibit activity of phosphoinositol-3-kinases (PI3Ks). PI3K activation results in the generation of phosphatidylinositol-3,4,5-trisphosphate (PIP₃) following phosphorylation of the D3 position of the inositol ring of PIP₂. PTEN directly dephosphorylates the D3 position, functionally antagonizing PI3K. The principal downstream activity of PIP₃ is the

phosphorylation and activation of Akt/Protein kinase B. The functional consequence of unopposed Akt activity is decreased apoptosis, increased proliferation, increased migration, and increased cell size, all of which are common in malignancies. However, it is clear that PTEN activity is also important in non-malignant diseases. For example, we have shown that PTEN loss or inhibition results in fibroblast-to-myofibroblast transdifferentiation and increased migration (10,11). Similarly, others have shown loss of PTEN in invasive synovial fibroblasts in rheumatoid arthritis (12).

PTEN is a 403 amino acid protein with molecular mass of 54 kDa that consists of a phosphatase domain, a C2 domain, and a tail domain. The C2 domain is involved in membrane binding, whereas the tail domain is instrumental in negative regulation of the PTEN protein. The tail domain consists of a number of serine and threonine residues which are constitutively phosphorylated (by Casein Kinase II), rendering the PTEN molecule inactive and relatively stable. Following dephosphorylation by an as yet unidentified phosphatase, PTEN localizes to the plasma membrane where it catalyzes the dephosphorylation of PIP₃.

Is PTEN important for regulating lung fibroblast motility and myofibroblast differentiation?

Eric S. White: PTEN activity appears to be important for both regulation of fibroblast motility and myofibroblast differentiation. Early studies demonstrated that homozygous *pten*-null cells are significantly more motile than wild-type cells (13) due to increased activity of the small GTPases Rac1 and Cdc42. Interestingly, focal

adhesion kinase (FAK) activity was not similarly increased (13) although subsequent reports have demonstrated that PTEN is capable of dephosphorylating FAK as well (14). Further study demonstrated that Akt/PKB may lie downstream of Rac1 and Cdc42 to facilitate *pten*-null fibroblast migration (15). Data from our lab has

demonstrated that in lung fibroblasts derived from patients with usual interstitial pneumonia (UIP), the histologic correlate of the progressive and often fatal disease idiopathic pulmonary fibrosis (IPF), PTEN expression is downregulated, associated with an increased migratory capacity (16). Further investigation by our group has demonstrated that increasing the activity of PTEN in both *pten*-null and wild-type cells can inhibit FGF-induced fibroblast migration (10). Most recently, we have shown that fibroblast motility *in vivo* may also be regulated by PTEN, since fibroblasts located within airspaces of patients with UIP have markedly deficient PTEN expression compared to interstitial fibroblasts (11).

When compared with quiescent lung fibroblasts, activated lung fibroblasts (myofibroblasts) also seem to be relatively deficient in PTEN levels (11). Moreover, we have found that loss of PTEN expression is necessary for myofibroblast differentiation in response to a potent fibroblast activator, transforming growth factor- β (TGF- β) (11). Additionally, we have shown that in patients with UIP, activated myofibroblasts within airspaces express little or no PTEN compared with surrounding cells, which correlates with increased expression of the myofibroblast marker α -smooth muscle actin (11). Thus, our data show *in vivo* relevance of PTEN loss in fibroproliferative disease.

Is Prostaglandin E2 involved?

Eric S. White: Prostaglandin (PG) E₂ is a biologically important lipid involved in many cellular processes. PGE₂ is known to inhibit fibroblast migration when signaling through the EP2 receptor. Interestingly, lung fibroblasts from patients with UIP have a diminished capacity to both produce PGE₂ (17-20) and to respond to exogenously applied PGE₂ (21). Because of this observation, we investigated the possibility that PGE₂ inhibited fibro-

blast migration through induction of PTEN activity. Upon stimulation with PGE₂ or an EP2-specific agonist (butaprost), we observed an increase in PTEN activity in lung fibroblasts that resulted in decreased migration (10). Mechanistically, this appeared to be due to changes in PTEN tyrosine phosphorylation, not PTEN levels. Thus, it appears that multiple means of PTEN regulation may be sufficient to decrease fibroblast migration.

Can your findings influence the development of drugs against idiopathic pulmonary fibrosis?

Eric S. White: We think so. Currently, no effective therapy for IPF exists. Standard therapies (corticosteroids and immunosuppressants) are ineffective at halting disease progression. Our collective data imply that increasing PTEN levels or enhancing the activity of PTEN within fibroblasts might

be an effective approach to halting tissue fibrosis by decreasing fibroblast migration and myofibroblast differentiation. It remains to be seen whether PTEN reconstitution in fibroblasts *in vivo* will ameliorate fibrosis in animal models, but experiments are currently underway.

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