Cancer-associated PTEN: Structural and Functional Characterization

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Abstract
Phosphatase and tensin homolog detected on chromosome ten (PTEN) is a tumor suppressor gene protecting cells from developing cancer and forming tumors (Leslie and den Hertog). *In addition, PTEN* mediates cell cycle arrest, adhesion, migration, and apoptosis(Sun, Lesche et al. 1999). The complete loss or mutations in the PTEN gene has been implicated in many human cancers from mammary carcinoma to developmental abnormalities and autism. It is in fact the most common mutation in human cancers and are inherited in an autosomal dominant manner (Yamada and Araki 2001). In their recent Cell paper, Papa et al (Papa, Wan et al. 2014) shows a novel homodimerization characteristic of PTEN that renders it active. Mechanistically, the homodimerized active conformation exerts its phosphatase activity on downstream targets such as AKT, PIP3, and PI3K and decreases their oncogenic activity. The two mutated PTEN isoforms characterized in this study have a reversed functionality compared to wild-type protein. These findings have great implications in developing drug agents that target PI3K pathway and sensitive to PTEN mutations.

Key words: AKT signaling, cancer, PI3K signaling, PTEN, tumor suppression

PTEN got more attention after its discovery in 1997 (Yamada and Araki 2001). The PTEN gene was found mutated very early during tumorigenesis or arise later through somatic mutations later on in advanced cancers to promote malignancy(Li, Podsypanina et al. 2001). PTEN has been characterized to be part of a complex signaling system and its regulation is highly sensitive to the intracellular environment (Gimm, Attié-Bitach et al. 2000).

The crystal structure of PTEN contains a phosphatase domain resembling protein tyrosine phosphatases but contain an enlarged active site that can account for its ability to bind PtdIns(3,4,5)**P**3 (Lee, Yang et al. 1999).

Structurally, the PTEN enzyme belongs to the class I Cys-based protein tyrosine phosphatases and more specifically to VH1-like family. The consequence of complete loss of PTEN in mice is embryonic lethal, therefore studying loss of PTEN function in vivo hampers developmental investigation. Lower PTEN expression also results in severe cancer phenotypes while transgenic overexpression of PTEN in mice exerts tumor suppressive properties. Human patient data further shows that complete loss of PTEN is more deleterious than truncated mutations in the PTEN gene.

Functionally, PTEN can be regulated through posttranslational modifications. These modifications can change the cellular localization as well as protein-protein interactions of PTEN. In the past, researchers have shown that the modification of specific serine and threonine residues of PTEN modulated its activity and stability (Vazquez, Ramaswamy et al. 2000; Torres and Pulido 2001). In fact, there is evidence that dephosphorylation or deletion of the tail results in enhanced phosphatase activity and rapid degradation (Vazquez, Ramaswamy et al. 2000). Papa et al shows for the first time that PTEN can exist in a dimeric complex through pull-down experiments. They also showed that PTEN is catalytically more active in its dimer state with respect to its monomer isoform. To study the conformational status of PTEN, Papa et al performed phosphomimetic and phosphodead

mutant-tail gel filtration assays and found that by expressing non-phosphorylatable PTEN prevents an upward shift on the gel further. These results indicate that PTEN tail-phosphorylation is important for facilitating its monomer formation and prevent its dimerization.

Papa et al also hypothesized that mutant PTEN cannot form dimers and hence have lowered catalytic activity. To study this, they made two of the most common cancer associated PTEN mutants: MycPTENC124S and MycPTENG129E and looked at their dimerization potential. To their surprise, these mutants could form homodimers and heterodimers in non-reducing conditions as well as in co-IP experiments, similar to wild-type (WT) PTEN. However, they observed that the catalytically inactive PTEN heterodimers could inhibit the activity of WT PTEN protein toward PIP3 dephosphorylation.

In vivo analysis of PTEN mutant knock-in (KI) mice revealed an exacerbated tumor spectrum in the pituitary gland with anterior lobe adenomas. These mice had severe lymphoproliferation from a young age. The older mice showed increase in spleen weight in addition to hematopoiesis and lymphoid hyperplasia. Similarly, PTEN KI mice developed adenomas of the thyroid, adrenal, and gallbladder, with 2 out of 29 PtenC124S/+, and 3 out of 37 PtenG129E/+ male mice also developing invasive adenocarcinoma of the thyroid in addition to neuronal development. In addition, these KI mice had higher sensitivity to growth factor stimulation and increased AKT activation that persisted in older mice. These observations came from PTEN KI MEF cells showing acute hyperphosphorylation of AKT (phosphor-AKT) as well as PI3K under Insulin-like growth factor stimulation through western blotting techniques. Of course, AKT hyperactivation resulted in many downstream AKT targets from mTORC1 to FoxO1 through negative feedback loops, leading to accelerated tumor formation. This, however, did not change the phosphorylation status of FAK and Src. Similarly through immunofluorescent studies of MEF cells under IGF stimulation, Papa et al found an accumulation of PIP3 at the membrane protrusions inducing cell migration and invasion. This observation was also consistent with recruitment of PTEN KI mutants to the plasma membrane through IP and immunofluorescent studies. They further show that the inactive hetero- and homodimers of PTEN can be recruited to the plasma membrane and displace and outcompete the WT PTEN homodimers. This also increases phosphorylation of AKT/PI3K (enhancing oncogenic signaling), a prediction for higher sensitivity to human cancers.

In Summary, the authors show that PTEN dimerization state can affect and modulate the activity of PIP3 and PI3K/AKT pathways that are instrumental in cell growth, proliferation, and metabolism. Other substrates of PTEN include PTEN itself, focal adhesion kinase (FAK), as well as c-SRC. Papa et al. first reveals that PTEN interaction with itself to form dimers can further activate its phosphatase properties that can in turn inhibit downstream signaling of PI3K and AKT pathways. They show that partial loss of normal PTEN tumor suppressor function can be compounded by additional disruption caused by the expression of inactive mutant PTEN protein. These data have significant therapeutic implications for patients with PTEN gene mutations which make them susceptible to certain cancer types (Steelman, Bertrand et al. 2004) and especially those who don't respond to AKT modulations. To elaborate, PTEN serves to counterbalance the effect of PI3K that normally phosphorylates PIP2 into PIP3. This conversion then phosphorylates AKT and downstream proteins involved in regulation of apoptosis and cell cycle progression. PTEN removal of phosphate from PIP3 inhibits this pathway by preventing its localization to cell membrane and decreasing cancer prevalence (Steelman, Bertrand et al. 2004). Therefore drugs that modulate PTEN activity seem to have a high therapeutic potential. However, it is also important to note that an additional source of complexity posed to this research is the fact that PTEN is not the only key phosphatase in cellular signaling and function and so studies only

focusing on PTEN as a therapeutic are inadequate and only partial in affectivity.

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