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Review Article

Link between PI3K/AKT/PTEN Pathway and NOX Protein in Diseases

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ABSTRACT: Accumulating evidence has revealed that the PI3K/AKT/PTEN pathway acts as a pivotal determinant of cell fate regarding senescence and apoptosis, which is mediated by intracellular reactive oxygen species (ROS) generation. NADPH oxidase (NOX) family of enzymes generates the ROS. The regulation of NOX enzymes is complex, with many members of this family exhibiting complexity in terms of subunit composition, cellular location, and tissue-specific expression. Cells are continuously exposed to the ROS, which represent mutagens and are thought to be a major contributor to several diseases including cancer and aging process. Therefore, cellular ROS sensing and metabolism are firmly regulated by a variety of proteins involved in the redox mechanism. In this review, the roles of oxidative stress in PI3K/AKT/PTEN signaling are summarized with a focus on the links between the pathways and NOX protein in several diseases including cancer and aging.

Key words: PTEN, PI3K, AKT, ROS, PPAR, WRN, SIRT1, cell signaling

Oxidative stress results from an imbalance of an excess of oxidants relative to antioxidant capacity [1]. The oxidants derive from various oxidase enzymes, including xanthine oxidase, lipoxygenase, or nicotinamide adenine denucleotide phosphate (NADPH) oxidase (NOX). An endogenous reactive oxygen species (ROS) mainly comes from mitochondria during the process of oxidative phosphorylation to produce energy in the form of ATP [2]. The ROS are also produced by intracellular membrane oxidases. Inflammation is typical source of those ROS at the sites of tissues. Some ROS function as signaling molecules that mediate various cellular responses. Experimental evidences suggest that free radical production in cells is critical to intracellular signal transduction and regulate various physiological processes including proliferation, differentiation, apoptosis and migration [3, 4]. The NOX family is considered one of the sources of ROS which participates in the induction of important signaling pathways. For example, it has been reported that p42/44 ERK activation is NOX dependent [5]. In addition, ROS modify the activity of several key enzymes for cell-dynamics, resulting in the reorganization of actin cytoskeleton, adhesion and cell migration [6]. On the other hand, ROS may react with cellular constituents including proteins, lipids, and DNA to generate an array of oxidative lesions. Protein and lipid oxidation by ROS is often proposed as a crucial determinant of health. These lesions may compromise genome stability which is critical for cellular homeostasis and progeny [7]. Increased levels of oxidative stress results in macromolecular damage and is implicated in various disease states such as atherosclerosis, diabetes, and cancer [8]. Accumulating evidence suggest that NOX-derived ROS may elevate the risk for genomic instability [9].

It is important for cells to neutralize ROS before they can damage cellular macromolecules. Therefore, cellular

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ROS metabolism is firmly regulated by a range of proteins involved in the redox mechanism. However, less is known about the initial regulation of signaling molecules for ROS production. NOX enzymes play central roles as they can stimulate other enzymatic sources of ROS. PI3K/AKT signaling pathway seems to play important roles in the NOX activation. It has been shown that the initial step in a NOX-activation requires PI3K by using a dominant negative construct. *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene that is frequently deleted or mutated in a variety of human cancers. It has been demonstrated that up-regulation of PTEN causes modulation of PI3K/AKT signaling to reduce ROS generation in cells [10]. The PI3K/AKT/PTEN pathway may be involved in the mechanisms of several diseases. Accordingly, investigation of the interactions of redox- and non-redox-based signaling pathways could lead to better pharmacological control over the diseases. In this review, NOX family proteins and its roles in oxidative stress are summarized with a focus on the molecular links and interplay between NOX and the PI3K/AKT/PTEN signaling pathway.

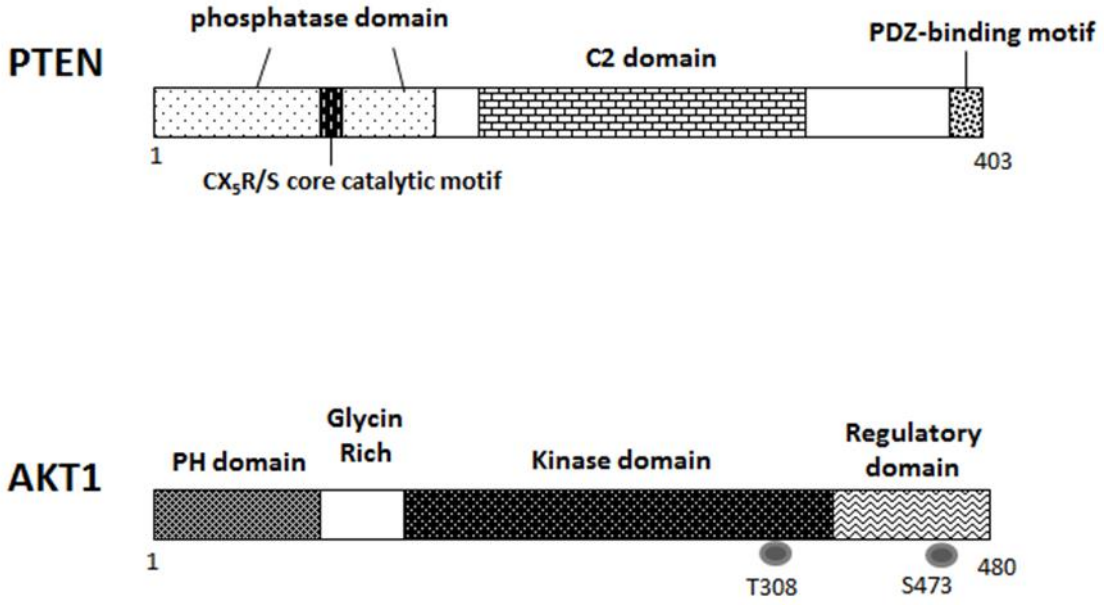

Figure 1. Schematic structures of human PTEN and AKT1 protein. The predicted consensual domain structures for each protein are depicted. Note that the sizes of protein are modified for clarity. C2 domain= a protein structural domain involved in targeting proteins to cell membranes; PDZ= a common structural domain in signaling proteins (PSD95, Dlg, ZO-1, etc); PH domain= pleckstrin homology domain

Characteristics of PI3K/AKT/PTEN pathway and NOX proteins

The PIP3 is the major second messenger of the PI3K pathway that mediates receptor tyrosine kinase signaling to the proliferation and survival kinase AKT [11]. PTEN negatively regulates the activity of PI3K/AKT signaling through converting PIP3 to PIP2, by which PTEN exerts its tumor-suppressive effect. Increased levels of PIP3 at the membrane cause PH domain-containing protein kinases to co-localize, resulting in the kinases-mediated phosphorylation and activation [12]. For example, the activated AKT phosphorylates target proteins involved in cell survival, cell cycling, angiogenesis, and metabolism [13]. PTEN acts as regulator of maintaining basal levels of PIP3 opposing to those signaling activation too much, as the increased proliferation, survival and motility are main cellular effects that contribute to tumorigenesis. Actually, dysregulation of the PI3K/AKT pathway has been found in many malignant cancers [14]. Human PTEN is a 53 kDa protein with homology to tensin and protein tyrosine phosphatases. Schematic structure of the PTEN protein is shown in Figure 1. The structure provides PTEN with its preference for acidic phospholipid substrates such as PIP3. Tumor suppressor p53 and peroxisome proliferator activated receptor γ (PPARγ) can

transcriptionally upregulate *PTEN* expression [15, 16]. Interestingly, it is reported that rosemary extract represses *PTEN* expression in K562 leukemic culture cells [17]. Overexpression of *PTEN* induces growth suppression by promoting G1 arrest [18]. AKT activation leads to Hypoxia-inducible factor-1 (HIF-1) stabilization, whereas PTEN attenuates the hypoxia-mediated HIF-1 stabilization [19]. The HIF-1 is a critical element for the transcriptional regulation of genes important for adaptation to low oxygen conditions. The instability of mutant PTEN and the reduction of HIF1 degradation have been shown to involve protein interactions [20]. PTEN has been shown to act as a tumor suppressor whose function includes important roles in regulating oxidative stress, indicating a potential role in oxidative damage-associated diseases [21].

NOX family proteins are committed reactive oxygen species-generating enzymes, which also regulate redox-sensitive signaling pathways [22]. The NOX family has been categorized into seven homologues (NOX1- NOX5 and DUOX1- DUOX2) depending on the membrane-associated flavor-proteins. NOXs are multimeric enzymes that consist of the cytosolic subunits p22phox, p40phox, p47phox, p67phox, and Rac1 [23] **(**Figure 2**)**. Upon activation, all of them as well as the transmembrane glycoprotein gp91phox and p22phox translocate to the membrane [23]. NOX-related cytosolic proteins associate with integral membrane subunits to form the functional enzyme, which generates ROS. NOX catalyses a one-electron transfer to molecular oxygen to form the radical superoxide anion, which can be reduced to hydrogen peroxide. It has been reported that loss of fluid shear stress, ischemia, and inflammation cause plasma membrane depolarization via ATP-sensitive K channel closure, initiating a signaling cascade which leads to NOX activation and ROS production [24].

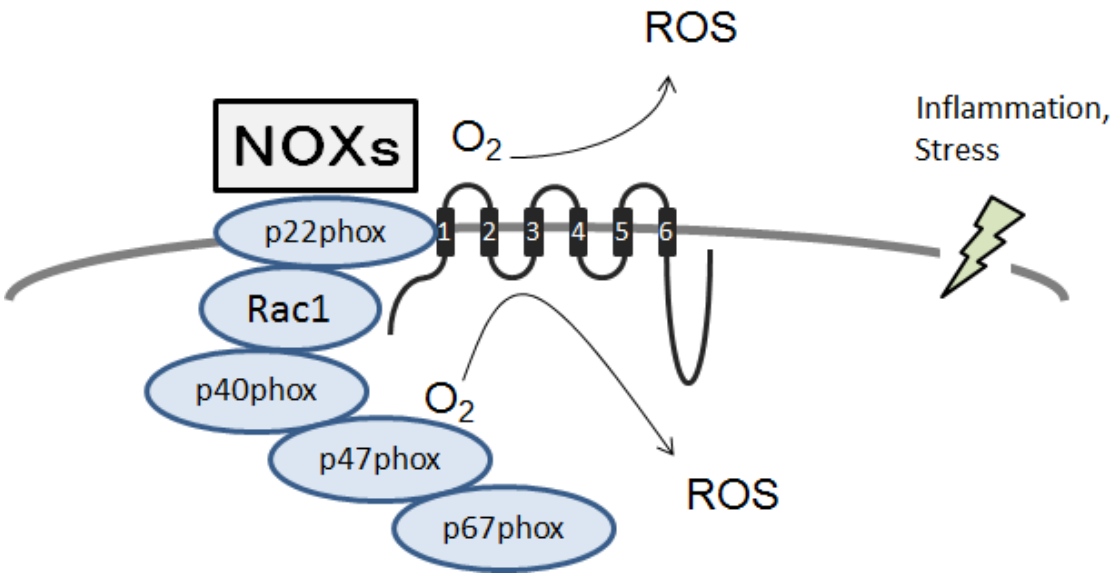

Figure 2. NOXs are trans-membrane protein and can form a complex with p22phox, Rac1, p40phox, p47phox and p67phox. NOXs are responsive to the extracellular fluid surrounding the inflammation and stress, and generate ROS. Note that some critical molecules have been omitted for clarity.

Roles of PI3K/ AKT /PTEN and NOX for the ROS production

Since wortmannin, a PI3K/AKT inhibitor, considerably inhibits the translocation of NOX subunits and reduces ROS production, in which the role of PI3K signaling might be involved [25, 26] **(**Figure 3**)**. It has also been reported that cell membrane depolarization, which is followed by PI3K/AKT and PKC activation causes NOX assembly and ROS production. The ROS production is abolished by the wortmannin as well as the PKC inhibitor H7 [24]. In addition, the combination of the wortmannin and H7 does not have a larger effect. In AKT1-null cells, the ROS production is reduced compared with wild-type

cells [27]. Although wortmannin prevents the NOX activation, wortmannin has no effect on the cell membrane depolarization. Activation of NOX is also diminished by dominant negative PI3K [28, 29]. Accordingly, AKT phosphorylation follows cell membrane depolarization, and it precedes the activation of NOX. In other words, PI3K/AKT and PKC activations are downstream of cell membrane depolarization but upstream of the NOX activation. It has also been shown that ROS could play a critical role in pancreatic islet β-cell dysfunction through PTEN-dependent JNK activation and AKT inhibition [30]. These results indicate that PI3K/AKT and PKC are in the same pathway and are required for ROS production. However, the intermediate steps that link depolarization and the NOX activation are not well understood.

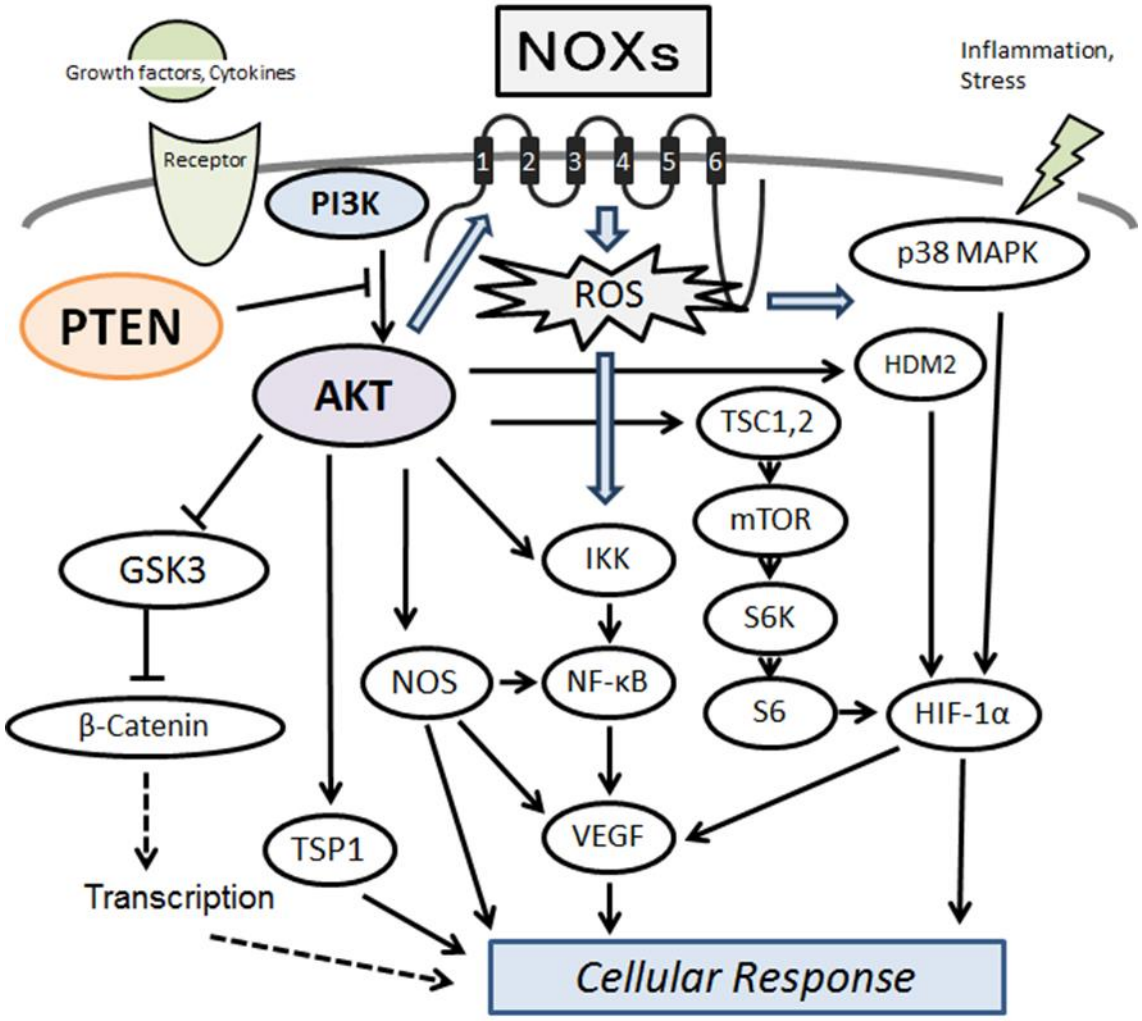

Figure 3. Schematic representation of PI3K/AKT/PTEN signaling, which modulate NOXs-derived ROS. NOXs sense hypoxia and can help to mediate activation of angiogenic pathway, while maintaining unregulated cell growth and apoptotic cell death. Examples of molecules known to act on the regulatory pathways are shown. Note that some critical pathways have been omitted for clarity.

ROS directly interact with signaling molecules to modulate the signaling in a variety of cellular processes such as proliferation and survival. Actually, the catalytic activity of PTEN is also modulated by ROS, and cellular PTEN phosphatase activity is inhibited by the oxidative stress [31]. The PTEN inactivation then causes an increase in cellular PIP3 levels, PIP3 accumulation occurs at the plasma membrane and activation of the downstream PIP3 target including AKT, indicating a part of functional role for elevated intracellular ROS. Furthermore, activated PI3K/AKT signaling causes increased expression of several genes for cell survival. Endogenous oxidant production in macrophages inactivates a fraction of the cellular PTEN, and this is associated with an oxidant-dependent activation of downstream signaling [32]. The phosphorylated PTEN inactivation and the consequent AKT activation in cells are inhibited by antioxidant treatment. ROS levels are increased in the retinal pigment epithelium cells in association with phosphorylation and inactivation of PTEN [33]. Mitochondrial PTEN protein levels are increased by H_2O_2, although ROS does not affect the PTEN expression [34]. The increased PTEN in mitochondria may further promote ROS production in cells. However, exposure of the PTEN to H_2O_2 results in inactivation of PTEN [35]. Various cysteine mutants in the active site of PTEN have indicated that the essential Cys residue specifically forms a disulfide bond during oxidation [36]. Uncontrolled generation of ROS might contribute to the cell proliferation by inhibiting PTEN function.

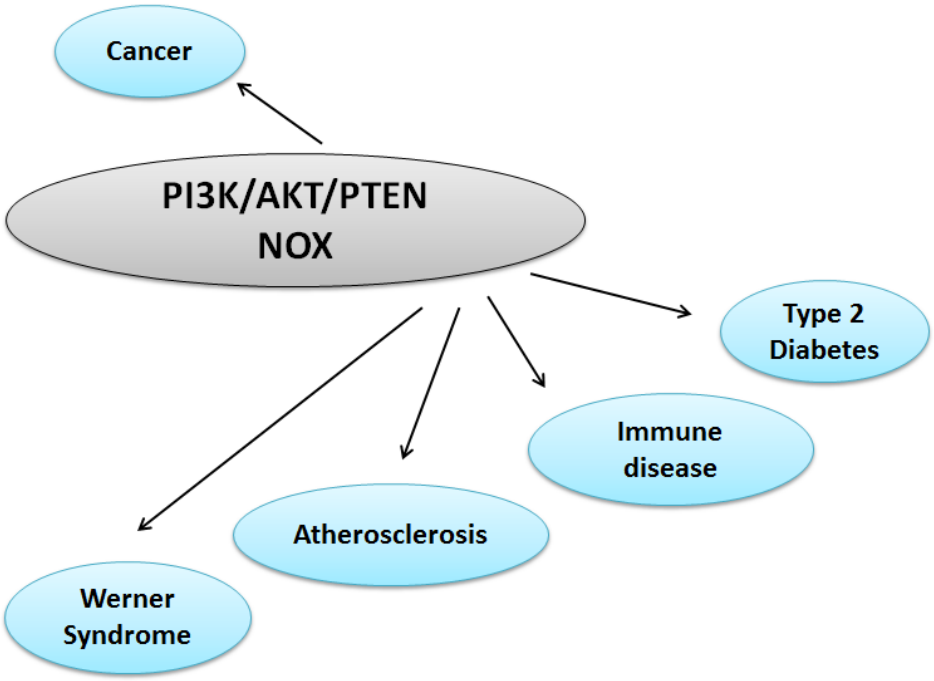

Figure 4. The PI3K/AKT/ PTEN pathway and ROS may be involved in several diseases besides cancer and aging.

Link between PI3K/AKT/PTEN pathway and NOX protein involved in several Diseases

Intracellular signaling is initiated at low ROS concentration, whereas oxidative stress is induced at high ROS concentration. Therefore, imbalance of NOX activities could easily be the potential cause of some diseases **(**Figure 4**)**. With regard to functional relationships between NOX isoforms and pathogenesis, it is of particular interest to study whether they are involved in the development in several diseases, because overproduction of ROS has long been implicated as a risk factor. For example, multiple genetic and epigenetic alterations by oxidative stress are required for cellular

transformation and carcinogenesis. Inactivation of the PTEN is implicated in the tumorigenesis of prostate cancer, in which PIP3 and oxidants seem to be produced as carcinogens. It has been reported that causal relationship between NOX and cancer is also in Ras oncogene-induced cell transformation [37, 38]. NOX family of genes appears to be required for survival and growth of a subset of human cancer cells [37]. In addition, NOX-derived ROS are known to play a central role in each step of the metastatic cascade including invasion, adhesion, angiogenesis and proliferation. ROS attack guanine bases in DNA and form 8-hydroxydeoxyguanosine (8-OHdG), which can bind to thymidine rather than cytosine, based on which, the level of 8-OHdG is generally regarded as a biomarker of mutagenesis consequent to oxidative stress [38, 39]. Higher levels of 8-OHdG are noted in Helicobacter pylori infection in atrophic gastritis as well as in gastric cancer [38, 39]. They act at specific cellular microdomains through the activation of oncogenes and the inactivation of tumor suppressor proteins. The produced ROS surrounding the tumor environment will continue unregulated cell growth, progress, facilitate invasion, and make metastasis.

Defects in insulin secretion and decreased sensitivity of target tissues to insulin action are the key features of type 2 diabetes. Dysfunction of β-cell in pancreas is one of major characteristics in the pathogenesis of type 2 diabetes [40]. It has been shown that excessive generation of ROS is linked to the β-cell dysfunction. Oxidative stress has also been implicated in the pathogenesis of free fatty acids (FFAs)-induced β-cell dysfunction. The combination of obesity and type 2 diabetes is associated with elevated plasma FFAs that lead to apoptosis of β-cells through PTEN-dependent AKT inhibition, followed by reduced insulin expression and secretion [41]. Although NOX-generated ROS are the well characterized in mammalian cells, ROS are also generated by the oxidative metabolism of arachidonic acid that is released from the membrane phospholipids via the activity of cytosolic phospholipase A2 (PLA2) [42, 43]. The interaction between ROS and inflammation also plays an important role in some other disease pathogenesis. For example, chronic airway diseases such as asthma are linked to oxidative environmental factors and are associated with increased production of ROS [44]. Oxidative stress seems an important contributing factor to asthma, and therefore, antioxidant strategies may be useful in the treatment of asthma. It is not surprising that the contributions of NOX-derived ROS to various aspects of asthma development and progression are diverse. Thyroid cells contain antioxidants to protect cells from the ROS-mediated oxidative damage [45]. NOX4, a homolog of the NOX family, is an intracellular source of ROS in the human thyroid gland. Papillary thyroid carcinoma, the most common thyroid cancer, is closely linked to the increased ROS production by NOX4 [46]. Imbalance of ROS may result in thyroid cell dysfunction and thyroid diseases, which provides us with valuable tools for a better understanding of pathophysiology of prevalent thyroid diseases. Furthermore, ROS regulates immune system and enhances atherosclerosis [47, 48].

Werner syndrome, which is characterized by the early onset of premature age-associated pathologies including elevated cancer incidence, is a rare autosomal disease [49]. Werner syndrome is caused by the lack of a functional nuclear RecQ-like DNA helicase, named WRN protein. The DNA damage, including oxidative DNA damage at telomeres, which plays a critical role in optimizing double strand breaks repair mechanisms due to its end-processing helicase and exonuclease activities. Studies indicate that WRN protein regulates HIF-1 activation by affecting the mitochondrial ROS production [50]. The depletion of WRN protein leads to increased HIF-1 complex stabilization and activation [50]. HIF-1 is implicated in the molecular mechanisms of ageing, whose activation in the absence of WRN involves the generation of mitochondrial ROS. Activation of PI3K/AKT pathway also leads to HIF-1 stabilization [51], whereas PTEN attenuates the HIF-1 stabilization [52]. Mutation of PTEN can destabilize the HIF-1. The deletion of AKT protects against the age-dependent defects, raising the possibility that modulation of the PTEN/AKT pathway could protect against premature aging syndromes. Down-regulation of PI3K/AKT pathway by PTEN expression may protect against age-dependent DNA damage and cancer, including the premature disorders observed in Werner syndrome. Vitamin C supplementation also rescues the shorter mean life span of the *WRN* mutant mice and reverses several abnormalities [53].

Discussion and Perspective

Induction of ROS is likely to play a key role in cell growth inhibitory responses. It is accepted that one of the symbols of aging is the accumulation of DNA damage caused by the ROS, which is known to induce genomic alterations such as point mutations and deletions, then inhibit tumor suppressor genes and/or activate oncogenes. It has been shown that tumor suppressor p53 also promotes ROS production and participates in the induction of apoptosis. Increasing ROS can also enhance insulin signaling to attenuate the development of insulin resistance. The enhanced ROS-dependent insulin signaling may be attributable to the oxidation and inhibition of PTEN. Given the importance of PTEN in regulating metabolism, there are many linking PTEN to diabetes and senescence. In addition, PTEN and PI3K/AKT are in the same

pathway and are supposed to be required for ROS production. As ROS can regulate PTEN, the role of PTEN in modulating ROS may form part of a feedback loop. However, this may paradoxically carry damaging consequences in the setting of persistent environmental stressors and during the process of aging. A better understanding of the complicated relationships between ROS and aging via NOX and PTEN/AKT pathway may provide clues to ROS-mediated pathogenesis-pathways for new drug discovery. Future appropriate experiments will determine which pathways are responsible for the redox imbalance.

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Competing interests statement

The authors declare that they have no competing financial interests.

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