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Forum Review

The Redox Regulation of PI 3-Kinase–Dependent Signaling

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ABSTRACT

Signal transduction via PI 3-kinases plays an important role in regulating the cellular processes of cell growth, survival, proliferation, and motility. The stimulated generation of reactive oxygen species is a necessary component of the signal transduction mechanisms by which many growth factors and cytokines activate this signaling pathway and elicit their cellular responses. Evidence now supports the oxidative inactivation of both tyrosine phosphatases acting upstream of PI 3-kinase, and of the lipid phosphatase PTEN as components of the normal stimulated regulation of PI 3-kinase signaling. However, the effects of chronic oxidative stress appear rather different, particularly a proposed role for nitrosylation of Akt and other targets leading to inhibition of PI 3-kinase signaling during diabetic insulin resistance in muscle. Recently, evidence has also begun to emerge, indicating that physiological redox signaling may display the same tight spatial and temporal specificity as seen with many other signal transduction systems in terms of targeting individual proteins for modification, and of enzymatic reversal mechanisms. This review will focus upon the details of these and other roles for reactive oxygen and nitrogen species in the regulation of PI 3-kinase signaling, both during acute stimulation and chronic oxidative stress, and the evidence for their significance. *Antioxid. Redox Signal.* 8, 1765–1774.

INTRODUCTION

THE COMPLEX BEHAVIOR of the cells of higher multicellular organisms is regulated by multilayered signal transduction pathways and networks that integrate information from several sources to control a cellular response. This review will focus upon the redox-dependent regulation of the PI 3-kinase signaling pathway, due to the significance of the pathway in regulating critical biological processes, such as cell growth, survival, proliferation, and motility, and also because of the role of redox modification in regulating the activity of the signaling pathway. Not only is there good evidence that the stimulated production of reactive oxygen species is required for the normal activation of PI 3-kinase–dependent signaling by many stimuli, but there are also suggestions that this signaling pathway, as the principal mediator of the cellular responses to insulin and insulin-like growth factors (IGF1), may play a special role in responding to the local redox environment and rates of metabolism at the level of the organism (31).

The PI 3-kinase signaling pathway

Interest has focused on PI 3-kinase signaling due to the activation of this pathway by many growth factors, hormones, cytokines, and extracellular matrix components, and the requirement for signaling via PI 3-kinase in the promotion by these stimuli of cellular responses such as cell growth, survival, proliferation, and motility (5, 30, 80). PI 3-kinase signaling is of particular interest in the field of cancer research, as at least half of all human tumors are believed to display elevated activity of this signaling pathway, many activators and positive components of the pathway are oncogenes, and the principal cellular inhibitor of the pathway, the lipid phosphatase, PTEN, is an important tumor suppressor (71, 81).

The phosphoinositide 3-kinase (or PI 3-kinase) signaling pathway as it will be considered in this review comprises the class I PI 3-kinase enzymes that catalyze the synthesis of the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3), and also the downstream targets for

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activation by this second messenger, the best characterized of which is probably the serine/threonine kinase Akt, also known as protein kinase B (PKB) (Fig. 1) (6, 40, 80). The broader PI3-kinase family comprises a rather diverse set of enzymes, including the Class II and III PI 3-kinase enzymes, but as these latter enzymes do not make PtdIns(3,4,5)P_3 or regulate the same signaling targets as the Class I enzymes (83), they will not be considered further here.

The pathway involves the stimulated synthesis of the second messenger PtdIns(3,4,5)P_3 at low concentrations from the relatively abundant membrane phospholipid phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. PtdIns(3,4,5)P_3 can then regulate downstream signaling through the action of proteins that are able selectively to recognize and bind this lipid, most commonly through lipid binding protein domains of the pleckstrin homology class (PH domains), a subset of which bind specifically to PtdIns(3,4,5)P_3 (see Fig. 1) (80). These PtdIns(3,4,5)P_3 binding targets include the serine/threonine kinases Akt/PKB and PDK1, the tyrosine kinases of the Tec/Btk family, several guanine nucleotide exchange factors for the GTPases rac and cdc42 and several other proteins of uncertain function (30, 68, 82). PtdIns(3,4,5)P_3 is in turn metabolized by at least two classes of phosphatases. The tumor suppressor, PTEN, is a principal regulator of both basal and stimulated PtdIns(3,4,5)P_3 levels, as it dephosphorylates the 3 position of this lipid, converting it back to PtdIns(4,5)P_2 (44, 74). Contrastingly, another unrelated group of phosphatases, the 5-phosphatases, including SHIP, SHIP2, and SKIP, dephosphorylate the 5 position of PtdIns(3,4,5)P_3 to make significant amounts of PtdIns(3,4)P_2, which may not only remove the PtdIns(3,4,5)P_3 signal, but have interesting signaling properties of its own (15). Indeed, cellular treatment with hydrogen peroxide generally leads to a greater rise in the levels of PtdIns(3,4)P_2 than PtdIns(3,4,5)P_3, suggesting that this former lipid may play a significant role mediating cellular responses to generated ROS (79). Another route of synthesis of PtdIns(3,4,5)P_3 in response to oxidative stress via the action of a PI(3,4)P_2 5-kinase has also been proposed, although the physiological significance of this route in higher organisms is currently unclear (23, 57).

EVIDENCE FOR THE REDOX REGULATION OF PI 3-KINASE–DEPENDENT SIGNALING

It has become clear over the last 10 years or so, that reactive oxygen species (ROS) are not simply toxic by-products of metabolism and are used not only in a specialized microbicidal role during phagocytosis, but also play crucial roles in many signal transduction networks (14, 63). This conclusion first arose from studies showing that numerous cellular signals, such as growth factors and cytokines, induced the cellular production of ROS, usually via a nonphagocytic NADPH oxidase complex, and then from later work showing that these ROS were required for efficient signal transduction (18, 62). The cellular stimuli for which ROS production is implicated as a necessary component of their signal transduction mechanism, include several stimuli known to activate the PI 3-kinase-Akt axis, such as PDGF, EGF, insulin, bFGF, TNF, angiotensin II, LPA, and LPS (4, 8, 22, 35, 43, 46, 54, 75).

Most of this experimental evidence for the accumulation of ROS in stimulated cells comes from the use of the ROS-responsive fluorescent dyes such as dichloro fluorescein diacetate and dihydrorhodamine (4, 46), although other methods such as electron paramagnetic resonance (EPR) spectroscopy (27) and chemical assays of released extracellular ROS (36) have also been used. The significance of a role for stimulated ROS production in specific signaling pathways has generally been supported by experiments affecting downstream signaling by intervention to control either the stimulus driven production of ROS, such as by using DPI, a small molecule inhibitor of the regulated NADPH oxidase activity that produces superoxide, or alternatively experimental intervention to change (usually enhance) the removal of ROS, for example, by overexpression of the hydrogen peroxide metabolizing enzyme catalase.

Specifically, a requirement for ROS production to activate Akt has been shown for angiotensin II, insulin, and EGF. In these experiments, overexpression of catalase causes a dramatic inhibition of the activation of Akt by insulin and angiotensin

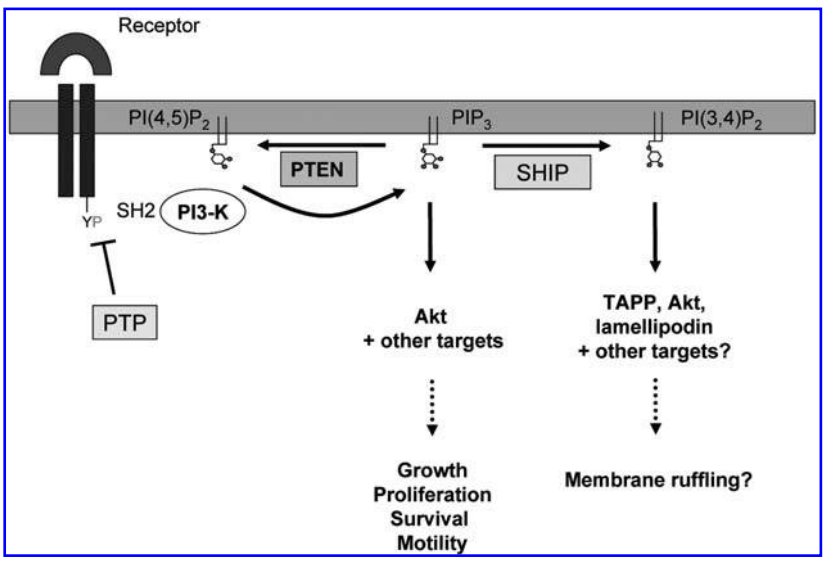

FIG. 1. A simplified model for PI 3-kinase signaling downstream of a tyrosine phosphorylated receptor. Receptor activation and phosphorylation lead to the recruitment and activation of PI 3-kinase enzymes. These phosphorylate the abundant phospholipid PtdIns(4,5)P_2 to generate PtdIns(3,4,5)P_3. This lipid signal in turn binds to its downstream target proteins. The activity of the pathway is opposed both by the action of tyrosine phosphatases that inhibit the activation of PI 3-kinase, and by the action of PTEN, a PtdIns(3,4,5)P_3 3-phosphatase, that converts this signal back to PtdIns(4,5)P_2.

II (55, 78) and overexpression of peroxiredoxin II inhibits EGF-induced Akt phosphorylation (38). Similarly, insulin-stimulated Akt phosphorylation has also been shown to be blocked by DPI treatment (48). A recent study has expanded these points in some detail, showing that in fibroblasts overexpressing the NADPH oxidase component, Nox1, PDGF stimulated PI 3-kinase activity was unaffected, but cellular PtdIns(3,4,5)P_3 levels in PDGF-stimulated cells were greatly increased relative to control cells without Nox1 expression (38). Similarly, overexpression of an inactive mutant of the hydrogen peroxide metabolizing enzyme peroxiredoxin II (PrxII) increased EGF stimulated PtdIns(3,4,5)P_3 levels, whereas overexpression of wild-type PrxII led to a small decrease in both EGF stimulated PtdIns(3,4,5)P_3 levels and Akt phosphorylation (38). Another strategy to address the role of ROS in Akt activation has been the use of two similar neuroblastoma cell lines that differ in their ability to generate ROS in response to stimuli. In these experiments, while both cell lines efficiently activated PI 3-kinase in response to insulin stimulation, only the cell line that accompanied insulin stimulation with stimulus driven ROS generation also efficiently activated Akt (69).

It is worth noting that recent evidence has begun to emerge, supporting that as with many other signal transduction mechanisms, specificity exists within the targets for ROS, regulating the PI 3-kinase and other signaling pathways. For example, stimulation of primary endothelial cells with angiopoietin-1 (Ang-1) leads to the transient accumulation of ROS, as with many other ligands for receptor tyrosine kinases (RTK) and this ROS accumulation appears to play a role in controlling downstream responses to Ang-1 stimulation (25). However, although many downstream signaling responses, such as Ang-1-stimulated PAK1 and MAPK activation, and cell migration, were affected by several methods of experimental antioxidant intervention, Ang-1-stimulated Akt phosphorylation was unaffected by overexpression of catalase, superoxide dismutase, or treatment with peptides that block the activation of NADPH oxidase in these cells (25). Similarly, it has recently been shown that PrxII is specifically recruited to the activated PDGF-R and has a role in the attenuation of specific downstream signals including PLCγ activation (10). MEFs lacking the gene for PrxII display enhanced PDGF stimulated receptor activation, PLCγ activation, proliferation, and migration, but appear unaffected in the phosphorylation of many tyrosine residues on the receptor, and the downstream activation of MAPK and Akt (10).

In addition to a role for low levels of endogenously generated ROS in the normal activation mechanisms of PI 3-kinase signaling by many stimuli, it is clear that chronic oxidative stress has an opposing effect, inhibiting the activation of PI 3-kinase signaling, particularly by insulin and IGF1. There is a strong correlation between chronic oxidative stress and insulin resistance, often seen in diabetic patients, and evidence showing that antioxidant treatment can greatly reduce this resistance, both in cultured cells and in mice and humans (17, 19, 47). However, this broad therapeutic strategy is somewhat inconsistent, and although some molecular mechanisms have been proposed, more research is clearly required to address the mechanisms of oxidative stress-induced insulin resistance and to validate antioxidant intervention as a therapeutic approach (19, 29, 67, 85). Currently, the best supported mechanism appears to be obesity-associated nitrosylation of components of the insulin, PI 3-kinase Akt pathway, although this may be specific to skeletal muscle (7, 61, 85).

Nitric oxide (NO) is a free radical gas that has been appreciated for some years to act as an important biological signaling molecule. When produced by cells, it appears to mediate many of its effects through interaction with the heme group of soluble guanylyl cyclase. However, the regulated generation of nitric oxide has also been proposed to regulate several biological processes through the generation of other reactive nitrogen species (RNS) and the direct oxidation of cellular protein targets. It has become clear that NO plays an important role in muscular insulin resistance and the activation of PI3-kinase-Akt signaling by insulin. The most significant evidence for this is the finding that in mice, deletion of the gene for inducible nitric oxide synthase (iNOS) causes a dramatic protection from obesity-linked insulin resistance in muscle (61). Although the mechanism for this effect is uncertain, candidates have recently been proposed, including nitrosylation of Akt and other pathway components (see below) (7, 85).

It is important to note that in some ways the study of redox signaling is a field in its infancy. The very reversible nature of the modification of cellular components (in most cases described here, protein thiol groups) makes these redox state changes far more difficult to analyze experimentally than, for example, more stable (or stabilizable) posttranslational protein modifications, such as phosphorylation or acetylation. Many studies, by necessity, involve the treatment of cells with high doses of exogenous reactive oxygen and nitrogen species that cause nonspecific oxidation of many cellular components. Although it appears that physiological redox regulation of specific proteins may exhibit the same tightly controlled specificity seen with other signaling systems, sensitive experimental approaches to address these features are only just emerging (10, 24, 28, 56).

MECHANISMS OF REDOX REGULATION

It has been known for several years that cellular stimulation with hydrogen peroxide leads to the rapid activation of the protein kinase, Akt (33, 70), that appears to be reproducible in many cell types. Soon after, it was also shown that hydrogen peroxide stimulation leads to a rapid, though transient, rise in the levels of PtdIns(3,4,5)P_3, and a more sustained, and much larger accumulation of PtdIns(3,4)P_2 (79), both of which lipids can bind to the PH domain of Akt. Similarly, the rapid activation of cellular Akt by nitric oxide donors and peroxynitrite treatment has also been shown (32), although there seems to be slightly less consistency in these effects (85). Further studies described above have indicated that ROS play a role in the normal activation of PI 3-kinase signaling by many stimuli, and are beginning to give us some insight into the mechanisms by which these effects are mediated. These studies strongly suggest that targets for oxidation by ROS exist both above and below the level of PI 3-kinase enzymes. Specifically most of these studies implicate cysteine-dependent phosphatases of the protein tyrosine phosphatase family that act both on tyrosine phosphorylated receptors and

adaptors that oppose the activation of PI 3-kinase (21, 55), and the distantly related PtdIns(3,4,5)P_3 phosphatase PTEN, that directly metabolizes this lipid second messenger (43, 69) (Fig. 2).

There is a clear contrast between the regulation of the signal transduction pathways of higher organisms, and the mechanisms of regulation of simple, direct redox responsive systems, exemplified by the bacterial transcription factor OxyR, which is a direct target for oxidative modification, and regulates target genes participating in an antioxidant response. In contrast, it appears that higher signal transduction networks, such as the PI 3-kinase pathway, or stress activated protein kinase cascades, are often regulated at several points by redox dependent modifications, both through the tightly regulated localized generation of reactive oxygen and nitrogen species, and through cellular oxidative stress (1, 28, 76). It would appear that this reflects the need of such signal transduction networks to integrate information from multiple sources to determine a response.

Cysteine-dependent phosphatases: PTEN and PTPs

Research addressing mechanisms for the redox regulation of PI 3-kinase signaling has largely focused upon the family of cysteine-dependent phosphatases (CDPs), which includes the PI 3-phosphatase, PTEN, and the classical protein tyrosine phosphatases. Strong evidence now supports a physiological role for these molecules as targets of ROS in the normal regulation of PI 3-kinase signaling by many stimuli (45, 63, 64, 77).

The CDPs comprise a large diverse family of enzymes that share a conserved catalytic mechanism based upon a highly reactive cysteine residue in the context of a $C(X)_5R$ motif. The human genome encodes around a hundred separate proteins that appear to be members of this phosphatase family, as well as several proteins lacking significant enzymatic activity, that have sequence identity to these active phosphatases (2, 3). These enzymes include firstly the classical protein tyrosine phosphatases (PTPs; 38 enzymes) that have activity specifically against phosphotyrosine, and the dual specificity PTPs (61 enzymes) capable of dephosphorylating a wider range of substrates, including tyrosine, serine, or threonine residues and some phosphoinositide lipids. This latter group includes PTEN, the myotubularin phosphatases, and possibly the Sac and 4-phosphatase enzymes. In addition, the low molecular weight and cdc25 groups of phosphatases share the minimal catalytic motif and a rather similar three-dimensional structure, but display very different organizational topologies, and so would appear to have evolved independently (2).

It is the conserved catalytic mechanism of the CDPs that makes them good candidates as mediators of cellular redox signaling. The local environment around the active site of these enzymes lowers the pKa of the catalytic cysteine, from around 8 for most cysteine residues, to around 5.5. This is believed to ensure that this cysteine exists as a thiolate anion at physiological pH, and to enhance its reactivity as a nucleophile during catalysis (64, 72) (Fig. 3A). However, these same properties that form the basis for the catalytic mechanism of the CDPs also make the active site cysteine reactive towards oxidizing agents such as reactive oxygen species. This enhanced sensitivity of the CDP active site cysteine relative to most cellular cysteine residues makes redox-dependent inactivation likely as a common mechanism of regulation of these phosphatases, and makes the family likely candidates as direct mediators of many cellular redox signaling pathways (13, 64, 77). Most significantly, a growing body of evidence supports both the role of oxidation in the regulation of these enzymes and their significance in mediating the effects of ROS and RNS on cellular signaling and function.

Several CDPs play important roles in regulating PI 3-kinase signaling, which split into two functional divisions (see Figs. 2 and 3B). The lipid PI 3-phosphatase PTEN metabolizes cellular PtdIns(3,4,5)P_3 and acts in direct opposition to PI 3-kinase, thus plays a principal cellular role inhibiting PI 3-kinase–dependent signaling. Additionally, since the Class 1A PI 3-kinase enzymes are defined by their activation through tyrosine kinase based signaling mechanisms, these activation mechanisms are opposed by protein tyrosine phosphatases,

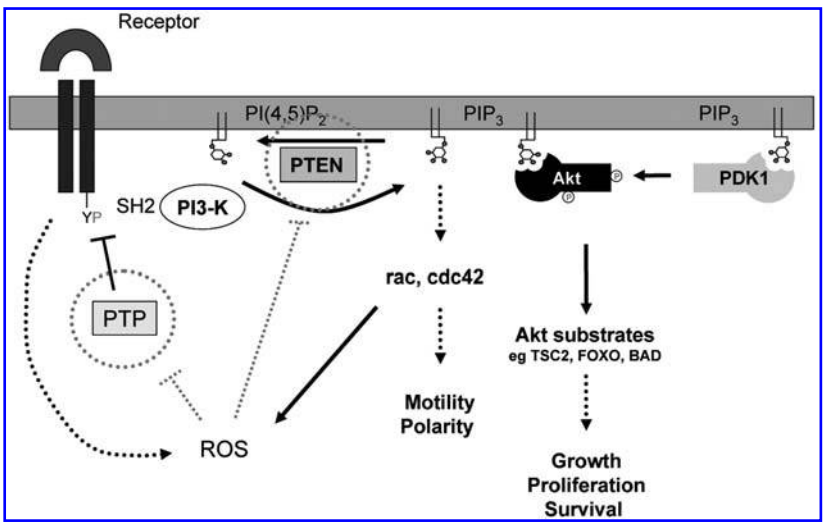

FIG. 2. Role of phosphatase oxidation in the activation of PI 3-kinase signaling. A simplified model for the activation of downstream signaling by PtdIns(3,4,5)P_3 is represented, highlighting the potential inhibitory roles of the oxidant sensitive protein tyrosine phosphatases (PTP) and PTEN. Evidence indicates that these phosphatases can be inactivated by reactive oxygen species (ROS) that are generated in response to receptor activation. The inhibition of PTPs and PTEN may be, at least in part, a temporary positive feedback process, as the receptor-stimulated rac-dependent generation of ROS is in some systems PI 3-kinase dependent, although PI 3-kinase–independent pathways to rac activation and ROS generation appear to exist downstream of some receptors.

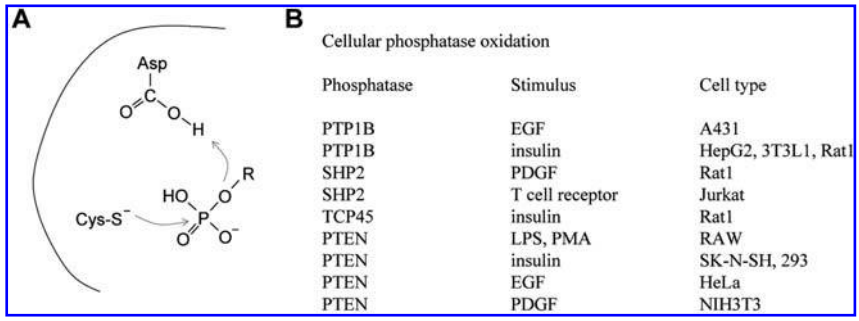

FIG. 3. Cysteine-dependent phosphatase family catalysis and their oxidation relevant to PI 3-kinase signaling. (**A**) The proposed catalytic mechanism of the cysteine-dependent phosphatase family is shown, including the cysteine nucleophile and acidic residue, usually an aspartic acid, required for activity. (**B**) The cellular phosphatases relevant to PI 3-kinase signaling are shown that have been reported to become oxidized upon cellular stimulation, and the cell type in which these experiments were performed. These are detailed further in the text.

and several such phosphatases have been identified as targets in the redox regulation of PI 3-kinase signaling.

PTEN

PTEN is a principal regulator of cellular PtdIns(3,4,5)P_3 levels and inhibitor of PI 3-kinase signaling, and an important tumor suppressor (44, 74). It has been shown that PTEN is sensitive to rapid inactivation *in vitro* by hydrogen peroxide, and in this case can form a stable intramolecular disulfide bond between the active site cysteine (C124) and another proximal cysteine residue (C71), only about 5Å apart in the PTEN crystal structure (42). PTEN has also been shown to be oxidized *in vitro* by *S*-nitrosothiols (87). Significantly, several studies have now shown that PTEN becomes oxidized in cells through endogenous generation of reactive oxygen species (and possibly reactive nitrogen species). This was first addressed in the murine macrophage cell line, RAW 264.7, in which the stimulated production of hydrogen peroxide and other ROS species by the phagocytic NADPH oxidase complex has been well studied. In these macrophages, stimulation with lipopolysaccharide and phorbol ester caused the oxidation of a fraction of the endogenous PTEN protein, as assessed using an alkylation protection assay and detected using a biotinylated alkylating agent, causing this apparent oxidized fraction to rise from 5% to 16% (43).

Most recently, and very significantly, the oxidation of PTEN has been demonstrated in cells stimulated with a range of peptide growth factors, in which the stimulated production of ROS is at greatly lower levels than found in macrophages. PTEN oxidation could be identified in neuroblastoma cells or HEK293 cells stimulated with insulin, in HeLa cells stimulated with EGF, and in fibroblasts stimulated with PDGF, using bandshift assays in addition to biotinylation and alkylation protection assay methods (38, 69). Despite the limited degree of PTEN oxidation in all of these studies, significant data support the functional significance of this PTEN oxidation. In PTEN null cells experimentally re-expressing PTEN from a virus, as with many other cell lines, acute stimulation with hydrogen peroxide caused a robust increase in cellular PtdIns(3,4,5)P_3 levels and downstream Akt/PKB activation. However, the original PTEN null cells neither accumulated PtdIns(3,4,5)P_3 nor activated Akt/PKB upon hydrogen peroxide stimulation (although turnover of the lipid is almost certainly increased), indicating the significance specifically of PTEN oxidation in the regulation of PtdIns(3,4,5)P_3 levels in these experiments (43). Similarly, in neuroblastoma cells in which insulin-stimulated PTEN oxidation could be detected, although the activation of PI 3-kinase was unaffected by insulin-stimulated ROS production, the activation of Akt/PKB was greatly reduced in cells not able to produce ROS upon insulin stimulation (69). These data indicate that insulin-stimulated ROS production appears not to affect the PI 3-kinase–dependent synthesis of PtdIns(3,4,5)P_3, but rather reduce the degradative capacity, strongly suggesting functionally significant PTEN inhibition.

PTEN oxidation has also been identified in cells overexpressing MnSOD, leading to downstream regulation of VEGF expression and angiogenesis (11). For the first time, this work implicates mitochondrial H_2O_2 in regulating PTEN, and also provides a novel mechanism by which ROS may regulate angiogenesis (11, 37, 51). Together, all these data support the hypothesis that PTEN is a direct target for oxidative inactivation by ROS produced in response to diverse cellular stimuli, and that this inactivation is significant in the regulation of downstream signaling responses.

Protein tyrosine phosphatases: PTP1B, SHP-2, and TC45

The PI 3-kinase signaling pathway as considered here relies upon the synthesis of PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2 by the action of Class I PI 3-kinase enzymes (80). The class IB p110γ enzyme is characterized by its activation by G-protein coupled receptor signaling through the interaction of G-protein βγ subunits with the p101 or p84 PI 3-kinase regulatory subunits (73). On the other hand, Class IA PI 3-kinases (p110α, β, and δ) are characterized by their recruitment and activation by tyrosine kinase signaling pathways via the SH2 domains of their corresponding regulatory subunits (p85 or p55) (80). This activation by tyrosine kinases is counteracted and suppressed in the unstimulated state by the action of PTP enzymes that, as described above, allows redox regulation through inhibition of these phosphatases (9, 84). Since these PTPs act on the signaling pathways stimulating the activation of PI 3-kinase, their actions are usually specific to particular stimuli, and as such, experimental approaches have tended to address the role of particular phosphatases in the regulation

by a particular ligand of several downstream signaling pathways including the PI 3-kinase/Akt axis.

Oxidation of PTPs and the regulation of PI 3-kinase activation by growth factors

Several studies have identified PTP1B and recently TC45 as targets for ROS-dependent oxidation during the activation of the PI 3-kinase pathway by insulin and other growth factors. PTP1B was the first CDP demonstrated to become oxidized by endogenously produced ROS, in A431 cells stimulated with EGF. This important study used radiolabeled iodoacetic acid to alkylate the active site cysteine of PTP1B, and showed that stimulation with EGF reduced the labeling by around 30% (41). Further studies have shown PTP1B oxidation in response to insulin, TC45 oxidation by insulin stimulation, SHP2 oxidation in response to PDGF stimulation or T-cell receptor engagement. and indeed inhibition of total PTP activity in response to insulin (39, 48, 49, 55, 56). These studies have used a number of different approaches in addition to loss of alkylating agent labeling, including loss of assayable activity after lysis and purification in anaerobic conditions and an apparent ROS-dependent loss of assayable activity despite the presence of reducing agents in the lysis buffer (which could be taken to indicate irreversible oxidation). One development in this technology was the use of an assay by Meng *et al*., who lysed cells in buffers containing unlabeled iodoacetic acid to alkylate any reduced PTPs. These phosphatases could then separated from the alkylating agent by gel electrophoresis and washing, before reducing and assaying in gel for the activity of phosphatases that had been protected from alkylation by the formation of a stable oxidized species (56). This, and subsequent adaptations to the method, have allowed the identification of phosphatase oxidation as a gain of assayable signal from a low background, rather than a less sensitive loss of total cellular activity.

DIRECT OXIDATION OF PATHWAY KINASES

PKB/Akt

Several studies have proposed that the PtdIns(3,4,5)P_3-dependent serine/threonine kinase Akt/Protein Kinase B is directly regulated through oxidation. The determination of the crystal structure of Akt revealed the unusual feature that the kinase domain of Akt contained an intermolecular disulphide bond between two cysteine residues (Cys297 and Cys311 of Akt2) at each end of the functionally critical activation loop (26). Further work on this aspect of the regulation of Akt has shown that the formation of this disulfide is induced in cultured cells during oxidative stress induced by 100 μ*M* hydrogen peroxide, but significantly that formation of this disulfide was inhibited by cellular overexpression of glutaredoxin (GRX) (58). An insight into how this disulfide regulates Akt function was also provided by this study, as although this oxidation did not appear to affect the kinase activity of purified Akt *in vitro*, oxidative stress induced not only the oxidation of Akt, but its interaction with the protein phosphatase PP2A, believed to dephosphorylate and inactivate Akt. Akt oxidation, inactivation, and PP2A recruitment were all inhibited by glutaredoxin overexpression, suggesting that only the oxidized disulfided kinase is able to interact with PP2A, and that this interaction regulates the phosphorylation and activity of the kinase (58). Since these two cysteine residues in the Akt activation loop are conserved in several other kinases, it will be interesting to see whether this redox regulation of protein kinase activity by PP2A phosphatase recruitment is a widespread phenomenon.

In accordance with this model for a redox-regulated disulfide-dependent interaction of Akt with PP2A, this interaction has also been shown to be blocked by the thiol alkylating agent *N*-ethylmaleimide (86). However, these authors generally found that thiol alkylation blocked Akt activation, although the target(s) for this effect was not identified (86).

Most recently, Yasukawa *et al.* proposed that Cys244 is a site for regulatory nitrosylation and that this nitrosylation could be important in insulin resistance in diabetic and obese muscle. This study showed that incubation of cells with the nitric oxide donor, SNAP, caused Akt nitrosylation, inhibited its kinase activity, and blocked the phosphorylation of the Akt substrate Bad without affecting PI 3-kinase activity or the activating phosphorylation of PKB on Thr308 and Ser473. Further analysis identified Cys244 as a functionally significant site of nitrosylation, as mutation of Cys244 to seine blocked the nitrosylation and inactivation of PKB by SNAP (85). This study also revealed enhanced nitrosylation of Akt in the skeletal muscle of diabetic genetically obese mice that appeared to play a part in the insulin resistance of Akt in these mice (85). These results appear to be closely linked to work identifying nitrosylation of the insulin receptor, the insulin receptor substrate 1 (IRS1) and Akt in obese muscle, and may provide an important insight into the mechanisms by which chronic oxidative stress induced insulin resistance (7). This latter study also found that the obesity-associated nitrosylation of the insulin receptor, IRS1, and Akt were all reversed by antisense knockdown of iNOS expression (7). Together, these studies strongly suggest a role for reactive nitrogen species in obesity-associated insulin resistance in muscle.

It is worthy of note that both of these studies saw a reduction in Akt activity upon cellular treatment with the NO donors GSNO or SNAP, and no evidence for effects on Akt protein levels over periods up to 24 h (7, 85). Other studies have indicated that peroxynitrite treatment can activate Akt (32), and that ROS (not RNS) may be involved in the regulation of Akt protein stability, although the role of ROS in these studies may only be as an inducer of caspases and other apoptotic proteolytic programmes (50, 53).

PI 3-kinase p85 regulatory subunit

The p85 subunit of PI 3-kinase has recently been proposed as a direct target for tyrosine nitration. In a study addressing the oxidative mechanisms by which high glucose hyperglycemia induces the apoptosis of endothelial cells, the authors demonstrated that either high glucose incubation (25 m*M*) or peroxynitrite incubation (500 μ*M*) blocked the activation of the PI

3-kinase-Akt cell survival pathway (16). They also showed that these treatments caused the dissociation of the p85 and p110 subunits of PI 3-kinase, but not in cells treated with the peroxynitrite decomposition catalyst FeTPPS. Although the antibodies available for the detection of nitrotyrosine are rather poor, they also showed convincing direct data for the increased tyrosine nitration of p85 in response to either high glucose or peroxynitrite (16).

mTOR

The mammalian target of rapamycin (mTOR) kinase plays a central role in the regulation of cell growth through nutrient availability in eukaryotes. The relationship of mTOR with PI 3-kinase and Akt is complex, as mTOR is regulated at least in part through upstream PI 3-kinase and Akt signaling via the TSC proteins and Rheb, and mediates many of the effects of PI 3-kinase on cell growth, but also may play a role in the activation of Akt (52, 65). The regulation of mTOR has proved technically difficult to study, due to its large size, complexity, and the difficulty of assaying its activity *in vitro*. However, structural analysis of the C-terminal FATC domain revealed a disulfide bond between two conserved cysteine residues that appeared to regulate the flexibility of a loop within this domain, correlating with an apparent change in the stability of the whole protein (12). These authors proposed that this could be a mechanism by which cellular redox state can regulate the abundance and thus activity of mTOR (12).

CONCLUSION

It should be clear that we have begun to uncover mechanistic aspects explaining the roles of ROS and RNS in cell signaling. However, it remains the case that very few direct targets for redox modification have been identified that can be tied to individual biological effects, despite the strong evidence that such targets exist. Significantly, proteomic approaches to the identification of redox modified proteins are beginning to bear fruit (20, 60, 66).

An area of great general interest that seems almost certain to continue in the future, is the role of ROS and PI 3-kinase signaling in the regulation of the aging process. Several cellular studies have indicated that signaling via PI 3-kinase and Akt, through inhibition of FOXO transcription factors, suppress cellular antioxidant activity, particularly through inhibiting the expression of MnSOD (34) and catalase (59). It has been proposed that these effects may play an important role in the regulation of aging within the whole organism (31, 34), but although it seems clear that the insulin/IGF pathway through PI 3-kinase plays a role in regulating life span, molecular details of the aging process remain elusive.

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ABBREVIATIONS

CDP, cysteine-dependent phosphatase; EGF, epidermal growth factor; GSNO, *S*-nitrosoglutathione; IGF, insulin-like growth factor; iNOS, inducible nitric oxide synthase; PDGF, platelet derived growth factor; PI 3-kinase, phosphoinositide 3-kinase; PLCγ, phospholipase C gamma; PTP, protein tyrosine phosphatase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SOD, superoxide dismutase.

REFERENCES

1. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, and Ronai Z. Regulation of JNK signaling by GSTp. *EMBO J* 18: 1321–1334, 1999.
2. Alonso A, Sasin J, Bottini N, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, and Mustelin T. Protein tyrosine phosphatases in the human genome. *Cell* 117: 699–711, 2004.
3. Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R, Tonks NK, and Moller NP. A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. *FASEB J* 18: 8–30 2004.
4. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, and Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* 272: 217–221, 1997.
5. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 296: 1655–1657, 2002.
6. Cantrell DA. Phosphoinositide 3-kinase signaling pathways. *J Cell Sci* 114: 1439–1445, 2001.
7. Carvalho–Filho MA, Ueno M, Hirabara SM, Seabra AB, Carvalheira JB, de Oliveira MG, Velloso LA, Curi R, and Saad MJ. *S*-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt: a novel mechanism of insulin resistance. *Diabetes* 54: 959–967, 2005.
8. Chen Q, Olashaw N, and Wu J. Participation of reactive oxygen species in the lysophosphatidic acid-stimulated mitogen-activated protein kinase kinase activation pathway. *J Biol Chem* 270: 28499–28502, 1995.
9. Chiarugi P and Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 28: 509–514, 2003.
10. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C, Bae YS, Lee BI, Rhee SG, and Kang SW. Regulation of PDGF signaling and vascular remodelling by peroxiredoxin II. *Nature* 435: 347–353, 2005.
11. Connor KM, Subbaram S, Regan KJ, Nelson KK, Mazurkiewicz JE, Bartholomew PJ, Aplin AE, Tai YT,

Aguirre–Ghiso J, Flores SC, and Melendez JA. Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation. *J Biol Chem* 280: 16916–16924, 2005.
12. Dames SA, Mulet JM, Rathgeb–Szabo K, Hall MN, and Grzesiek S. The solution structure of the FATC domain of the protein kinase target of rapamycin suggests a role for redox-dependent structural and cellular stability. *J Biol Chem* 280:20558–20564, 2005.
13. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 533–542, 1998.
14. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
15. Dyson JM, Kong AM, Wiradjaja F, Astle MV, Gurung R, and Mitchell CA. The SH2 domain containing inositol polyphosphate 5-phosphatase-2: SHIP2. *Int J Biochem Cell Biol* 37:2260–2265, 2005.
16. el-Remessy AB, Bartoli M, Platt DH, Fulton D, and Caldwell RB. Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. *J Cell Sci* 118: 243–252, 2005.
17. Evans JL, Goldfine ID, Maddux BA and Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 52: 1–8, 2003.
18. Finkel T. Redox-dependent signal transduction. *FEBS Lett* 476: 52–54 2000.
19. Frank GD, Eguchi S, and Motley ED. The role of reactive oxygen species in insulin signaling in the vasculature. *Antioxid Redox Signal* 7: 1053–1061, 2005.
20. Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M, Bonetto V, Mengozzi M, Duffieux F, Miclet E, Bachi A, Vandekerckhove J, Gianazza E, and Ghezzi P. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc Natl Acad Sci USA* 99: 3505–3510, 2002.
21. Goldstein BJ, Mahadev K, and Wu X. Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* 54: 311–321, 2005.
22. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
23. Halstead JR, Roefs M, Ellson CD, D'Andrea S, Chen C, D'Santos CS, and Divecha N. A novel pathway of cellular phosphatidylinositol(3,4,5)–trisphosphate synthesis is regulated by oxidative stress. *Curr Biol* 11: 386–395, 2001.
24. Hanson GT, Aggeler R, Oglesbee D, Cannon M, Capaldi RA, Tsien RY, and Remington SJ. Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators. *J Biol Chem* 279: 13044–13053, 2004.
25. Harfouche R, Abdel–Malak NA, Brandes RP, Karsan A, Irani K, and Hussain SN. Roles of reactive oxygen species in angiopoietin-1/tie-2 receptor signaling. *FASEB J* 19: 1728–1730, 2005.
26. Huang X., Begley M, Morgenstern KA, Gu Y, Rose P, Zhao H, and Zhu X. Crystal structure of an inactive Akt2 kinase domain. *Structure (Camb)* 11: 21–30, 2003.
27. Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, Fearon ER, Sundaresan M, Finkel T, and Goldschmidt–Clermont PJ. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. *Science* 275: 1649–1652, 1997.
28. Kamata H, Honda S, Maeda S, Chang L, Hirata H, and Karin M. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120: 649–661, 2005.
29. Kaneto H, Kawamori D, Nakatani Y, Gorogawa S, and Matsuoka TA. Oxidative stress and the JNK pathway as a potential therapeutic target for diabetes. *Drug News Perspect* 17: 447–453, 2004.
30. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, and Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 17: 615–675, 2001.
31. Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell* 120: 449–460, 2005.
32. Klotz LO, Schieke SM, Sies H, and Holbrook NJ. Peroxynitrite activates the phosphoinositide 3-kinase/Akt pathway in human skin primary fibroblasts. *Biochem J* 352: 219–225, 2000.
33. Konishi H, Matsuzaki H, Tanaka M, Takemura Y, Kuroda S, Ono Y, and Kikkawa U. Activation of protein kinase B (Akt/RAC-protein kinase) by cellular stress and its association with heat shock protein Hsp27. *FEBS Lett* 410: 493–498, 1997.
34. Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, and Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419: 316–321, 2002.
35. Krieger–Brauer HI and Kather H. Antagonistic effects of different members of the fibroblast and platelet-derived growth factor families on adipose conversion and NADPH-dependent H2O2 generation in 3T3 L1-cells. *Biochem J* 307: 549–556, 1995.
36. Krieger–Brauer HI and Kather H. Human fat cells possess a plasma membrane-bound H2O2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. *J Clin Invest* 89: 1006–1013, 1992.
37. Kuroki M, Voest EE, Amano S, Beerepoot LV, Takashima S, Tolentino M, Kim RY, Rohan RM, Colby KA, Yeo KT, and Adamis AP. Reactive oxygen intermediates increase vascular endothelial growth factor expression *in vitro* and *in vivo*. *J Clin Invest* 98: 1667–1675, 1996.
38. Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, and Rhee SG. Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. *Proc Natl Acad Sci USA* 101: 16419–16424, 2004.
39. Kwon J, Qu CK, Maeng JS, Falahati R, Lee C, and Williams MS. Receptor-stimulated oxidation of SHP-2 promotes T-cell adhesion through SLP-76-ADAP. *EMBO J* 24: 2331–2341, 2005.
40. Lawlor MA and Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J Cell Sci* 114: 2903–2910, 2001.
41. Lee SR, Kwon KS, Kim SR, and Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431

cells stimulated with epidermal growth factor. *J Biol Chem* 273: 15366–15372, 1998.
42. Lee SR, Yang KS, Kwon J, Lee C, Jeong W, and Rhee SG. Reversible inactivation of the tumor suppressor PTEN by H2O2. *J Biol Chem* 277: 20336–20342, 2002.
43. Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, and Downes CP. Redox regulation of PI 3-kinase signaling via inactivation of PTEN. *EMBO J* 22: 5501–5510, 2003.
44. Leslie NR and Downes CP. PTEN: The down side of PI 3-kinase signaling. *Cell Signal* 14: 285–295, 2002.
45. Leslie NR, Lindsay Y, Ross SH, and Downes CP. Redox regulation of phosphatase function. *Biochem Soc Trans* 32: 1018–1020, 2004.
46. Lo YY and Cruz TF. Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. *J Biol Chem* 270: 11727–11730, 1995.
47. Maddux BA, See W, Lawrence JC Jr, Goldfine AL, Goldfine ID, and Evans JL. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of alpha-lipoic acid. *Diabetes* 50: 404–410, 2001.
48. Mahadev K, Wu X, Zilbering A, Zhu L, Lawrence JT, and Goldstein BJ. Hydrogen peroxide generated during cellular insulin stimulation is integral to activation of the distal insulin signaling cascade in 3T3-L1 adipocytes. *J Biol Chem* 276: 48662–48669, 2001.
49. Mahadev K, Zilbering A, Zhu L, and Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b *in vivo* and enhances the early insulin action cascade. *J Biol Chem* 276: 21938–21942, 2001.
50. Martin D, Salinas M, Fujita N, Tsuruo T, and Cuadrado A. Ceramide and reactive oxygen species generated by H2O2 induce caspase-3-independent degradation of Akt/protein kinase B. *J Biol Chem* 277: 42943–42952, 2002.
51. Maulik N. Redox signaling of angiogenesis. *Antioxid Redox Signal* 4: 805–815, 2002.
52. McManus EJ and Alessi DR. TSC1-TSC2: a complex tale of PKB-mediated S6K regulation. *Nat Cell Biol* 4: E214–E216, 2002.
53. Medina EA, Afsari RR, Ravid T, Castillo SS, Erickson KL, and Goldkorn T. Tumor necrosis factor-{alpha} decreases Akt protein levels in 3T3-L1 adipocytes via the caspase-dependent ubiquitination of Akt. *Endocrinology* 146: 2726–2735, 2005.
54. Meier B, Radeke HH, Selle S, Younes M, Sies H, Resch K, and Habermehl GG. Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumor necrosis factor-alpha. *Biochem J* 263: 539–545, 1989.
55. Meng TC, Buckley DA, Galic S, Tiganis T, and Tonks NK. Regulation of insulin signaling through reversible oxidation of the protein tyrosine phosphatases TC45 and PTP1B. *J Biol Chem* 279: 37716–37725, 2004.
56. Meng TC, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol Cell* 9: 387–399, 2002.
57. Mitra P, Zhang Y, Rameh LE, Ivshina MP, McCollum D, Nunnari JJ, Hendricks GM, Kerr ML, Field SJ, Cantley LC, and Ross AH. A novel phosphatidylinositol(3,4,5)P3 pathway in fission yeast. *J Cell Biol* 166: 205–211, 2004.
58. Murata H, Ihara Y, Nakamura H, Yodoi J, Sumikawa K, and Kondo T. Glutaredoxin exerts an antiapoptotic effect by regulating the redox state of Akt. *J Biol Chem* 278: 50226–50233, 2003.
59. Nemoto S and Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295: 2450–2452, 2002.
60. Paron I, D'Elia A, D'Ambrosio C, Scaloni A, D'Aurizio F, Prescott A, Damante G, and Tell G. A proteomic approach to identify early molecular targets of oxidative stress in human epithelial lens cells. *Biochem J* 378: 929–937, 2004.
61. Perreault M and Marette A. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med* 7: 1138–1143, 2001.
62. Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med* 31: 53–59, 1999.
63. Rhee SG, Kang SW, Jeong W, Chang TS, Yang KS, and Woo HA. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr Opin Cell Biol* 17: 183–189, 2005.
64. Salmeen A and Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. *Antioxid Redox Signal* 7: 560–677, 2005.
65. Sarbassov DD, Guertin DA, Ali SM, and Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307: 1098–1101, 2005.
66. Saurin AT, Neubert H, Brennan JP, and Eaton P. Widespread sulfenic acid formation in tissues in response to hydrogen peroxide. *Proc Natl Acad Sci USA* 101: 17982–17987, 2004.
67. Scott JA and King GL. Oxidative stress and antioxidant treatment in diabetes. *Ann NY Acad Sci* 1031: 204–213, 2004.
68. Seminario MC and Wange RL. Signaling pathways of D3-phosphoinositide-binding kinases in T cells and their regulation by PTEN. *Semin Immunol* 14: 27–36, 2002
69. Seo JH, Ahn Y, Lee SR, Yeol Yeo C, and Chung Hur K. The major target of the endogenously generated reactive oxygen species in response to insulin stimulation is phosphatase and tensin homolog and not phosphoinositide-3 kinase (PI-3 kinase) in the PI-3 kinase/Akt pathway. *Mol Biol Cell* 16: 348–357, 2005.
70. Shaw M, Cohen P, and Alessi DR. The activation of protein kinase B by H2O2 or heat shock is mediated by phosphoinositide 3-kinase and not by mitogen-activated protein kinase-activated protein kinase-2. *Biochem J* 336: 241–246, 1998.
71. Stephens L, Williams R, and Hawkins P. Phosphoinositide 3-kinases as drug targets in cancer. *Curr Opin Pharmacol* 5: 357–365, 2005.
72. Stone RL and Dixon JE. Protein-tyrosine phosphatases. *J Biol Chem* 269: 31323–31326, 1994.
73. Suire S, Coadwell J, Ferguson GJ, Davidson K, Hawkins P, and Stephens L. p84, a new Gbetagamma-activated regulatory subunit of the type IB phosphoinositide 3-kinase p110gamma. *Curr Biol* 15: 566–570, 2005.
74. Sulis ML and Parsons R. PTEN: from pathology to biology. *Trends Cell Biol* 13: 478–483, 2003.
75. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270: 296–269, 1995.

76. Takeda K, Matsuzawa A, Nishitoh H, and Ichijo H. Roles of MAPKKK ASK1 in stress-induced cell death. *Cell Struct Funct* 28: 23–29, 2003.
77. Tonks NK. Redox redux: revisiting PTPs and the control of cell signaling. *Cell* 121: 667–670, 2005.
78. Ushio–Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, and Griendling KK. Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. *J Biol Chem* 274: 22699–22704, 1999.
79. Van der Kaay J, Beck M, Gray A, and Downes CP. Distinct phosphatidylinositol 3-kinase lipid products accumulate upon oxidative and osmotic stress and lead to different cellular responses. *J Biol Chem* 274: 35963–35968, 1999.
80. Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, and Waterfield MD. Synthesis and function of 3-phosphorylated inositol lipids. *Annu Rev Biochem* 70: 535–602, 2001.
81. Vivanco I and Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2: 489–501, 2002.
82. Welch HC, Coadwell WJ, Stephens LR, and Hawkins PT. Phosphoinositide 3-kinase-dependent activation of Rac. *FEBS Lett* 546: 93–97, 2003.
83. Wymann MP and Pirola L. Structure and function of phosphoinositide 3-kinases. *Biochim Biophys Acta* 1436: 127–150, 1998.
84. Xu D, Rovira II, and Finkel T. Oxidants painting the cysteine chapel: redox regulation of PTPs. *Dev Cell* 2: 251–252, 2002.
85. Yasukawa T, Tokunaga E, Ota H, Sugita H, Martyn JA, and Kaneki M. *S*-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. *J Biol Chem* 280: 7511–7518, 2005.
86. Yellaturu CR, Bhanoori M, Neeli I, and Rao GN. *N*-Ethylmaleimide inhibits platelet-derived growth factor BB-stimulated Akt phosphorylation via activation of protein phosphatase 2A. *J Biol Chem* 277: 40148–40155, 2002.
87. Yu CX, Li S, and Whorton AR. Redox regulation of PTEN by *S*-nitrosothiols. *Mol Pharmacol* 68: 847–854, 2005.

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3. Ji Hye Kim , Seung-Yong Song , Sang Gyu Park , Sun U. Song , Ying Xia , Jong-Hyuk Sung . 2012. Primary Involvement of NADPH Oxidase 4 in Hypoxia-Induced Generation of Reactive Oxygen Species in Adipose-Derived Stem Cells. *Stem Cells and Development* **21**:12, 2212-2221. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
4. Jue Cui, Ying Xiao, Yong-Hui Shi, Bin Wang, Guo-Wei Le. 2012. Lipoic acid attenuates high-fat-diet–induced oxidative stress and B-cell–related immune depression. *Nutrition* **28**:3, 275-280. [CrossRef]
5. Linlin Li, Yinan Hua, Jun Ren. 2012. Short-Chain Fatty Acid Propionate Alleviates Akt2 Knockout-Induced Myocardial Contractile Dysfunction. *Experimental Diabetes Research* **2012**, 1-10. [CrossRef]
6. Mohamed Kodiha, Ursula Stochaj. 2012. Nuclear Transport: A Switch for the Oxidative Stress—Signaling Circuit?. *Journal of Signal Transduction* **2012**, 1-18. [CrossRef]
7. Lide Arana, Patricia Gangoiti, Alberto Ouro, Io-Guané Rivera, Marta Ordoñez, Miguel Trueba, Ravi S. Lankalapalli, Robert Bittman, Antonio Gomez-Muñoz. 2011. Generation of reactive oxygen species (ROS) is a key factor for stimulation of macrophage proliferation by ceramide 1-phosphate. *Experimental Cell Research* . [CrossRef]
8. Sang Gyu Park, Ji Hye Kim, Ying Xia, Jong-Hyuk Sung. 2011. Generation of reactive oxygen species in adipose-derived stem cells: friend or foe?. *Expert Opinion on Therapeutic Targets* 1-10. [CrossRef]
9. Rajindar S. Sohal, William C. Orr. 2011. The redox stress hypothesis of aging. *Free Radical Biology and Medicine* . [CrossRef]
10. María Rodríguez-Muñoz , Elena de la Torre-Madrid , Pilar Sánchez-Blázquez , Javier Garzón . 2011. NO-released Zinc Supports the Simultaneous Binding of Raf-1 and PKC# Cysteine-Rich Domains to HINT1 Protein at the Mu-Opioid Receptor. *Antioxidants & Redox Signaling* **14**:12, 2413-2425. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. Jan Lewerenz , Pamela Maher . 2011. Control of Redox State and Redox Signaling by Neural Antioxidant Systems. *Antioxidants & Redox Signaling* **14**:8, 1449-1465. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Janel E. Le Belle, Nicolas M. Orozco, Andres A. Paucar, Jonathan P. Saxe, Jack Mottahedeh, April D. Pyle, Hong Wu, Harley I. Kornblum. 2011. Proliferative Neural Stem Cells Have High Endogenous ROS Levels that Regulate Self-Renewal and Neurogenesis in a PI3K/Akt-Dependant Manner. *Cell Stem Cell* **8**:1, 59-71. [CrossRef]
13. Ercüment Aksakal, Saltuk Bu#rahan Ceyhun, Orhan Erdo#an, Deniz Ekinci. 2010. Acute and long-term genotoxicity of deltamethrin to insulin-like growth factors and growth hormone in rainbow trout. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **152**:4, 451-455. [CrossRef]
14. Thomas E. Rohan, Lee-Jun Wong, Tao Wang, Jonathan Haines, Geoffrey C. Kabat. 2010. Do Alterations in Mitochondrial DNA Play a Role in Breast Carcinogenesis?. *Journal of Oncology* **2010**, 1-11. [CrossRef]
15. Georg T. Wondrak . 2009. Redox-Directed Cancer Therapeutics: Molecular Mechanisms and Opportunities. *Antioxidants & Redox Signaling* **11**:12, 3013-3069. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
16. Samuel W. Fan, Richard A. George, Naomi L. Haworth, Lina L. Feng, Jason Y. Liu, Merridee A. Wouters. 2009. Conformational changes in redox pairs of protein structures. *Protein Science* **18**:8, 1745-1765. [CrossRef]
17. Masashi Hasegawa, Tohru Ogihara, Hiroshi Tamai, Mayo Hiroi. 2009. Hypothermic inhibition of apoptotic pathways for combined neurotoxicity of iron and ascorbic acid in differentiated PC12 cells: Reduction of oxidative stress and maintenance of the glutathione redox state. *Brain Research* **1283**, 1-13. [CrossRef]
18. T. Cindrova-Davies. 2009. Gabor Than Award Lecture 2008: Pre-eclampsia – From Placental Oxidative Stress to Maternal Endothelial Dysfunction. *Placenta* **30**, 55-65. [CrossRef]
19. Andrean L. Simons, Arlene D. Parsons, Katherine A. Foster, Kevin P. Orcutt, Melissa A. Fath, Douglas R. Spitz. 2009. Inhibition of Glutathione and Thioredoxin Metabolism Enhances Sensitivity to Perifosine in Head and Neck Cancer Cells. *Journal of Oncology* **2009**, 1-10. [CrossRef]
20. Jue Cui, Guowei Le, Ruili Yang, Yonghui Shi. 2009. Lipoic acid attenuates high fat diet-induced chronic oxidative stress and immunosuppression in mice jejunum: A microarray analysis. *Cellular Immunology* **260**:1, 44-50. [CrossRef]
21. Bing#Hua Jiang, Ling#Zhi LiuChapter 2 PI3K/PTEN Signaling in Angiogenesis and Tumorigenesis **102**, 19-65. [CrossRef]

22. N R Leslie, I H Batty, H Maccario, L Davidson, C P Downes. 2008. Understanding PTEN regulation: PIP2, polarity and protein stability. *Oncogene* **27**:41, 5464-5476. [CrossRef]

23. Dunyaporn Trachootham , Weiqin Lu , Marcia A. Ogasawara , Nilsa Rivera-Del Valle , Peng Huang . 2008. Redox Regulation of Cell Survival. *Antioxidants & Redox Signaling* **10**:8, 1343-1374. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

24. Xinjiang Wang, Xuejun Jiang. 2008. PTEN: a default gate-keeping tumor suppressor with a versatile tail. *Cell Research* **18**:8, 807-816. [CrossRef]

25. Cecilia Hidalgo , Paulina Donoso . 2008. Crosstalk Between Calcium and Redox Signaling: From Molecular Mechanisms to Health Implications. *Antioxidants & Redox Signaling* **10**:7, 1275-1312. [Abstract] [Full Text PDF] [Full Text PDF with Links]

26. Daphne Haas-Kogan, David Stokoe. 2008. PTEN in brain tumors. *Expert Review of Neurotherapeutics* **8**:4, 599-610. [CrossRef]

27. Jae-Woong HWANG, Ji-Won JUNG, Yong-Soon LEE, Kyung-Sun KANG. 2008. Indole-3-Carbinol Prevents H2O 2-Induced Inhibition of Gap Junctional Intercellular Communication by Inactivation of PKB/Akt. *Journal of Veterinary Medical Science* **70**:10, 1057-1063. [CrossRef]

28. Jean-Marc Zingg. 2007. Modulation of signal transduction by vitamin E. *Molecular Aspects of Medicine* **28**:5-6, 481-506. [CrossRef]

29. Masaki Kashiwada, Ping Lu, Paul B. Rothman. 2007. PIP3 pathway in regulatory T cells and autoimmunity. *Immunologic Research* **39**:1-3, 194-224. [CrossRef]

30. James A. McCubrey , Richard A. Franklin . 2006. Reactive Oxygen Intermediates and Signaling Through Kinase Pathways. *Antioxidants & Redox Signaling* **8**:9-10, 1745-1748. [Citation] [Full Text PDF] [Full Text PDF with Links]