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Induction of mitochondrial ROS production by electrophilic lipids: a new pathway of redox signaling?

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REACTIVE OXYGEN SPECIES (ROS), such as superoxide or hydrogen peroxide, are usually thought of as damaging agents that nonspecifically disrupt proteins, lipids, and nucleic acids (1). However, there is now considerable evidence that ROS also act as signaling agents in a range of biological pathways (2, 5, 6). An example of redox signaling is the OxyR pathway in *Escherichia coli,* which responds to hydrogen peroxide by increasing the expression of antioxidant defense enzymes (5, 10). The OxyR protein contains exposed thiols that react with hydrogen peroxide to form an intraprotein disulfide. The oxidized OxyR protein acts as a transcription factor increasing the transcription of a range of antioxidant pathways (5). The disulfide bonds on oxidized OxyR are reduced by intracellular reductants such as thioredoxin or glutaredoxin, so once the oxidative stress has passed, OxyR returns to its inactive, reduced state, enabling a reversible response to oxidative stress (10). Whereas it is clearly important to sense and respond to ROS for the induction of antioxidant defenses, ROS signaling plays a wider role in metabolism, and ROS production is thought to be regulated as part of redox signaling pathways (5, 7). One way this occurs is by the activation of cell surface NADPH oxidases that produce superoxide that dismutates rapidly to hydrogen peroxide (5, 7). ROS production by these oxidases is activated by a number of cell surface receptors, such as those for epidermal growth factor and platelet-derived growth factor, via G proteins such as Rac (7, 8). The ROS produced then acts on a number of downstream signaling pathway components, such as phosphatases, transcription factors, and kinases (8). In many situations, these proteins sense increased ROS production by the reversible modification of reactive cysteine residues, just as occurs for OxyR (5).

The mitochondrial respiratory chain is the major source of ROS within most cells, and the ROS produced from respiratory chain complexes I and III are of pathological importance in a range of degenerative diseases (6, 14, 15). However, ROS production by mitochondria is increasingly being implicated in redox signaling (6), although its significance and mechanism of production remain obscure. A particularly intriguing aspect of mitochondrial ROS production is that ROS from the plasma membrane or from exogenous oxidants seem to induce further ROS production from the respiratory chain (3, 12, 13, 17). The reasons for this are unclear, but it may be a mechanism to amplify or sustain an initiating ROS signal from outside the mitochondrion (13).

In this issue of *American Journal of Physiology-Heart and Circulatory Physiology*, the paper by Landar et al. (9) is a particularly interesting contribution to our understanding of mitochondrial redox signaling. In earlier work (16), these authors had shown that exposure of endothelial cells to oxidized low-density lipoprotein (oxLDL) increased mitochondrial ROS production. They hypothesized that as the breakdown products of lipid peroxidation mimic the toxic effects of oxLDL, these lipid breakdown products might be the oxLDL component that increased mitochondrial ROS production (11). The lipid peroxidation products of most interest were electrophilic lipids, which have reactive carbon atoms and thereby react with nucleophiles to form stable products. Most typically, electrophilic lipids react with the exposed thiols of protein cysteine residues to form adducts. High levels of electrophilic lipids cause cell death, but at lower concentrations they can act as signaling molecules by modifying reactive thiols on proteins in much the same way as ROS (11). For example, the cytosolic Kelch-like ECH-associated protein-1 (Keap-1) contains reactive thiols that react with electrophilic lipids to form an adduct (4, 11). This leads to the release from Keap-1 of the transcription factor NF-E2-related factor 2 that migrates to the nucleus and increases expression of a number of genes such as heme oxygenase-1 and those involved in glutathione synthesis (4).

To determine whether electrophilic lipids increased mitochondrial ROS production by endothelial cells, Landar et al. (9) tested three electrophilic lipids, 4-hydroxynonenal (4-HNE) and two cyclopentenones, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), and 15-J_2-isoprostane (15-J2-IsoP). 4-HNE and 15-J_2-IsoP are produced by the nonspecific oxidation of lipids, whereas 15d-PGJ_2 is the product of the enzyme cyclooxygenase. They first compared the ability of these electrophilic lipids to induce expression of heme oxygenase-1 and the enzymes of glutathione synthesis in endothelial cells and found that cyclopentenones were more potent than 4-HNE. They then used the fluorescent ROS probe dichlorofluorescein (DCF) to see whether exogenous electrophilic lipids increased ROS production. This was indeed the case in cells, and this ROS production was localized to mitochondria, with the two cylclopentenones again being more potent than 4-HNE. Related lipids lacking the electrophilic carbon were ineffective, suggesting that the increase in ROS was secondary to the formation of adducts. To confirm that the ROS increase was due to the mitochondrial respiratory chain, the authors used cells lacking a functional respiratory chain and showed that in this case, exogenous electrophilic lipids did not increase ROS production. To see whether the electrophilic lipids were acting directly or indirectly on the mitochondria, the authors constructed a fluorescently labeled derivative of one of the electrophilic lipids (15d-PGJ_2) and showed that it was taken up selectively by mitochondria within cells. Pretreatment of the cells with unlabeled 15d-PGJ_2 blocked this binding. Furthermore, immunoblots indicated that the tagged version of 15d-PGJ_2 was binding to proteins. They concluded that exogenous electrophilic lipids can be taken up into mitochondria within cells and there increase ROS production.

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This is an exciting finding for a number of reasons. It may help explain how oxLDL increases mitochondrial ROS production and is thus an important contribution to our understanding of endothelial cell damage during inflammation and atherosclerosis. Perhaps more importantly, this work suggests that electrophilic lipids are an important mechanism of modulating mitochondrial ROS production. It may be that the increase in ROS production by mitochondria after exposure to exogenous ROS is due to the formation of electrophilic lipids. The possibility that electrophilic lipids play a role in modulating mitochondrial ROS production in both redox signaling and pathological situations now needs to be considered. One limitation to electrophilic lipids as a redox signals is that it is difficult to see how the adduct formed between the protein and the lipid could be reversed, suggesting that the signal would have to be switched off by protein degradation. However, signal inactivation by protein degradation is not unprecedented, and the Keap-1 electrophilic lipid response is already well established.

It is likely that the proteins are modified by electrophilic lipids through adduct formation on reactive protein thiols. Low pK_a thiols will be particularly reactive and because the pK_a of most cysteine residues is ~8.5, the greater reactivity of mitochondrial thiols with electrophilic lipids may be partly due to the higher pH of the mitochondrial matrix (~8 vs. ~7.2 in the cytosol). This may also help explain the intriguing finding that electrophilic lipid binding to mitochondria was decreased in the absence of a functional respiratory chain, because under those conditions the mitochondrial matrix pH would be similar to that of the cytosol.

Of course, many questions remain to be addressed. Among these is the mechanism by which electrophilic lipids increase mitochondrial ROS production. This is probably secondary to adduct formation on mitochondrial proteins, but how this occurs is unclear. It could be that the electrophilic lipids bind directly to proteins in the respiratory chain to modify their superoxide production. Alternatively, electrophilic lipids might inactivate antioxidant defenses such as peroxiredoxin, thioredoxin, or thioredoxin reductase, all of which have reactive thiols or selenols that may be susceptible to reaction with electrophilic lipids. In such a scenario, ROS production by the respiratory chain would be unaltered but the amount diffusing from mitochondria to the rest of the cell would increase. The identification of the proteins affected by the electrophilic lipids is clearly a priority, and the availability of biotin-tagged electrophilic lipids should facilitate rapid progress. The source of the electrophilic lipids is also intriguing, and it will be interesting to see whether electrophilic lipids produced within oxidatively damaged mitochondria also modulate mitochondrial ROS production.

To summarize, Landar et al. (9) provide a timely stimulus to investigate the role of electrophilic lipids in mitochondrial ROS production in a range of physiological and pathophysiological situations. Electrophilic lipids may prove to be an important component of redox signaling pathways linking the mitochondrion to the rest of the cell.

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