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DJ-1 regulation of mitochondrial function and autophagy through oxidative stress

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The dysregulation of mitochondrial function has been implicated in the pathogenesis of Parkinson disease. Mutations in the parkin, PINK1 and DJ-1 genes all result in recessive parkinsonism. Although the protein products of these genes have not been fully characterized, it has been established that all three contribute to the maintenance of mitochondrial function. PINK1 and parkin act in a common pathway to regulate the selective autophagic removal of depolarized mitochondria, but the relationship between DJ-1 and PINK1- and/or parkin-mediated effects on mitochondria and autophagy is less clear. We have shown that loss of DJ-1 leads to mitochondrial phenotypes including reduced membrane potential, increased fragmentation and accumulation of autophagic markers. Supplementing DJ-1-deficient cells with glutathione reverses both mitochondrial and autophagic changes suggesting that DJ-1 may act to maintain mitochondrial function during oxidative stress and thereby alter mitochondrial dynamics and autophagy indirectly.

Reduced mitochondrial complex 1 activity accompanied by increased oxidative stress has been linked to Parkinson disease by many previous studies. The identification of the early-onset recessive parkinsonism genes DJ-1, PINK1 and parkin, which are associated with mitochondria, further support the idea that changes in mitochondrial function can cause neurodegeneration as seen in Parkinson and related disorders. The serine/threonine kinase PINK1 is localized to the outer mitochondrial membrane, whereas parkin, an E3 ubiquitin ligase and DJ-1 can each be recruited to mitochondria following either depolarization of the mitochondrial membrane in the case of parkin or increased cellular oxidative stress in the case of DJ-1. In addition to their localization, these proteins also contribute to protection against mitochondrial toxins including the complex 1 inhibitors rotenone or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

We characterized the effects of DJ-1 deficiency on mitochondria and also investigated the relationship between DJ-1 and PINK1-parkin. We found multiple mitochondrial abnormalities in DJ-1-deficient cells including decreased mitochondrial membrane potential, increased numbers of fragmented mitochondria and reduced mitochondrial connectivity. Similar mitochondrial phenotypes are seen in PINK1- and parkin-deficiency models. Furthermore, parkin overexpression can rescue PINK1 deficiency, supporting the idea of a common pathway in which PINK1 is genetically upstream of parkin. We found that DJ-1 expression in mammalian cells does not rescue mitochondrial fragmentation resulting from the absence of either PINK1 or parkin, but its expression is able to protect against mitochondrial fragmentation resulting from rotenone treatment in PINK1-deficient cells. We also found that PINK1 or parkin overexpression reverses mitochondrial fragmentation in DJ-1-deficient cells. These results suggest that DJ-1 works in a parallel pathway to PINK1 and parkin to limit mitochondrial damage in response to oxidant stress.

There is also evidence of altered autophagy in Parkinson disease. Selective

recruitment of parkin to depolarized mitochondria promotes mitochondrial clearance through the mitophagy pathway. The relocalization of parkin from the cytosol to damaged mitochondria is dependent on PINK1 kinase activity. In addition, PINK1-deficient cells accumulate autophagic vesicles. Therefore, we measured LC3 levels (a marker of enhanced autophagic sequestration or reduced clearance) in DJ-1-deficient cells and observed an increase in GFP-LC3 positive puncta that are closely associated with mitochondria. Whether this represents a primary effect of loss of DJ-1 or an attempted compensatory event in the cells was not immediately clear from these data.

DJ-1 is closely involved in oxidative stress responses and cells deficient in DJ-1 are reported to have higher levels of reactive oxygen species (ROS). This led us to hypothesize that the phenotypes of DJ-1-deficient cells are primarily caused by enhanced oxidative stress. We also hypothesized that the effects on either mitochondrial morphology and/or autophagy may be secondary events. We confirmed that oxidative stress is increased in our DJ-1-deficient cells and demonstrated that treatment with cell-permeable glutathione analogues rescues the reduced mitochondrial membrane potential, changes in mitochondrial morphology and markers of autophagy. Therefore, many of the events in the DJ-1-deficient cells were likely consequences of a primary defect in oxidative stress predominantly generated in mammalian cells by mitochondria themselves.

Increases in mitochondrial fusion followed by fission events are thought to be a protective mechanism against oxidative stress. This process leads to the generation of uneven mitochondria where oxidized and damaged proteins are sequestered into mitochondria with lower membrane potential and are subsequently eliminated through mitophagy. Overexpression of mitochondrial fusion proteins or inhibition of fission proteins counteracts this process and is sufficient to reduce mitophagy. In our system, we found that DJ-1-deficient cells had lower rates of mitochondrial fusion.

Elevated ROS levels influence autophagy independent of their effects on mitochondrial dynamics. ROS-mediated regulation of autophagy has been demonstrated under many circumstances, but the specific targets of ROS are still unclear. One pathway by which autophagy is repressed by cells under conditions of increased oxidative stress is through ROS-dependent activation of the kinase mTOR by NFκB. A recent study reports that DJ-1 negatively regulates the deubiquitinating enzyme Cezanne that functions to inhibit NFκB activation. When DJ-1 is absent, NFκB signaling is reduced, which we predict would lessen NFκB-dependent suppression of autophagy via mTOR. This is an addressable hypothesis as many suitable reagents for mTOR and NFκB signaling are available.

Despite intense investigation of PINK1, parkin and DJ-1, the mechanisms by which these proteins have an impact upon mitochondrial function, morphology and mitophagy still require a great deal of further elucidation. PINK1 and parkin, which are both enzymes, probably regulate mitochondrial function through their substrates, which are the subject of intensive investigation. For DJ-1, it is clear that the protein is involved in oxidative stress responses but its precise function remains elusive. Although mitochondrial phenotypes or changes in autophagy may be secondary effects of loss of DJ-1, they provide useful phenotypes by which to measure function of the protein. The key point is that these assays can help us to understand cellular functions of all three genes for recessive parkinsonism and the relationships between them.

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