Nitric Oxide Links Mitochondrial Fission to Alzheimer's Disease

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Abstract

Mitochondrial dysfunction is a hallmark of amyloid-β (Aβ)-induced neuronal injury in the pathogenesis of Alzheimer's disease. Neurotoxic Aβ peptide, thought to be a key mediator of Alzheimer's disease, may be imported into human brain mitochondria where it inhibits key enzymes of respiratory metabolism. Nitric oxide (NO) produced in response to Aβ induces S-nitrosylation of the mitochondrial division protein, dynamin-related protein 1 (Drp-1), which leads to excessive mitochondrial fission, synaptic loss, and neuronal damage. Furthermore, brains of patients with Alzheimer's disease contain high amounts of S-nitrosylated Drp-1. Aβ-dependent mitochondrial fragmentation likely enhances the decline in bioenergetic capacity of damaged mitochondria and therefore contributes to neuronal injury and pathogenesis of Alzheimer's disease.

Mitochondria are amazingly dynamic organelles. In many eukaryotic cell types, they continuously move around, fuse with one another, and then split apart again (*1*, *2*). This dynamic behavior serves a multitude of different cellular functions: Active movement distributes mitochondria within cells, partitions the organelles during cell division, and positions them at sites of high energy demand in differentiated cells (*3*). In particular, the intricate balance of fusion and fission is a major determinant of cell life and death. Fusion mixes organellar contents and unifies the mitochondrial compartment, thereby allowing the efficient exchange of metabolites and mitochondrial DNA, and thus ensuring efficient production of ATP in metabolic active cells (*4*). Fission, on the other hand, creates numerous small morphologically and functionally distinct organelles. Fragmentation of the

intracellular mitochondrial network by the mitochondrial fission machinery is an integral step of the intrinsic pathway of apoptosis, and enhanced fission is sufficient to induce cell death (*5*). In addition, an imbalance between mitochondrial fusion and fission contributes to neurodegenerative diseases (*6*). The vulnerability of neurons to mitochondrial defects likely reflects their high energy demands and their strict spatial and functional requirements for mitochondria in different cell regions, such as soma, synaptic regions, and dendritic extensions (*7*). A study now reveals a direct link between excessive mitochondrial fission activity and neuronal damage in Alzheimer's disease (*8*).

Mitochondrial function and energy metabolism are impaired early in the course of Alzheimer's disease. Respiratory chain activity is severely compromised in mitochondria isolated from autopsied brain samples from Alzheimer's disease patients (*9*), and patients' biopsy specimens exhibit several mitochondrial abnormalities, including increased amounts and partial mislocalization of both mitochondrial DNA and cytochrome *c* oxidase (*10*). The amyloid hypothesis provides a widely accepted explanation for the pathogenesis of the disease (*11, 12*). This hypothesis states that accumulation of the toxic amyloid-β (Aβ) peptide in the brain is the primary factor driving pathogenesis. Aβ peptides are produced by proteolytic processing of amyloid precursor protein (APP) by the γ-secretase complex, which includes presenilin 1 and 2 (PS1 and PS2), in the plasma membrane of neurons. Missense mutations in genes encoding APP, PS1, or PS2 increase the production of a toxic Aβ variant that has an increased propensity for aggregation. Accumulation of extracellular Aβ plaques then contributes to progressive neuronal injury, ultimately leading to widespread neuronal dysfunction and cell death and causing dementia (*11, 12*). What might the role of mitochondria be in this scenario? As neurons in human brains accumulate toxic Aβ intracellularly prior to the deposition of extracellular plaques, Aβ may also exert cytotoxic effects from inside the cell (*13*). Indeed, there are several lines of evidence pointing to mitochondria as potential targets of Aβ toxicity. Aβ is imported into mitochondria and localizes to the inner membrane cristae (*14*), where it can inhibit key enzymes of respiratory metabolism (*15-17*). Aβ-binding alcohol dehydrogenase (ABAD) is

another mitochondrial target for Aβ toxicity; its direct interaction with Aβ promotes mitochondrial dysfunction and cell death in the brains of Alzheimer's disease patients (*18*). Thus, Aβ-induced mitochondrial damage appears to contribute to Alzheimer's disease progression (*19*).

The paper by Cho *et al.* (*8*) adds another twist to the connection between mitochondria and Alzheimer's. Previous work has shown that treatment of neurons with nitric oxide (NO) triggers mitochondrial fragmentation (*20*). NO normally acts as a signaling molecule (*21*), but when produced in excess, is highly neurotoxic, induces mitochondrial damage, and contributes to the pathogenesis of neurodegenerative diseases (*20*). Intriguingly, mitochondrial fragmentation similar to that observed upon NO treatment is seen in neurons exposed to Aβ (*20, 22*). In both cases, excessive mitochondrial fission depends on Drp-1, a dynamin-related guanosine triphosphatase (GTPase) that is a core component of the mitochondrial division machinery (*20, 22*). These observations point to a common mechanism for Aβ– and NO-induced mitochondrial fragmentation and prompted Cho *et al*. to investigate whether these processes are functionally linked in Alzheimer's disease (*8*).

A possible mechanism of NO-dependent modulation of mitochondrial fission activity was suggested by the observation that Drp-1 homologs involved in endocytosis become covalently modified by S-nitrosylation upon exposure to NO (*23, 24*). Cho *et al*. tested whether Drp-1 was also a direct target for S-nitrosylation. They treated neurons with an exogenously added NO donor and transfected cells with a neuronal NO synthase construct. In both cases, Drp-1 was covalently modified by S-nitrosylation in the presence of elevated NO concentrations. Strikingly, treatment of neurons with Aβ induced S-nitrosylation of Drp-1 in a similar manner, concomitant with mitochondrial fragmentation. If this reaction is relevant to the pathogenesis of Alzheimer’s disease, it would be expected that brains of Alzheimer's patients would contain higher amounts of S-nitrosylated Drp-1 compared to non-afflicted patients, which was exactly what the authors found. Thus, formation of S-nitrosylated Drp-1 is a characteristic feature of Alzheimer's disease progression.

Next, the authors identified a critical cysteine residue (Cys^{644}) in the GTPase effector domain of Drp-1, which affects GTPase activity, higher order assembly, and mitochondrial division activity (*25*). Nitrosylation of Drp-1 at this residue increased dimer formation and GTPase activity. In contrast, NO did not induce S-nitrosylation, dimerization, or increased GTPase activity of Drp-1 when the Cys^{644} residue was mutated to Ala (C644A). Moreover, NO-induced mitochondrial fragmentation in neurons was abrogated by the expression of the C644A Drp-1 variant defective for S-nitrosylation (*8*). Thus, S-nitrosylation in response to NO or Aβ augmented Drp-1 activity. But is this relevant in the disease state? To answer this question, the authors examined the effect of Drp-1 nitrosylation on neurotoxicity and found that expression of the C644A Drp-1 mutant protected neurons from NO-induced death. Furthermore, exposure of cortical neurons to Aβ decreased the number of synaptic spines (*8*), which is a characteristic feature of Aβ-mediated neurotoxicity (*26*). Again, expression of the Drp-1 mutant had a protective effect, suggesting that S-nitrosylation of Drp-1 is a critical step in Aβ-dependent neuronal damage (*8*).

In summary, these data support a model that links the accumulation of Aβ, nitrosative stress, mitochondrial fragmentation, and neuronal damage (*8*). The formation of Aβ might increase nitrosative stress in neurons, which then leads to enhanced S-nitrosylation of Drp-1. This in turn augments Drp-1 activity, resulting in fragmentation of mitochondria. It is possible that mitochondrial fragmentation triggers synaptic damage, because dysregulation of the mitochondrial fusion and fission equilibrium induces loss of synapses and dendritic spines (*27*). Moreover, relatively similar amounts of S-nitrosylated Drp-1 were detected in Aβ-treated neurons in culture and in human Alzheimer's disease brains, suggesting that this pathway might be pathophysiologically relevant (*8*).

Mitochondria clearly play a role in Alzheimer's disease, and it is likely that several negative effects exerted by Aβ on mitochondria potentiate each other (Fig. 1). Direct inhibition of mitochondrial enzymes and respiratory complexes may lead to loss of respiratory capacity (*15-18*). If bioenergetic failure is combined with fragmentation of mitochondria due to

excessive fission (*8*, *20*, *22*), the energy supply of neurons will rapidly decline below a critical threshold, leading to neuronal damage. Repair processes and complementation of gene products present in individual mitochondria that are damaged in different molecular complexes will be hampered by fragmentation of the organellar network, resulting in a decline in total bioenergetic capacity. Furthermore, fragmented mitochondria will be less efficiently transported and distributed within neurons, leading to further depletion of the energy supply in large areas of the cell. Thus, mitochondrial defects combine to induce cumulative neuronal injury and dysfunction. The work by Cho *et al*. adds Drp-1 to the list of potential drug targets for therapeutic treatment of Alzheimer's disease. A screen for chemical inhibitors of mitochondrial division in yeast has identified a compound that attenuates Drp-1-dependent mitochondrial division in mammalian cells (*28*). It will be exciting to see whether further research on mitochondrial dynamics can aid in the development of additional therapeutic strategies for Alzheimer's disease.

Fig. 1. A model for Aβ-induced impairment of mitochondrial function in Alzheimer's disease. Proteolytic processing of amyloid precursor protein (APP) leads to the release of toxic Aβ peptides, some of which end up in the cytosol of neurons. Some Aβ is taken up by mitochondria where it inhibits key respiratory enzymes. Aβ also increases nitrosative stress, which enhances the modification of a critical cysteine residue in the mitochondrial division protein, dynamin-related protein 1 (Drp-1), with nitric oxide (NO). S-nitrosylation augments Drp-1 fission activity and leads to fragmentation of mitochondria in neurons, impairing exchange of metabolites, and complementation and repair processes between damaged mitochondria. This leads to the formation of numerous morphologically and functionally distinct mitochondria, which exhibit decreased respiratory capacity (indicated by the gray color) due to the accumulation of irreversible Aβ-induced damage. Together with collateral mitochondrial transport and distribution defects, Aβ-induced mitochondrial damage may cause a decline in the overall respiratory capacity of neurons, ultimately resulting in synaptic damage and neuronal injury.

References and Notes

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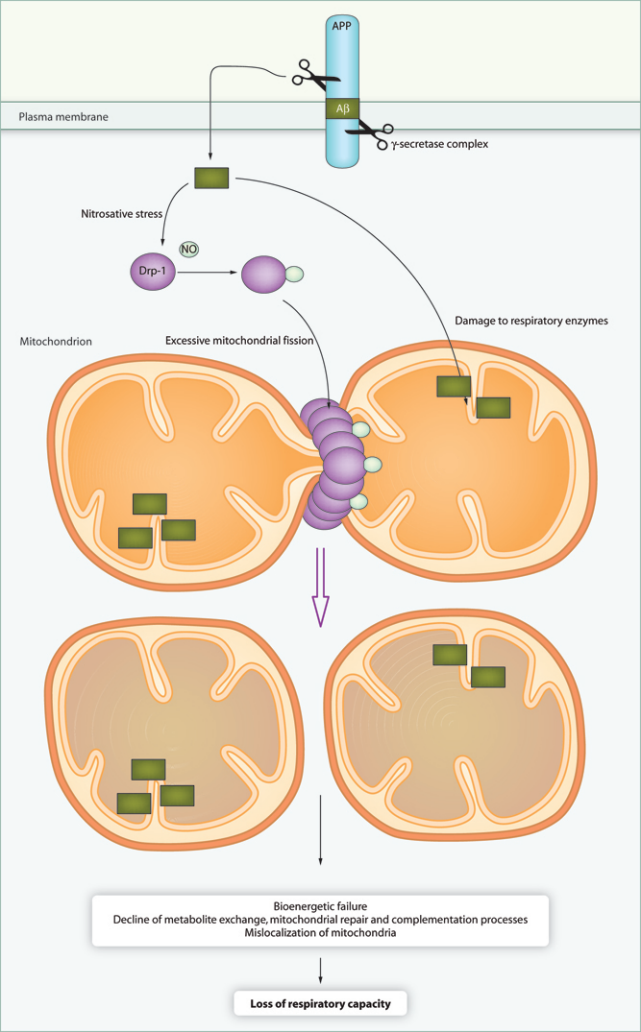
APP
Aβ
Plasma membrane
γ-secretase complex
Nitrosative stress
NO
Drp-1
Damage to respiratory enzymes
Mitochondrion
Excessive mitochondrial fission
Bioenergetic failure
Decline of metabolite exchange, mitochondrial repair and complementation processes
Mislocalization of mitochondria
Loss of respiratory capacity