Could successful (mitochondrial) networking help prevent Huntington’s disease?

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Polyglutamine expansions in huntingtin (Htt) are known to cause the profound neurodegenerative disorder, Huntington’s disease (HD). Mitochondrial dysfunction has long been implicated in the pathophysiology of HD, but the underlying mechanism remains obscure. An article by Costa et al in this months edition describes a smooth mechanistic cascade from the well-accepted upstream event that mutant Htt is associated with Ca\(^{2+}\) handling abnormalities, through to apoptotic neuronal death. The proposed cascade implicates calcineurin, activated by abnormal Ca\(^{2+}\) levels, in the dephosphorylation of dynamin-1-like protein (Drp1), increasing its association with mitochondria and promoting fission, cristae disruption, cytochrome \(c\) release and apoptosis (Fig 1). Together with the recent reports of increased mitochondrial fission in striatal neurons from HD patients, the article by Costa et al provides a compelling case for the role of abnormal mitochondrial networking in HD pathogenesis.

Scientists of a certain age will remember being taught that mitochondria were discrete, static organelles present at large numbers in virtually all eukaryote cells. Although some highly impressive serial electron micrographs of budding yeast had suggested that mitochondria could form large reticular networks, it was not until the 1990s that researchers began to realize that mitochondria could bud and fuse. This field of mitochondrial dynamics has been revolutionized by time-lapse imaging of fluorescent mitochondrialotropic factors and the identification of numerous proteins that can promote mitochondrial fusion and fission of both the outer and inner mitochondrial membranes. The resultant dynamic mitochondrial network has become commonplace to cell biologists, but what is its physiological function? Although the exact role of mitochondrial fission and fusion has yet to be resolved, it is clear that mitochondrial dynamics are essential for cell viability. Further, mutations in two key pro-fusion players mitofusin 2 (Mfn2) (essential for outer membrane fusion) and optic atrophy protein 1 (Opa1) (inner membrane fusion) are known to cause the peripheral neuropathy Charcot–Marie–Tooth type IIA and autosomal dominant optic atrophy, respectively. Is it possible that defective mitochondrial dynamics could modulate other neurological defects? With this in mind, Costa et al have studied mitochondrial dynamics in numerous cell lines from patients with HD or models of this hereditary neurodegenerative disorder (Costa et al, 2010).

Nearly two decades have passed since the HD causing mutation was identified as an unstable expansion-biased trinucleotide repeat. The mutation results in an expanded polyglutamine tract in the Htt protein, which is widely expressed throughout the brain but, remarkably, leads to preferential degeneration of GABAergic medium spiny neurons in the striatum. In spite of significant progress, the pathogenic puzzle stands unsolved and HD remains as incurable now as upon the time of George Huntington’s first report over a century ago.

What is the link between mutant Htt and mitochondrial abnormalities?

Mitochondrial dysfunction has long been proposed to play a crucial role in Huntington’s disease (HD) pathogenesis, as striatal degeneration can be recapitulated with systemically administered mitochondrial inhibitors and decreased activity of mitochondrial respiratory complexes has been found in post-mortem striatum from HD patients. Further, mounting evidence suggests that mutant huntingtin (Htt) or its polyQ-containing N-terminal fragments can affect mitochondrial function, either in soluble or aggregate form. A wide spectrum of pathogenic mechanisms...
has been proposed, ranging from direct Htt–mitochondria association to indirect transcriptional dysregulation affecting mitochondrial composition (Oliveira, 2010). While the focus has traditionally been on altered mitochondrial bioenergetics compromising adenosine triphosphate (ATP) generation and Ca$^{2+}$ handling, the spotlight has recently turned towards changes in mitochondrial dynamics. Reduced mitochondrial biogenesis, impaired mitochondrial movement along neuronal processes and fusion–fission imbalance have all been proposed to play a role in HD pathogenesis.

What are the specific findings of Costa et al?

It has previously been reported that HeLa cells expressing truncated Htt protein containing a large polyQ expansion (polyQ74) had reduced mitochondrial fusion and movement when compared to cells expressing Htt with a shorter expansion (polyQ28). Further, it had been shown that this fragmentation could be ameliorated by overexpressing a dominant negative version of the fusion protein dynamin-1-like protein (Drp1) or the fusion protein Mfn2 (Wang et al, 2009). It was concluded that the cytotoxicity induced by expressing polyQ expanded Htt was likely to be mediated in some way by increased mitochondrial fragmentation. Costa et al have now confirmed these observations in a variety of cell types including HD patient lymphoblasts and primary striatal neurons from a mouse model (YAC128) of HD but were able to extend our understanding further. In addition to mitochondrial fragmentation, expression of the polyQ length expansion also clearly led to disruption of cristae, the invaginations of the inner membrane that are now known to help maintain a discrete submitochondrial environment. Another intriguing observation was the increased levels of Drp1 in the mitochondria from these cell lines, which was consistent with mitochondrial fragmentation. Perhaps more controversial, but consistent with a decrease in phosphorylated Drp1, was the apparent increase in calcineurin activity in HD cells. Calcineurin, a Ca$^{2+}$-dependent phosphatase, has been shown to dephosphorylate Drp1 in the cytosol, leading to Drp1 mitochondrial translocation (Cereghetti et al, 2008). Although levels of calcineurin and its inhibitor regulator of calcineurin 1-isoform 1L (RCAN1-1L) were similar across wild-type and HD cells, Costa et al associate the increased calcineurin activity with the enriched Ca$^{2+}$ stores in HD cells. While stored Ca$^{2+}$ levels cannot be causally linked to cytosolic calcineurin activity, the proposal does fit into the literature framework of abnormal Ca$^{2+}$ homeostasis in HD cellular models (Oliveira, 2010). Furthermore, the central role for calcineurin in the mitochondrial fragmentation and cristae remodelling found in the HD cell lines was supported.
by reconstitution of the mitochondrial network on inhibition of calcineurin by the pharmacological inhibitor FK506 and by expression of the dominant negative forms of calcineurin (which led to inhibition of Drp1 translocation to the mitochondrion) and Drp1. Indeed, in a similar fashion to previous reports, Costa et al showed that by manipulating the levels of various pro-fission and pro-fusion proteins, the mitochondrial network could be restored in the various HD cells.

HD cell lines are sensitive to apoptosis inducing factors
Polyglutamine expansions in the HD cells clearly lead to fragmentation of the mitochondrial network and a shortening of cristae. Whilst this is intriguing, what does this observation have to do with the increased cell death seen in the spiny neuron population of the striatum in individuals with HD? It has been known for some time that these neurons are lost due to apoptosis. Could the mitochondrial fragmentation somehow lead to increased apoptosis? Costa et al did not show this directly, but report an increased susceptibility to factors that induce mitochondrially mediated apoptosis through oxidative and DNA damage (hydrogen peroxide, etoposide) or staurosporine-induced caspase activity. Crucially, rescuing the fusion deficiency by simply modulating the levels of pro-fusion and fission components was not necessarily sufficient to protect against the apoptosis inducers. The authors show that it is the successful remodelling of the cristae that is essential for this protection. This was highlighted by the contrasting effects of overexpressing two pro-fusion proteins in the HD cells, Mfn1 and Opa1. The former is a key factor in mediating mitochondrial outer membrane fusion, whilst the latter is present in the inner membrane, where oligomers play a crucial role in maintaining tight cristae junctions. Although increased expression of Mfn1 promoted mitochondrial elongation, susceptibility to apoptosis inducing factors remained high and there was no evidence of cristae remodelling. Increased Opa1 expression, however, led not only to reformation of the mitochondrial network but also to marked increase in cristae length and protection against apoptosis induction. Why is it that well-defined mitochondrial cristae appear to help protect against apoptotic inducers? One of the key initiators in mitochondrially mediated apoptosis is cytochrome c, which, on release from mitochondria, causes caspase activation. It has been estimated that more than 85% of mitochondrial cytochrome c is stored in the intracristal space of some cell types. Costa et al show that on disruption of cristae in the HD lines, cytochrome c is more readily released in response to apoptotic inducers, although the exact mechanism of this release is unclear.

The strength of this current paper by Costa et al is that viewing the data in toto now leads to a very credible story to link the mitochondrial fragmentation and cristae loss seen in various HD cells with a credible molecular mechanism for increased susceptibility to mitochondrially mediated apoptosis, the cellular pathology that has been known to underlie HD for some years. This study, together with that of Kim et al (Kim et al, 2010), strongly implicates excessive fission in HD pathogenesis. But can mitochondrial fission inhibition (via Drp1 or calcineurin inhibition) help prevent HD? The strikingly abnormal neurodevelopment and lethality in Drp1 knockout mice cautions about the consequences of preventing mitochondrial fission. Indeed, whilst Drp1-dependent mitochondrial fission may be dispensable for non-polarized cells, it is crucial for extremely polarized cells such as neurons, where mitochondria must be shaped into small enough entities capable of distributing throughout narrow neurites where they are essential to assist synapse formation and proper function (Fig 1). Thus, while Drp1 inhibition may rescue neurons from staurosporine-induced apoptosis, as shown in the Costa et al study, neurons are likely to be functionally compromised due to fission inhibition. Concerning calcineurin inhibition, Costa et al address the contrasting literature that claims either protective or worsening effects in HD models. The protection of HD lymphoblasts by calcineurin inhibition suggests that the increased activity of this phosphatase is unlikely to be restricted to neurons, and does not explain preferential striatal vulnerability. Nonetheless, the study by Costa et al adds to our current knowledge of HD pathophysiology, and fuels the alluring hypothesis that the fine-tuning of mitochondrial networking may yield therapeutic benefit.

The authors declare that they have no conflict of interest.

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