

## Addenda

# Protective Roles for Induction of Autophagy in Multiple Proteinopathies

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## KEY WORDS

autophagy, Huntington's disease, polyglutamine disease, spinocerebellar ataxia, tau, Parkinson's disease, Alzheimer's disease

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## Addendum to:

### *Rapamycin Alleviates Toxicity of Different Aggregate-Prone Proteins*

Z. Berger, B. Ravikumar, F.M. Menzies, L. Garcia Oroz, B.R. Underwood, M.N. Pangalos, I. Schmitt, U. Wullner, B.O. Evert, C.J. O'Kane and D.C. Rubinsztein

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and

### *Rapamycin Pre-Treatment Protects Against Apoptosis*

B. Ravikumar, Z. Berger, C. Vacher, C.J. O'Kane, and D.C. Rubinsztein

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## ABSTRACT

Many late-onset neurodegenerative diseases, including Parkinson's disease, tauopathies, Huntington's disease and forms of spinocerebellar ataxia, are caused by aggregate-prone proteins. Previously we showed that mutant huntingtin is an autophagy substrate and that autophagy induction reduced soluble and aggregated huntingtin levels and attenuated its toxicity in cell, fly and mouse models of disease. We have recently shown in cell and fly models that autophagy induction may have general protective effects across a range of diseases caused by aggregate-prone intracellular proteins. First, we showed that this strategy reduces the levels of the primary toxin, the aggregate-prone mutant protein. Second, our recent work suggests that autophagy induction may have additional cytoprotective effects by protecting cells against a range of subsequent pro-apoptotic insults.

The accumulation and aggregation of misfolded proteins is a feature of a group of diseases known as proteinopathies, or protein conformation disorders. Each individual disease is characterized by the particular protein that aggregates and the cell types in which this occurs. The exact mechanism of cellular toxicity caused by these proteins remains to be elucidated. However, it is generally accepted that a toxic gain-of-function is involved. Loss-of-function mutations often show phenotypes distinct from that seen in the disease, if any at all, whilst disease severity appears to correlate with expression level of the mutant protein. The role of aggregates in these diseases is controversial and they have been suggested to be both toxic and protective in the cell. Aggregate toxicity may result from blockage of axonal transport or from the sequestration of transcription factors leading to transcriptional dysregulation.<sup>1</sup> Equally, aggregates may protect the cell by sequestering putatively harmful soluble species of the mutant protein.<sup>2</sup> However, no studies have shown the aggregates to be as beneficial as the wild-type protein. Thus, even when aggregates appear to be less toxic than the soluble mutant protein, they may be deleterious relative to the wild-type protein. Regardless of which of these scenarios is correct, reducing the load of toxic proteins in the cell is likely to be beneficial. Mechanisms by which this can be achieved are therefore of great interest from a therapeutic standpoint.

A subset of proteinopathies are caused by codon reiteration mutations, in which tracts of repeated amino acids become expanded, such as the polyglutamine tract of huntingtin in Huntington's disease (HD). Our initial study using either an exon 1 fragment of Huntingtin with 74 glutamines, or 19 alanine repeats fused to GFP as model aggregate-prone proteins, demonstrated that they can be cleared by autophagy in cell culture.<sup>3</sup> Indeed, this may be the preferential route of clearance of these proteins as it has been demonstrated that the proteasome is unable to cleave within the polyglutamine tract,<sup>4,5</sup> and proteasomes cannot clear aggregated species of mutant huntingtin.<sup>6</sup> Subsequent to our initial observations, it has been shown that other aggregate-prone proteins are autophagy substrates, namely forms of  $\alpha$ -synuclein, the major component of Lewy bodies in Parkinson's disease,<sup>7</sup> peripheral myelin protein 22, associated with demyelinating peripheral neuropathies<sup>8</sup> and mutant  $\alpha$ 1-antitrypsin Z, which accumulates in the ER causing  $\alpha$ 1-antitrypsin deficiency.<sup>9</sup>

The observation that clearance of many of these proteins can be increased by autophagy offers an exciting therapeutic possibility in the form of rapamycin, an inhibitor of mTOR that results in the upregulation of autophagy. Rapamycin or the rapamycin ester, CCI-779, have previously been shown to reduce toxicity in both *Drosophila* and mouse models expressing mutant huntingtin.<sup>10</sup> Thus, it was important to establish if this protective effect was confined to huntingtin or could be more widely applied to aggregate-prone proteins. Our recent data demonstrate that rapamycin treatment may indeed be effective in the

clearance of a diverse range of aggregate-prone proteins.<sup>11</sup> We have shown that, not only can rapamycin induce the clearance of a range of polyglutamine or polyalanine-containing proteins (including proteins mutated in certain spinocerebellar ataxias), but also the clearance of mutant tau associated with fronto-temporal dementia/tauopathy. Furthermore, we have shown that rapamycin reduces toxicity of these aggregate-prone proteins in *Drosophila*. These findings suggest a much broader potential for rapamycin therapy, beyond HD.

In addition to the protective effect of rapamycin via enhanced clearance of aggregate-prone proteins, our recent studies suggest that rapamycin can have other cytoprotective effects.<sup>12</sup> Rapamycin pretreatment can protect cells and *Drosophila* against subsequent diverse apoptotic insults. This protective effect is, however, lost when autophagy is inhibited. Although a role for autophagy has been implicated in both apoptotic and nonapoptotic cell death, the exact functional significance of autophagy in these pathways is poorly understood. There are two major apoptotic cascades in a cell, namely the intrinsic and extrinsic pathways (Fig. 1). The intrinsic pathway requires mitochondrial-dependent cytochrome-c release for activation of downstream caspases, whereas, the extrinsic or death-receptor mediated pathway occurs independent of the mitochondria. However in certain cell types, designated as type-II cells, induction of death receptor-mediated apoptosis results in death that proceeds through the mitochondrial pathway.

We found that pretreatment with rapamycin protected against apoptosis that occurred via the mitochondrial pathway, suggesting the involvement of mitochondria in this protective mechanism. No protective effect was seen in a death receptor cell-death paradigm that was independent of mitochondria. Consistent with these data, the protective effect of rapamycin was lost when cytochrome-c release from mitochondria was blocked. We provided a plausible mechanism for the protective effect exerted by rapamycin. We observed decreased levels of several mitochondrial proteins upon rapamycin pretreatment suggesting enhanced clearance of mitochondria, which are degraded via autophagy. After pro-apoptotic insults, we observed decreased levels of cytosolic cytochrome c and activated caspases in cells treated with rapamycin, consistent with reduced mitochondrial load. We propose that the protective effect of rapamycin can be accounted for by enhanced clearance of mitochondria by autophagy, thereby reducing cytosolic cytochrome-c release and downstream caspase activation after pro-apoptotic insults.

Pro-autophagic treatments may thus be useful in certain disease conditions, like some neurodegenerative diseases, where a slow but increased rate of apoptosis is evident. Inducing autophagy may have two distinct beneficial effects in protein conformational diseases. First, it can be beneficial by clearing the primary toxin causing these diseases—the aggregate-prone protein. Second, enhanced autophagy attenuates apoptotic responses to various insults. In Huntington's disease and various other neurodegenerative conditions, such insults may include excitotoxicity and elevated levels of reactive oxygen species.

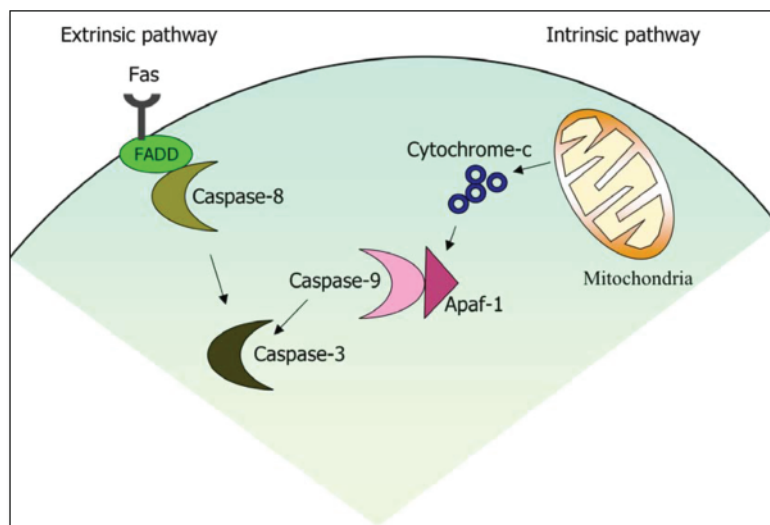


Figure 1. Pathways of apoptosis. In the intrinsic pathway, pro-apoptotic insults result in activation of mitochondrial dependent cytochrome-c release which further activates the effector caspase, caspase-9 which then activates caspase-3. On the other hand, activation of the extrinsic pathway (death-receptor pathway) involves direct activation of the effector caspase, caspase-8 (without involving mitochondria) which then signals to caspase-3. In some cells types (type-II), however, activation of the extrinsic pathway leads to death that proceeds via mitochondria.

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