Huntington’s disease (HD) is a progressive neurodegenerative condition caused by the abnormal expansion of a polyglutamine tract in the N-terminus of the huntingtin protein. Over the last 20 years, HD pathogenesis has been explained by the generation of N-terminal fragments containing the polyglutamine stretch. A new study from Frederic Saudou’s group now investigates the function of the C-terminal fragments generated upon cleavage and shows that these products may also contribute to cellular toxicity in HD (El-Daher et al, 2015).

See also: M-T El-Daher et al (September 2015)

Huntington’s disease (HD) is a late-onset neurodegenerative disorder caused by the progressive loss of neurons in the striatum and the cortex, which results in movement disorders, cognitive decline and psychiatric symptoms. The disease arises from an abnormal expansion in the polyglutamine (polyQ) tract at the N-terminus of the huntingtin (HTT) protein, which causes the protein to misfold and to accumulate in intracellular aggregates.

Since the observation in 1997 that N-terminal fragments of HTT accumulate in nuclear inclusions and dystrophic neurites in HD patients’ brains (DiFiglia et al, 1997), many studies have supported the idea that the generation of N-terminal fragments plays a critical role in HD pathogenesis. Mouse and cell models specifically expressing N-terminal HTT fragments with polyQ expansions recapitulate the phenotypes observed in full-length HTT models and show an accelerated onset of the pathology. The higher toxicity of these fragments may arise from their increased propensity to aggregate, or to form nuclear versus possibly less toxic cytoplasmic aggregates (Lunkes & Mandel, 1998; Mangiarini et al, 1996).

HTT is a large protein of 3,144 amino acids. It contains two proteolysis-prone domains: the region between 460 and 600, with multiple sites for caspases and calpains, of which residue 586 is of particular importance for HD since mutations in this site ameliorate the phenotype in a HD mouse (Graham et al, 2006), and the region comprising residues 104–114 and 146–214 (Lunkes et al, 2002). The formation of N-terminal fragments may also result from alternative splicing of the first exon of HTT, which correlates with polyQ length (Sathasivam et al, 2013). Thus, both protein- and RNA-dependent mechanisms may be involved in the generation of N-terminal fragments.

Protease cleavage of HTT should also generate the corresponding C-terminal fragments. While these products have been observed in post-mortem HD brain samples, where generation of both N-terminal and C-terminal fragments is increased relative to control brains (Mende-Mueller et al, 2001), the lack of the pathogenic polyQ signature in these fragments has deviated the attention towards the N-terminal fragments. Though reasonable, this may have hindered our understanding of any possible contribution of these fragments to disease.

El-Daher et al (2015) have established an elegant and clean approach to address the contribution of different cleaved forms of HTT to pathogenesis in model systems, avoiding the use of protease inhibitors that would affect additional substrates. They generated a regulatable cleavable version of full-length HTT by introducing tobacco etch virus (TEV) protease cleavage sites into previously reported domains of enzymatic proteolysis. To enable the exact timing of cleavage, a system comprising the N- and C-terminal domains of TEV fused to FRB and FKBP, respectively, allowed its rapid dimerization and therefore activation upon addition of the heterodimerizer, rapamycin.

Using this system to study the contribution of HTT proteolysis to cellular toxicity, they observed that double cleavage of HTT at 167 and 586 or 513 sites resulted in higher toxicity in a striatal cell line and flies than single cleavage. More importantly, this was observed not only when expressing HTT constructs with 100Q, but also with wild-type HTT bearing the physiological Q23 length. These findings provided evidence for some common toxicity as a consequence of HTT cleavage that is independent of the polyQ tract.

When the relevant N- and C-terminal fragments, HTT 1–167, HTT 1–586 and HTT 587–3144, were expressed in cells and flies, the C-terminal fragment HTT 587–3144 was not only toxic by itself but also potentiated the toxicity of the short N-terminal fragment HTT 1–167. However, its toxicity was abolished when expressed together with the non-toxic larger N-terminal fragment comprising residues 1–587. In further co-immunoprecipitation experiments, the authors demonstrated that the C-terminal 587–3144 fragment interacts with the N-terminal 1–586 stretch, but not with the shorter HTT 1–167, suggesting that the toxicity of the C-terminal fragment is quenched when interacting with its N-terminal binding partner. Because it cannot interact with shorter N-terminal HTT, the authors suggest that when the N-terminal is highly protelysed, the C-terminal would remain free and toxic (Fig 1).

But what is the contribution of the polyQ to cellular toxicity in this model? Using the TEV system, they report that the presence of the mutant polyQ makes the construct more prone to be cleaved and more toxic. They observe a highest rate of cleavage of mutant HTT relative to wild type, not only in cells using the TEV construct but also in human HD post-mortem striatal brain samples. Since the N-terminal cleavage results in the loss of the HTT 1–586 fragment, they hypothesize...
that the protective interaction between C-terminal HTT 587–3144 and N-terminal HTT 1–586 fragments is reduced in HD brains.

El-Daher et al. (2015) then address a mechanistic explanation for the toxicity of the C-terminal HTT 586–3144 fragments. In the absence of any interaction, this product induces ER vacuolization in striatal cells that is also observed in brains of HD knock-in mice, which may induce ER stress and toxicity. They found that a highly conserved C-terminal region of HTT interacts with the ER, compared to those containing either the C-terminal or N-terminal fragments. Overexpression of wild-type dynamin 1 rescued toxicity when co-expressed with the C-terminal HTT fragment, and targeting a dynamin 1 dominant-negative mutant to the ER was sufficient to cause ER vacuolization, which correlated with cell death. However, the role of dynamin 1 at the ER remains to be elucidated, as this localization has not been reported before, and it is possible that toxicity could also arise from the disruption of other well-known functions of dynamin 1 in membrane trafficking.

It would also be interesting to discern whether the interaction of the C-terminal HTT fragment with dynamin 1 has any physiological relevance for wild-type HTT. Other roles of the C-terminus of HTT have been described recently. For example, HTT binds through its C-terminal region to p62, a protein that enhances the sequestration of various cargoes into autophagosomes for degradation. Concomitantly, the middle region of HTT interacts with the autophagy regulator ULK1, suggesting that HTT may act as a scaffold in selective autophagy (Rui et al., 2015).

To summarize, Saudou’s group have defined a novel potential toxic role for the C-terminal fragments generated upon cleavage of HTT. As they focussed on cleavages at S86 (caspase-6-mediated) and S53 (caspase-3-mediated), further studies will be necessary to explore the possible effects of other C-terminal fragments generated after proteolysis by other proteases at different sites.

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