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COMMENT

A possible non-proteolytic role of ubiquitin conjugation in alleviating the pathology of Huntingtin's aggregation

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Ubiquitin conjugation to target proteins followed by their subsequent proteasomal degradation has become the hallmark of the mechanism by which cells specifically remove regulatory proteins, tune their activity, or destroy damaged proteins. Along the years, it has become clear that different modes of ubiquitin conjugation (e.g., length of the ubiquitin chain or the different internal lysines that serve as linking anchors to the ubiquitin moieties) can serve numerous nonproteolytic functions, such as modulating signaling, metabolism and neuronal activity. A recent study from our laboratories describes yet another nonproteolytic role of ubiquitin modification—attenuation of the pathogenic effects of polyQ-expanded aggregate-prone Huntingtin (Htt), the culprit protein behind Huntington's disease (HD).

HD is a progressive and fatal neurological disorder, caused by a genomic expansion of a Cytosine-Adenine-Guanine (CAG) repeat in the gene encoding for Htt. The disease is associated with severe motor symptoms, as well as cognitive and psychiatric symptoms that often appear years before motor symptoms manifest [1]. HD symptoms are attributed to a massive degeneration of striatal neurons, yet neurons in the neocortex and other brain structures are also affected, explaining, perhaps, the extensive range of motor, cognitive and psychiatric symptoms associated with HD.

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HD is a relatively rare disease (considered an orphan disease in the USA); yet, research on HD (12,794 hits in Pubmed, at the time of writing) far exceeds its clinical centrality. This is due, in part, to the unequivocal identification of its genetic cause, and to commonalties with vastly more prevalent neurological diseases (e.g., Alzheimer and Parkinson diseases), in which the precipitating causes are mostly elusive. One such commonalty is the presence of abnormal protein aggregates in all these diseases, which have been implicated to varying degrees in the disease etiology. Consequently, the hope is that insights gained on protein aggregation in HD and its relationships with HD etiology, will be informative in broader contexts as well.

Htt is a large (~350 kDa) protein which seems to play multiple roles in cellular physiology [1, 2], although much is yet to be learned in this regard. What is clear, however, is that the HD-associated CAG expansion, which translates into an abnormally long stretch of glutamines (polyQ), alters mutated Htt (mHtt) properties to the point that it gains additional, nonphysiological 'functions' [1]. Most of mHtt's pathological features relate to HTT exon 1, which contains the aforementioned CAG expansion; in fact, expression of this fragment in animal models is sufficient to cause an HD-related phenotype [3–5]. The abnormally long poly-Q stretch alters Htt's physicochemical properties, its folding and its tendency to aggregate, resulting in the appearance of mHtt cytoplasmic aggregates and nuclear inclusion bodies. These aggregates are often positive for ubiquitin and components of the Ubiquitin-Proteasome System [4, 6, 7], which seems to indicate that mHtt is marked for proteasomal degradation, but fails to undergo proper degradation and consequently accumulates in the form of large aggregates. As degradation rates of native Htt are not slowed by effective UPS inhibitors [8], ubiquitination and subsequent degradation might mainly pertain to newly synthesized but and cotranslationally misfolded mHtt molecules destined for degradation by cellular quality control mechanisms [9]. Accordingly, ubiquitin-positive mHtt aggregates might reflect an overwhelming and ultimately the failure of

cellular quality control systems [10]. A similar mechanism has been described also in another polyQ-expanded disease–Spinocebellar Ataxia Type 1 ([11])—and it therefore might apply to all polyQ-expanded diseases, and possibly to other neurodegenerative diseases associated with aggregate-prone proteins.

In agreement with this possibility, two independent studies, carried out in different HD animal models, reported that mHtt, but not WT Htt, is selectively ubiquitinated on two lysine residues located at the N-terminus of this protein (K6 and K9 [12, 13]). Moreover, in both studies, the majority of the ubiquitinated mHtt resided in insoluble protein fractions. These lysines are part of the conserved 17 amino acid N-terminal segment of Htt, that resides upstream of the polyQ stretch. This segment has been shown to play crucial roles in mHtt oligomerization and fibril nucleation [14], in manners sensitive to post-translational modifications of lysines 6,9 (and 15) through SUMOylation [15] (note that the authors showed that the same two lysine residues can be also modified by ubiquitin) or acetylation [16] (see also Ref. 17). If, as suggested above, ubiquitination at these sites targets misfolded mHtt to UPS-mediated degradation, it might be expected that elimination of these ubiquitination sites would exacerbate mHtt aggregation. A recent study suggests, however, that the outcome is more complex [13]. Here, expression vectors encoding for human mHtt Exon-1 with a 134 glutamine repeat (Htt-134Q) were prepared in which lysine 6 and 9 were (or were not) mutated to arginine. These mHtt variants, fused to EGFP, were expressed in rat cortical neurons in culture, and followed continuously by time-lapse microscopy for about two weeks. Surprisingly, elimination of these ubiquitination sites significantly delayed the appearance of discernable mHtt aggregates and reduced the total numbers of aggregates observed at the end of this period. Paradoxically, however, expressing the same fusion proteins in cell lines revealed that cell death increased and cell survival decreased in preparations expressing the lysine-less mHtt forms.

This apparent paradox was resolved by subjecting extracts of the same cells to biochemical analyses. These revealed that lysine elimination increased the fraction of insoluble mHtt at the expense of the soluble fraction, resulting in minute aggregates detectable by filtration methods but not by the light microscopy techniques used in that study [13]. These observations are congruent with the realization that the most toxic forms of mHtt might not be the large, readily discernable mHtt aggregates, but rather minuscule oligomers or 'protofibrils' of misfolded mHtt [9, 18]. Such oligomers have been shown to impair cell viability by inducing apoptosis [18], or by crippling protein quality control systems such as ER associated degradation [9]. Interestingly, while large aggregate appearance rates were reduced by lysine 6 and 9 elimination, individual aggregate growth kinetics were not, indicating that ubiquitination accelerates a rate limiting process at the beginning of aggregate formation such as nucleation [19] or fibril formation [20]. This interpretation would be in good agreement with the importance of the N-terminal segment in mHtt oligomerization and fibril nucleation [14]. It is worth noting, however, that other forms of post-translational modification (e.g., phosphorylation, acetylation) within this region tend to suppress, rather than enhance aggregate formation [17] as does the addition of lysine residues when aggregation is tested in cell free conditions [20]. It is also worth noting that in the studies describing mHtt ubiquitination mentioned above, it was not reported whether modifications were of the mono or oligo/poly type, and if the latter, which internal ubiquitin lysine(s) were involved. This information is important (although difficult to obtain due to the destruction of ubiquitin chains during the tryptic digestion necessary for mass spectrometric analysis) as it may explain why the adducts are not degraded, and what changes conferred by ubiquitination render mHtt more aggregate-prone (Fig. 1).

The observation that ubiquitination enhances mHtt aggregation–and probably more efficient sequestration of aggregates away from cellular machineries—is not without precedent. Thus for example, ubiquitination has been shown to directly promote the formation of large aggregates of α-Synuclein [21, 22], a very different protein that forms insoluble fibrils in pathological conditions such as Parkinson's disease. This brings up an interesting point related to ubiquitin (and ubiquitin-like) conjugations: Beyond their obvious influences on protein targeting and trafficking, they also affect the physicochemical properties of the modified proteins, promoting formation of large aggregates in some cases or functionally important interactions in others [23, 24]. For example, NEDDylation of PSD-95, a key postsynaptic scaffolding protein, is essential for its function in spatially organizing postsynaptic molecules and complexes. Inhibiting NEDDylation resulted in declustering of the protein, and impaired dendritic spine development and stability, probably via loss of interactions with other proteins [25]. Perhaps this less studied effect of ubiquitination might prove to be informative in understanding relationships between ubiquitination, aggregate formation, and cell death in settings beyond Htt and HD.

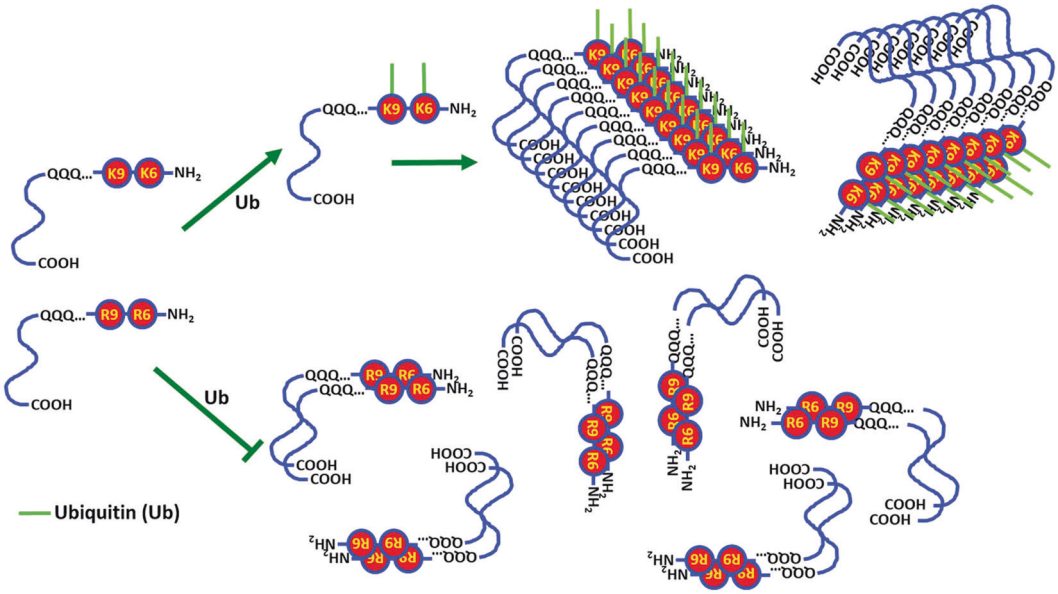

Fig. 1 Site specific ubiquitination of Huntingtin on lysine residues 6 and 9 results in the formation of fewer but larger, probably multi-adduct aggregates. Substituting the ubiquitination sites with arginine residues that cannot be modified, results in the formation of numerous smaller, probably oligo-adduct aggregates—many of which cannot be seen microscopically—that are more toxic to the cell.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Ghosh R, Tabrizi SJ. Huntington disease. Handb Clin Neurol. 2018;147:255–78.
2. Vitet H, Brandt V, Saudou F. Traffic signaling: new functions of Huntingtin and axonal transport in neurological disease. Curr Opin Neurobiol. 2020;63:122–30.
3. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell. 1996;87:493–506.
4. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 1997;90:537–48.
5. Yang H, Yang S, Jing L, Huang L, Chen L, Zhao X, et al. Truncation of mutant huntingtin in knock-in mice demonstrates exon1 huntingtin is a key pathogenic form. Nat Commun. 2020; 11:2582.
6. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science. 1997;277: 1990–3.
7. Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, et al. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Mol Biol Cell. 2001;12:1393–407.
8. Hakim V, Cohen LD, Zuchman R, Ziv T, Ziv NE. The effects of proteasomal inhibition on synaptic proteostasis. EMBO J. 2016;35:2238–62.
9. Shacham T, Sharma N, Lederkremer GZ. Protein misfolding and ER stress in Huntington's Disease. Front Mol Biosci. 2019;6:20.
10. Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI. Progressive disruption of cellular protein folding in models of polyglutamine diseases. Science. 2006;311:1471–4.
11. Cummings CJ, Reinstein E, Sun Y, Antalffy B, Jiang Y-H, Ciechanover A, et al. Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. Neuron. 1999;24:879–92.
12. Sap KA, Guler AT, Bezstarosti K, Bury AE, Juenemann K, Demmers JAA, et al. Global proteome and ubiquitinome changes in the soluble and insoluble fractions of Q175 Huntington mice brains. Mol Cell Proteom. 2019;18:1705–20.
13. Hakim-Eshed V, Boulos A, Cohen-Rosenzweig C, Yu-Taeger L, Ziv T, Kwon YT, et al. Site-specific ubiquitination of pathogenic huntingtin attenuates its deleterious effects. Proc Natl Acad Sci USA. 2019;117:18661–9.
14. Arndt JR, Chaibva M, Legleiter J. The emerging role of the first 17 amino acids of huntingtin in Huntington's disease. Biomol Concepts. 2015;6:33–46.

15. Steffan JS, Agrawal N, Pallos J, Rockabrand E, Trotman LC, Slepko N, et al. SUMO modification of Huntingtin and Huntington's disease pathology. Science 2004;304:100–4.
16. Chaibva M, Jawahery S, Pilkington AW 4th, Arndt JR, Sarver O, Valentine S, et al. Acetylation within the first 17 residues of huntingtin exon 1 alters aggregation and lipid binding. Biophys J. 2016;111:349–62.
17. Schaffert LN, Carter WG. Do post-translational modifications influence protein aggregation in neurodegenerative diseases: a systematic review. Brain Sci. 2020;10:232.
18. Ramdzan YM, Trubetskov MM, Ormsby AR, Newcombe EA, Sui X, Tobin MJ, et al. Huntingtin inclusions trigger cellular quiescence, deactivate apoptosis, and lead to delayed necrosis. Cell Rep. 2017;19:919–27.
19. Scherzinger E, Sittler A, Schweiger K, Heiser V, Lurz R, Hasenbank R, et al. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. Proc Natl Acad Sci USA. 1999; 96:4604–9.
20. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren VM, Byeon IJ, et al. Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. Nat Struct Mol Biol. 2009;16:380–9.
21. Rott R, Szargel R, Haskin J, Shani V, Shainskaya A, Manov I, et al. Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. J Biol Chem. 2008;283:3316–28.
22. Lee JT, Wheeler TC, Li L, Chin LS. Ubiquitination of alpha-synuclein by Siah-1 promotes alpha-synuclein aggregation and apoptotic cell death. Hum Mol Genet. 2008;17:906–17.
23. Ma Q, Ruan H, Peng L, Zhang M, Gack MU, Yao WD. Proteasome-independent polyubiquitin linkage regulates synapse scaffolding, efficacy, and plasticity. Proc Natl Acad Sci USA. 2017;114:E8760–E8769.
24. Rott R, Szargel R, Shani V, Hamza H, Savyon M, Abd Elghani F, et al. SUMOylation and ubiquitination reciprocally regulate α-synuclein degradation and pathological aggregation. Proc Natl Acad Sci USA. 2017;114:13176–81.
25. Vogl AM, Brockmann MM, Giusti SA, Maccarrone G, Vercelli CA, Bauder CA, et al. NEDDylation inhibition impairs spine development, destabilizes synapses and deteriorates cognition. Nat Neurosci. 2015;18:239–51.