Austin Journal of Anatomy

Open Access

Austin Publishing Group

Mini Review

Endoplasmic Reticulum Homeostasis in Huntington's Disease

Elena N Fazio[1], Yuwei Jiang[1] and Patrick Lajoie[1]*

[1]Department of Anatomy and Cell Biology, University of Western Ontario, London, Ontario, Canada

***Corresponding author:** Patrick Lajoie, PhD, Department of Anatomy and Cell Biology, University of Western Ontario London, Ontario N6A 5C1, Canada

Received: September 16, 2014; **Accepted:** November 05, 2014; **Published:** November 10, 2014

Abstract

Huntington's disease (HD) is a neurodegenerative pathology associated with an increased number of polyglutamine repeats (polyQ) within the mutant huntingtin protein (mHtt). Expanded polyQ repeats increase the propensity of the mHtt protein to form aggregates, resulting in the subsequent formation of larger, insoluble, amyloid-like aggregates termed inclusion bodies. Over the years several studies have identified perturbation of endoplasmic reticulum homeostasis (ER stress) as a determinant of polyQ toxicity in various HD models, from yeast cells to patient post-mortem samples. Importantly, targeting ER stress in experimental models of HD has resulted in increased neuronal cell viability both *in vitro* and *in vivo*. Here, we discuss the recent advances in the field that link ER stress signaling pathways to mHtt aggregation and toxicity.

Keywords: Huntington's disease; Endoplasmic reticulum stress; Protein misfolding; Unfolded protein response

Abbreviations

ER: Endoplasmic Reticulum; ERAD: ER-Associated Degradation, FRAP: Fluorescence Recovery after Photobleaching; GFP: Green Fluorescent Protein; HD: Huntington's Disease, mHtt: mutant Huntingtin; Htt^{ex1}: Htt exon 1; PolyQ: Polyglutamine; UPR: Unfolded Protein Response

Huntington's Disease

Huntington 's disease (HD) is an autosomal neurodegenerative pathology associated with mutations in a gene encoding the Htt protein [1]. The result is an increased number of polyglutamine repeats (polyQ) within a stretch of the first exon of Htt (Htt^{ex1}) [2]. In asymptomatic individuals the *HTT* gene encodes less than 35 repeats, while an increase in this number leads to the appearance of HD symptoms in patients, including cognitive loss, involuntary movements and diminished coordination [3] (Figure 1). PolyQ repeat expansion leads to an increased propensity of mutant Huntingtin (mHtt) to aggregate into cytoplasmic, non-soluble, amyloid-like-fibrils called inclusion bodies [4,5]. Various aspects of neuronal cell homeostasis are impaired in HD, including transcription [6], calcium signaling [7], mitochondrial activity [8] and protein degradation pathways [9-11], which can all be traced back to a dysfunctional ER stress response. Thus, expression of mHtt impairs normal cellular functions, especially the ability of cells to properly regulate stress response pathways.

The Unfolded Protein Response

In eukaryotic cells nearly a third of the proteome consists of secreted proteins that enter the secretory pathway via the ER, where quality control machinery assists and monitors the proper folding of the nascent peptides. When environmental insults or mutations perturb the ER folding environment, the result is an increase in the ER misfolded protein burden. Aberrant accumulation of misfolded proteins in the ER is detrimental to cells and triggers an ensemble of coping mechanisms called the Unfolded Protein Response (UPR) [12]. UPR activation results in the initiation of a transcriptional program that leads to the up-regulation of ER chaperones [13] and transient global attenuation of protein translation to decrease the amount of newly synthetized proteins [14]. While the UPR has been conserved throughout evolution, the complexity of the UPR signaling network has evolved over time; one ER stress sensor is present in yeast (Ire1), while mammals have three (IRE1, PERK, and ATF6) [15] (Figure 2). In the budding yeast *S. cerevisiae*, the ER-resident transmembrane protein, inositol-requiring enzyme 1 (Ire1), splices *HAC1* mRNA [16], allowing the translation of the transcription factor Hac1 which increases the expression of a vast number of UPR target genes [17]. These UPR-driven genes include ER chaperones, degradation machinery and genes regulating lipid homeostasis and ER membrane proliferation [17]. Unresolved UPR during prolonged

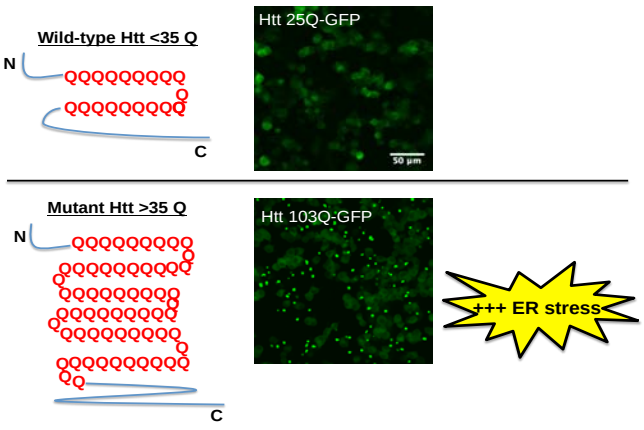

Figure 1: Htt toxicity at the cellular level. Expansion of the number of polyQ repeats within the first exon of Htt results in the appearance of HD symptoms in humans. PolyQ repeat expansion results in increased mHtt aggregation. PC12 cells expressing GFP-tagged Htt exon1 with either 25 or 103 repeats are shown.

Lajoie et al. © All rights are reserved

Citation: Fazio EN, Jiang Y and Lajoie P. Endoplasmic Reticulum Homeostasis in Huntington's Disease. Austin J Anat. 2014;1(5): 1025.

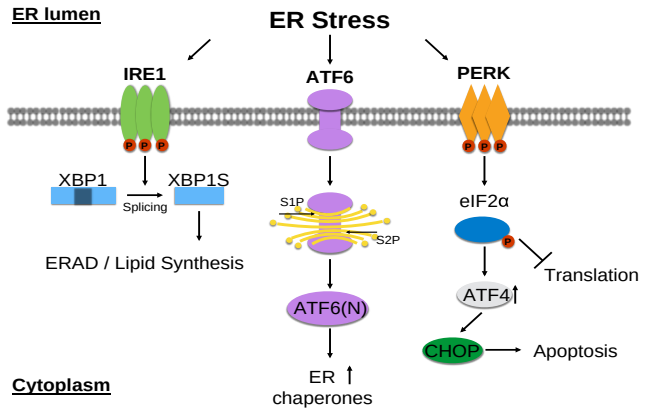

Figure 2: The Unfolded Protein Response. In mammals, the UPR consists of 3 ER stress sensor proteins (IRE1, PERK and ATF6) and their specific signaling cascades that monitor changes in the ER misfolded protein burden.

ER stress can lead to sustained Ire1 signaling, which is ultimately detrimental to yeast cells and leads to growth arrest and death [18,19]. In higher eukaryotes, accumulation of misfolded proteins in the ER over a certain threshold activates cell death signaling pathways, including several caspases, and results in induction of apoptosis [20-23]. Ire1 activation in both yeast and mammals has traditionally been associated with direct binding of misfolded proteins to the lumenal domain of Ire1. However, recent studies including our own, showed that perturbation of the lipid composition of the ER membrane can trigger UPR activation independently of misfolded protein accumulation [24-26]. It appears that the lumenal domain of yeast and mammalian Ire1, as well as PERK, can sense changes in lipids. Thus, multiple mechanisms can lead to UPR sensor activation [25,26]. The physiological relevance of these different activation modes to ER stress-associated diseases such as HD will be the focus of future studies.

In mammals, accumulation of misfolded proteins in the ER also leads to activation of ER stress sensors (Figure 2). During ER stress, ATF6 enters the Golgi apparatus where the Site-1 protease (S1P) and Site-2 protease (S2P) release the ATF6 N-terminal domain that translocates to the nucleus to upregulate genes involved in maintaining homeostasis of the ER folding environment [27,38,39]. PERK and IRE1 homo-oligomerize, autophosphorylate and activate specific downstream signaling cascades [27,28]. PERK phosphorylates eIF2α resulting in global attenuation of translation and increased expression of Activating Transcription Factor (ATF) 4, which then upregulates transcription of ER chaperones [29,30]. However, ATF4 activation can also lead to activation of pro-apoptotic pathways that depend on the production of the CHOP/GADD153 transcription factor [31]. Interestingly, deletion of CHOP increases cell survival during ER stress [32].

Similar to what happens in yeast, mammalian IRE1 splices *XBP1* mRNA allowing the translation of the transcription factor which upregulates specific UPR targets [27,33]. Mammalian IRE1 also degrades mRNA targeted to the ER to reduce the amount of newly synthesized secretory proteins during ER stress in a process termed regulated Ire1-dependent decay (RIDD) [34]. A recent study showed that CHOP activation during prolonged ER stress leads to activation of the cell death receptor 5 (DR5). However, IRE1 can transiently induce DR5 mRNA decay, indicating that different UPR signals have opposing effects [23]. Moreover, IRE1 has also been shown to degrade micro RNAs to derepress caspase 2 expression, leading to cell death [35]. Furthermore, IRE1 regulates other pro-apoptotic molecules including JNK [36] and BCL-2 family members [20]. Thus, the mammalian UPR response is a rather complex paradox, simultaneously inducing both an adaptive response and pro-apoptotic pathways [37]. Regardless, UPR signaling is at the root of various neurodegenerative diseases where misfolded proteins accumulate, including HD, and targeting the UPR in these disorders has been the focus of several studies [40, 41].

UPR Activation in Huntington's Disease

Multiple studies have identified the impairment of ER quality control as a modulator of mHtt toxicity. Striatal cells from the *Htt* knock-in mouse, expressing 111 polyQ repeats, display signs of ER stress [42], as do striatal neurons in the R6/2 mouse, which expresses the Htt^{ex1} containing 150 polyQ repeats [43]. Furthermore, expression of mHtt in knock-in mouse models of HD resulted in activation of ER stress pathways and early upregulation of Rrs1, a regulator of ribosome synthesis [44]. The same study also showed that UPR markers such as BiP are upregulated in post-mortem samples of HD patients [44]. mHtt expression has been shown to both induce ER stress and impair misfolded protein degradation [45,46] and live-cell imaging assays have shown that expression of mHtt in the cytoplasm can trigger accumulation misfolded proteins in the ER [47]. Studies have also shown that the Htt protein can associate with the ER and can potentially play a direct role in induction of ER stress [48,49]. Interestingly, knockdown of the ER stress transcription factor XBP1 targeted to the striatum resulted in a decreased propensity to exhibit HD pathology, with decreased mHtt expression and improved neural survival and motor performance [50]. Finally, treatment of mice expressing mHtt with the bile acid tauroursodeoxycholic acid, a compound known to attenuate ER stress [51], decreased striatal atrophy and apoptosis [52]. Taken together, these findings illustrate a major role for the UPR in HD pathology, making the role of ER homeostasis in cell survival a priority in HD research.

The ER Misfolded Protein Burden and mHtt Expression

Accumulation of misfolded proteins in the ER is a hallmark of perturbed ER homeostasis. However, it was unclear how a cytoplasmic protein such as mHtt could induce changes in the ER misfolded protein load. BiP/GRP78 is a protein folding chaperone that is upregulated during ER stress to help cope with the accumulation of unfolded proteins [53]. In order to monitor the misfolded protein burden in cells expressing toxic mHtt proteins, we developed a live cell imaging technique to quantify misfolded protein burden in real time by expressing a BiP-GFP fluorescent fusion protein in cells. Upon induction of ER stress, BiP binds to unfolded proteins forming a complex that decreases the lateral mobility of the BiP-GFP reporter [47,54]. Fluorescence recovery after photobleaching (FRAP) allows us to photobleach a region of interest (ROI) in BiP-GFP expressing cells and subsequently monitor the diffusion of unbleached fluorescent BiP molecules into the bleached area over time. The diffusion coefficient (*D*) is then calculated using the diffusion rate at which fluorescent signal repopulates the ROI [55].

We previously co-transfected Neuro2a cells with BiP-GFP and mHtt proteins of varying polyQ length [47]. This approach revealed that cells expressing cytoplasmic mHtt show decreased BiP-GFP mobility, suggesting that mHtt indirectly affects the flux of misfolded peptides out of the ER for degradation as previously demonstrated [45]. For the first time, changes in misfolded protein flux in and out of the ER could be quantified in living HD neuronal cells. Thus, this assay can be used to test the ability of small molecules to restore ER homeostasis in HD cells.

ER-Associated Degradation and Huntington's Disease

ER-associated degradation (ERAD) is a degradation mechanism integral to ER protein quality control. ERAD alleviates the misfolded and unfolded protein load in the ER lumen through ERAD-specific factors, transporting proteins to the cytosol for ubiquitin-mediated degradation by the proteasome [56]. We and others have shown that expression of expanded mHtt proteins in yeast, PC12 and mouse striatal cells is toxic due to ER dysfunction [10,46,47]. The mechanism of mHtt toxicity involves the sequestration of the ERAD transport chaperones p97, Npl4 and Ufd1, leading to inefficient transport of unfolded proteins to the cytosol. Unfolded proteins then accumulate in the ER and are not degraded, sensitizing cells to other forms of stress, ultimately leading to cell death [45]. Furthermore, overexpression of Npl4 and Ufd1 in cells expressing mHtt proteins leads to amelioration of toxicity, confirming that mHtt toxicity is elicited through ERAD inhibition [45]. Also, mHtt has been shown to sequester Gp78, an E3 ligase that plays an important role in ERAD [57]. Interestingly, soluble mHtt oligomers, and not inclusion bodies, have been associated with mHtt toxicity. Recently, it was shown that these oligomers are indeed the species responsible for induction or ER stress and ERAD impairment in mHtt-expressing cells [46].

Conclusions

Several research groups have now established a clear link between UPR activation and mHtt toxicity in various models of HD. Recent studies show that modulation of UPR can improve mHtt-expressing cell survival. Interestingly, striatum-targeted knockdown of the ER stress transcription factor XBP1 in mice improves HD phenotypes [50], indicating that preventing excessive UPR activation can be beneficial for HD cells. Moreover, small molecule inhibition of PERK improved survival of striatal cells expressing mHtt [58]. New specific inhibitors of IRE1 have been developed [22,59,60] and have been shown to be beneficial in animal models of multiple myeloma [60] and diabetes [22]. However, their impact on mHtt toxicity remains to be tested. Furthermore, pharmacological inhibition of protein disulfide isomerase (PDI), a key component in protein folding, has been shown to improve cell viability in HD models, indicating that targeting the ER protein control machinery can reduce mHtt toxicity [61]. Thus, targeting UPR sensors and their downstream effectors represents an exciting avenue to improve our ability to manage HD.

Acknowledgements

PL is supported by a Schulich Strategic Support Award from the University of Western Ontario and an operating grant from the Canadian Institutes for Health Research (CIHR). ENF is supported by the CIHR Strategic Training Program in Vascular Research. The authors thank Dr. Robert Cumming (UWO) for the PC12 Htt-GFP cells.

References

1. MacDonald ME, Ambrose CM, M.P D, Gusella JF. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's disease Collaborative Research Group. Cell. 1993; 72: 971-983.
2. Zheng Z, Diamond MI. Huntington disease and the huntingtin protein. Prog Mol Biol Transl Sci. 2012; 107: 189-214.
3. Gusella JF, MacDonald ME. Huntington's disease: seeing the pathogenic process through a genetic lens. Trends Biochem Sci. 2006; 31: 533-540.
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: 537-548.
5. 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: 1990-1993.
6. Riley BE, Orr HT. Polyglutamine neurodegenerative diseases and regulation of transcription: assembling the puzzle. Genes Dev. 2006; 20: 2183-2192.
7. Giacomello M, Oliveros JC, Naranjo JR, Carafoli E. Neuronal Ca (2+) dyshomeostasis in Huntington disease. Prion. 2013; 7: 76-84.
8. Costa V, Scorrano L. Shaping the role of mitochondria in the pathogenesis of Huntington's disease. EMBO J. 2012; 31: 1853-1864.
9. Finkbeiner S, Mitra S. The ubiquitin-proteasome pathway in Huntington's disease. ScientificWorldJournal. 2008; 8: 421-433.
10. Duennwald ML. Yeast as a platform to explore polyglutamine toxicity and aggregation. Methods Mol Biol. 2013; 1017: 153-161.
11. Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. Science. 2001; 292: 1552-1555.
12. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011; 334: 1081-1086.
13. Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature. 1988; 332: 462-464.
14. Pavitt GD, Ron D. New insights into translational regulation in the endoplasmic reticulum unfolded protein response. Cold Spring Harb Perspect Biol. 2012; 4.
15. Mori K. Signalling pathways in the unfolded protein response: development from yeast to mammals. J Biochem. 2009; 146: 743-750.
16. Cox JS, Walter P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell. 1996; 87: 391-404.
17. Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell. 2000; 101: 249-258.
18. Chawla A, Chakrabarti S, Ghosh G, Niwa M. Attenuation of yeast UPR is essential for survival and is mediated by IRE1 kinase. J Cell Biol. 2011; 193: 41-50.
19. Rubio C, Pincus D, Korennykh A, Schuck S, El-Samad H, Walter P. Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. J Cell Biol. 2011; 193: 171-184.
20. Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, et al. Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. Science. 2006; 312: 572-576.
21. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Biol. 2011; 13: 184-190.
22. Ghosh R, Wang L, Wang ES, Perera BG, Igbaria A, Morita S, et al. Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress. Cell. 2014; 158: 534-548.

23. Lu M, Lawrence DA, Marsters S, Acosta-Alvear D, Kimmig P, Mendez AS, et al. Cell death. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis. Science. 2014; 345: 98-101.

24. Lajoie P, Moir RD, Willis IM, Snapp EL. Kar2p availability defines distinct forms of endoplasmic reticulum stress in living cells. Mol Biol Cell. 2012; 23: 955-964.

25. Promlek T, Ishiwata-Kimata Y, Shido M, Sakuramoto M, Kohno K, Kimata Y . Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. Mol Biol Cell. 2011; 22: 3520-3532.

26. Volmer R, van der Ploeg K, Ron D. Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proc Natl Acad Sci U S A. 2013; 110: 4628-4633.

27. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007; 8: 519-529.

28. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol. 2000; 2: 326-332.

29. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell. 2000; 6: 1099-1108.

30. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature. 1999; 397: 271-274.

31. Ma Y, Brewer JW, Diehl JA, Hendershot LM. Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. J Mol Biol. 2002; 318: 1351-1365.

32. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, et al. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev. 1998; 12: 982-995.

33. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol. 2003; 23: 7448-7459.

34. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS . Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol. 2009; 186: 323-331.

35. Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, et al. IRE1α cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. Science. 2012; 338: 818-822.

36. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 2000; 287: 664-666.

37. Rutkowski DT1, Arnold SM, Miller CN, Wu J, Li J, Gunnison KM, Mori K . Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. PLoS Biol. 2006; 4: e374.

38. Shen J1, Chen X, Hendershot L, Prywes R . ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Dev Cell. 2002; 3: 99-111.

39. Rutkowski DT1, Kaufman RJ . A trip to the ER: coping with stress. Trends Cell Biol. 2004; 14: 20-28.

40. Hetz C, Glimcher LH. Protein homeostasis networks in physiology and disease. Curr Opin Cell Biol. 2011; 23: 123-125.

41. Vidal R, Caballero B, Couve A, Hetz C. Converging pathways in the occurrence of endoplasmic reticulum (ER) stress in Huntington's disease. Curr Mol Med. 2011; 11: 1-12.

42. Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, et al. Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. Hum Mol Genet. 2000; 9: 2799-2809.

43. Cho KJ, Lee BI, Cheon SY, Kim HW, Kim HJ, Kim GW. Inhibition of apoptosis signal-regulating kinase 1 reduces endoplasmic reticulum stress and nuclear huntingtin fragments in a mouse model of Huntington disease. Neuroscience. 2009; 163: 1128-1134.

44. Carnemolla A, Fossale E, Agostoni E, Michelazzi S, Calligaris R, De Maso L, et al. Rrs1 is involved in endoplasmic reticulum stress response in Huntington disease. J Biol Chem. 2009; 284: 18167-18173.

45. Duennwald ML, Lindquist S . Impaired ERAD and ER stress are early and specific events in polyglutamine toxicity. Genes Dev. 2008; 22: 3308-3319.

46. Leitman J, Ulrich Hartl F, Lederkremer GZ. Soluble forms of polyQ-expanded huntingtin rather than large aggregates cause endoplasmic reticulum stress. Nature communications. 2013; 4: 2753.

47. Lajoie P, Snapp EL. Changes in BiP availability reveal hypersensitivity to acute endoplasmic reticulum stress in cells expressing mutant huntingtin. J Cell Sci. 2011; 124: 3332-3343.

48. Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol. 2011; 7: 453-460.

49. Atwal RS, Xia J, Pinchev D, Taylor J, Epand RM, Truant R. Huntingtin has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. Hum Mol Genet. 2007; 16: 2600-2615.

50. Vidal RL, Figueroa A, Court FA, Thielen P, Molina C, Wirth C, et al. Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. Human molecular genetics. 2012; 21: 2245-2262.

51. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science. 2006; 313: 1137-1140.

52. Keene CD, Rodrigues CM, Eich T, Chhabra MS, Steer CJ, Low WC. Tauroursodeoxycholic acid, a bile acid, is neuroprotective in a transgenic animal model of Huntington's disease. Proc Natl Acad Sci U S A. 2002; 99: 10671-10676.

53. Kozutsumi Y, Normington K, Press E, Slaughter C, Sambrook J, Gething MJ. Identification of immunoglobulin heavy chain binding protein as glucose-regulated protein 78 on the basis of amino acid sequence, immunological cross-reactivity, and functional activity. J Cell Sci Suppl. 1989; 11: 115-137.

54. Lai CW, Aronson DE, Snapp EL . BiP availability distinguishes states of homeostasis and stress in the endoplasmic reticulum of living cells. Mol Biol Cell. 2010; 21: 1909-1921.

55. Snapp EL, Altan N, Lippincott-Schwartz J. Measuring protein mobility by photobleaching GFP chimeras in living cells. Curr Protoc Cell Biol. 2003; Chapter 21: Unit 21.

56. Bernasconi R, Molinari M. ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER. Curr Opin Cell Biol. 2011; 23: 176-183.

57. Yang H, Liu C, Zhong Y, Luo S, Monteiro MJ, Fang S. Huntingtin interacts with the cue domain of gp78 and inhibits gp78 binding to ubiquitin and p97/VCP. PLoS One. 2010; 5: e8905.

58. Leitman J, Barak B, Benyair R, Shenkman M, Ashery U, Hartl FU, et al. ER stress-induced eIF2-alpha phosphorylation underlies sensitivity of striatal neurons to pathogenic huntingtin. PLoS One. 2014; 9: e90803.

59. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, et al. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. Proc Natl Acad Sci U S A. 2012; 109: E869-878.

60. Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, et al. Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. Blood. 2011; 117: 1311-1314.

61. Hoffstrom BG, Kaplan A, Letso R, Schmid RS, Turmel GJ, Lo DC. Inhibitors of protein disulfide isomerase suppress apoptosis induced by misfolded proteins. Nat Chem Biol. 2010; 6: 900-906.