A SYSTEMS BIOLOGY APPROACH TOWARDS DECIPHERING THE UNFOLDED PROTEIN RESPONSE IN HUNTINGTON´S DISEASE

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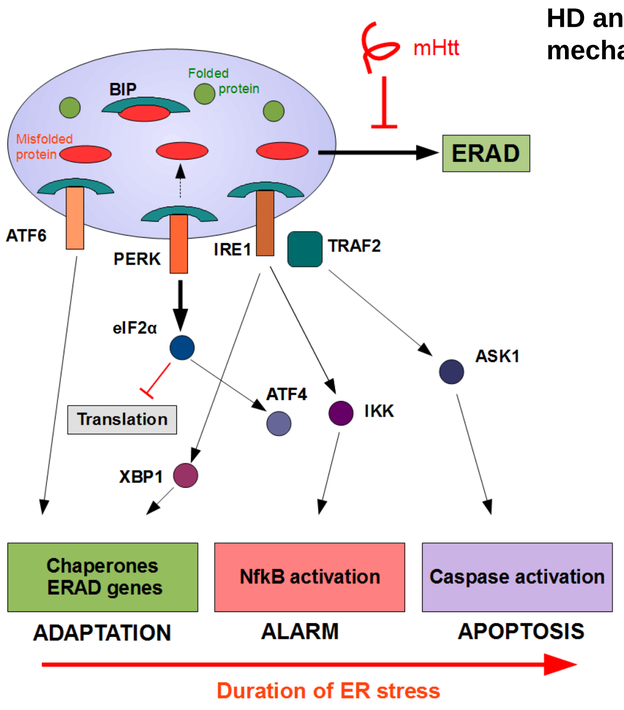
Introduction

Although the disease-causing gene (*huntingtin*) has been known for some time, the exact cause of neuronal cell death during Huntington´s Disease (HD) is still unknown. One potential mechanism contributing to the massive loss of neurons in HD brains might be the unfolded protein response (UPR) with is activated by accumulation of misfolded proteins in the endoplasmatic reticulum (ER).

Here, we examined the activation of UPR during HD, and its connection to neuroinflammation and apoptosis to elucidate it potential role as a disease-relevant process. Due to the complexity of these molecular mechanisms, a system biology approach was pursued.

ER Stress and UPR



HD and possible UPR-associated mechanisms during disease progression

mHtt: mutant Huntingtin

BIP: Binding protein (glucose-regulated protein, 78kDa, HSP70a5)

ERAD: ER-associated protein degradation

ATF6: activating transcription factor 6

PERK: protein kinase RNA (PKR)-like ER kinase

IRE1: Inositol-requiring protein-1

XBP1: X-box binding protein 1

eIF2α : α subunit of eukaryotic translation initiation factor-2

TRAF2: TNF receptor-associated factor 2

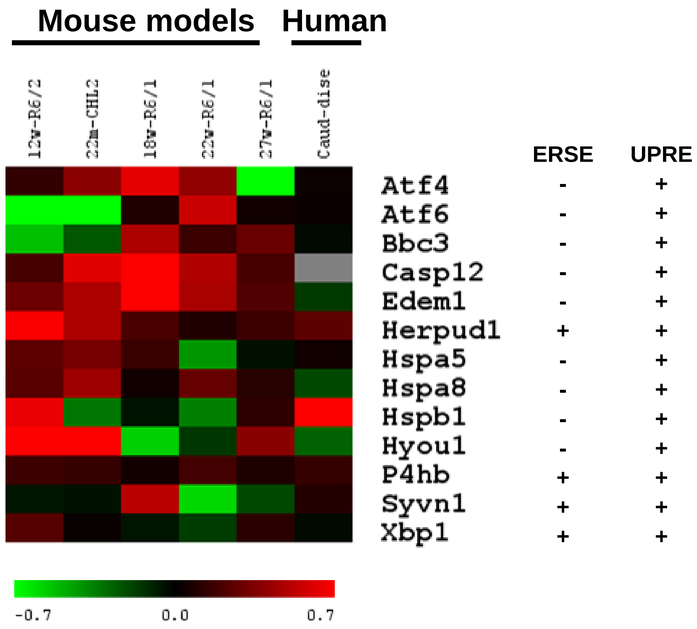
ASK1: apoptosis signal-regulating kinase 1

Mutant Huntingtin can interfere with ER-associated degradation resulting in accumulation of misfolded protein in the ER and thus in the activation of the UPR (Duennwald & Lindquist, 2008). As an adaptive response, the UPR up-regulates transcription of chaperones, temporarily attenuates new translation, and activates protein degradation via the proteasome. Persistent levels of ER stress, however, may trigger inflammatory pathways and can ultimately lead to the induction of neuronal cell death.

Expression of UPR genes in HD

To examine whether the UPR is activated in HD, we assemble a set of genes that are known to be involved in UPR (such as ATF6 and XBP1) or to be expressed under ER stress (such as HSPA5 and HERPUD1). Available gene expression data of HD mouse models (Kuhn *et al.* 2006 and Hodges *et al.* 2008) and human HD brain samples (Hodges *et al.* 2006) were examined for disease-associated changes. Additionally, promoter sequences (-5000 kb to +1000kb) were inspected for the presence of known ER stress elements (ERSE) and UPR elements (UPRE).

Our analysis indicates the activation of several UPR-associated genes, especially during the early stages of HD pathogenesis.



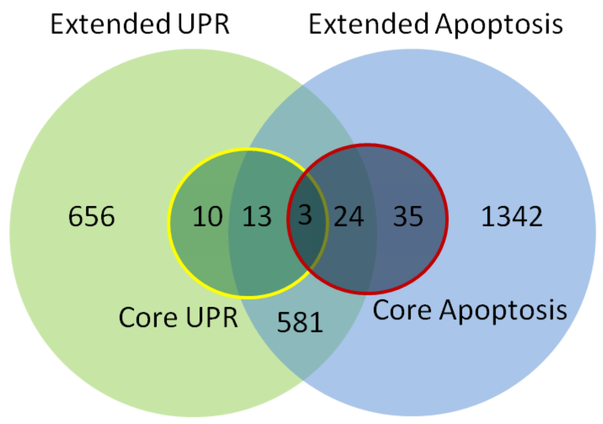
	ERSE	UPRE
Atf4	-	+
Atf6	-	+
Bbc3	-	+
Casp12	-	+
Edem1	-	+
Herpud1	+	+
Hspa5	-	+
Hspa8	-	+
Hspb1	-	+
Hyou1	-	+
P4hb	+	+
Syvn1	+	+
Xbp1	+	+

Expression of UPR–associated genes in HD mouse models (1-5 columns from left) and human HD patients (6th column). Up-regulation is represented by shades of red, down-regulation is represented by shades of green. Additionally, the presence of ERSE and UPRE in the promotor regions is indicated : +'= present; '-'= absent

Crosstalk between UPR and Apoptosis

To identify proteins linking UPR and apoptosis, we first defined a core UPR by assembling a network of the main effectors PERK, ATF6, ATF4 and XBP1 and their interaction partners (n=26). A network for the core apoptosis pathway was obtained from the KEGG database and comprises 62 proteins.

The direct overlap between UPR and apoptosis networks was constituted by three proteins only. For an increased coverage of possible cross-links between UPR and apoptosis, the core networks were extended by protein interaction partners from the UniHI database (*www.unihi.org*). This resulted in a considerably larger overlap.



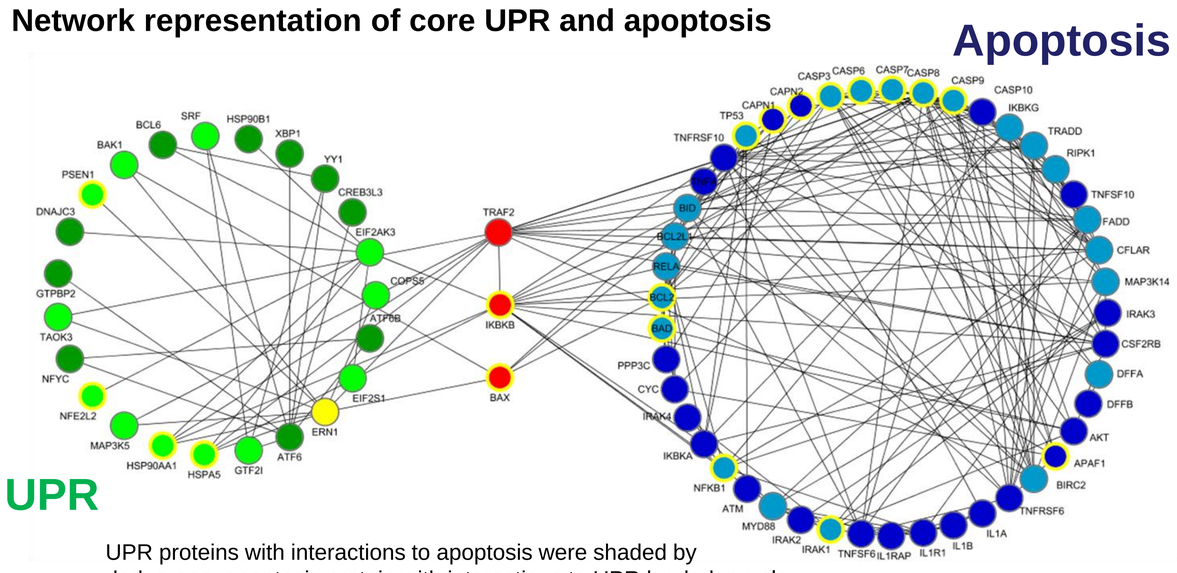
Venn diagram for core and extended UPR and apoptosis network

The overlap between the core UPR and apoptosis networks consisted of :

- **TRAF2** (TNF receptor-associated factor 2): ER stress-induced neuronal death can be activated by binding of ASK1 to the complex of IRE1 and TRAF2 (Nishitoh H *et al.* 2002).
- **IKBKB** (IκB-kinase beta): Phosphorylation of IkB proteins by IKBKB leads to their ubiquitination and destruction , thereby allowing activation of the NF-kappa-B complex.
- **BAX (**BCL2-associated X protein): This protein forms a heterodimer with BCL2, and acts as an apoptotic activator.

To enable visual inspection of the interconnection between UPR and apoptosis, a graphical representation of the core networks was generated and different types of proteins were highlighted.

Network representation of core UPR and apoptosis



UPR proteins with interactions to apoptosis were shaded by dark green, apoptosis protein with interactions to UPR by dark purple. Interconnecting proteins are shown in blue.
HD associated genes are highlighted by yellow circles.

Conclusions and Future Direction

Several studies indicated the activation of the UPR in Huntington´s disease as well as in related neurodegenerative disease. Its relevance for disease progression has remained unclear.

Our analysis of publicly available expression data showed the up-regulation of several UPR marker genes in different mouse models as well as in HD brains. More detailed analysis of the expression profile as well as the promotor regions of UPR genes may clarify their temporal activation.

Examination of the physical interaction network revealed a strong coupling between UPR and apoptosis. Inspection of network structures suggested a important role of TRAF2, IKBKB and BAX for connecting both processes. For a more complete representation of UPR-induced apoptosis, a dynamic network representation will be constructed. Such model may also serve as rationale basis for experimental studies.

Acknowledgement

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