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Review

Defining the role of the Bcl-2 family proteins in Huntington's disease

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B-cell lymphoma 2 (Bcl-2) family proteins regulate survival, mitochondria morphology dynamics and metabolism in many cell types including neurons. Huntington's disease (HD) is a neurodegenerative disorder caused by an expanded CAG repeat tract in the *IT15* gene that encodes for the protein huntingtin (*htt*). *In vitro* and *in vivo* models of HD and HD patients' tissues show abnormal mitochondrial function and increased cell death rates associated with alterations in Bcl-2 family protein expression and localization. This review aims to draw together the information related to Bcl-2 family protein alterations in HD to decipher their potential role in mutated *htt*-related cell death and mitochondrial dysfunction.

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Facts

- The B-cell lymphoma 2 (Bcl-2) family proteins regulate the outer mitochondrial membrane (OMM) permeability thus controlling the mitochondrial potential, mitochondrial morphology dynamics and the balance between survival and death in many cell types including neurons.
- HD is a neurodegenerative disease characterized by progressive neuronal dysfunction and death. In patients with HD and in *in vitro* and *in vivo* HD models, mutated *htt* expression leads to OMM permeabilization, loss of mitochondrial potential and mitochondrial fragmentation.
- HD cell culture models and HD mouse brain models show alterations in Bcl-2 family protein expression and localization. Autopsy studies in patients' brain have detected Bcl-2 family protein alterations in HD.

Open Questions

- We still need more precise evidence on the role of Bcl-2 family proteins in HD-related cell death.
- We do not know the role of Bcl-2 family proteins in HD-related mitochondrial dysfunction.
- Nor do we know through which molecular pathway Bcl-2 family proteins can cause mitochondrial dysfunctions and cell death in HD.

Introduction

Proteins belonging to the Bcl-2 family are key regulators of the apoptotic mitochondrial pathway. Growing evidence shows that Bcl-2 family proteins also modulate mitochondrial morphology^{1–3} and cellular metabolism⁴ independently from cell death mechanisms. Given that HD typically leads to progressive neuronal death causing neuronal cells to display several mitochondrial dysfunctions (including decreased mitochondrial potential ($\Delta\psi$), abnormal calcium handling, decreased ATP synthesis capacity and increased mitochondrial fragmentation), research has for long investigated Bcl-2 family proteins in HD models and tissues from patients with HD. These studies highlighted evidence that mutated *htt* dysregulates Bcl-2 family protein levels and localization. Considering the complex Bcl-2 network, a literature review could help to elucidate these proteins' role in mutated *htt*-related mitochondrial dysfunction and cell death.

What Evidence Exists for Dysregulated Bcl-2 Family Proteins in HD?

Research has to date identified over 20 Bcl-2 family members classified, according to their function, in three Bcl-2 family subgroups: the pro-survival proteins, the pro-apoptotic BH3-only proteins and the pore-forming proteins. Here, we

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Abbreviations: HD, Huntington's disease; *htt*, huntingtin; OMM, outer mitochondrial membrane; ER, endoplasmic reticulum; $\Delta\psi$, mitochondrial potential; BH3, Bcl-2 Homology 3; CNS, central nervous system; OPA1, optic atrophy-1 protein; UPR, unfolded protein response; SGC, superior cervical ganglion; BDNF, brain-derived neurotrophic factor; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; Bad, Bcl-2 antagonist of cell death; Bim, Bcl-2 interacting mediator of cell death; Bid, Bcl-2 interacting domain death agonist; Bnip3, Bcl-2/adenovirus E1B 19 kDa interacting protein 3; Puma/Bbc3, p53-upregulated modulator of apoptosis/Bcl-2 binding component 3; Bax, Bcl-2-associated X-protein; Bak, Bcl-2 antagonist/killer

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review the three subgroups, focusing on the Bcl-2 family members relevant for HD and gathering data on the cell culture model SThdH,⁵ cell culture models obtained by transient htt transfection, in genetic mouse models R6/1, R6/2,⁶ Tet/HD94,⁷ N171-82Q,⁸ YAC128⁹ and human cells from patients with HD.

The Pro-survival Bcl-2 Proteins: Bcl-2 and Bcl-xL

Bcl-2 and B-cell lymphoma-extra large (Bcl-xL) function to repress programmed cell death.^{10,11} They are stably inserted in intracellular membranes (OMM and the endoplasmic reticulum (ER) membrane) and inhibit cell death by sequestering the pro-apoptotic Bcl-2 family proteins into inactive complexes.¹² In the normal central nervous system (CNS), Bcl-2 is widespread expressed during the embryonic stage, whereas after birth it progressively declines with aging. Studies in adult monkey and adult human CNS disclosed, apart from rare Bcl-2 positive neurons, that most neurons and astrocytes express no Bcl-2 and that most Bcl-2 protein in adult brain results from microglial expression.¹³ Bcl-xL is encoded by the *Bcl-x* gene and, differently from Bcl-2, retains its expression in adult neurons at a relatively high level, predominantly in Purkinje cells in the cerebellum, in cerebral cortical neurons and in hippocampal neurons.^{14,15}

Bcl-2 level alterations in HD remain controversial. Bcl-2 levels were first investigated in HD cell culture models: mutated htt expression significantly decreased Bcl-2 protein levels in the neuroblastoma cell line Neuro2A¹⁶ and significantly decreased Bcl-2 transcript and Bcl-2 protein levels in immortalized striatal cells.^{17,18} Although these results strongly suggest that mutated htt expression decreases Bcl-2 levels, they provide no definitive proof given that the proto-oncogene *Bcl-2* is highly dysregulated in many tumor cell lines including those of neural origin.^{19,20} Hence, data from immortalized cell cultures might not correspond to Bcl-2 levels in post-mitotic neurons.

Others have investigated Bcl-2 levels also in brains from HD mouse models. A first study detected no Bcl-2 protein level changes in the total brain or in mitochondrial fractions from R6/2 mice as compared with littermate controls.²¹ Accordingly, two independent studies reported no differences in Bcl-2 mRNA and protein levels between R6/1 and control mice.^{22,23} Conversely, more recent studies disclosed decreased Bcl-2 mRNA and protein levels in R6/2 mouse brains^{17,24} and N171-82Q mice.²⁵

After reappraising these seemingly contradictory findings, we find it difficult to provide a definitive answer about whether Bcl-2 levels are dysregulated in HD mouse models. To understand the potential protective role of Bcl-2 in HD, Zhang *et al.*²¹ crossed R6/2 mice with transgenic mice selectively overexpressing Bcl-2 in neurons: double transgenic R6/2-Bcl-2 mice survived longer than R6/2 littermates and their motor deficits had a significantly later onset. Notwithstanding contradictory data about Bcl-2 expression in HD mouse models, this result suggests that Bcl-2 overexpression can protect neurons from toxicity elicited by mutated htt.

Bcl-2 expression was analyzed by immunostaining in post-mortem controls and the HD patients' caudate nucleus.

Control neurons had low or negative Bcl-2 signals, in agreement with a previous report,¹³ whereas Bcl-2 labeling was stronger in HD neurons, and Bcl-2 expression reached maximum in the brains of HD patients with longer disease duration.²⁶ In accordance with a potential neuroprotective role of Bcl-2 in HD, the investigators suggested that HD patients' neurons may increase Bcl-2 expression in an attempt to survive. An alternative hypothesis is that Bcl-2 protein in HD brains is cleaved by caspase proteases activated by mutated htt expression.²⁷ If so, the caspase-dependent cleavage inactivates Bcl-2 antiapoptotic function and converts Bcl-2 into a Bcl-2 associated X-protein (Bax)-like protein that enhances cell death.²⁸ According to this hypothesis, rather than inhibiting cell death, high Bcl-2 levels in HD cells could worsen neurodegeneration. The Bcl-2 upregulation in HD patients seems to be brain-specific given that no differences were found between Bcl-2 protein levels in peripheral blood cells from patients with HD and from controls.²⁹

Few data are available about the other pro-survival protein, Bcl-xL in HD: Bcl-xL protein levels were investigated in the brain of HD mice, but no significant difference was found in total-brain lysates, striatum lysates or mitochondrial fractions in HD mice and controls.^{21,22,30} To our knowledge, only one report suggests a role of Bcl-xL in HD. The authors showed that Bcl-xL expression prevented the htt proteolysis induced by DNA damage and hypothesized that blocking Bcl-xL may activate the caspases that cause htt proteolysis.³¹

The Pro-apoptotic BH3-only Proteins

The pro-apoptotic BH3-only proteins, by responding to specific death and survival signals, function as sensors of cellular damage. Activating one or more among the BH3-only proteins culminates in allosteric activation of the pore-forming proteins Bax and Bcl-2 antagonist/killer (Bak).

BH3-only proteins could activate Bax and Bak through two mechanisms. The 'direct activation' model posits that certain BH3-only proteins termed 'activators' (eg, Bcl-2 interacting mediator of cell death (Bim), Bcl-2 interacting domain death agonist (Bid)/truncated Bid (tBid) and p53 upregulated modulator of apoptosis (Puma)) bind to Bax and Bak directly triggering their oligomerization in the OMM, whereas other BH3-only proteins, termed 'sensitizers' (eg, Bcl-2 antagonist of cell death (Bad), Bcl-2/adenovirus E1B 19 kDa interacting protein 3 (Bnip3) and Puma) would bind only to pro-survival Bcl-2 family members, thus liberating 'activator' proteins. Instead, the 'indirect activation' model posits that all the BH3-only proteins bind to pro-survival Bcl-2 family members, thereby preventing them from binding to and neutralizing Bax or Bak.³²

Bcl-2 Antagonist of Cell Death

Bad has a double function: it separately regulates apoptosis and glucose metabolism in multiple cell types including neurons.^{33,34} Investigating *in vitro* HD models, Rigamonti *et al.*³⁵ showed that Bad overexpression in immortalized striatal cells induces apoptosis only in clones co-expressing

mutated htt, thus suggesting that mutated htt toxicity induces Bad-dependent cell death. For HD mouse models, no differences in striatal Bad protein levels were found between R6/1 and littermate controls.²² Also in the R6/2 mouse, no change was found in the brain Bad protein levels. Conversely, the investigators found a significant decrease in phosphorylated Bad (pBad) during the late-HD stages in R6/2 mice.²¹ Because pBad promotes mitochondrial respiration and ATP production, whereas non-pBad binds the anti-apoptotic partners Bcl-2 and Bcl-xL eliciting Bax and Bak activation,³⁶ the low pBad/Bad ratio found in R6/2 brain lysates suggests that Bad activity shifts from positive metabolic to negative apoptotic function in HD cells.

Bcl-2 Interacting Mediator of Cell Death

The BH3-only pro-apoptotic protein Bim is expressed in three major isoforms generated by alternative splicing: Bim short (BimS), Bim long (BimL) and Bim extra-long (BimEL). All these isoforms neutralize activity in pro-survival Bcl-2-like proteins, but they differ in pro-apoptotic potency, BimS being the most effective and BimEL the least effective killer.³⁷ In the mouse and human CNS, Bim is expressed primarily in neurons, and the most expressed isoform in the brain is BimEL.^{38,39}

Several observations show that mutated htt causes BimEL accumulation in various cell types. In particular, mutated htt expression induces BimEL accumulation in HEK293T cells, in Neuro-2a cells,⁴⁰ in sympathetic superior cervical ganglion neurons⁴¹ and in immortalized striatal cells.⁴² BimEL silencing in Neuro-2a cells significantly reduces cell death elicited by mutated htt.⁴⁰

In the mouse model R6/2, high BimEL levels were found in total-brain lysates and in mitochondrial fractions specifically at the late stages of disease.^{21,40} These data found further confirmation in two other HD mouse models showing increased striatal BimEL in R6/1 mice at the late stages of disease and in the conditional model Tet/HD94.²² Collectively, these results demonstrate that mutated htt causes BimEL accumulation and translocation to mitochondria.

Bcl-2 Interacting Domain Death Agonist

The BH3-only pro-apoptotic protein Bid is involved in neuronal cell death in many neurological disorders such as stroke,⁴³ ischemia⁴⁴ and Alzheimer's disease.⁴⁵ Full-length Bid has extremely weak pro-apoptotic activity but reaches its strongest pro-apoptotic activity after proteolytic cleavage by several proteases that produce truncated Bid (tBid).⁴⁶ Bid protein is widely expressed in embryonic and postnatal brain, and its expression in post-mitotic neurons in the limbic system, basal ganglia, mesencephalic tectum and cerebellum persists at a high level into adulthood.¹⁵

Several lines of evidence show that mutated htt causes Bid and tBid accumulation in neurons and in mitochondrial neuronal fractions. In particular, western blot analysis for Bid and tBid detection in HeLa cells and Neuro2A cells disclosed that mutated htt transfection causes Bid cleavage.¹⁶

Increased Bid cleavage was also detected in brain lysates from R6/2 mice at the middle stage of HD.²¹ Different results

come from the R6/1 model in which Garcia-Martinez *et al.*²² found no tBid accumulation, but instead found full-length Bid at high levels in the striatum and in striatal mitochondrial fractions. The Bid protein increase correlated with enhanced Bid mRNA expression. Increased full-length Bid protein levels were also found in the striatum in the conditional mouse model of HD, Tet/HD94: Bid accumulation specifically depended on mutated htt expression, given that transgene suppression completely reverted Bid protein to wild-type levels.²²

Bcl-2/adenovirus E1B 19kDa Interacting Protein 3

Bnip3 has had an emerging role in human health, as convincing evidence implicated its death, inducing activity in heart diseases, whereas Bnip3 loss of function was associated with tumor growth.^{47,48} Current knowledge shows that Bnip3 is involved in cell death, autophagy and programmed mitochondrial clearance.⁴⁹ Bnip3 induces cell death through at least two distinct mechanisms: Bnip3 can engage anti-apoptotic Bcl-2 family members to trigger Bax-Bak-dependent OMM permeability⁵⁰ or can induce a novel mitochondrial leak pathway by interacting with the optic atrophy-1 protein (OPA1).^{51,52}

Because Bnip3 is expressed in the brain and skeletal muscle,^{53,54} in recent years, we undertook a study aimed to investigate the potential role of Bnip3 in HD by assessing Bnip3 level and localization in htt-transfected neuronal cells, brain in HD mouse models and in muscle cells from HD patients.⁵⁵ We observed that mutated htt expression causes Bnip3 accumulation in whole-cell lysates and in the mitochondrial fraction of SHSY5Y and HEK293T cells. We also found higher Bnip3 protein levels in mitochondrial fractions from R6/2 mice and in striatum from YAC128 mice than in littermate controls. Finally, we observed that Bnip3 mainly co-localized with the mitochondria in HD patients' muscle cells, whereas in control cells it mainly localized in the cytosol and nucleus. We also observed that the expression of a dominant-negative Bnip3 named Bnip3 Δ TM^{56,57} rescued the mitochondrial membrane potential loss in HD muscle cells.⁵⁵ Overall, these data suggest that mutated htt enhances Bnip3 activity in neuronal and muscular cells.

p53-upregulated Modulator of Apoptosis/Bcl-2 Binding Component 3 (Puma/Bbc3)

Puma/Bbc3 is a BH3-only protein identified in 2001.^{58,59} Puma/Bbc3 appears not to be expressed in the normal adult brain, but its expression is strongly induced in some brain diseases such as status epilepticus⁶⁰ and cerebral ischemia.⁶¹ Originally identified as a transcriptional target of p53,^{58,59} Puma/Bbc3 is also transcriptionally induced in neuronal cells undergoing ER stress-induced unfolded protein responses (UPR).⁶² Few data are available about Puma/Bbc3 expression in HD models: in SCG neurons, mutated htt expression leaves Puma/Bbc3 mRNA unchanged,⁴¹ whereas in PC12 cells it increases Puma/Bbc3 protein sixfold. Although Puma/Bbc3 protein accumulation in HD may depend on mutated htt-induced ER stress, because this datum remains unconfirmed in other HD models, the possible

pathogenetic role of Puma/Bbc3 in HD needs further clarification.

The Pore-forming Proteins Bax and Bcl-2 Antagonist/Killer (Bak)

Bax and Bak act downstream of the pro-survival and BH3-only members and have a key role in the mitochondrial apoptotic pathway. Bak is normally inserted in the OMM; Bax is predominantly cytosolic, but, once activated, it translocates from the cytosol into the OMM where together with Bak it forms the apoptotic pore, the point of no return in mitochondrial apoptosis.⁶³ Besides inducing apoptosis, Bax and Bak influence mitochondrial fission–fusion dynamics in healthy cells.^{1,2} Both Bax and Bak are expressed in neurons: Bax is expressed in many regions in the human CNS^{64,65} including the human caudate nucleus,²⁶ and Bak is expressed in the human brain at the fetal, adult and elder stages.⁶⁶

Ample evidence underlining the link between Bax activation and HD pathogenesis comes from research assessing Bax protein levels and localization in neuronal cell culture models of HD and in the brains of HD mouse models. Mutated htt transfection in PC12 cells increased Bax protein levels fourfold as compared with wt htt transfection,⁶⁷ in Neuro2A

cells, mutated htt expression induced Bax translocation from the cytosol to the mitochondria.⁴⁰ Only one *in vitro* study argues against a Bax role in mutated htt-induced cell death: King *et al.*²¹ showed that in sympathetic superior cervical ganglion (SCG) neurons mutated htt induced Bax-independent cell death.

Studies in HD mouse models showed increased Bax levels in the brain mitochondrial fractions from R6/1 and R6/2 mice^{21,22} and increased Bax mRNA in the cortex and cerebellum from R6/1 mice, brain areas in which Bax mRNA correlated with the number of apoptotic nuclei.²³ Bax expression was also analyzed in the caudate nucleus from HD patients. The cytoplasmic Bax signal was stronger in caudate neurons from HD patients than from controls. Bax expression was already maximal in HD brains at disease onset and the highest in the most severely compromised shrunken and dark neurons.²⁶ Hence, data from HD mouse models and human tissues converge to indicate that mutated htt expression causes Bax protein accumulation and Bax translocation to the OMM in neurons. Bax dysregulation in HD seems not to be brain specific insofar as Bax protein levels were also higher in lymphocytes and monocytes from HD patients than from controls.²⁹ This finding probably depends on the fact that htt is ubiquitously expressed in human tissues.⁶⁸

Table 1 Evidence for Bcl-2 family protein dysregulation in cell culture models of HD (Significant differences between HD and control cells are highlighted in bold)

Cell culture model	Observations / Results	Reference
Neuro2A cells transfected with htt-exon1 (16Q vs 40Q)	↑tBid and ↓ Bid protein in total lysates ↓Bcl-2 protein in total lysates	Majumder <i>et al.</i> ¹⁶
Neuro2A cells transfected with htt-exon1 (25Q vs 103Q)	↑Bax protein in mitochondrial fractions ↑BimEL protein in total lysates and in mitochondrial fractions ↓Bid protein in total lysates	Leon <i>et al.</i> ⁴⁰
STHdh cells expressing full-length htt (7Q vs 111Q)	↑BimEL protein in total lysates	Kong <i>et al.</i> ⁴²
STHdh cells expressing full-length htt (7Q vs 109Q)	↓Bcl-2 mRNA and Bcl-2 protein in total lysates	Ju <i>et al.</i> ¹⁷
STHdh cells expressing full-length htt (7Q vs 111Q)	↓Bcl-2 protein and ↓Bcl-2/Bax protein ratio in total lysates	Ruiz <i>et al.</i> ¹⁸
HEK293 cells transfected with full-length htt (Q17 vs Q47)	↑Bnip3 protein in total lysates and in mitochondrial fractions	Sassone <i>et al.</i> ⁵⁵
HEK293 cells transfected with htt-exon1 (25Q vs 72/103Q)	↑BimEL protein and ↑pSer69-BimEL in total lysates ↑BimEL mRNA (with htt 103Q)	Leon <i>et al.</i> ⁴⁰
PC12 cells transfected with htt N171 fragment (18Q vs 82Q)	↑Bax protein in total lysates ↑Puma/Bbc3 protein in total lysates	Bae <i>et al.</i> ⁶⁷
sympathetic superior cervical ganglion neurons infected with htt-exon1 (25Q vs 97Q)	↑BimEL protein in total lysates No change in Puma/Bbc3 mRNA in basal condition	King <i>et al.</i> ⁴¹
SHSY5Y cells transfected with htt-exon1 (9Q vs 60Q)	↑Bnip3 protein in total lysates and in mitochondrial fractions	Sassone <i>et al.</i> ⁵⁵
HeLa cells transfected with htt-exon1 (16Q vs 40Q)	↓Bcl-2 mRNA ↑tBid and ↓Bid protein in total lysates	Majumder <i>et al.</i> ¹⁶

Little is known about Bak levels in HD cells: Garcia-Martinez *et al.*²² analyzed the R6/1 mouse striatum and found equal Bak levels in R6/1 mice and littermate controls. Although these data may indicate that Bak has no role in HD cell death and mitochondrial dysfunction, others observed that immortalized striatal cells overexpressing wt htt are completely protected from Bak-induced death.³⁵ Whether and, if so, how Bak intervenes in the pathogenic pathway induced by mutated htt therefore remains an intriguing topic for further investigation.

Through Which Molecular Pathway Could Bcl-2 Family Proteins Cause Mitochondrial Dysfunctions and Cell Death in HD?

By summarizing the data about Bcl-2 family proteins in cell culture models of HD (Table 1), mouse models of HD (Table 2) and cells and tissues from HD patients (Table 3), we formulated a hypothetical model for explaining Bcl-2 family protein activation in HD (Figure 1).

Because many results converge to demonstrate that mutated htt causes BimEL activation (Tables 1 and 2), we

hypothesize that BimEL, a protein that in healthy cells associates with cellular microtubule complexes,⁶⁹ in HD neurons localizes to mitochondria where, according to the 'direct activation' model (paragraph 2.2), it would trigger Bax activation. This hypothesis receives support from evidence that both BimEL and Bax accumulate in the mitochondrial fractions from R6/1 and R6/2 mouse models. Alternatively, according to the 'indirect activation' model, BimEL in HD cells may bind to Bcl-2, thereby preventing it from neutralizing Bax. In this context, Bcl-2 may have a neuroprotective role in HD. This hypothesis receives support from evidence that Bcl-2 overexpression in neurons slows down neurodegeneration in the R6/2 model.²¹ What remains unclear is how mutated htt activates BimEL. An interesting clue comes from evidence that BimEL expression is negatively regulated by the brain-derived neurotrophic factor (BDNF).^{70,71} By wide consensus, the HD mutation results in lower BDNF levels in the brain⁷² because htt is indirectly involved in transcriptional control over the *BDNF* gene.⁷³ Mutated huntingtin might therefore elicit BimEL accumulation/activation by inhibiting BDNF expression. BimEL is also a well-known downstream target of ER stress.⁷⁴ Insofar, as proteins with an abnormally long

Table 2 Evidence for Bcl-2 family protein dysregulation in mouse models of HD (Significant differences between HD and control cells are highlighted in bold)

Mouse model	Observations / Results	Reference
R6/1	At 18 weeks no change in Bcl-xL protein in the striatum.	Hansson <i>et al.</i> ³⁰
R6/1	At 8 and 12 weeks no change in Bid and BimEL protein in the striatum. At 16 weeks ↑Bid and ↑BimEL protein in the striatum. At 16 weeks no change in Bcl-2, Bcl-xL, Bax, Bak and Bad protein in the striatum. At 16 weeks no change in Bid and BimEL protein in the cortex. At 30 weeks ↑Bid, ↑Bax and ↑BimEL protein in the striatal mitochondrial fractions. At 30 weeks no change in Bcl-2 and Bcl-xL protein in the striatal mitochondrial fractions. At 30 weeks ↑Bid and ↑BimEL protein in the striatal cytosolic fractions. At 30 weeks ↑Bid mRNA, no change in BimEL mRNA in the striatum. At 30 weeks no change Bax, Bcl-2 and Bcl-xL protein in the striatal cytosolic fractions.	Garcia-Martinez <i>et al.</i> ²²
R6/1	At 12, 24 and 36 weeks no change in Bcl-2 mRNA in cerebellum and cortex. At 12, 24 and 36 months ↑Bax mRNA in cerebellum and cortex.	Teles <i>et al.</i> ²³
R6/2	At 9 weeks no change in Bad, pBad, Bcl-2, Bcl-xL, BimEL and Bax protein in total brain. At 9 weeks ↓Bid and ↑Bid protein in total brain. At 12 weeks no change in Bad, Bcl-2, Bcl-xL and Bax protein in total brain. At 12 weeks ↑BimEL and ↓pBad protein in total brain. At 9 and 12 weeks no change in Bcl-2 and Bcl-xL protein in mitochondrial fractions. At 9 and 12 weeks ↑Bax protein in mitochondrial fractions. At 12 weeks ↑BimEL protein in mitochondrial fractions.	Zhang <i>et al.</i> ²¹
R6/2	At 13 weeks ↑BimEL protein in the striatum.	Leon <i>et al.</i> ⁴⁰
R6/2	At 10 weeks ↓BNip3 protein in total lysates and ↑in striatal mitochondrial fractions. At 10 weeks no change in BNip3 protein in the cortex.	Sassone <i>et al.</i> ⁵⁵
R6/2	At 10.5 and 12 weeks ↓Bcl-2 protein in the striatum.	Chiang <i>et al.</i> ²⁴
R6/2	At 12 weeks ↓Bcl-2 mRNA and Bcl-2 protein in total brain.	Ju <i>et al.</i> ¹⁷
Tet/HD94	At 22 months, transgene ON ↑Bid and ↑BimEL protein in the striatum. At 22 months, transgene OFF no change in Bid and ↑BimEL protein.	Garcia-Martinez <i>et al.</i> ²²
N171-82Q	At 12 weeks ↓Bcl-2 protein in the striatum.	Duan <i>et al.</i> ²⁵
YAC128	At 6 weeks ↑BNip3 protein in striatum and striatal mitochondrial fractions.	Sassone <i>et al.</i> ⁵⁵

polyglutamine expansion cause ER stress,⁷⁵ BimEL activation in HD may also depend on mutated htt induced-UPR. A question awaiting further research is whether Puma/Bbc3, the other potent BH3-only protein activated in response to ER stress, has a role in HD.

Another BH3-only protein potentially involved in HD pathogenesis is Bid. In HD models, mutated htt elicits Bid cleavage or full-length Bid accumulation or both events (Tables 1 and 2). Bid activation was suggested as a key event in many neurodegenerative diseases because Bid is highly expressed in neurons.⁷⁶ In HD cells, full-length Bid and tBid, by migrating to mitochondria, could sustain Bax activation,⁷⁷ thus amplifying the mitochondrial damage.

The molecular mechanism by which mutated htt causes full-length Bid to accumulate is undefined. Because full-length

Bid pro-apoptotic activity is extremely weak, high Bid expression levels are nevertheless unlikely to cause cell death in HD. Conversely, because tBid exhibits strong pro-apoptotic activity, it may have a key role in HD cell death. This possibility receives support also from evidence that mutated htt enhances activity of caspase-8^{78, 79} and calpain⁸⁰ that both enzymes are able to cleave full-length Bid.⁴⁶

The most recently BH3-only protein potentially implicated in HD pathogenesis is Bnip3. Our results provide evidence that Bnip3, an apoptotic regulator that in healthy cells localizes to the cytosol or the nuclei, in neuronal and non-neuronal cells expressing mutated htt mainly localizes to the mitochondria.⁵⁵ Because Bnip3 activation causes $\Delta\psi$ loss, mitochondrial fragmentation and mitophagy,^{51,81} Bnip3 could be implicated in the mitochondrial dysfunction in HD. Precisely, how

Table 3 Evidence for Bcl-2 family protein dysregulation in HD patients' cells (Significant differences between HD and control cells are highlighted in bold)

Cells/Tissues	Observations / Results	Reference
Caudate nucleus from 18 HD patients	↑Bcl-2 and ↑Bax protein in neurons	Vis et al. ²⁶
Peripheral blood mononuclear cells from 10 HD patients	No change in Bcl-2 protein ↑Bax protein in B/T lymphocytes and in monocytes	Almeida et al. ²⁹
Primary muscular cells from 4 HD patients	↑Bnip3 protein in total lysates and in mitochondrial fractions	Sassone et al. ⁵⁵

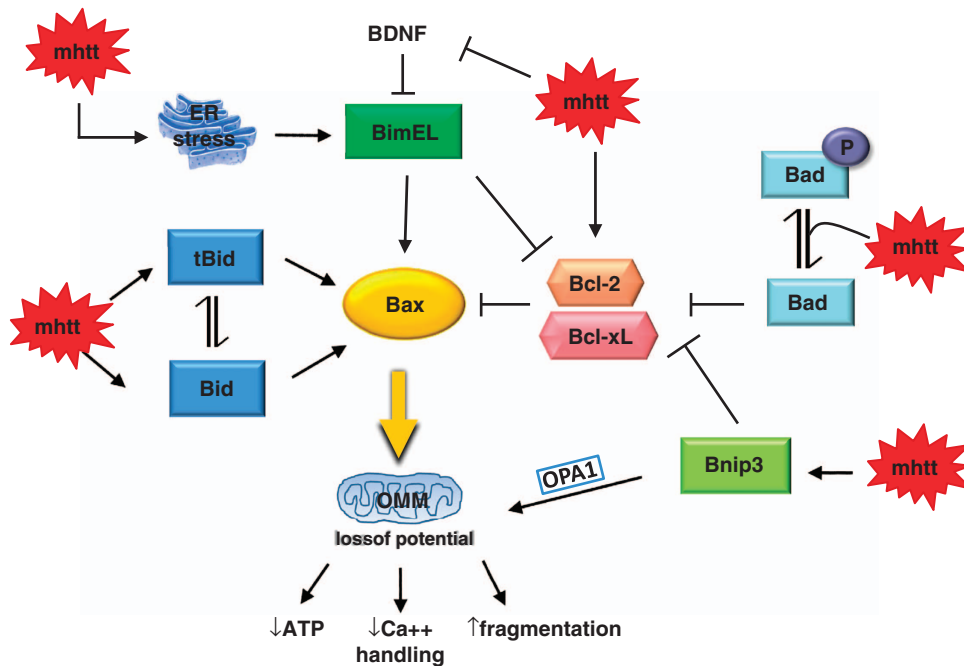


Figure 1 Hypothetical model explaining the pathway through which mutated htt (mhtt) causes OMM permeabilization. Bax activation in HD may depend on the 'activators' BH3-only proteins and the 'sensitizers' that bind only to pro-survival proteins Bcl-2 and Bcl-xL. Mutated htt may interact with the Bcl-2 protein network at multiple levels by modulating Bcl-2 expression, inducing Bid accumulation and Bid cleavage, promoting Bnip3 activation and increasing non-pBad levels. BimEL accumulation/activation may depend on htt control over BDNF expression or ER stress-induced UPR or both mechanisms

mutated htt induces Bnip3 activation remains an unanswered question. Previous reports show that the Bnip3–mitochondria association is strongly stabilized by acidosis^{82,83} or by an increased cytosolic calcium concentration,⁸⁴ conditions that may both take place in HD cells owing to mitochondrial respiration inhibition. An alternative possibility is that htt binds directly or indirectly to Bnip3, but this issue awaits elucidation in future studies.

Summary and Perspective

Current evidence, derived from research efforts by many investigators over a lengthy time-span, increasingly implies that mutated htt adversely influences Bcl-2 family protein levels and localization. Studies conducted *in vitro* and *in vivo* provide convincing evidence that mutated htt expression activates at least four BH3-only proteins. Although mutated htt may upregulate each of these four proteins through different mechanisms, their activation culminates in an identical consequence, namely Bax activation. Bax activation, by promoting cytochrome *c* release, may underpin the progressive neuronal apoptosis in HD patients' brains. Although this hypothesis receives support from early studies that identified apoptotic-like cells in the HD striatum,^{85,86} evidence for HD cell death arising through apoptosis alone is controversial.⁸⁷ An alternative possibility is that Bcl-2 protein family dysregulation alters HD mitochondrial dynamics in an apoptosis-independent manner. Especially interesting in this context is a report that Bax-induced mitochondrial fission and Bax-initiated cytochrome *c* release are separable events and that Bcl-2 family proteins can influence mitochondrial fission–fusion dynamics in HD cells independently of apoptosis.^{1,2} If true, Bcl-2 family proteins in HD may be responsible for the fragmented mitochondrial morphology, changes in mitochondrial ultrastructure and impaired mitochondrial trafficking demonstrated in *in vitro* and *in vivo* models of HD.^{88–90}

Future studies should aim to go beyond analyzing Bcl-2 family protein levels because their expression level often poorly indicates protein activation, especially given that most BH3-only members undergo strong regulation by post-translational mechanisms. Future studies aimed to elucidate the molecular mechanisms underlying the Bcl-2 protein interactions documented in *in vitro* and *in vivo* HD models will exploit innovative fluorescence techniques⁹¹ that may more clearly illustrate the Bcl-2 role in HD. Equally important are the genetic approaches such as crossing HD mouse models onto mice knockout for *Bcl-2* genes that will provide definitive proof that Bcl-2 family members are pathogenetically involved in HD.

Finally, future studies involving innovative human cellular models such as iPS from patients with HD, will clarify whether Bcl-2 family proteins, already molecular targets in cancer therapy,⁹² may also be a therapeutic target for HD.

Conflict of Interest

The authors declare no conflict of interest.

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