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Genetic Manipulations of Mutant Huntingtin in Mice: New Insights into HD Pathogenesis

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Abstract

This year (2013) marks the twentieth anniversary of the identification of the causal genetic mutation for Huntington's disease (HD), a landmark discovery that has heralded the study of the biological underpinnings of this most common dominantly inherited neurodegenerative disorder. Among the variety of model organisms used to study HD pathogenesis, the mouse model has been by far the most commonly used mammalian genetic model organism. Much of our current knowledge regarding mutant Huntingtin (mHtt)-induced disease pathogenesis in mammalian models has been gained from studying transgenic mouse models expressing mHtt N-terminal fragments, full-length murine or human mHtt. In this review, we will focus on recent progress in using novel HD mouse models with targeted mHtt expression in specific brain cell types. These models help to address the role of distinct neuronal and non-neuronal cell types in eliciting cell-autonomous or non-cell-autonomous disease processes in HD. We will also describe several mHtt transgenic mouse models with targeted mutations in Htt *cis*-domains to address specific pathogenic hypotheses, ranging from mHtt proteolysis to post-translational modifications. These novel mouse genetic studies, through direct manipulations of the causal HD gene, provide a reductionist approach to systematically unravel the cellular and molecular pathways that are targeted by mHtt in disease pathogenesis and to potentially identify novel targets for therapy.

Keywords

Huntington's disease; posttranslational modification; mouse models; nuclear translocation; caspase 6; genetic manipulation; cell-cell interaction; genomic transgenic mice

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive neurological symptoms, including involuntary movement (e.g. chorea, dystonia and gait abnormalities), cognitive deficits and psychiatric manifestations. Neuropathology reveals selective neurodegeneration and neuroinflammation occurring foremost in the striatum and to a lesser extent in the cortex [1]. HD is caused by an expansion of CAG repeats translating into an elongated polyglutamine (polyQ) repeat near

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the amino-terminus of the huntingtin (Htt) protein. While the polyQ length normally ranges from 6 to 34 repeats, individuals with 40 or more repeats invariably develop HD [2]. The CAG repeat length correlates inversely with the age of motor symptom onset, a criterion for disease diagnosis [3]. A critical, but yet unanswered question in HD is how the widely expressed mHtt protein can cause age-dependent, selective neurodegeneration. Despite ubiquitous expression of mHtt in the brain and body, the striatal medium spiny neurons (MSNs) and deep-layer cortical projection neurons (CPNs) are the most vulnerable to degeneration in HD patients [1]. Another important related question is whether degeneration of these specific neuronal types is due to purely intrinsic toxicities of mHtt in these neurons (cell-autonomous toxicities), or whether mHtt acting in other cell type(s) in turn contributes to the demise of these vulnerable neurons (i.e. non-cell-autonomous toxicities). The third, and arguably the most pressing question, is what molecular targets, beyond mHtt itself (its mRNA or protein), could be engaged to prevent or slow the disease process.

The onset of the plethora of behavioral and neuropathological phenotypes takes decades in HD patients, and the disease relentlessly progresses for 10–20 years from the motor symptom onset to patient's death. To elucidate the causal, mHtt-induced disease mechanisms during the long disease course from presymptomatic to symptomatic phases, it will be insufficient to perform patient studies alone and will require the development of genetic animal models that recapitulate salient genetic, behavioral and neuropathological features of HD. If such models are available, in principle, a “two-step reductionist” approach can then be employed to unravel cellular logic and elucidate molecular mechanisms underlying selective pathogenesis in the disease model. The first step would be to determine in which brain cell types the expression of mHtt is necessary or sufficient to elicit critical aspects of the disease, either through cell-autonomous or non-cell-autonomous mechanisms. The second step would then be to further identify the precise molecular mechanisms within HD-relevant cell types, as defined by the first step, which can modify the course of the disease. In this review, we will summarize recent progress in mouse genetic studies on HD pathogenesis that fits this overall strategy. In particular, we will focus on novel mouse genetic studies that systematically manipulate mHtt itself to ask questions related to the spatiotemporal requirement of mHtt expression in eliciting HD-like phenotypes in mouse models and novel mouse models that aim to address the impact of specific huntingtin *cis*-domains or post-translational modifications (PTMs) on disease pathogenesis.

Genetic Mouse Models of Huntington's Disease: A Brief Update

Htt is a large protein consisting of 3144 amino acids. Beyond the first exon that contains the polyQ stretch, Htt consists of dozens of HEAT repeats that are thought to mediate protein-protein interactions. Deletion of murine *huntingtin* (*Hdh*) in mice leads to embryonic lethality [4–6], while conditional knockout of *Hdh* in post-mitotic neurons leads to progressive neurodegeneration in the cortex of aged mice [7]. These data support an essential role of Htt in both embryonic development and in adult neuronal survival. Although it remains plausible that partial loss of normal Htt function could contribute to HD pathogenesis (reviewed by [8]), polyQ expanded mHtt can fully substitute endogenous wildtype Htt during embryonic development, and hence mHtt gain-of-function toxicities are likely key to the disease process [9–11]. Consistent with this conclusion, it has been demonstrated through genome wide gene expression analysis that polyQ length-correlated genes and *Hdh*-null-altered genes are either in the same pathways or in unique, but interconnected pathways. A close examination of energy and lipid metabolism categories revealed that mHtt-induced gene expression changes were distinct from, but related to, the effects of the lack of Htt, suggesting a gain-of-function mechanism through a property of polyQ expansion [12].

The HD field is richly endowed with a plethora of genetic mouse models expressing mHtt (Table 1, also see review in [13]) which together have been instrumental in understanding many aspects of HD pathogenesis. By and large, there are three major different types of HD mouse models. The first is mHtt mouse models that express a toxic N-terminal fragment of mHtt. Indeed, the first *in vivo* evidence supporting the hypothesis that the polyQ expansion in an Htt fragment can elicit neurotoxicity in mice is from the overexpression of a human mHtt-exon1 fragment with 144Q (*i.e.* R6/2 and R6/1) by Gill Bates's group [14]. The R6 models were instrumental in the discovery of *in situ* aggregate formation by mHtt N-terminal fragments [15], which were subsequently confirmed in HD patient post-mortem brains [16]. The subsequent development of additional mHtt fragment models, such as N171-82Q model expressing mHtt N-terminal 171 amino acids with 82Q [17], was key to demonstrating the reproducible and robust toxicities of mHtt N-terminal fragments. These fragment models often exhibit progressive and severe motor impairment, global brain and body weight loss, and prominent intranuclear and neuropil mHtt aggregation, features that are similar to patients. However, fragment models often have brain atrophy that is relatively global, and with only modest neuronal loss [ibid; 18, 19, 20]. Certain features of the fragment models, such as the early age-of-onset of behavioral symptoms, rapid and often lethal disease progression, and presence of seizures in the R6/2 model, suggest that these models may capture certain features of Juvenile-onset HD [13, 21].

Since HD pathogenesis in patients is elicited by full-length mHtt over decades, to study such slowly progressive pathogenic processes, two types of full-length mHtt models have also been developed and are increasingly being used in the study disease pathogenesis. The first type of model is the knock-ins, in which expanded CAG repeats or human mutant *HTT* exon1 are used to replace corresponding sequences in the endogenous murine *Hdh* locus (reviewed by [21]). There is a series of such so-called mHtt knock-in (KI) models with increasing polyQ length repeats, with *Hdh*-Q111 [22], CAG140 [23], Q150 [24–26], and now zQ175 [27, 28] being the models most used for HD molecular pathogenesis and therapeutic research (reviewed by [21]). The allelic series of mHtt-KI models have the most precise genomic context and confer endogenous levels of full-length mHtt expression [29]. However, it should be noted that the KI mice express a hybrid of mostly murine Htt protein with human mHtt exon1, under the regulation of murine promoters and genomic regulatory elements, hence there are still subtle differences at the levels of *Hdh/HTT* genomic DNA and protein sequences between KI mice and HD patients [13, 30]. The KI mice are valuable to study progressive mHtt accumulation and aggregation [31] and molecular changes in affected striatal and cortical neurons [26]. Several mHtt-KI models, particularly those with human *HTT*-exon1 and with repeats ranging from 140Q-200Q, exhibit multiple behavioral deficits and late-onset brain atrophy that are relatively restricted to the same brain regions affected in patients, *i.e.* striatum and cortex [23, 32–35].

Although the majority of KI mice exhibit behavioral deficits and degenerative pathology at relatively late time-points, a recently characterized mouse KI model, zQ175, has been shown to exhibit early and robust disease phenotypes [27, 28]. The zQ175 mice were obtained as a result of spontaneous expansion of the CAG repeat to about 175 from the previously generated CAG 140 KI mice [23]. Both homozygotes and heterozygotes were characterized, with the homozygotes developing motor and grip strength deficits as early as 4–8 weeks, and progressive behavioral impairment, including rotarod and cognitive deficits, at 6 months and 10 months of age. Importantly, heterozygous zQ175 mice showed locomotor deficits as early as 4.5 months of age, and striatal gene expression changes at 6 and 10 months [28]. Importantly, both homozygotes and heterozygotes exhibit progressive whole brain, cortical and striatal volume loss at 3 and 4 months of age, with evidence of striatal neuronal loss starting at 4.5 months of age in the homozygotes (about 15% reduction by stereological cell counting [28]). Additional characterization of the model also revealed progressive hyper-

excitability in striatal MSNs in acute brain slices and changes in MR spectroscopy consistent with those seen in patients. Since brain volume loss in the homozygote (but not heterozygote) zQ175 mice occurs relatively early (e.g. 6 weeks of age), it remains possible that two copies of *Hdh* with 175Q may elicit some developmental deficits [27]. Overall, the gene-dosage-dependent, relatively early and progressive behavioral, electrophysiological, pathological and molecular changes support the use of this new KI model for HD pathogenic and preclinical studies.

The third type of HD transgenic model is the human genomic transgene mouse models expressing fl-mHtt from the human genomic locus transgenes using either a yeast artificial chromosome (YAC) [36, 37] or a bacterial artificial chromosome (BAC) [9]. Both YAC and BAC HD models introduce a large (> 200kb) segment of the human *HTT* genomic locus, including the 5'- and 3'-UTRs, into the mice, providing relatively intact human genomic regulatory elements and protein context within the span of the transgene. The YAC HD model lines, including YAC18, YAC46, YAC72, and YAC128, are named after the size of the polyQ repeat in the human *HTT* gene, while BACHD mice express mHtt harboring 97Q. As a group, the fl-human *mutant HTT* genomic transgenic mouse models demonstrate slowly progressive but relatively robust motor dysfunction (i.e., rotarod deficits), psychiatric-like and cognitive deficits, and selective atrophy in the striatum and cortex that spares the cerebellum [9, 36, 38]. The latter pattern of brain atrophy is reminiscent of those seen in the HD patients. One major difference between YAC128 and BACHD mice is the DNA sequence encoding the polyQ: YAC128 has a relatively pure CAG repeat, while BACHD has a mixed CAG/CAA repeat. The latter repeat is genetically stable in germline and somatic tissues, including the striatum, cortex, and cerebellum, hence demonstrating that repeat instability, at least at a relatively long repeat range, is not necessary for fl-mHtt to elicit disease in this model [9]. The BACHD mice have also been shown to exhibit relatively robust motor deficits and brain atrophy, as measured by power analyses of several phenotypic readouts, while YAC128 mice generally confer milder deficits [9, 34, 37]. These may be due in part to the relatively high levels of mHtt expression (i.e. an estimated 75% of transgene expression in YAC128 vs. 150–200% in BACHD compared to the endogenous murine Htt protein levels). However, even with higher mHtt expression, BACHD mice exhibit fewer mHtt aggregates than YAC128 mice do [9, 39]. The discrepancy between the level of aggregates and severity of behavioral deficits suggests the aggregates detected by these antibodies may not represent a toxic species that contributes to the HD pathogenesis [39, 40]. One shared phenotype commonly observed in these human *HTT* genomic transgene mice is body weight gain, which is unlike HD patients and is likely due to the Htt dosage effect on the IGF-1 pathway, since YAC or BAC mice overexpressing wildtype Htt also show weight gain [41; Gu and Yang, unpublished data]. An alternative explanation is the hypothalamic toxicities elicited by mHtt leading to weight gain these models [42]. Several studies suggest that the behavioral deficits in HD genomic transgenic models cannot be simply attributed to body weight changes [9, 34]. Moreover, these mice still exhibit HD-like brain and testicular atrophy [9, 43], demonstrating HD-like pathogenic processes in these models. Despite the overall similarities in behavioral deficits and selective brain atrophy, BACHD and YAC128 models have a few notable differences, including the timing and extent of nuclear mhtt accumulation and striatal gene expression changes [39], which could be due to the levels of mHtt expression or the nature of repeats encoding the polyQ. However, further investigation by our group (Cantle and Yang, unpublished data) and others [44] found that 12-month old BACHD mice showed similar reductions in expression of multiple striatal genes (e.g. *Actn2*, *Darpp-32*, *Ddit4l* and *Pcdh20*), which are comparable to those altered in YAC128 striata [45]. Thus, these data suggest molecular, behavioral and pathological phenotypic similarities between YAC128 and BACHD mice.

Conditional Expression of Mutant Huntingtin to Illuminate Cellular Targets in HD Pathogenesis

In the brain, complex neural circuitry regulates normal functions of neurons in various regions. In HD patients, severe and progressive neurodegeneration is observed in two highly connected regions, cortex and striatum, suggesting cell-cell interaction among MSNs and CPNs may play an important role in HD pathogenesis. To address this question, we developed a conditional mouse model, called RosaHD, in which the expression of a mHtt-exon1 fragment with 103Q can be precisely turned on in specific cell types [46, 47]. To achieve conditional expression, mutant *HTT*-exon1 was targeted to the murine Rosa26 locus with two LoxP sites flanking a transcription termination (STOP) sequence that is strategically placed before the *HTT*-exon1 sequence. Thus, the expression of this toxic mHtt fragment is dependent on Cre-mediated excision of the STOP sequence. By comparing RosaHD mice crossed with predominantly striatal-selective *Dlx5/6-Cre* (which is also expressed in a population of cortical interneurons) with predominantly cortical glutamatergic neuron-selective *Emx1-Cre*, or to a pan-neuronal *Nestin-Cre* mouse line, we could assess relative contributions of cell-autonomous vs. non-cell-autonomous toxicities of mHtt-exon1 in disease pathogenesis in the cortex and striatum. We found that mHtt-exon1 can form aggregates in a cell-autonomous manner in both cortex and striatum. However, motor deficits and neurodegenerative pathology (e.g. dystrophic neurites, dark degenerating neurons, and reactive gliosis) were only observed in the cortex and striatum of RosaHD/*Nestin-Cre* (RN) mice at one year of age, and not in RosaHD/*Emx1-Cre* (RE; selective expression of mHtt-exon1 in CPNs) and mice RosaHD/*Dlx5/6-Cre* mice (RD; selective expression of mHtt-exon1 in striatal MSNs and cortical interneurons). These studies suggest, at least in the context of mHtt-exon1 induced disease, that purely cell-autonomous toxicities in either the cortical or striatal neurons alone are not sufficient to induce the full extent of the disease within the vulnerable neuronal populations, and full-scale neuropathogenesis in either the CPNs or MSNs would require non-cell-autonomous neuronal toxicities from mHtt expressed in other neuronal or glial cell types. The precise cellular origins of such toxicities were not defined in these initial studies, but they provide strong support to the notion that non-cell-autonomous toxicity could be relevant in HD pathogenesis.

The question of cell-autonomy versus non-cell-autonomy in HD pathogenesis is far from being fully understood. Our original studies of RE and RD mice suggested that even the cell-autonomous expression of mHtt-exon1 in CPNs or MSNs is able to yield modest toxicities, analyzed by sensitive readouts such as EM or electrophysiology [46, 47]. Another study reported by Ehrlich and colleagues showed that transgenic mice (namely DE5) that selectively express an N-terminal 171 amino acid fragment of mHtt with 98Q selectively in MSNs (via the *DARPP-32* promoter) lead to cell-autonomous mHtt aggregation, forebrain atrophy, age-dependent motor deficits and striatal gene expression changes [48, 49]. These studies support cell-autonomous toxicities of this mHtt fragment in MSNs. However, compared to the pan-neuronal expression of the same mHtt fragment driven by the prion promoter in another transgenic mouse model, N171-82Q, the striatal pathology and overall disease phenotypes of N171-82Q mice are much more severe than DE5, despite the higher level striatal mHtt fragment expression in the latter model [17]. Overall, the studies of RosaHD model series and DE5 mice support the likelihood of contributions from both cell-autonomous and non-cell-autonomous toxicities in striatal and cortical pathogenesis in HD fragment models.

Glial cells play an essential role in maintaining brain homeostasis to support proper neuronal function. In HD, reactive astrogliosis and microgliosis are consistently found in the striatum and cortex of postmortem HD brains [50, 51]. Furthermore, elevated proinflammatory cytokines such as IL-6 and TNF are found in the blood and CSF of HD patients [52],

suggesting that peripheral and central inflammatory reactions could mark or actively contribute to ongoing disease processes [53]. The activation of astrocytes and microglia could be a double-edged sword, possibly having protective and harmful roles in neurodegenerative diseases [54]. An elegant example of glia or microglia contributing non-cell-autonomously to neuropathogenesis in the case of familial amyotrophic lateral sclerosis (ALS) elicited by mutant SOD1 has been demonstrated by Don Cleveland and colleagues [55–57]. In HD, since mHtt is broadly expressed in both neuronal and non-neuronal cells, and mHtt aggregates and glial dysfunction, such as reduced glutamate transporter expression, have been found in HD mouse models, it is conceivable that glial mHtt may elicit cell-autonomous or non-cell-autonomous disease processes in HD. To address such a question, Li and colleagues developed a transgenic mouse model with astrocyte-specific expression of a mHtt N208 fragment with 160Q repeats [58]. These mice develop age-dependent neurological phenotypes, including motor impairment, body weight loss, and reduced survival, while mice expressing the N208 fragment with short (23Q) polyQ repeats are spared of the disease. The mutant mice also exhibit some pathology, such as mHtt aggregation and gliosis, but do not show neuronal death. These results suggest that mHtt in astrocytes can contribute to neurological symptoms through dysfunction rather than degeneration of the neuronal systems. A second study by the same group of investigators further strengthens this view by showing that astrocyte-specific expression of mHtt N208 fragment with 98Q by itself does not show overt phenotypes but can exacerbate the disease caused by prion promoter driven N171-82Q in double transgenic mice [59]. These studies provide compelling evidence that mHtt fragments in astrocytes can contribute to HD pathogenesis.

One caveat of the published studies examining the cell-type-specific roles of mHtt in HD is the exclusive use of mHtt fragment models, which may not have ideal construct validity when compared to the full-length mHtt mouse models [60]. Therefore, studies using mouse models with conditional expression of full-length mHtt in specific neuronal or non-neuronal cell types in genetic mouse models of HD will be valuable to further study the cellular targets of mHtt in HD. Such studies will provide new insights into how full-length mHtt elicits pathological interactions between cortical and striatal neurons to contribute to disease pathogenesis.

Mouse Genetic Models to Study Huntingtin Mutant Huntingtin Proteolysis and Post-Translational Modifications in HD Pathogenesis

Another fruitful approach to dissecting HD pathogenesis using mouse genetic models is to develop models carrying specific mHtt *cis*-domain mutations that reside outside the polyQ domain. These mutations either mimic or block potential pathogenic events that directly affect mHtt itself. Such studies may provide rigorous evidence of a particular Htt domain or its PTM modifying the plethora of pathogenic consequences resulting from the expression of mHtt. *HTT* is a very large gene, covering a 170 kb genomic region in human and encoding a protein with >3144 amino acids that has been shown to be capable of interacting with several hundred mammalian brain proteins [61, 62]. Therefore, Htt *cis*-domains, their PTMs and/or interacting proteins may play crucial roles in the production, trafficking, function and clearance of Htt itself. A precise understanding of such mechanisms, and a rigorous evaluation of their role in HD pathogenesis, could constitute a rational strategy towards Htt-targeted therapy for HD.

One rigorous line of research in this area is the study of mHtt proteolysis, which creates smaller and more toxic mHtt N-terminal fragments that are known to accumulate as nuclear inclusions (NIs) and cytoplasmic/neuropil aggregates in the brains of HD patients and mouse models [15, 63, 64]. Full-length mHtt can be cleaved into a large number of N-

terminal fragments, and a subset of the enzymes that generate N-terminal mHtt fragments of varying sizes having been determined [65]. They include caspases 3 and 6 (Casp3 and Casp6, respectively) [66, 67], calpain [68], matrix metalloproteinase 10 (MMP-10) [69], and a yet to be defined aspartyl protease [70]. Interestingly, a recent study by Bates and colleagues also showed a potential genetic mechanism to produce a mHtt-exon1 product independent of proteolysis [71]. This study showed that aberrant splicing of mutant *HTT*-exon1, in a CAG repeat length dependent manner, can yield a short polyadenylated *HTT* mRNA transcript that is translated into mHtt-exon1 protein product. The precise contribution of many of the distinct proteolytic mechanisms or the newly identified alternative splicing mechanism to the overall disease pathogenesis remains to be clarified. One of these mechanisms, the cleavage of mHtt into an N-terminal 586 fragment of mHtt, has been rather thoroughly investigated using mouse genetic models. Casp6 can cleave mHtt at residue 586 [67], with the subcellular compartment for such cleavage appearing to be in the nucleus [72]. The original mouse genetic experiment demonstrating the significance of Casp6 cleavage in HD pathogenesis, performed by Graham, Hayden and colleagues, showed that YAC128 transgenic mice expressing a genomic copy of human fl-mHtt carrying a mutation that blocks Casp6 cleavage lead to prevention of disease pathogenesis compared to the baseline YAC-128Q model, with such an effect not seen in YAC mice with mHtt mutations that blocked Casp3 cleavage [73]. Activation of Casp6 is an early pathogenic event in HD mice, and its level of activation is directly correlated with CAG repeat length and inversely correlated with age of onset in HD patients [74]. Recent studies have shown that transgenic expression of mHtt caspase fragments in mice can elicit disease that appears to be more severe than fl-mHtt models but less severe than models expressing smaller mHtt fragments, with these mice appearing to accumulate predominantly cytoplasmic mHtt aggregates [75, 76]. Thus, these transgenic experiments confirm the toxicity of the Casp6 fragment of mHtt, but suggest this cleavage product may be an intermediate that can be further processed into smaller and likely more toxic mHtt fragments. These studies provide initial proof-of-concept that specific mHtt cleavage events, at least through *cis*-mutations, can prevent the onset of both neuronal dysfunction and neurodegeneration in fl-mHtt expressing HD mice.

Recent advances in studying the role of Casp6 in HD pathogenesis have moved towards genetic validation of Casp6 itself a potential target for HD therapy, primarily by crossing various HD mouse models with *Casp6* deficient mice [65, 77]. However, these studies so far do not support Casp6 alone being responsible for mHtt cleavage at residue 586, since crossing *Casp6* null alleles into either HdhQ150 or BACHD mice does not show reduced levels of cleaved mHtt fragments at this site, and extracts from *Casp6* null mouse brains still can generate the mHtt 586 amino acid fragment from fl-mHtt [77, 78]. Unexpectedly, BACHD/*Casp6*^{-/-} mice showed a reduced overall level of mutant and wildtype Htt, and modest improvement of behavioral deficits but no effect on neurodegeneration [77]. The modest benefit of Casp6 reduction is correlated with activation of mHtt clearance, likely through the autophagy and the ubiquitin proteasome systems. Taken together, the data so far suggest that Casp6 cleavage site mutations around residue 586 may block the disease via inhibition of mHtt cleavage by several potential proteases, including Casp6. Therefore, further identification of the set of proteases that can mediate this critical cleavage of mHtt will be crucial to move forward on this path towards therapeutics. Moreover, it is equally important to evaluate, using rigorous mouse genetic means, the pathogenic significance of alternative mechanisms in the generation of mHtt N-terminal fragments, such as MMP-10 [69] and aberrant splicing of mHtt-exon1 [71].

Another exciting avenue in investigating the pathogenesis of HD and related polyQ disorders is the use of novel mouse genetic models to assess the roles of PTMs on the disease-causing protein [30, 79, 80]. An elegant example of PTM as an important

mechanism in HD pathogenesis is the acetylation of lysine residue 444, which is a signal to traffic mutant and wildtype Htt to the autophagosome for selective clearance [81]. Importantly, rendering mHtt incapable of being acetylated (via K444R mutation) results in dramatic accumulation and neurodegeneration in cultured neurons and in mouse brain, suggesting that boosting Htt-selective clearance mechanisms could be beneficial in HD.

Another domain of Htt that is an intensive area of ongoing research in the context of PTMs in HD pathogenesis is the first 17 amino acid domain of Htt (N17) immediately preceding the polyQ domain [82]. The N17 domain of Htt is highly conserved amongst all vertebrate Htt paralogs, but not present in invertebrates [83]. It forms an amphipathic α -helical structure [84, 85] that can accelerate polyglutamine length dependent aggregation *in vitro* [86, 87]. Several elegant studies have shown that an important cellular function of N17 is to target Htt and its small N-terminal fragments in the cytosol, likely through distinct mechanisms of mediating association with cytoplasmic membranous structures (e.g. ER or mitochondria) [84, 88] and by facilitation Crm1-dependent nuclear export [89, 90]. The precise molecular cascades underlying N17-mediated nuclear-cytoplasmic shuttling of Htt, including its N-terminal fragments, and its relevance to HD, are currently under intensive investigation. Candidate N17 interacting proteins such as 14-3-3 [91, 92], Tpr [93] and Tcp1 [86] have already been implicated in modulating mHtt subcellular localization and/or aggregation. One impressive aspect of N17 biology, despite its small domain size, is the demonstration of >10 possible PTMs including ubiquitination, SUMOylation, acetylation, phosphorylation and oxidation [84, 94–97]. The pathogenic significance of some of these PTMs has been explored in model organisms. For example, the ubiquitination and SUMOylation of the lysine residues (K6, K9 and K15) appear to exert opposing roles in the mHtt-exon1 toxicity in a fly model [96]. Our laboratory was the first to explore the pathogenic significance of serine 13 and 16 phosphorylation in the context of fl-mHtt induced pathogenesis in HD mice [98]. We generated BAC transgenic mice expressing fl-mHtt-[97Q] (same as BACHD) with either phospho-mimetic (S13D and S16D; or “SD”) or phospho-resistant mutations (S13A and S16A; or “SA”). We demonstrated that both forms of fl-mHtt are functional in rescuing murine *Hdh* KO lethality. SA mice reproduce all the key disease features originally observed in the BACHD model, including age-dependent motor deficits (rotarod and open field exploration), two psychiatric-like behavioral deficits (depression-like and anxiety-like deficits), and selective forebrain atrophy, while two independent SD transgenic mouse lines do not exhibit these disease phenotypes at all [9, 98; Gu and Yang, unpublished data]. The neuroprotective effects of the SD mutations or actual serine 13 and 16 phosphorylation have also been shown in cell and mouse brain slice models [94, 97]. One surprising finding from our mouse study is that SD but not SA mice no longer exhibit mHtt aggregation at 12 months of age [98]. This finding was supported by *in vitro* studies using mHtt-exon1 peptides with SD mutations or with phosphorylated serine 13 and 16 residues, which were both shown to block oligomer formation by mHtt fragments, leading to retardation of mHtt aggregation and inhibition of amyloid fibril formation [87, 98, 99]. Another explanation for reduced aggregation by S13 and S16 phosphorylation is the evidence that these PTMs can promote mHtt clearance via the ubiquitin proteasome and autophagy pathways [97]. Together, these studies suggest that S13 and S16 phosphorylation may act as a molecular switch to critically modulate mHtt trafficking or clearance, and reduce mHtt toxicity in cell and animal models of HD. The precise kinases mediating N17 phosphorylation are being actively investigated, with candidate kinases including IKK [97] and CK2 [94]. A long-term goal of this line of investigation, besides elucidating the precise biological functions and regulatory pathways for the Htt N17 domain, is to explore such new knowledge for HD therapy. Encouraging developments along this line include the discovery of small molecules that appear to boost S13 and S16 phosphorylation *in vitro* [94] and a small ganglioside compound (GM1) that exert neuroprotection in YAC128 mice and concomitant enhancement of S13 and S16 phosphorylation on mHtt [100]. The precise

causal relationship between these small molecules enhancing N17 phosphorylation and disease suppression need to be further investigated, and potentially can be demonstrated by differential effects of such compounds on HD mice expressing mHtt with wildtype N17 or N17 with phospho-resistant mutations (e.g. BACHD vs. SA mice).

Conclusion

Since the discovery of *HTT* as the causal gene in HD, tremendous effort has been deployed to study the pathological role of mHtt at molecular, cellular and organismal levels to gain insights that can inform novel therapeutic strategies. At the present time, our sophisticated understanding of Htt biology and the advancement of tools to study *in vivo* disease proteins have lead to the development of a series of novel mouse models that allow molecular and spatial manipulations of mHtt itself. This new generation of mHtt-expressing models has allowed the systematic and rigorous testing of pathogenic hypotheses related to age-dependent, progressive and selective neuronal pathogenesis in HD. The data so far support the use of Cre/LoxP conditional mouse models and cell-type-specific promoter driven mHtt models to address the cell-autonomous vs. non-cell-autonomous toxicities of mHtt (Fig. 1A). Moreover, mouse models with mHtt *cis*-domain mutations, particularly those addressing specific pathogenic contributions of distinct PTMs, have also proven to be valuable in validating disease-modifying mechanisms (Fig. 1B). Together, by using the two-step reductionist approach, the HD research field is beginning to unravel the cellular and molecular targets of mHtt in HD pathogenesis, with such knowledge likely to offer new insights in HD therapeutic development.

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Abbreviation List

HD	Huntington's disease
Htt	huntingtin
mHtt	mutant huntingtin
polyQ	polyglutamine
MSN	medium spiny neuron
CPN	cortical projection neuron
PTM	post-translational modification
BAC	bacterial artificial chromosome
YAC	yeast artificial chromosome
NI	nuclear inclusions
Casp3	Caspase 3
Casp6	Caspase 6

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Figure 1.

(A) Selective expression of mHtt-exon1 expression in only either cortical or striatal neurons does not elicit the full extent of neuropathology and behavior deficits observed in mice expressing mHtt in both regions. Astroglial expression of mHtt-N208 exacerbates the disease caused by prion promoter driven N171-82Q in double transgenic mice. These results suggest the role of cell-cell interaction in HD pathogenesis and that mHtt may induce both cell-autonomous and non-cell-autonomous toxicity. (B) Illustration of PTMs and caspase-cleaving sites of Htt. Several mouse models have been generated carrying point mutations to mimic or block PTM or caspase cleavage. These mutations may exacerbate (red), ameliorate (blue) or have no significant effect on the pathogenesis of HD.

Table 1

Summary of Commonly Used Genetic Mouse Models of HD

Model	Construct Design		Behavioral Phenotypes		Neuropathology		Citations
	Promoter and construct	Poly Q Length	Onset	Severity	Specificity	Severity	
Fragment Transgenic Models							
R6/2	Hu <i>HTT</i> Promoter, Exon 1	144Q (variable)	Early	+++	Widespread	+++	[14]
N171-82Q	Mu Prion Promoter, 1-171 a.a.	82Q	Early	+++	Widespread	+++	[17]
GFAP-HD	Hu GFAP Promoter, 1-208 a.a.	160Q	Adult	++	n/a	n/a	[58]
RosaHD	Flox-STOP <i>Rosa</i> locus, Exon1	103Q	Promoter-dependent	Promoter-dependent	Promoter-dependent		[46, 47]
Full-length Human Genomic Transgenic Models							
YAC128	Hu <i>HTT</i> Locus	128Q	Adult	++	Region-specific	++	[36]
BACHD	Hu <i>HTT</i> Locus w/ Exon 1 floxed	97Q	Adult	++	Region-specific	++	[9]
Knock-in Models							
Hdh ^{Q111}	Hu Exon 1	111Q	Late Adult	+	Region-specific	+	[22]
CAG140	Hu-Mo Exon 1 hybrid	140Q	Late Adult	+	Region-specific	+	[23]
Hdh ^{(CAG)150}	Mo Exon 1	150Q	Late Adult	+	Region-specific	+	[24]
zQ175	Hu-Mo Exon 1 hybrid	175Q	Adult	++	Region-specific	++	[28]