The challenge in translating basic research discoveries to treatment of Huntington disease

Daria Mochly-Rosen, Marie-Helene Disatnik & Xin Qi

To cite this article: Daria Mochly-Rosen, Marie-Helene Disatnik & Xin Qi (2014) The challenge in translating basic research discoveries to treatment of Huntington disease, Rare Diseases, 2:1, e28637, DOI: 10.4161/rdis.28637

To link to this article: http://dx.doi.org/10.4161/rdis.28637
The challenge in translating basic research discoveries to treatment of Huntington disease

Daria Mochly-Rosen1, *, Marie-Helene Disatnik1, and Xin Qi2

1Department of Chemical and Systems Biology; Stanford University School of Medicine; Stanford, CA USA; 2Department of Physiology & Biophysics; Center of Mitochondrial Disease; Case Western Reserve University School of Medicine; Cleveland, OH USA

Huntington disease is a rare neurodegenerative disease resulting from insertion and/or expansion of a polyglutamine repeats close to the N-terminal of the huntingtin protein. Although unequivocal genetic test has been available for about 20 years, current pharmacological treatments do not prevent or slow down disease progression. Recent basic research identified potential novel drug targets for the treatment of Huntington disease. However, there are clear challenges in translating these discoveries into treatment strategies for these patients. The following is a brief discussion of these challenges using our recent experience as an example.

Introduction

Huntington disease (HD), an dominantly inherited fatal neurodegenerative disorder, is caused by expansion of polyglutamine (polyQ, CAG) repeat within the first exon of the protein huntingtin (Htt).2 HD prevalence is 5–10 per 100 000 individuals in Western Europe and North America, and the age of disease onset is inversely proportional to the length of the CAG repeat; affected individuals usually live for 15–20 years after the initial onset of symptoms.3 Clinical characteristics of HD are devastating and include progressive cognitive impairment, abnormal movements, psychiatric disturbances, dementia, and death.2 Even though the genetic mutation of HD has been identified 20 years ago, a cure or even disease-modifying treatments remain elusive.

At least three factors contribute to the challenges in identifying drug targets and developing new treatments for HD.

The Critical Cellular and Molecular Pathological Pathways that Should Be Targeted For HD Therapy Development are Still Unclear

Many cellular and molecular pathological pathways have been suggested to contribute to HD. These include a role of polyQ aggregates and misfolded mutant huntingtin (mHtt) in neurotoxicity;4 a role of a variety of mHtt fragments in the pathology;3 and a role of mHtt in mitochondrial dysfunctions, including imbalanced fusion and/or fission (also called mitochondrial dynamics), increased mitochondrial immobility in the neurons, inhibition of mitochondrial biogenesis, and dysregulated mitochondrial bioenergetics.5 Genomic analysis also demonstrates the association between HD and changes in the expression of many genes,6 suggesting that mHtt may regulate transcription. Which of these pathways and molecular events have a pivotal role in the pathology? How many of these processes should be blocked to prevent HD pathology? What molecular tools can be used to test these possibilities? Current studies in many laboratories examine these issues, but a consensus has not yet been formed; even the role of wild type...
huntingtin protein is far from being fully understood.

Wild type Htt is a very large multi-domain protein of 3144 amino acids. Recent proteomic studies demonstrate that wild type Htt interacts directly or indirectly with hundreds of proteins. Which of the interacting protein(s) is dysregulated in HD is not known. Further, it may be a challenge to affect the dysregulated interaction(s) without disturbing other roles of Htt. It is also unclear whether HD pathology represents a gain of function or a loss of function of the Htt protein. Specifically, insertion of a long polyQ repeat in Htt may result in a gain of function due to, e.g., poly-Q-dependent release of huntingtin-interacting protein (HIP) from Htt and its activation of apoptosis. Expansion of a polyQ repeat may also cause a loss of function, e.g., due to mislocalization of Htt and the Htt-binding proteins, increased proteolysis of Htt and/or of Htt-binding proteins, etc. PolyQ repeat-containing proteins, like mHtt, form polymers, fibrils, and large inclusion bodies, which are thought to contribute to or trigger neurotoxicity.

Lack of information on which of the above molecular events is required and sufficient to induce the HD-associated pathology makes it difficult to use a rational search for strategies to prevent or inhibit the progression of the disease.

**Lack of Optimal Animal Models to Test Therapeutic Approaches for HD**

It is difficult to create a proper transgenic model for HD because of the size of the mutated gene, the difference in the number of polyQ repeats between patients and the finding that mHtt creates pathology in only selected areas in the brain. Further, the slow progression, age-dependency, and the cognitive pathologies of the disease are difficult to recapitulate and assess in animal models. Multiple models of HD have been created with fragments and full-length mHtt. These models vary in the type of pathologies that they mimic and the rate of disease progression (e.g., see Table 1 and references within). Expression of full-length and N-terminal fragments (which contain the polyQ repeat) of mHtt with or without untranslated upstream regions of the gene induces only some of the HD pathologies as determined in a variety of animal models (11). Some of the animal models exhibit cognitive and behavioral abnormalities, sleep pattern abnormalities, and a shorter lifespan. The extent of regional or general neurodegeneration varies between models. In discussing which of the models is most suitable to study HD, it is apparent that each of the models has its limitations. Thus, in agreement with others, utilization of multiple HD models to test preclinical drug candidates is likely required to compensate for the limitations of each of these models.

**The Challenge in Developing Clinical Trials in a Rare Chronic Progressive Neurodegenerative Disease**

Some of the challenges in developing clinical trials for HD are typical for any clinical trials in patients with a rare disease: limited number of patients, geographical challenge for patients to access trial sites, the number of competing clinical trials for the same patient populations, etc. Other challenges relate to the nature of HD. HD progresses over a long period (years). There are differences in the severity and in the rate of disease progression. The clinical assessment for HD is not sufficiently quantitative, objective and robust and there are no surrogate markers to assess disease progression and therapeutic benefit over a short trial time. Basic research in academia thrives to help address this unmet clinical need. In the following, we reflect on our recent experience in addressing the above challenges.

**What Target to Be Chosen for HD Drug Development?**

In academia, the choice of testing the validity of a particular drug target often relates to current basic research and strength of a particular laboratory rather than as a result of a systematic assessment of all potential drug targets. Our laboratory was intrigued by the findings that mHtt interacts with the mitochondrial fission machinery and specifically with dynamin-related protein 1 (Drp1). Further evidence that mitochondrial fission and fusion are dysregulated peaked our interest. Because we developed expertise and tools to examine the role of abnormal mitochondrial dynamics and excessive mitochondrial fission in neurodegeneration, we opted to focus on determining whether inhibition of excessive fission, by inhibiting excessive Drp1 activation, would reduce mHtt-induced pathology.

**When Identifying a Possible Therapeutic Strategy, What Models of HD Should Be Used?**

We recently identified an inhibitor of excessive Drp1 function, which inhibited pathology and neuronal cell death in models of another neurodegenerative disease, Parkinson disease. Using six models of HD, we then tested whether mitochondrial excessive fission occurs and the effect of treatment with our Drp1 inhibitor. Each of the models that we used and its limitations is listed below.

1. Mouse striatal cells that express a full-length human mHtt containing 111 CAG repeats (HdhQ111), which were compared with mouse striatal cells carrying 7 CAG repeats (HdhQ7; wild-type). This model measures direct neurotoxicity of mHtt. However, mHtt is overexpressed and its expression is not regulated as it would be in patients. Further, the cultured striatal-like cells lack the influence of other brain cells on the pathology, and the extent of protein aggregation in this model is limited.

2. We also used HEK293T cells expressing a fragment of the human Htt gene containing 73 polyglutamine repeats (73Q). If some of the toxicity of mHtt relates to domains other than the one encoded by the first exon, these will not be represented in this cell culture model. Further, since HEK293T are not neuronal cells, in
addition to the limitation of the use of culture models mentioned above, the cytotoxicity measured in these cells may not mimic neurotoxicity.

(3) We next used dermal fibroblasts obtained from HD patients and compared their mitochondrial abnormalities and responses to treatment with the Drp1 peptide inhibitor, P110, with that of dermal fibroblasts from control (healthy) subjects. This model has the same limitations as the HEK293 cells have in cell culture studies of non-neuronal cells. However, since the dermal cells are derived from human patients, the regulation of mHtt gene expression, and thus potential contribution of changes in expression of mHtt and other genes, were undisturbed.

(4) We also used neurons derived from control and HD patient-iPS cells, which conserve the same number of CAG repeats in patients and all the other benefits of model 3, above; but better represent the organ where HD pathology is noted.

(5) Further, we selected HD patient-derived GABAergic neurons and medium spiny neurons (MSNs) that are most affected in HD patients and are largely degenerated in the late stage of the disease. These patient-derived neuronal cells represent a unique human disease-specific cellular system to elucidate disease mechanisms.

Table 1. List of commonly used rodent models in Huntington disease

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Transgene</th>
<th>CAG repeat</th>
<th>Onset of behavior symptoms</th>
<th>Limitations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>R6/1 mice</td>
<td>Human Htt-67 amino acids</td>
<td>116</td>
<td>15-21 wk</td>
<td>Mild symptoms. *More representative of the juvenile than the adult HD phenotype.</td>
<td>Mangiarini et al. 34</td>
</tr>
<tr>
<td>R6/2 mice</td>
<td>Human Htt-67 amino acids</td>
<td>144</td>
<td>3.5-6 wk</td>
<td>Limited for early disease mechanism. Diabetes and death occur at 12-15 wks. *As above.</td>
<td>Mangiarini et al. 34</td>
</tr>
<tr>
<td>N171-Q82 mice</td>
<td>Human cDNA-171 amino acids</td>
<td>82</td>
<td>10 wk</td>
<td>Due to high variability, large number of mice is required. Expression of mutant protein restricted to neurons.</td>
<td>Schilling et al. 35</td>
</tr>
<tr>
<td>CAG 140 mice</td>
<td>Chimeric human Htt exon 1: mouse Htt</td>
<td>140</td>
<td>12 months</td>
<td>No brain cell loss. No neuropathology signs of HD. Normal life span.</td>
<td>Menalled et al. 36</td>
</tr>
<tr>
<td>HdhQ92 mice/ HdhQ111 mice</td>
<td>Chimeric human Htt exon 1: mouse Htt</td>
<td>92/111</td>
<td>96 wk</td>
<td>No brain cell loss. No neuropathology signs of HD. Normal life span.</td>
<td>Wheeler et al. 37</td>
</tr>
<tr>
<td>HdhQ150 mice/ HdhQ200 mice</td>
<td>Chimeric human Htt exon 1: mouse Htt</td>
<td>150/200</td>
<td>6 months</td>
<td>No behavioral deficit until 25 wk. Normal life span. Late mHtt aggregation (~14 months) with no neuron loss.</td>
<td>Lin et al. 38, Heng et al. 39</td>
</tr>
<tr>
<td>BACHD mice</td>
<td>Human Htt</td>
<td>97</td>
<td>12 wk</td>
<td>Normal life span. Few aggregates appear at 12 months.</td>
<td>Gray et al. 40</td>
</tr>
<tr>
<td>YAC128 mice</td>
<td>Human Htt</td>
<td>128</td>
<td>8-12 wk</td>
<td>Normal life span. Behavioral deficit significant at 12 months.</td>
<td>Slow et al. 41</td>
</tr>
<tr>
<td>YAC 48 mice</td>
<td>Human Htt</td>
<td>48</td>
<td>-</td>
<td>No symptoms up to 20 months.</td>
<td>Hodgson et al. 42</td>
</tr>
<tr>
<td>YAC 72 mice</td>
<td>Human Htt</td>
<td>72</td>
<td>6-10 months</td>
<td>Degeneration of brain cells at 12 months.</td>
<td>Hodgson et al. 42</td>
</tr>
<tr>
<td>BACHD rat</td>
<td>Human Htt</td>
<td>97</td>
<td>3-5 months</td>
<td>-</td>
<td>Yu-Taeger et al. 43</td>
</tr>
<tr>
<td>HD rat</td>
<td>Rat Htt-fragment mHtt</td>
<td>51</td>
<td>40-50 wk</td>
<td>Lack of full length mHtt; pathology restricted to striatum, recapitulating only some of human pathology (Note: later same group developed transgenic BACHD rat)</td>
<td>von Horsten et al. 44</td>
</tr>
</tbody>
</table>
underlying HD; they were also suggested to be a useful platform of patient cells for screening therapeutic candidates.\textsuperscript{22} However, this cultured model exhibits only some of the HD neuropathology, and cannot replicate the overall pathology as seen in intact brains of HD patients.

(6) Finally, we used the R6/2 mice, which express exon 1 of the human mHtt gene carrying more than 120 CAG repeats.\textsuperscript{23} This mouse model allowed us to test the importance of the pathway for HD pathology in vivo. However, since only a part of the mHtt protein is expressed, it has many limitations, including the presence of wild-type Htt, the lack of regulated expression, and the use of human mHtt that might not interact optimally with mouse Htt-interacting partners. (Table 1 describes other transgenic rodent models for HD and lists some of their advantages and limitations).

**Is Inhibition of Excessive Drp1 Activation a New HD Drug Target?**

Using the six models listed above, we found mHtt-induced Drp1 activation (as measured by its association with the mitochondria) relative to cells that do not express mHtt.\textsuperscript{10,17,18} Importantly, we showed that inhibition of Drp1 hyper-activation by P110 rescued mHtt-induced mitochondrial fragmentation, corrected defects in mitochondrial function, and reduced cell death in multiple HD cell culture models derived from either mice or patients, including patient GABAergic striatal neurons differentiated from patient-derived pluripotent stem cells (HD-iPS cells).\textsuperscript{1} Moreover, in HD transgenic R6/2 mice, sustained treatment with the Drp1 peptide inhibitor corrected mitochondrial fragmentation, cristae disruption and respiratory inactivity. These, in turn, were associated with reduced HD-related motor deficits, striatal neuronal loss, and mHtt aggregate accumulation, and increased animal survival.\textsuperscript{1} An important feature of the Drp1 peptide inhibitor is that its effect is specific for activated Drp1 during excessive fission, as shown in R6/2 mice; the inhibitor has no effect on basal level Drp1 activity and non-pathological mitochondrial fission as demonstrated in cultured cell studies and in vivo, using wild type mice.\textsuperscript{1} Together, our data provide a proof-of-concept for the use of the Drp1 inhibitor, such as P110, to inhibit or slow down HD progression.

**Why Would Inhibition of One Aspect of HD Pathology (Mitochondrial Excessive Fragmentation) Be Effective?**

It is surprising that inhibition of mHtt-induced Drp1-mediated excessive mitochondrial fragmentation appears to be sufficient to suppress HD-related cell death in culture and to prevent selective neuronal loss in the striatum in vivo. We suggest that cell-type-specific mitochondrial characteristics might help explain the efficacy of the above treatment. The striatum is particularly sensitive to defects in mitochondrial oxidative phosphorylation (OXPHOS) due to an increased reliance on OXPHOS function in this area.\textsuperscript{24} Thus, one can imagine that striatal neurons can be preferentially affected by functional decline in mitochondrial activity relative to that in neurons residing in other brain regions. The loss of PGC-1alpha, a master regulator of mitochondrial biogenesis, in medium spiny neurons and not in other neurons (e.g., interneurons)\textsuperscript{25} may also explain the causal role of mitochondrial damage in selective loss of striatal neurons. Further, mitochondrial Ca\textsuperscript{2+} buffering affects striatal medium spiny neurons more severely than that in other areas, such as the cortex.\textsuperscript{26} Finally, medium spiny neurons have long projections, which might render them more vulnerable to trafficking and mitochondrial fission and fusion defects.\textsuperscript{27}

It is also possible that inhibition of mitochondrial fission impairment can re-balance the fusion and fission cycle and improve the overall quality of mitochondria, ensuring proper mitochondrial transportation to nerve terminals for optimal energy supply. Thus, selective protection of mitochondria might preferentially protect striatal neurons from various toxicities directly or indirectly induced by mHtt. Indeed, we found that preventing mHtt-induced excessive mitochondrial fission by treatment with the Drp1 peptide inhibitor reduced the formation of dysfunctional mitochondria (as evidenced by reduced mitochondrial depolarization and mitochondrial reactive oxygen species) and improved overall mitochondrial functions (as measured by for example ATP content).\textsuperscript{1} Healthier mitochondria can provide more ATP to the neurons, and fewer dysfunctional mitochondria can reduce the burden of oxidative stress within the cells. Supporting this possibility is our finding that treatment with the Drp1 peptide inhibitor improved neuronal morphology as evidenced by increased neurite outgrowth and inhibited cell death rate in patient-derived neurons. It is likely that increasing the cellular energy reserves may enable the neurons to withstand other injurious effects of mHtt. These in turn can translate to inhibition of mHtt-induced neurological symptoms and lethality, in vivo, that we observed.\textsuperscript{1}

Whether a Drp1 inhibitor or inhibitors of other molecular events associated with HD pathology are sufficient to alleviate the burden of HD disease in patients will need to be tested in clinical trials. This aspect of drug development is often driven and funded by industry. However, academic research can help identify surrogate biomarkers for HD.

**Lack of Biomarkers for HD to Assist in Patient Assessments during Clinical Trials Hinders Drug Development for the Disease**

Identification of biomarkers for diagnosis prior to clinical symptoms and for use during the development of therapeutics should be considered as a high priority for the success of translational research in this field. (Biomarkers are cellular, biochemical, or molecular indicators that can be used to evaluate pharmacological responses to a therapeutic treatment.\textsuperscript{28}) So far only clinical examinations, which are not sensitive enough to detect small
changes over short period of times, are used to assess disease progression.\textsuperscript{28,29} Sensitive surrogate HD biomarkers will allow following the beneficial effect of a tested drug prior to the manifestation of clinical symptoms.

Some biomarkers have been suggested. These include plasma creatine kinase (CK-BB isozyme), whose levels are reduced in R6/2 mice and in humans with HD.\textsuperscript{30} Other biomarkers include 8-hydroxy-2-deoxyguanosine (8-OH-2-dG), an indicator of oxidative injury to DNA, which was found to be highly elevated in HD patients; this marker can be detected in the urine and blood and brain tissue of patients.\textsuperscript{31} Measurements of oxidized molecules in blood and urine, levels of endogenous anti-oxidants, and branch chain amino acids as products of metabolism have also been suggested as potential biomarkers.\textsuperscript{32}

Another potential biomarker to monitor HD is the overexpression of H2AFY, a member of the H2A histone family.\textsuperscript{33} An unbiased gene expression profiling identified it to be elevated in the blood of HD patients. The authors suggest H2AFY as a potential biomarker associated with disease activity and pharmacodynamic response. Whether any of the above biomarkers is indeed predictive of disease progression and is a useful surrogate for treatment efficacy remains to be determined in future studies. Further unbiased studies as well as rational studies, using patient samples and animal models are underway; these are likely to shorten the length (and cost) of clinical trials for HD patients and will contribute an important component to ensuring the success of developing effective HD treatments.

\section*{Perspectives}

HD and other aging-related neurodegenerative diseases, such as Parkinson disease and Alzheimer disease, share the major features of late-onset and selective neuronal loss, as well as accumulation of protein aggregates.\textsuperscript{5} Because of its well-characterized mutation in a single gene, the Htt gene, HD was expected to be an “easier” disease among neurodegenerative diseases, to elucidate the pathology and to identify the pathway that can be modulated for treatment. Yet the research in the past 20 years suggests that the task is far from being completed. Several recent preclinical studies, including our own, suggest that pharmacological agents that protect mitochondrial functions may provide effective means to slow down or inhibit the progression of HD and possibly other neurological diseases. We described some of the challenges in developing new therapeutics for HD and believe that academic research can greatly assist in addressing these challenges.

\section*{Disclosure of Potential Conflicts of Interest}

A patent on the design and application of mitochondrial fission peptide inhibitor has been filed. The authors declare no conflicts of interest.

\section*{References}


15. Muzio-Sanjian I, Bates GP. The importance of integrating basic and clinical research toward the development of new therapies for Huntington disease. J Clin Invest 2011; 121:476-83; PMID:21285520; http://dx.doi.org/10.1172/JCI43536


www.landesbioscience.com

Rare Diseases

e28637-5

Downloaded by [Case Western Reserve University] at 09:29 14 June 2016
22. Robinotn DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. Nature 2012; 481:295-305; PMID:22258608; http://dx.doi.org/10.1038/nature10761


