

Hereditary Disease Foundation

Tripping up Triplets – Prevention, Intervention, Basic Understanding

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Abstract

At a meeting preceding the annual meeting of the American Society for Human Genetics in Philadelphia, some 25 scientists studying triplet repeat diseases met to discuss how their work fits into a program of finding a cure. Several suppressors of polyglutamine pathology, including chaperone proteins, were proposed as potential targets for drug screening. In addition, new models were discussed, including cDNA, knock-in, and transgenic mouse models. Mechanistic investigations are also proceeding in an effort to better understand the pathogenesis of polyglutamine diseases and to reveal new therapeutic targets. Research suggests the importance of proteolysis, dendritic pathology, protein interactions, and a conformational shift in the mutant protein.

In advance of the annual meeting of the American Society for Human Genetics, some 25 scientists studying a group of neurodegenerative triplet repeat diseases, including Huntington's disease, Kennedy's disease, and the Spinocerebellar Ataxias, met in Philadelphia to consider how their research fits into a program for finding cures. Ethan Signer, executive director of the Hereditary Disease Foundation's Cure Huntington's Disease Initiative, posed this challenge to the group: "How do you go about curing a disease?" For a relatively small number of diseases, said Signer, a protein is found that interferes with normal cellular function or damages the cells, and a small molecule is developed to interfere with that protein. However, for most diseases including Huntington's disease and most of the other triplet repeat diseases, the

offending protein is known, but the mechanism by which that protein causes damage is not.

For these diseases, Signer outlined two general strategies for finding cures: one can either work to understand what the protein does; or one can make no assumption about the protein's function but find a surrogate property that occurs along with the deleterious effect and hope that by abrogating the surrogate property, you will also abrogate the property that causes the pathology. The trick, he said, is finding an assay.

"Can you get some sort of condition either *in vitro* among protein molecules or *in vivo* among cells that you can point to and say, 'This is something that the huntingtin disease protein [mutant huntingtin] does that ordinary huntingtin protein doesn't do,' and then pass that assay through hundreds of thousands of compounds to find a candidate that has activity against a surrogate marker." After this initial high-throughput screening test, the candidate would then be tested in flies, worms, and mice, with the hope of finding something that might have efficacy in humans. Signer noted that the Hereditary Disease Foundation has formed a partnership with the biotechnology company, Aurora Biosciences, to develop assays for high-throughput screening of compounds that might interfere with some surrogate marker of HD.

Identifying targets for drug discovery

Nancy Bonini discussed work indicating that the chaperone protein HSP70 plays an important role in polyglutamine pathology. She has created a transgenic *Drosophila* model of Machado-Joseph disease (MJD), also

known as spinocerebellar ataxia type 3 (SCA-3). Expression of the truncated form of the MJD gene in flies confers a late-onset, progressive neurodegenerative phenotype that mimics human polyglutamine disease. In testing candidate genes that might interfere with this process, Bonini's group found that overexpression of HSP70 suppresses neurodegeneration. Moreover, her work indicates that the disease process is sensitive to both up-regulation and down-regulation of HSP70, suggesting a critical role for this protein.

The mechanism through which HSP70 might suppress degeneration was further discussed by Bonini and Edwin Chan, who works with her. They found that even when neurodegeneration is suppressed, aggregates still remain. However, Chan found that there is a profound difference in solubility properties of the disease protein. Severe degeneration is associated with low levels of monomeric protein, while mild degeneration or suppression is associated with high levels of monomeric protein.

“That suggests that, indeed, chaperones are modulating the solubility of the protein, and presumably that reflects some aspect of toxicity,” said Bonini. Further, it supports the hypothesis that visible aggregates may not be the direct cause of neurodegeneration.

Huda Zoghbi has also shown, in a *Drosophila* model of SCA-1, that expression of the mutant gene with 80 polyglutamines induces a neurodegeneration phenotype that mimics what is seen in mammalian cells. She used these flies in a suppressor screen, which identified at least eight genes in the chaperone-proteasome

pathway, along with other genes. Zoghbi's work indicates that HSP40, HSP70, and other chaperone proteins are good suppressors of neurodegeneration. She also found that haplo-insufficiency of ubiquitin (Ub) or Ub-conjugating enzyme enhances both aggregation and neurodegeneration, suggesting that drugs which enhance the activity of Ub or Ub-conjugating enzyme may be worthwhile candidates to test for therapeutic effect.

Parsa Kazemi-Esfarjani also reported on suppressor studies in flies, using a line of flies he created that express 127 polyglutamine repeats. He looked for genes that when overexpressed can suppress degeneration. By screening for P-element insertions, Kazemi-Esfarjani obtained 30 suppressor lines and 29 enhancer lines. Two of the suppressor lines were very effective in restoring normal eye function, and in both of these the suppressor was found to be a chaperone-related gene: HDJ-1 (HSP40) and tetratrichopeptide repeat protein 2 (TTR2). Both of these proteins have a “J” domain, which is required for interaction with HSP70. This characteristic may explain the suppressor effect.

He has also identified a suppressor of polyglutamine toxicity not related to chaperones. Myeloid leukemia factor (MLF) is homologous to MLF-1 in humans, which is involved in chromosomal translocation and is associated with leukemia. Kazemi-Esfarjani suggested that MLF-1 may be involved in a cell survival pathway, and which might explain its ability to rescue polyglutamine toxicity.

Josephine Dorsman also briefly described the use of *C. elegans* for

screening of modifiers and suppressors of polyglutamine pathology.

While the use of flies and worms will undoubtedly speed up the search for rational therapeutic targets, neither these nor any of the models precisely replicate the human disease. Lesley Jones and Nancy Wexler described preliminary efforts to search the human genome for modifiers of the HD gene. Jones works with a population of HD individuals in the United Kingdom. She has begun looking at people with a set repeat length but with various ages of onset, searching for polymorphisms that might explain the difference. Wexler and colleagues, working with people with HD in Venezuela, are doing the same, and are also searching for modifiers that affect age of onset.

New models

Danilo Tagle described mouse models his group has generated that attempt to address the issue of truncated vs. full-length protein. A full-length cDNA model shows progression, clear neurological abnormalities, and cell loss. Subsequently, they generated other mice that express truncated protein (the first three exons) with the same polyglutamine repeat lengths (16, 48, 89) as the full-length model. In both the truncated and full-length models, they saw early neurological abnormalities and hyperactivity, but the truncated model became stuck at that stage while the full-length model progressed to cell loss and hypoactivity. They did not see any remarkable pathology in the mice expressing truncated protein and only a slight increase in aggregates.

“My interpretation is that you can in some ways compartmentalize the effect of the mutation,” said Tagle. “One is

clearly a pure polyglutamine effect, which is probably in common with a lot of polyglutamine diseases. And what we think is happening is that we have the sequences that flank the protein that may actually confer some disease-specific or region-specific cell loss. And that could be by way of other mechanisms such as interacting proteins.”

Wojtek Auerbach described a knock-in model with an expanded polyglutamine tract (Q111). These mice show only subtle neurological but no behavioral phenotype. They also show characteristic cellular changes including nuclear mislocalization of htt and formation of nuclear inclusions and insoluble aggregates. A possible explanation, said Auerbach, is that cell dysfunction could be caused by the depletion of normal htt in the cytoplasm, while cell death results from polyglutamines in the nucleus. Blocking the transfer of htt from the cytoplasm to the nucleus might slow the progression of the disease.

Two transgenic mouse models of SCA7 were described by Albert La Spada and Gaël Yvert. La Spada’s mice express full-length cDNA under the control of the Prp promoter and show retinal degeneration that parallels what is seen in humans. The transgene is expressed in all layers of the retina with degeneration of cones preceding that of rods. La Spada said there is some evidence of proteolytic processing in certain populations of cells.

Yvert’s mice express full-length cDNA with 10 or 90 repeats under the control of either the rhodopsin promoter or the pcp2 promoter. In these mice, the N-terminal portion of the protein accumulates, while C-terminal immunoreactivity decreases with age.

When this transgene is overexpressed in rods, the mutation caused sprouting alterations of dendrites of post-synaptic interneurons, suggesting that dendritic regeneration reported in the medium spiny neurons in early HD stages may be a trans-neuronal response. Jean-Louis Mandel noted that these mice are available for therapeutic trials in the eye.

Understanding mechanism

Mechanistic investigations are likely to provide not only new information about the pathogenesis of the polyglutamine diseases, but also may reveal potential therapeutic targets.

Proteolysis

Albert La Spada discussed the role of proteolytic processing of mutant proteins. He cited recent work from Xiao-Jiang Li's lab¹ using Peggy Shelbourne's knock-in mice, which prior to the onset of the phenotype show some electrophysiological abnormalities. Li demonstrated that proteolytically-cleaved peptides appear in the cytosol and have an effect on processes occurring at the synapse. LaSpada said this finding ties into extensive literature on excitotoxicity in HD as well as studies in other polyglutamine diseases that indicate an important role for proteolytic cleavage. "Proteolysis may be a very important process in releasing a toxic peptide that causes the disease pathology; that's my hypothesis," said LaSpada.

Diane Merry's lab has also been interested in proteolysis. Working with full-length and truncated forms of the androgen receptor (AR), she has shown that overexpression of the truncated form results in aggregates and repeat-length-dependent proteolysis. AR with 112 repeats results in proteolytic

fragments, while AR with normal repeats does not. Merry said the cleavage site appears to be in the polyglutamine region of the protein, distinguishing it from the caspase site. The proteolytic fragment is localized in the nucleus and is exclusively associated with cell death. Further understanding of this cleavage event may yield clues about the relationship between proteolysis and cell death, she said.

Jean-Louis Mandel's lab has also been studying proteolysis in both HD and SCA7. His research indicates that short fragments are more toxic than long fragments, although it has been difficult to map the proteolytic sites. He also suggested that the preferential sensitivity of different neurons may be due to differences in proteolytic cleavage in those cells.

Dendritic pathology

James Eberwine discussed the role of dendrites in the pathogenesis of many neurodegenerative diseases. "I think many neurodegenerative diseases initiate at the dendrites and move into the cell body," he said. "In the dendrites translation can occur, and if you muck that up, perhaps that's one of the initiating factors associated with many aspects of neurodegenerative diseases."

Danilo Tagle said his mice do show dendritic pathology. Early on, these mice show proliferative changes, followed by degenerative changes later. He added that Marian DiFiglia has seen, in human studies, proliferative changes in grade 2 cases but degenerative changes in later stages. This indicates the loss of dendritic spines, which would be suggestive of an initial loss of presynaptic connections in the medium spiny neurons, followed by some attempt to reestablish connections. In other

words, the proliferation may be secondary to a loss of presynaptic input.

Protein interactions

Andrew Lieberman discussed work suggesting that in SBMA, alterations in function between normal AR and expanded repeat AR might be due to an interaction of the mutant protein with transcriptional co-activators. His group has done expression analysis looking at androgen responsive genes in a motor neuron neuroblastoma line. They have shown that polyglutamine expansion results in a loss of AR function. There are genes responsive to wild type but not mutant AR; another set that are responsive to both; and genes responsive to mutant but not wild type. They are now looking at AR with smaller repeats to see the effect on gene expression.

CREB-binding protein (CBP), a transcriptional regulator, has been implicated in HD pathogenesis by several groups. One hypothesis is that expanded polyglutamine binds to short polyglutamine regions of other proteins like CBP and interferes with their function. CBP is a good candidate, said Christopher Ross, because it is known to be present in low levels and known to be necessary for neuronal survival. His lab, in collaboration with others, has shown that CBP is normally present diffusely in the nucleus, but in cells transfected with mutant htt, CBP is “sucked away” from its normal location to coaggregate with htt in both the nucleus and cytoplasm. They looked at CBP-mediated transcription and found that in cells transfected with normal htt, there is no effect on transcription, but in cells transfected with expanded htt, transcription is blocked. However, coaggregation will not take place when

the short polyglutamine stretch of CBP is deleted. These same results were obtained with atrophin.

They have also looked *in vivo* and have detected CBP in aggregates from both transgenic mice and human patients, said Ross. “The evidence looks pretty good that this may be a mediator of htt-induced toxicity.” He added that this model might provide a unifying explanation for why polyglutamine is toxic.

Conformational shift in protein

Huda Zoghbi presented provocative evidence indicating that neurodegeneration and aggregate formation occur not only when protein (ataxin) with expanded polyglutamine is present, but also when the wild-type protein is overexpressed. She suggested that ataxin might exist in two conformations, and that at any point both conformations exist in equilibrium in the nucleus. The equilibrium could be shifted in response to increased protein level, increased numbers of glutamine residues, the presence of chaperones, or other factors.

Albert LaSpada suggested calling this model the “Dr. Jekyll and Mr. Hyde model,” noting that proteolysis could also shift the equilibrium to the “bad” conformation. Zoghbi proposed that the altered conformation is step one of the pathogenic process, and all the other observations (aggregation, inclusions, altered gene expression, etc.) are downstream effects. Modulation of any or all of these steps may offer therapeutic targets.

Human studies

Russell Margolis discussed preliminary data concerning a large pedigree with a disorder resembling HD.

Before gene testing was available, family members with this autosomal dominant condition were assumed to have HD; however, these patients do not have repeat expansion of the htt gene. “We’ve cloned the expansion and we don’t know anything about it,” said Margolis. “It’s too small a fragment to know much about the protein except that it’s a CAG expansion that we can test for, that causes a CAG phenotype just like HD both clinically and pathologically.” Comparing the protein product of this new disease gene to htt may provide useful information about motifs and interacting factors common to the two proteins, and hence likely to be involved in disease pathogenesis, said Margolis.

Time for drug screens?

Drug screens have already been initiated by several groups, including the collaborative effort with Aurora Biosciences. Yet, there is still little consensus on which targets are most likely to yield compounds effective in preventing, delaying, or curing HD in an animal model or a human trial.

Mechanistic studies are still needed that will produce more knowledge on the clinical, cellular, and molecular aspects of polyglutamine disorders. It may be difficult to narrow the range of possibilities. “The biology of these diseases is very complicated,” said Huda Zoghbi. “We’re not going to lose those neurons for a single reason.”

Allan Tobin predicted that by next year a number of targets will have been identified and screens will detect “dozens and dozens” of compounds to be tested. The problem will then become one of controlling the profusion of data and carrying out these screens with efficiency.

Peter Harper suggested using as fully as possible agents that have already been approved by the FDA. Ethan Signer said there are about 1500 such compounds and a library, called the “FDA2000 library,” is being put together by Steven Gullans at Brigham and Women's Hospital in Boston, (sgullans@rics.bwh.harvard.edu). This library will be available on microtiter plates for high-throughput screening. The Hereditary Disease Foundation will support anyone who wants access to that library, he said.

Christopher Ross said better models are still needed, noting that there is no clearly established model with selective degeneration of the medium spiny neurons. “Having so many good compounds makes having good models more important, not less,” he said.

¹ H Li, S -H Li, H Johnston, P F Shelbourne and X -J Li. 2000, Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity 1989, *Nature Genetics*, 25(4): 385-389