

Hereditary Disease Foundation

P53 & Huntington's disease – A Relationship?

June 26-27, 2000 • New York, New York

Prepared by Lisa J. Bain

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Abstract:

Following a report suggesting functional role for the tumor suppressor factor, p53, in Huntington's disease (HD), scientists who study p53 and its role in transcriptional regulation and tumor suppression joined with those that focus on HD to consider the p53-Huntington's connection. Research in Leslie Thompson's lab found that p53 interacts *in vitro* with the exon 1 protein (httexp1) and copurifies with p53 in cells grown in tissue culture. They also showed that expanded httexp1 interacts with the coactivator CREB binding protein (CBP) and the corepressor mSin3a, and that the expression of expanded httexp1 repressed transcription of genes regulated by p53. Discussion of the paper included suggestions for additional controls as well as other techniques to assess the effect of htt on transcriptional repression. There was also more general discussion about the effect of htt on apoptosis and nuclear inclusion formation, and whether a conformational change in the protein might play a role in neurodegeneration. Participants agreed that crossing one or more of the HD mice with a p53 null mouse would help clarify the role of p53 in HD and that further study is needed into the structure and function of both the mutant and the wild-type protein. Therapeutic implications of this research were also discussed.

In June 2000, Leslie Thompson's lab at the University of California, Irvine, published a paper in PNAS¹ suggesting a functional role for the tumor suppressor factor, p53, in Huntington's disease (HD). Less than two months later on June 26-27, scientists from two separate realms -- those that study p53 and its role in transcriptional regulation and tumor suppression and those

that focus on Huntington's disease -- came together at Rockefeller University in New York City to consider the p53-Huntington's connection. Hosting the meeting, Arnold Levine, president of Rockefeller University and discoverer of the p53 protein in 1979, called it a "fortuitous meeting between the two groups," noting that the p53 field is beginning to coalesce around a hypothesis linking an overactive p53 with a variety of neurodegenerative diseases that occur over a lifetime. The p53 protein, he said, probably arose in evolution to surveil genotoxicity in the embryo and later became used to surveil cancer as well. "But it's dangerous and it had to be regulated very carefully. And the danger comes, I think, especially in the neurodegenerative areas."

The human side of HD

"I'm from Philadelphia and have Huntington's disease, and I also have a great deal of hope that as this process keeps going on, the picture for me and my kids is much more positive."

Before addressing the scientific questions raised by the Thompson paper, the group met two families affected by HD. One family member lost his first wife to the disease and has two children in later stages of the disease. He and his wife have devoted their energy and resources to finding a cure. A member of the second family, a 59-year-old real estate executive from Philadelphia who himself has HD, came to the meeting with his wife and daughter.

It was nearly 10 years ago that he began noticing the telltale problems associated with HD: dropping things, losing his balance, forgetting, and struggling with anger. He knew what was coming, having watched the progression of the disease in his

mother, who was diagnosed at age 40 and died at age 78; and in his sister who developed the disease at about age 40 as well. “I had always lived with this little dark shadow inside that said, ‘you’ve got a 50% chance that you’re going to get the disease,’” he said.

As the disease has progressed, he has noticed differences in his gait and balance and the effects on his speech and memory. “As time goes on, I’m more threatened by remembering names and articulating what the company’s vision is. For me, it’s probably not going to be too far down the road where I’m going to have to stop [working].”

Meanwhile, his family has faced difficulties of their own. “I’m the only one in our family who’s not at risk or doesn’t have Huntington’s,” said his wife. “It’s a unique position. It’s very difficult to be a mother who has four very able, intelligent, attractive children and knows every single one of them and every single grandchild could have Huntington’s. It’s certainly made my direction in life very different.”

Their children and brother face other problems. While watching their father’s and aunt’s conditions deteriorate, they worry about their own futures and those of their siblings, cousins, and children. “I think I’m a living time bomb, going to go off in two years,” said one of the daughters. She has chosen not to be tested to see if she has the gene, yet she remains “vehemently opposed” to passing on the disease to another generation. “It’s so hard to live with your convictions and try to do the right thing,” she added.

Addressing the assembled group of scientists who struggle with test tubes, mice, and gels to understand the pathobiology of

this devastating disease, Nancy Wexler said, “What we have before us is pretty lethal in terms of protecting the next generation. Even if Huntington’s is never in your DNA, everyone is affected and the changes are radical and irrevocable.”

Why study p53?

Leslie Thompson provided some background for those unfamiliar with HD. HD is an autosomal dominant neurodegenerative disorder characterized by psychiatric, cognitive, and movement dysfunction. Neurodegeneration occurs primarily in the medium spiny neurons of the striatum. The gene for HD was cloned in 1993 and was found to code for a 350 kd protein named huntingtin (htt). In the non-mutated form, htt is cytosolically located and is ubiquitously expressed throughout the body. The mutated form differs from wild type in that there is an expansion of glutamine residues. There are now known to be eight different so-called polyglutamine repeat diseases, with the polyglutamine expansion occurring in different genes, only two of which are of known function. Each of the polyglutamine diseases has a distinct pattern of cell loss. The implication, said Thompson, is that apoptosis may be involved.

A transgenic mouse model created by Gillian Bates had shown that the amino-terminal portion of the Huntington gene alone, containing huntingtin exon 1 with an expanded CAG repeat, results in an HD-like phenotype, characterized by neuronal dysfunction and late-stage neurodegeneration as well as motor and behavioral symptoms. Moreover, both in human HD and in transgenic mouse models, down-regulation of certain neurotransmitter receptor genes has been demonstrated,

suggesting transcriptional dysregulation as a possible pathogenic mechanism of HD. The PNAS paper examined the role of the exon 1 protein (httexp1) on transcription.

“We’ve been looking at this along these lines: that potentially changes in gene expression and transcription can lead to neuronal dysfunction and, ultimately, downstream cell death,” said Thompson. “So we went ahead to look at a potential role for alterations in gene expression.”

Joan Steffan, who initiated the experiments in Thompson’s lab summarized the results of their study. They found that p53 interacts *in vitro* with httex1p (both wild type and with expanded polyglutamines), and that it copurifies with p53 from cells grown in tissue culture, using a novel purification method developed by Alex Kazantsev in David Housman’s lab. The interaction requires the presence of the extreme carboxy-terminus of p53. They also showed that expanded httex1p interacts *in vitro* with the coactivator CREB binding protein (CBP), and the corepressor, mSin3a. CBP and mSin3a have both been found in intranuclear inclusions in HD transgenic mice in collaboration with Gillian Bates’ lab. Work from other investigators, several of whom were at the meeting, have shown that CBP and mSin3a interact with p53 and mediate p53 transcriptional regulation. Further, they demonstrated in cell culture, that expression of expanded httexp1 repressed transcription of genes regulated by p53, the *p21* and *MDR-1* luciferase reporters. These data suggest that expanded httex1p may behave similarly to the viral protein E1A, which interacts with CBP/p300, repressing transcription and leading to cell death.

Interaction of p53 with htt

There was much discussion about the technical aspects of the Steffan paper and additional controls that would increase confidence in the conclusion that htt interacts with p53 and modulates transcription. Maureen Murphy commented that the interaction between p53 and htt needs to be assessed more stringently, in light of evidence that both proteins have “sticky” proline-rich regions. There was also concern about some of the cell types used (293, SAOS-2). Carol Prives, for example, suggested that HeLa cells aberrantly control expression of p53; and Eileen White added that 293 and Cos cells may also produce artifactual results and may not accurately represent the physiological cell types affected in HD. Since these cells are transformed by oncogenes, they contain viral proteins that may aggregate or otherwise alter p53. She suggested developing neuronal cell lines that resemble cells that are affected in HD. Maureen Murphy suggested trying immunoprecipitation-western blot analysis using endogenous p53 and htt, with stringent washing condition such as high detergent and high salt.

Effect of htt on transcriptional repression

Several participants suggested that gene expression profiling of cells might be preferable to luciferase assays in determining whether p53-regulated genes are altered in their expression. Based on Steffan’s work, repression of key promoters would be predicted. In studying transcriptional repression, Murphy and others suggested avoiding cells that have been transiently transfected with proteins that ultimately kill the cell, as the results may be confounded by non-specific repression. It may be better to analyze repression of endogenous genes, both those

that are induced and those that are repressed by p53.

Akira Sawa has also been investigating whether the interaction of p53 and htt modulates gene expression. In particular, Sawa is interested in the influence of this interaction on mitochondrial function. With Tim Greenamyre at Emory University, he has reported mitochondrial dysfunction in HD lymphoblasts and fibroblasts. In addition, mitochondrial dysfunction has been reported in the brain tissue of HD patients. Mitochondria regulate apoptotic cell death, said Sawa, and many transcripts induced by p53 are related to mitochondria.

Gene expression profiling was also suggested in fibroblasts from HD patients to compare against gene expression in the striatum of HD patients.

Richard Morrison, who was unable to attend the meeting but faxed in his comments, suggested that it would be helpful to have a composite of proteins as well as genes that are regulated in a p53-dependent manner in neurons during the course of programmed cell death. His lab has been developing a proteomics approach to define p53-dependent changes in gene expression. His technique makes it possible to screen a complex mixture of proteins from different cells and tissues to determine which are expressed, and where.

Effect of htt on apoptosis and nuclear inclusion formation

The role of nuclear inclusions and sequestration of proteins in the pathogenesis of HD has remained an unanswered and perplexing question. According to Leslie Thompson, the role of inclusions is unclear. There is some evidence that the formation of inclusions precedes the onset of symptoms; however, there is still no consensus on this

point. One of the suggestions raised by the Steffan paper is the possibility that p53 could be a chaperone, mediating the transport of htt to the nucleus and the formation of nuclear inclusions.

Allan Tobin noted that it is somewhat paradoxical that a large protein like htt would exert its damaging effects in the nucleus. "So the unifying hypothesis is that some proteolytic event cleaves the N-terminal portion, allowing it to get into the nucleus."

Akira Sawa presented data that might help clarify this point. Working in lymphoblasts from HD patients, Sawa has shown that an increase in stress-induced apoptotic cell death is associated with caspase-3 activation. He has shown definitively that the same peptides that block caspase activity also prevent htt cleavage. Further, his work has demonstrated that caspase-cleaved htt sorts to the perinuclear region first and ultimately accumulates in the nucleus, thus lending support to theories that stress the importance of nuclear translocation and accumulation of cleaved htt. He has also demonstrated upregulation of p53 in tet-inducible PC12 cells following induction of an expanded repeat containing huntingtin protein.

Another hypothesis suggested that htt is sequestering p53 protein or somehow inactivating it. If that were true, said William Kaelin, there might be down-regulation of p53 target genes in the HD mouse. Gene expression studies might shed light on this question. Results from the Luthi-Carter, et al² paper where gene expression changes in R6/2 mice were profiled using GeneChip arrays were discussed.

De-Maw Chuang has been studying the

role of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) on apoptosis. His work indicates that GAPDH is translocated to the nucleus during the apoptotic cascade and upregulated in response to apoptotic stress. His laboratory recently showed that GAPDH is a downstream target of p53. Earlier work had demonstrated that the enzyme specifically binds to the polyglutamine region of htt, suggesting that GAPDH might be involved in transport of htt to the nucleus, the formation of nuclear inclusions, the formation of p53-htt aggregates, and/or the neurotoxicity associated with htt. Further study of the role of GAPDH might open up new avenues for therapy.

Eileen White and Scott Lowe described the mechanism whereby the viral protein E1A drives apoptosis. By interacting with CBP/p300, E1A represses transcription of genes that use those coactivator proteins. E1A also causes reduced expression of MDM2, a protein that is involved in the degradation of p53. Thus, p53 levels rise and the apoptotic cascade is initiated. The cascade can be stopped by overexpression of p300. Steffan attempted to determine whether httexp1 has similar effects of p53 in HeLa cells, but saw no increase in p53. Because of the problems with HeLa cells mentioned earlier, however, and because of Sawa's results showing an increase in p53 in PC12 cells, and in light of data highlighting the importance of proline rich regions in the interaction of htt with p53, CBP, and mSin3a, Steffan said she plans to continue to investigate similarities between E1A and expanded httexp1.

Arnold Levine commented that glutamine repeats can recruit heat shock proteins and that mutant p53 binds to heat shock proteins. He suggested that these

proteins could play a role as chaperones and genetic modifiers in the process of inclusion formation and/or apoptosis. Polymorphisms in the heat shock proteins might help explain some of the variation in progression and age of onset.

Might a prion-like interaction be involved?

Levine also suggested that a prion-like interaction may be involved in the process of neurodegeneration. Ethan Signer mentioned that monoclonal antibodies are available that recognize the expanded but not the short polyglutamine, adding presumptive evidence that there is a conformational shift when the polyglutamine stretch is expanded. This idea, that there are two possible conformations for the protein, lends support to the prion hypothesis, said Levine, suggesting that the conformational structure of the protein might recruit other proteins to assume such a structure. In addition, he noted that heat shock proteins play a big role in prion diseases. Heat shock proteins bind proteins and dissociate by ATP hydrolysis; and when they dissociate, they fold. Levine suggested looking to see if any of the heat shock proteins bind to polyglutamine.

There was additional interest in the idea that protein folding might be important in htt-induced apoptosis. According to Eileen White, one of the triggers of apoptotic cell death in some cell types is the inability to deal with a misfolded protein. If inclusions are only in cells that are dying, this might be a part of the explanation. Maureen Murphy suggested that HD might result from a defect in protein folding. She reasoned that the cell's attempts to refold the misfolded protein could lead to altered rates of ATP hydrolysis and accelerated "aging" of the affected neurons.

Bill Kaelin noted that malformed proteins also appear to play a role in the pathogenesis of von Hippel-Lindau (VHL) cancer syndrome, which is caused by mutations in the VHL tumor suppressor.

A number of related questions arose, the answers to which might help clarify the importance of p53 in HD. One of the key unanswered questions is whether HD is a disease of cell death, cell dysfunction, or apoptosis. Other important questions include: What is the normal function of htt? Why do people with the mutant gene express the disease relatively late in life? One factor that may play a role is that the efficiency with which ATP is produced in mitochondria declines with age. Another possibility is that there is something accumulating with age.

What experiments are needed to clarify the role of p53 in HD?

There was general agreement in the value of crossing one or more of the HD mice into the background of a p53 null mouse. Since p53 null mice get cancer at an early age, an HD transgenic mouse with rapid disease progression is preferable. Leslie Thompson noted that Gillian Bates has attempted to do so but was unsuccessful because of breeding problems that made it difficult to generate homozygous p53 $-/-$ females. However, Akira Sawa has had success generating these crosses using David Borchelt's transgenic mice. The cross-bred mice with knocked-down expression of p53 exhibit a less severe HD phenotype in terms of motor coordination at eight months compared with htt transgenic mice, suggesting an important causal role of p53 in HD pathology. Other possible means of investigating the role of p53 in mice would be to give p53 inhibitors to HD

transgenic mice or to challenge these mice with an apoptotic stress, such as irradiation.

According to Scott Lowe, this cross would answer what he called "the million dollar question": Is it a cause or a consequence? Does htt at higher levels create a pathologic state that eventually triggers p53 to kill, or is it more direct, in that HD is actually modulating p53 activity?"

This mouse could also yield important evidence through gene expression profiling experiments. If p53 does not have a role, expression profiling of a p53 $-/-$ mouse compared with a wild-type mouse should show recognizable overlap; whereas if p53 is involved, there should be significant differences in the gene expression profiles of striatum from HD transgenic mice in the absence of p53.

Lowe also suggested further experiments using fibroblasts from HD patients and normal controls as a means of understanding the function of normal and mutant htt and the role of p53 in HD pathology. He noted that while fibroblasts do not express the HD phenotype, they do express endogenous levels of the mutant and normal protein.

A new direction

A consensus emerged as the meeting progressed, that key pieces of information are missing in the attempt to understand the pathophysiology of the disease, including the role of p53. Namely, the structure and function of both the wild-type and mutant proteins is unknown. Understanding what happens conformationally to the protein when there are expanded numbers of polyglutamines might help explain some of the unanswered questions in HD research.

For example, why is it that a person with less than 34 repeats does not get the disease while a person with over 40 repeats does? David Housman presented data showing that 70-80% of the variance in age of onset is explained by repeat length. A person with greater than 50 repeats gets the disease at an earlier age, and a person with greater than 60 repeats always gets the disease prior to the age of 20. Nancy Wexler added, however, that there does not seem to be a correlation between repeat length and progression of disease.

“It’s a little surprising that there’s such a tremendous age dependence upon a few extra amino acids,” said Arnold Levine. “That is not what a structural biologist would have told you intuitively.” The implication, he said, is that genetic modifiers are working on the repeat length. “It seems to be a reasonable hypothesis that everything has to do with polyglutamines, not with proteins; and that conformation and length are important,” he said. He suggested looking at the structure and conformation of proteins with short and long polyglutamine repeats.

Scott Lowe returned with some exciting news. During the coffee break he had spoken with Nikola Pavletich, a structural biologist at Memorial Sloan Kettering Cancer Center. Pavletich joined the meeting and, after studying the sequence of huntingtin, made several interesting observations. The sequence suggests that htt plays a role in the cytoskeleton and that the region of AA500-600, a caspase cleavage site, is non-structural, he said. Pavletich expressed interest in studying structure of the HD protein using x-ray crystallography and NMR.

Therapeutic implications of p53 for

HD

Finding appropriate therapies will require a better understanding of whether HD is a disease of cell death by apoptosis or necrosis; or of cell dysfunction. If cells are dysfunctional but not dead, therapy may be directed at restoring function.

To determine whether apoptosis is the cause of HD pathogenesis, Eileen White suggested crossing an HD transgenic mouse into the Bcl-2 transgenic model of J.C. Martinou. This mouse overexpresses Bcl-2 in the brain, which prevents neuronal cell death. If the cross rescued the HD phenotype, the implication would be that apoptosis was involved. And if that proves to be the case, an approach for finding a treatment for HD would be to look at inhibitors of apoptosis. White noted that many drug companies are already developing anti-apoptosis agents for the treatment of neurodegenerative diseases, and that “the HD community may be able to bootstrap their way to a therapy on these already-ongoing efforts.”

Robert Fine has developed peptides that interact with the proline-rich region of p53, thereby activating p53- induced apoptosis. If these same peptides interact with the proline-rich region of htt, they may block transcriptional repression induced by httexp1 and apoptosis.

De-Maw Chuang suggested a different possible therapeutic strategy. He discussed the possibility that excitotoxicity might be involved in neurodegeneration in the striatum. When the excitotoxin quinolinic acid is injected into the striatum of rats, there is a selective loss of medium-sized spiny neurons, which resembles the pathology seen in the brain of people with HD. Associated with the injection of

excitotoxin, Chuang saw an activation of the transcription factor NF-kB and induction of p53, c-Myc, and cyclin D1. Pretreatment of the rats with lithium significantly reduced the size of striatal lesions. Lithium protection is accompanied by induction of the major cytoprotective protein, Bcl-2. “Although the neuroprotective mechanisms of lithium are still unclear,” he concluded, “Clinical trials using lithium to treat HD patients seem to be warranted.”

Scott Lowe also suggested further investigation of the role of genetic modifiers as a target for potential therapeutic strategies, even as the effort is made to better understand the structure.

Ethan Signer urged the group to begin how to consider therapeutic implications of the research. “The logical progression would be to say that we will understand how expanded polyglutamine works, and we will know what the target is and then we will design an assay that will let us screen for drugs. Well, if we do that we’re liable to have to wait for several more years and we don’t want to do that. So instead, we choose a property that we can demonstrate depends on expanded polyglutamine, and we use that to screen a library.”

¹ Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu Y, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM. (2000) The Huntington’s disease protein interacts with p53 and CREB-binding protein and represses transcription. *PNAS* 97:6763-6768.

²Luthi-Carter R, Strand A, Peters ML, Solano S, Hollingsworth ZR, Menon AS, Frey AS, Spektor BS, Penney EB, Schilling G, Ross CA, Borchelt DR, Tapscott SJ,

Young AB, Cha J-H, Olson J (2000) Decreased expression of striatal signaling genes in a mouse model of Huntington’s disease. *Human Molecular Genetics* 9(9): 1259-1271