



University of Kentucky
UKnowledge

University of Kentucky Doctoral Dissertations

Graduate School

2003

THE ROLE OF P53 IN OXIDATIVE STRESS AND POLYGLUTAMINE NEUROTOXICITY

Jay C. Dunn

University of Kentucky, jcdunn0@uky.edu

[Right click to open a feedback form in a new tab to let us know how this document benefits you.](#)

Recommended Citation

Dunn, Jay C., "THE ROLE OF P53 IN OXIDATIVE STRESS AND POLYGLUTAMINE NEUROTOXICITY" (2003).
University of Kentucky Doctoral Dissertations. 266.
https://uknowledge.uky.edu/gradschool_diss/266

This Dissertation is brought to you for free and open access by the Graduate School at UKnowledge. It has been accepted for inclusion in University of Kentucky Doctoral Dissertations by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

ABSTRACT OF DISSERTATION

Jay C. Dunn

The Graduate School
University of School
2003

THE ROLE OF P53 IN OXIDATIVE STRESS AND POLYGLUTAMINE
NEUROTOXICITY

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor
of Philosophy in the College of Arts and Sciences at the University of Kentucky

By
Jay C. Dunn

Lexington, Kentucky

Director of Dissertation: Sheldon M. Steiner, Professor of Biology

Lexington, Kentucky

2003

Copyright © JayChristopher Dunn 2003

ABSTRACT OF DISSERTATION

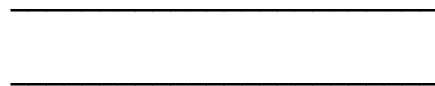
THE ROLE OF p53 IN POLYGLUTAMINE EXPANSION INDUCED NEURODEGENERATION

Polyglutamine expansion disorders are progressive neurodegenerative diseases that are caused by the pathological expansion of polyglutamine repeats. Huntington's disease (HD) is a polyglutamine disorder caused by the expansion of an existing polyglutamine tract in a novel protein, Huntingtin (Htt). Oxidative stress has been implicated in the neural dysfunction observed in multiple neurodegenerative conditions including HD. The tumor suppressor p53 is a multifunctional protein that has roles in the cell cycle, apoptosis and neurodevelopment. The role of p53 in HD-associated neurodegeneration has been studied but not fully elucidated, nor has the role of p53 in oxidative stress toxicity been fully elucidated.

Here I present work that demonstrates polyglutamine expansion induced alterations to p53 stability, localization, and activity. The transcriptional activity of p53 was found to have a role in oxidative stress mediated as well as polyglutamine mediated neurotoxicity *in vitro*. The expression of p53 was also altered *in vivo* in a mouse model of HD as well as in HD brain.

Taken together, these data demonstrate a role for p53 in polyglutamine and oxidative stress toxicity.

Keywords: Polyglutamine, Huntington's Disease, p53, oxidative stress, neuron



THE ROLE OF P53 IN OXIDATIVE STRESS AND POLYGLUTAMINE
NEUROTOXICITY

By

Jay Christopher Dunn

Sheldon M. Steiner

Director of Dissertation

Peter M. Mirabito

Director of Graduate Studies

Copyright © Jay Christopher Dunn 2003

RULES FOR THE USE OF DISSERTATIONS

Unpublished dissertations submitted for the Doctor's degree and deposited in the University of Kentucky Library are as a rule open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but quotations or summaries of parts may be published only with the permission of the author, and with the usual scholarly acknowledgments.

Extensive copying or publication of the dissertation in whole or in part also requires the consent of the Dean of the Graduate School of the University of Kentucky.

DISSERTATION

Jay C. Dunn

The Graduate School
University of Kentucky
2003

ROLE OF P53 IN OXIDATIVE STRESS AND POLYGLUTAMINE NEUROTOXICITY

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

Jay C. Dunn

Lexington, Kentucky

Director: Sheldon M. Steiner, Professor of Biology

Lexington, Kentucky

2003

ACKNOWLEDGEMENTS

I'd first like to thank Dr. Jeff Keller. He took me on as a student in 2001 when he didn't have to. He has been a friend for almost my entire graduate career, an advisor and teacher for the same period, and for the last 2 years he's been my dissertation advisor. He's been inspiring, entertaining, and has taught me more about the human brain and its diseases and disorders than anybody else I've known. Jeff took me to meetings, and this allowed me to expand the scope of my knowledge and meet people in the field. I was his first student to graduate, and I know I'm the first of many. I'm still not the world's best technical writer, but I have improved immensely thanks to the patience of Jeff, who must have gotten his from Job. Thanks for turning me on to neurodegenerative diseases Jeff, thanks for being my advisor and making me want to do that extra little bit, and may you always be successful.

I'd also like to thank Dr. Shelly Steiner. Shelly was my first advisor. We worked on microglia and the effects of lipid signaling on these cells. Shelly will always have a special place in my heart as he was instrumental in bringing me to UK and teaching me the ins and outs of research. I learned how to give a good presentation from Shelly, and his wife Marion, which is the bread and butter of science. If you can't explain your work to other people, then it has little meaning. I've enjoyed Shelly's stories, our endless debates over the issues outside of science, and just interacting with him. Thank you Shelly for your advice, most of which I've either taken or am saving it for another day, your understanding, and mostly your faith that I'd end up with one of these degrees. Someday I'd like to go catch the biguns with Shelly.

My committee members have been very helpful to me, whether as a function of their membership or individually. Dr. Brian Rymond is a unique individual in my experience. His courses were tough but some of the best I have ever had. I also spent a rotation in his lab working with things I will probably never work with again, and learned as much about doing science from him as I ever had in the previous 28 years of life. Controlled experiments are part of my lexicon even more since I've known this

man. Thanks for your insights into research and always being willing to educate me. Keep pushing folks Dr. Rymond, it's what makes you a good advisor.

Doug Harrison has been very helpful at times with microscopy, genetics, software, etc. Thanks for informative discussions about the ins and outs of science, and other subjects. Another contribution has been allowing his lab to be a place of refuge for me to commiserate with friends.

A special word about Pete Mirabito is needed. Pete has always demonstrated faith in me and my abilities. He's been tough as nails when he need to be, but has the best interest of the student at heart. I hope one day he can slow down enough so we can just hang out and watch football. Thanks for everything Pete, the advice, the hints, and the inspiration.

Many graduate students have contributed to my Ph.D. Without intention of missing any, Rongwen Xi, Jason Rawlings, Brian Dillon, Brandon Thomas, Kristin Morey, Jen Matlock, and Jennifer Klein were all on my end of the hall. Mark Lies, Sri Venkatram, and CK Prakash were all allies in the cause. You all have been good friends, compatriots, drinking buddies, and tent mates. May each of you get what you wish for in life, and if you ever need me I'll be somewhere where you can find me. My compadres from the Rymond lab, Mitch McLean, Becky Seipelt, Seyung and Neetu, Kevin Vincent, God Bless I thank you for your support during a rough first spring in grad school. Avoid the Jay Dunn virus.

I'd like to thank the members of the Keller lab, past and present who took me in and allowed me to make a place of my own there. Qunxing Ding has been a very good sounding board for me and I appreciate his advice, his humor, and his friendship. Ed Dlmayuga has been a source of entertainment and cells. Thank you for your hospitality and your prayers Ed. Janelle Reed, Sarah Martin, Vidya Nukula, Feng Fa Huang have all been good friends and have been very helpful all along. I hope all of you have much success. Brandon Lovell, a guy whom I've known the shortest amount of time, has made the biggest impact in some ways, by helping me finish some of the work described herein. A marvelous young man, Brandon will do well. Thank you from the

bottom of my heart. And my partner in the trenches, Jill Gee, is to be acknowledged for her counsel, her ear, and her willingness to share. Keep up the good fight Jill and you'll get one of these! Thanks for being there and putting up with me. Good luck in all you do.

I'd like to thank my family for raising and supporting me these last 33 years. My Dad is my best friend in so many ways. We talk almost everyday, sometimes several times a day. I learned how to be a man from him, which I had to learn how to do before I could do something like this. My mother is the quiet, faithful presence in my heart. Thank you for your love and your faith over the years, Mom, as well as your prayers. My brother Jon has missed most of this mission of mine being on a mission of his own in the U.S. Navy. God keep you my brother and bring you home to us. Thanks for being supportive and proud of me all these years. My grandparents Junior and Faye Dunn have also been a big part of my support system during grad school. Thanks for always being there.

Dr. Bob Francis was my undergraduate advisor. I may be the last to thank him in a forum such as this. Bob, it has been my honor to call you friend and advisor for over 10 years. I will always appreciate your strong support for me in time of need and in times of change for me personally and professionally. You are a true scholar and a gentleman.

Finally, I'd like to thank Jenine Martin. Jenine and I will be married in 2004. She has been a presence in my heart for 17 years and from this day forward as well. She has been a rock as I have been for her during her education. Now that we're done with school, I'm ready to start the rest of our lives. Thanks for waiting honey and I love you very much.

Table of Contents

ACKNOWLEDGEMENTS	ii
List of Figures	vi
List of Files	viii
Chapter One: I ntroduction/Background	1
Huntington’s disease	1
Neurodegeneration in HD	3
Oxidative Stress in HD	6
p53 in HD	8
Chapter Two: Polyglutamine induced changes to p53 expression, localization, and transcriptional activity	16
p53 expression and stability	17
p53 is modified in neural cells expressing polyglutamine expansion	26
Genetic Inhibition of p53 activity attenuates polyglutamine neurotoxicity	27
p53 in HD mice and human HD	29
Chapter 3: Oxidative Stress Mediated Alterations to p53 Expression and Transcriptional Actiiviity	35
Inhibition of p53 Attenuates H ₂ O ₂ Mediated Death in Neural Cells	40
A Genetic Inhibitor of p53 Attenuates H ₂ O ₂ induced neurotoxicity	42
Chapter 4: Discussion and Conclusions	45
Expression and regulation of p53	45
Polyglutamine Expansion Alters p53 Expression and Activity in neural cells ..	54
p53 in the R 6/2 mouse	56
Human HD	60
p53 and oxidative stress	63
Future Directions	72
Chapter 5: Materials and Methods:	78
Cell Culture	76
HD Mouse Model	77
Analysis of Protein Expression	78
Immunoprecipitation	80
Analysis of Neural Death	81
Statistical Analysis	82
References	83
Vita	99

List of Figures

Figure 1. Expression of p53 is altered in neural cells expressing polyglutamine expansion	17
Figure 2. p53 expression is altered in neural cells that express polyglutamine expansion.	19
Figure 3. Mdm2 expression is altered in neural cells expressing polyglutamine expansion	21
Figure 4. Polyglutamine expansion alters expression of p53 and Mdm2 in human neural cells	23
Figure 5. p53 interaction with Mdm2 is altered in polyglutamine expansion expressing cells	24
Figure 6. Decreased expression of MDM2 is sufficient to increase neural cell toxicity in differentiated and undifferentiated neural cells	25
Figure 7. p53 is modified in cells expressing polyglutamine expansion	27
Figure 8. Polyglutamine expansion alters p53 DNA binding in neural cells	28
Figure 9. Expression of p53 and post-translational modifications to p53 are altered in HD mice	32
Figure 10. The expression of p53 is altered in HD Brain	34
Figure 11. H₂O₂ induces dose dependent neural death	37
Figure 12. H₂O₂ alters p53 expression in rat cortical neurons	39
Figure 13. Pharmacological inhibition of p53 attenuates neural death induced by oxidative stress	40
Figure 14. Pharmacological inhibition of p53 attenuates cell death induced by oxidative stress in human neural cells	41
Figure 15. Pharmacological inhibition of p53 activity attenuates oxidative stress induced death of differentiated human neural cells	42
Figure 16. Loss of p53 DNA binding attenuates oxidative stress mediated neural death	43

Figure 17. Genetic inhibition of p53 DNA binding attenuates neural death induced by H₂O₂	44
Figure 18. There are many possible routes to HD through p53	73

List of Files

JayDunn1.pdf

1.7MB

Chapter One

Introduction/Background

Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disease that affects about 1 in 20,000 people of European descent (Rubinsztein, 2002). It is autosomal dominant in nature and was first described in detail in 1872 by George Huntington. In 1993, the genetic cause of Huntington's disease (HD) was defined. The Huntington's Disease Collaborative Research Group (HDCRG) identified the gene IT15, located on the short arm of chromosome 4, as the defective gene common to members of 75 families afflicted with HD. IT15 was found to have 67 exons, and code for a protein approximately 348 kD in size. This protein was of unknown function and had no relation to any known gene (HDCRG, 1993). Subsequently named huntingtin, this gene contains within exon 1, a region of CAG trinucleotide repeats. HD occurs in individuals who have an unstable genetic insertion of repeating CAG, which code for glutamine, sequences. Therefore HD is known as a trinucleotide repeat or polyglutamine expansion disease. Normal individuals have between 9 and 35 glutamines in the polyglutamine domain of the huntingtin gene (htt), whereas greater than 37 glutamines results in HD. Polyglutamine expansion is a common mutation in at least 8 other neurodegenerative disorders including spinocerebellar ataxia 1 (SCA1), and spinal and bulbar muscular atrophy (SBMA) (Koshy et al., 1997; Shahbazian et al., 2001; Zoghbi et al., 2000). The polyglutamine expanded proteins that cause each of these diseases are unique to their individual disease, and have no known relationship to

each other, other than the region of polyglutamine expansion. All of these diseases result in selective neurodegeneration.

The presence of polyglutamine expansion is thought to cause a deleterious gain of function in each of the polyglutamine disorders, which then directly causes increased neurotoxicity. For example, normal huntingtin protein (Htt) is required for embryonic development (Zeitlin et al., 1995). This toxicity can be rescued by expression of mutant Htt (Hodgson et al., 1996). Additionally, when wild-type htt is over-expressed *in vitro*, cellular toxicity in response to various stimuli is reduced (Ho et al., 2001; Leavitt et al., 2001). Htt therefore appears to be an essential protein, and the polyglutamine expansion does not appear to inactivate it (Wellington et al., 1997; Zoghbi et al., 2000).

Age of onset and severity of HD is inversely proportional to the length of the polyglutamine expansion, with most affected people displaying symptoms in their 30's or 40's. However, persons with very long polyglutamine repeats suffer early onset HD, even before the age of 20 (Koshy et al., 1997). People with HD exhibit symptoms of chorea, or uncontrollable movements such as grimacing, flexing and unflexing of the fingers, and movement of the shoulders (Zoghbi et al., 2000). Additional symptoms include slowness of voluntary movements and speech known as bradykinesia (Zoghbi et al., 2000). The reflex responses in HD patients are not affected. Late stage patients have difficulty with swallowing and become dystonic, rigid, and bedridden. In some patients, there are also psychiatric symptoms, including schizophrenia, mood disorders, and cognitive difficulties (Ho et al., 2001; Naarding et al., 2001). These data indicate that mutant Htt cause multiple neurophysiological disturbances.

Neurodegeneration in HD

The selective neurodegeneration seen in HD affects mostly the corpus striatum (Vonsattel et al., 1985). The corpus striatum consists of 3 regions of the brain, the caudate nucleus, the putamen, and the globus pallidus (Vonsattel et al., 1985). The neuropathology of HD is distinctive, highlighted by selective loss of striatal neurons, mostly in the caudate and putamen (Vonsattel et al., 1985). The loss of these neurons results in atrophy of the striatum. Other regions of the basal ganglia, such as the substantia nigra (SN), are reduced in size due to loss of striatal projections. Neurons from the striatum project processes into other parts of the brain including the SN, to regulate signals from the SN. Loss of these projections results in loss of SN volume. Some cortical and cerebellar atrophy have also been reported (Vonsattel et al., 1985). The majority of neurons lost in HD are medium spiny neurons, which are gamma -amino butyric acid (GABA) and glutamatergic neurons. The NADPH (nicotinamide adenine dinucleotide phosphate) diaphorase neurons are relatively spared. Upon autopsy, HD can be classified pathologically in Grades 0-4, based upon the severity of caudate atrophy (Vonsattel et al., 1985). Grade 0 brains have no appreciable neuron loss, whereas Grade 3 or Grade 4 brains have pronounced atrophy and neuron loss and exhibit increased astrogliosis, or increased growth of reactive astrocytes. The classification of neuropathology generally correlates with progression of the disease. However, some Grade 0 patients exhibit clinical symptoms of HD (Vonsattel et al., 1985). This suggests that HD symptoms occur previous to, or occur independently of, widespread neural death. This could suggest that neurons may be dysfunctional before

they die, and that loss of neurons is a byproduct of the disease, and not the cause of the disease phenotype.

Htt associates with itself as well as with other proteins, forming intracellular aggregates (IA's). IA's have been observed in cell nuclei and cytoplasm of postmortem human HD brains (Becher et al., 1998; Turmaine et al., 2000). Amino (N-) terminal fragments of expanded *htt* have been observed to aggregate in the nuclei and axons of post-mortem HD brain (DiFiglia et al., 1997). In fact, only N-terminal fragments of *htt* have been observed in both nuclear and cytoplasmic IA's (Cummings et al., 2000). Protein aggregates are much more common in dendrites than in the nucleus, and are more common in the cortex than in the striatum in HD brain (Gutkunst et al., 1999). These observations have been corroborated by studies in mice transgenic for full length human *htt* or N-terminal fragments of *htt* (Hodgson et al., 1999; Mangiarini et al., 1996) as well as in cell lines transfected with *htt* constructs (Cooper et al., 1998; Steffan et al., 2000; Wang et al., 1999). Studies incorporating polyglutamine fused to Green Fluorescent Protein (GFP) have also shown that pathological lengths of polyglutamine are sufficient to induce aggregate formation (Ding et al., 2002; Moulder et al., 1999). These data have been corroborated in HD "knock-in" models where the full length mutant huntingtin is under the control of the native *htt* promoter (Li et al., 2001). In these mice, aggregates in the axons of primary striatal neurons transfected with N-terminal fragment of *htt* were found to inhibit axonal transport. This inhibition took place prior to neuronal death, suggesting that aggregates inhibit neuronal function, which may eventually lead to death. In several models of polyglutamine disease, IA's have been found to contain several different proteins. These include ubiquitin, transcription factors

such as CREB (cyclic AMP response element binding protein) Binding Protein (CBP) (Nucifora et al., 2001; Steffan et al., 2000) and p53 (Suhr et al., 2001) , as well as a p53 regulator, Mouse Double-Minute-Protein 2 (Mdm2) (Suhr et al., 2001).

The contribution of aggregates to neurotoxicity is unclear. One possibility is that the nuclear aggregates are the toxic component of HD. This hypothesis is attractive in that transcription factors have been found in protein aggregates (Li et al., 2002; Nucifora et al., 2001; Suhr et al., 2001; Yu et al., 2002), and dysregulation of CBP mediated transcription has been found in mouse and cellular models of HD (Igarashi et al., 2003; Wytttenbach et al., 2001). CBP binds to CREB, which binds to the cyclic AMP response element (CRE) and mediates many pathways necessary for neuronal homeostasis (Freeland et al., 2001; Jin et al., 2001). Disruption of CREB signaling has been shown to lead to striatal neurodegeneration reminiscent of HD (Mantamadiotis et al., 2002) . Taken together, these data indicate that loss of transcription plays direct role in HD associated neurodegeneration. Some of the genes mediated by CBP include genes involved in growth signaling, such as retinoid receptor genes, and alterations in these pathways could be responsible for neuritic dystrophy observed in both mouse and human HD. Some studies have suggested that CBP, which also contains a polyglutamine repeat domain, is sequestered by protein aggregates, and that this depletes available nuclear CBP in HD and other polyglutamine disorders (McCampbell et al., 2000; Nucifora et al., 2001). However, other studies have found that CBP as well as other transcription factors , such as SP1 (Li et al., 2002), bind to soluble *htt*, and that CBP is not depleted by *htt* aggregates (Yu et al.,

2002). Alternatively, aggregates in the neurites may be toxic. They could interfere with intracellular transport, such as in the model described above (Li et al., 2001).

Oxidative Stress in HD

Oxidative stress has been implicated in the neural dysfunction and death observed in neurodegenerative conditions such as Alzheimer's disease (AD) (Butterfield et al., 2002; Butterfield et al., 2002; Markesbery, 1997), Parkinson's disease (PD) (Blum et al., 1997), as well as HD (Browne et al., 1999). Oxidative stress has also been observed to be a major component of neural dysfunction and death following ischemic stroke and traumatic brain injury (TBI) (Kaya et al., 1999; Martin et al., 2003; Mattson et al., 2001). Cells normally produce free radicals as byproducts of aerobic respiration and other metabolic processes (Grunewald et al., 1999). These free radicals include reactive oxygen species (ROS). ROS are highly reactive oxidants and can have deleterious effects on cellular lipids, proteins, and DNA (Heales et al., 2002). Cells normally have enzymes and coenzymes that act as antioxidants (Keller et al., 1998; Reiter et al., 2002). These are able to "neutralize" ROS and prevent them from causing damage (Heales et al., 2002). Oxidative stress occurs when the cell can no longer mitigate the effects of ROS, resulting in pathology that can include oxidized DNA, lipids, and proteins (Atlante et al., 2001; Mecocci et al., 1999). ROS include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO), and hydroxyl radical ($HO\cdot$) (Keller et al., 2001). The primary site of the production of these free radicals is the mitochondrion (Fleury et al., 2002). Superoxide is produced within the mitochondrion at complexes I, II, III, and IV of the electron transport chain (Klein et al., 2003). A single

electron is transferred from the electron transport chain at these sites to molecular oxygen. The reaction of superoxide with H^+ catalyzed by superoxide dismutase (SOD) produces H_2O_2 (Keller et al., 2001; Klein et al., 2003). H_2O_2 reacts with the transition metals Cu^+ or Fe^{2+} via the Fenton reaction to produce the hydroxyl radical . NO is produced by nitric oxide synthase (NOS), which catalyzes the conversion of arginine to citrulline and NO (Deckel, 2001). NO can react with superoxide to produce the peroxynitrite free radical (Deckel et al., 2001).

The effects of ROS on neuronal homeostasis have been extensively studied (Browne et al., 1999; Jenner, 2003; Markesbery, 1997). ROS generation can result from excitotoxicity, perturbances in calcium homeostasis, or disruptions to oxygen and glucose levels during ischemia (e.g. stroke). Elevations in intracellular calcium, such as that observed in glutamate excitotoxicity, can depolarize the mitochondrial membrane, resulting in the release of cytochrome C, which can lead to neural death (Mattson et al., 2001). Oxidative stress can also lead to DNA damage, which can lead to neural death. One marker of this DNA damage is the modified nucleotide 8-hydroxy-2-deoxyguanosine (8-OHdG), which has been shown to be produced by peroxynitrite (Markesbery, 1997).

It has been hypothesized that oxidative stress mediates neuronal dysfunction or death in HD. For example, DNA damage was detected in the form of 8-OHdG in a mouse model of HD (Bogdanov et al., 2001). Oxidative damage to mitochondrial DNA has also been observed in human HD (Polidori et al., 1999). Increased lipid peroxidation has been observed in striatum of HD transgenic mice (Perez-Severiano et al., 2000), and is correlated with a decrease in superoxide dismutase (SOD) activity and

increased NOS activity (Perez-Severiano et al., 2002). Decreased SOD activity would decrease H₂O₂ produced from excess superoxide. Increased superoxide production in concert with increased NOS activity, would result in increased peroxynitrite levels. In a chemical model of HD, treatment of rats with 3-nitropropionic acid, a complex II inhibitor, leads to striatal lesions, comparable to what is observed in HD (Brouillet et al., 1999). In these models, there is increased oxidative damage to DNA, membrane lipids, and proteins (Browne et al., 1999). Recent studies have demonstrated antioxidants promote neuroprotection, increased performance, and increased survival in animal models of HD (Feigin et al., 2002). For example, treatment with the antioxidants coenzyme Q10 and remacemide improved survival and motor performance in mouse models of HD (Ferrante et al., 2002). Additionally, an increase in brain weight attributable to decreased ventricle size was observed after treatment of mice with these compounds (Ferrante et al., 2002). Lipoic acid, a compound that naturally occurs in mitochondria, has been shown to attenuate oxidative damage, was also shown to increase mean survival in two mouse models of HD (Andreassen et al., 2001). Taken together, these data indicate a role for oxidative stress in mediating HD. and this may be alleviated by antioxidant therapy.

p53 in HD

The protein p53 has been implicated in a wide array of neurodegenerative disease and neural death due to injury (Blum et al., 1997; de la Monte et al., 1998; Duan et al., 2002; Kaya et al., 1999). It is a pleiotropic protein with many functional roles in the cell (Bargonetti et al., 2002). Originally, p53 was identified as an oncogene,

but is actually a tumor suppressor (Lane et al., 1979). It was detected as a host protein that co-precipitated with the large T antigen of SV40 when cells were transformed with that virus (Lane et al., 1979). Additionally, this protein was immunoprecipitated with large T antigen in uninfected cells (Crawford et al., 1981; DeLeo et al., 1979). After twenty plus years, it has been found that p53 mutations are present in more than half of human cancers (Oren et al., 1999). Acting as a sequence specific transcription factor, p53 activates genes involved in cell differentiation (el-Deiry, 1998; Maacke et al., 1997), apoptosis (el-Deiry, 1998), and the cell cycle (Flatt et al., 2000; Winters, 2002). Cell cycle checkpoints that are activated in response to genomic damage or other stresses are mediated by p53. For example, the transcription of p21^{WAF1/CIP}, a protein that induces a G1 cell cycle arrest in response to DNA damage or other stresses, is mediated by p53 (Balint et al., 2001; el-Deiry, 1998). The pro-apoptotic gene BAX is up regulated by p53 (Morrison et al., 2000) and is involved in release of Cytochrome C from the mitochondrion (Scorrano et al., 2003). Apoptosis protease activating factor-1 (APAF-1), another transcriptional target of p53, is activated by Cytochrome C and promotes neuronal apoptosis through activation of a caspase cascade (Fortin et al., 2001). Transcription of pro-apoptotic genes is mediated by p53 in response to excitotoxicity, trophic factor deprivation, or hypoxia, as well as in neurodevelopment (Miller et al., 2000; Morrison et al., 2000; van Lookeren Campagne et al., 1998). Not only does p53 induce pro-apoptotic genes, it also represses the promoters of genes that respond to growth signals (Fortin et al., 2001). An array of promoters involved in promoting cell growth, including interleukins 2, 4, and 6 as well as the insulin promoter, are repressed by p53.

As a transcription factor, p53 is regulated by post-translational modifications. Examples of these modifications include phosphorylation and acetylation (Gu et al., 1997; Ito et al., 2001; Vousden, 2002; Woods et al., 2001). These modifications are associated with changes in p53 activity (Ito et al., 2001), localization (Liang et al., 2001), and/or stability. There are several regulatory domains in p53, including an N-terminal transactivation domain, a nuclear localization sequence (NLS), nuclear export sequences (NES), an oligomerization domain, a sequence specific DNA binding domain, and a C-terminal regulatory domain (Balint et al., 2001; Woods et al., 2001). Modification or interference with these domains, either through post-translational modification or protein interaction, can greatly alter p53 stability, localization, and activity (Ashcroft et al., 1999; Gu et al., 1997; McLure et al., 1998). Because of its role in cell death and the cell cycle, p53 stability is tightly regulated. The half-life of p53 is normally about 15 minutes. Under normal conditions, p53 is bound by Mdm2 (Alarcon-Vargas et al., 2002). Mdm2 is an E3 ubiquitin ligase and targets p53 for proteolysis (Michael et al., 2003). Mdm2 may also sequester p53 away from the nucleus (Freedman et al., 1998). Mdm2 transcription is mediated by p53, which results in a negative autoregulatory loop (Barak et al., 1993; Oren, 2003; Vousden, 2002). However, in response to certain signals, such as DNA damage, p53 is phosphorylated on residues located within the region of Mdm2 association (Gao et al., 1999; Shieh et al., 1999). This has been suggested to attenuate the Mdm2-p53 interaction, resulting in reduced p53 proteolysis (Ashcroft et al., 1999; Shieh et al., 1999).

Acetylation occurs on several lysine residues of p53 (Gu et al., 1997; Vousden, 2002). The proteins that acetylate p53 are the histone acetyltransferases, or HAT

proteins (Prives et al., 2001). These include CREB Binding Protein (CBP), p300, and p300/CBP associated factor (P/CAF), which can also form complexes with p53 upstream of target genes (Grossman, 2001). The effect of p53 acetylation is unclear. Earlier data suggested that p53 DNA binding activity was increased as a direct result of acetylation (Gu et al., 1997). Recently though, it has been reported that p53 acetylation activates transcription through the recruitment of other coactivators or transcription factors (Barlev et al., 2001). Over expression of histone deacetylase proteins has been shown to attenuate p53 transcriptional activity, which would be consistent with both models (Juan et al., 2000). These observations are important for HD because treatment of animal models of HD with histone deacetylase (HDAC) inhibitors have been shown to ameliorate the polyglutamine-dependent neurodegeneration observed in these animals (Steffan et al., 2001). Additionally, in a cell model of SBMA, a disease caused by polyglutamine expansion within the androgen receptor gene (Zoghbi et al., 2000), polyglutamine dependent cell death was attenuated by CBP over expression (McCampbell et al., 2000). These studies were aimed at studying the role of CBP in polyglutamine disease in the context of CBP sequestration in protein aggregates. However, due to the effects of acetylation on p53 activity or stability, it is reasonable to ask whether HDAC inhibition might also affect p53 activity in models of polyglutamine disease and what effect on neurotoxicity such alterations might have.

The role of p53 in neuronal apoptosis has been studied extensively (Mattson et al., 2001). Characteristics of apoptosis include caspase activation, chromatin condensation and fragmentation, membrane blebbing, loss of cellular volume, and eventual phagocytosis (Zimmermann et al., 2001). Neural death mediated by p53 has

been implicated in a variety of disease and injury models including AD (de la Monte et al., 1997), PD (Blum et al., 1997; Duan et al., 2002), amyotrophic lateral sclerosis (ALS) (de la Monte et al., 1998), ischemia (van Lookeren Campagne et al., 1998), excitotoxic injury (Culmsee et al., 2001; Uberti et al., 1998; Uberti et al., 2000), and traumatic brain injury (Kaya et al., 1999; Napieralski et al., 1999). Studies have suggested that neural apoptosis may be dependent on p53 transactivation of a variety of genes. Some studies have used pharmacological inhibitors of p53, such as pifithrin- α , which blocks p53 transcriptional activity (Culmsee et al., 2001; Duan et al., 2002). Some of the genes implicated in p53 dependent neural apoptosis in both injury and disease models include APAF-1 (Fortin et al., 2001), BAX (Blum et al., 1997; Duan et al., 2002; Zhang et al., 2002), which code for proteins that have been implicated in oxidative stress mediated neurotoxicity.

The role of p53 in polyglutamine neurotoxicity has not been extensively studied. Several proteins were reported to aggregate with mutant Htt in intracellular inclusions including p53, Mdm2, CBP, and ubiquitin (Suhr et al., 2001). Thompson and colleagues (Steffan et al., 2000) also examined the relationship between Htt and p53 in non-neuronal cell lines that expressed *htt*. The proline rich region adjacent to the polyglutamine region of *htt* has been suspected to be involved in polyglutamine neurotoxicity (Steffan et al., 2000). *Htt* constructs were made with and without the proline rich region (Steffan et al., 2000). It was observed that *htt* and p53 coaggregate in intracellular inclusions irrespective of the proline rich region (Steffan et al., 2000). Glutathione-S-transferase (GST) pulldown experiments utilizing GST-Htt fusion proteins demonstrated a direct interaction *in vitro* between mutant Htt and p53 that was

dependent on the proline rich region of htt. However, this dependence was not observed in similar studies conducted in cell culture. Additionally, p53 mediated transcription, e.g. that of p21^{WAF/CIP}, was repressed in cells transiently transfected with a polyglutamine expanded htt construct. CBP and was also observed to be in intracellular inclusions in this study. The authors proposed a model whereby p53 mediated transcription was repressed due to the sequestration of CBP and other p53 coactivators by the inclusions. Together these data indicate direct interaction between mutant Htt and p53 and that p53 mediated transcription may be altered in HD.

Another study utilized cell lines derived from striatum of wild type or HD mice (Trettel et al., 2000). The HD mice were knock in mice where polyglutamine expanded htt was substituted for the wild type gene. Cell lines were immortalized using a temperature sensitive large T antigen from SV40 virus. One of the observations made was that cells from HD mice had a longer doubling time. Fluorescence Assisted Cell Sorting (FACS) analysis revealed that there was twice the DNA content in these cells, indicating blockage of the p53/SV40 large T antigen establishment pathway. At restrictive temperature, there was 2 fold greater large T antigen in the mutant Htt cells but 6 fold greater p53 expression compared to wild type cells, suggesting that polyglutamine expansion had a role in cellular stress that stabilized or induced p53. These data are important for HD because these studies were conducted in striatal neurons, which are the neurons most affected in HD, and demonstrated elevated p53.

Another study was conducted in inducible mouse neural cell lines stably transfected with either an expanded N-terminal htt fragment (60Q and 150Q) or nonpathological N-terminal htt fragment (16Q) (Jana et al., 2001). Analysis of protein

expression revealed more p53 in the 150Q cell line than the 16Q cell line, that was dependent on polyglutamine expansion mediated deficits in protein degradation. This increase was concomitant with increased levels of cytosolic cytochrome C, and cell death, suggesting a role for p53 in polyglutamine expansion mediated neural death.

One of the hallmarks of polyglutamine expansion disease is the loss of dendritic spines from affected neurons. Loss of dendritic spines results in a decrease in neural networking and is thought to contribute to neural dysfunction. For example, in a mouse model of SCA1, a polyglutamine expansion disease caused by expansion in the ataxin-1 protein (Zoghbi, 1995), Purkinje cells show a decrease in the number of dendritic spines (Shahbazian et al., 2001). This may be analogous to abnormal neurite development observed in differentiated cells expressing polyglutamine expansion (Li et al., 1999; Song et al., 2002; Wyttenbach et al., 2001). SCA1 mice that did not express p53 were found to have less neuropathology than SCA1 littermates that express p53 (Shahbazian et al., 2001). These mice were heterozygous for the SCA1 mutation and homozygous for p53 deletion. SCA1 mice that are homozygous for p53 deletion had no difference in cell number or in early motor performance. There was no difference in p53 expression between wildtype and SCA1 mice. These data indicate that p53 has a role in SCA1 other than mediating cell death, perhaps by suppressing pathways that mediate neurite outgrowth.

DNA damage and oxidative stress increase p53 expression and activity (Geller et al., 2001). Because both of these occur in HD (Bogdanov et al., 2001; Deckel et al., 2002; Feigin et al., 2002), it is likely they play a role in increased p53 expression. Taken together, these studies suggest that p53 is at least stabilized, if not

induced, in the presence of polyglutamine expansion. Additionally, p53 has been implicated in various models of neuronal injury and death. While the data in polyglutamine disorders indicate that p53 is stabilized, the role of p53 in the disease process is not clear. It may be made more active through other means than induction, such as modifications that make p53 more stable. The role of p53 in oxidative stress has not been fully elucidated, especially with regard to HD. Mitochondrial dysfunction has been implicated in HD and mutant Htt has been reported to directly interact with the mitochondrial membrane (Grunewald et al., 1999; Panov et al., 2002; Panov et al., 2003). Oxidative stress has been associated with p53 in neurons, and it is thought that neuron loss seen in HD may be partially mediated by oxidative stress. So it would be of interest to study p53 in context of oxidative stress and HD, and see if a role for p53 in these processes can be determined.

Neural death in HD is clearly caused by polyglutamine expansion in htt and is likely mediated in part by oxidative stress. My dissertation describes research that investigated two hypotheses. First, polyglutamine expansion and oxidative stress induce alterations in p53 expression, stability, and activity. Second, p53 plays a direct role in mediating oxidative stress toxicity, which is relevant to HD. Experiments investigating polyglutamine expansion mediated alterations to p53 are described in Chapter 2, and experiments investigating the role of p53 in oxidative stress are described in Chapter 3. The results of these experiments and how these data fit into what is known about HD, oxidative stress, and p53 are discussed in Chapter 4.

Chapter Two

Polyglutamine induced changes to p53 expression, localization, and transcriptional activity

Previous studies have suggested that p53 may have a role in polyglutamine disorders (Jana et al., 2001; Shahbazian et al., 2001; Steffan et al., 2000; Trettel et al., 2000). Mutant Htt has been shown to interact with p53 in one model of HD (Steffan et al., 2000) and the absence of p53 reduces the neuropathology seen in an SCA1 mouse model (Shahbazian et al., 2001). In this chapter, I describe research that investigated the hypothesis that polyglutamine expansion alters p53 expression, stability, and activity.

I utilized SH-SY5Y cells, a human neural cell line, that stably express a fusion of Green Fluorescent Protein (GFP) and either physiological or pathological lengths of polyglutamine (19-GFP and 56-GFP, respectively) (Ding et al., 2002). The expression of p53 mRNA and p53 protein was analyzed, and p53 stability assessed in these cells. The intracellular localization of p53 was visualized using immunocytochemical techniques. Additionally, post-translational modifications of p53 were noted, specifically, acetylation and phosphorylation. The possible involvement of Mdm2, a key protein in p53 biology, was also investigated. Mdm2 expression and localization were determined in 19-GFP and 56-GFP cells, and its interaction with p53 in these cells analyzed. In order to determine how p53 is altered *in vivo*, I conducted additional experiments. In these studies, I analyzed p53 expression in human HD brains as well

as in an HD mouse model. To determine modifications to p53 *in vivo*, I examined mouse and human brains for acetylated and phosphorylated p53.

p53 expression and stability

The expression of p53 was examined in SY5Y cells that stably express 19-GFP or 56-GFP (Figure 1). SY5Y cells express wild type p53, unlike many other tumor derived cell lines (Rodriguez-Lopez et al., 2001). SY5Y cells utilized in this study were either undifferentiated or differentiated. These cells can be differentiated with retinoic

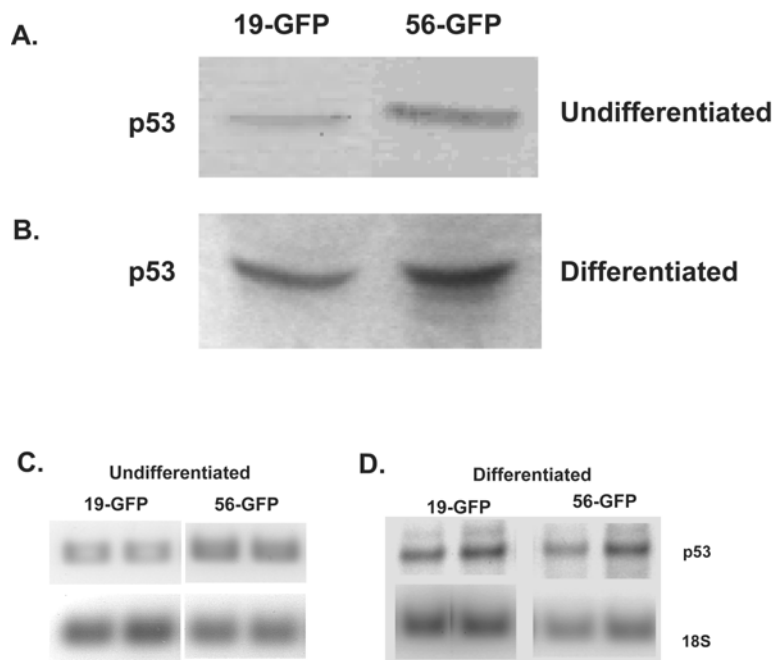


Figure 1. Expression of p53 is altered in neural cells expressing polyglutamine expansion. The expression of p53 protein was examined by Western blot analysis in undifferentiated (A) or retinoic acid differentiated (B) 19-GFP and 56-GFP expressing neural cells. p53 mRNA expression of duplicate samples was examined by RT-PCR in undifferentiated (C) or differentiated (D) 19-GFP and 56-GFP cells. p53 mRNA expression was normalized to that of 18s rRNA. Data shown are representative of results from 3 similar experiments.

acid. This induces a G1 cell cycle arrest and development of a neural phenotype (Tucholski et al., 2001). Undifferentiated 56-GFP expressing cells had approximately 120% more p53 protein expression than 19-GFP cells (Figure 1A). Differentiated 56-GFP cells had about 20 % more p53 protein expression than differentiated 19-GFP cells (Figure 1B). This data is not quantitative because there was no analysis conducted for loading. For example, the expression of actin or some other “housekeeping” protein could have been analyzed to control for loading. However, I conducted these experiments several times, and the increase in p53 expression was always observed in 56-GFP cells compared to 19-GFP cells. In differentiated 19-GFP and 56-GFP expressing cells, p53 mRNA expression was similar (Figure 1D). However, p53 mRNA was elevated in undifferentiated 56-GFP cells compared to undifferentiated 19-GFP expressing cells (Figure 1C). Because neurons are in a post-mitotic state in the brain, I decided to conduct subsequent experiments with terminally differentiated cells, except where noted.

The level of p53 protein in the cell can be increased, or stabilized by either transcriptional activation of the p53 gene, or stabilization of the p53 protein (Alarcon-Vargas et al., 2002; Ashcroft et al., 1999). Since p53 mRNA was not increased in differentiated 56-GFP cells, compared to 19-GFP cells, experiments were conducted to determine if p53 protein was stabilized in these cells. Differentiated 19-GFP and 56-GFP expressing SY5Y cells, as well as differentiated wild type SY5Y cells, were incubated with cycloheximide (Figure 2).

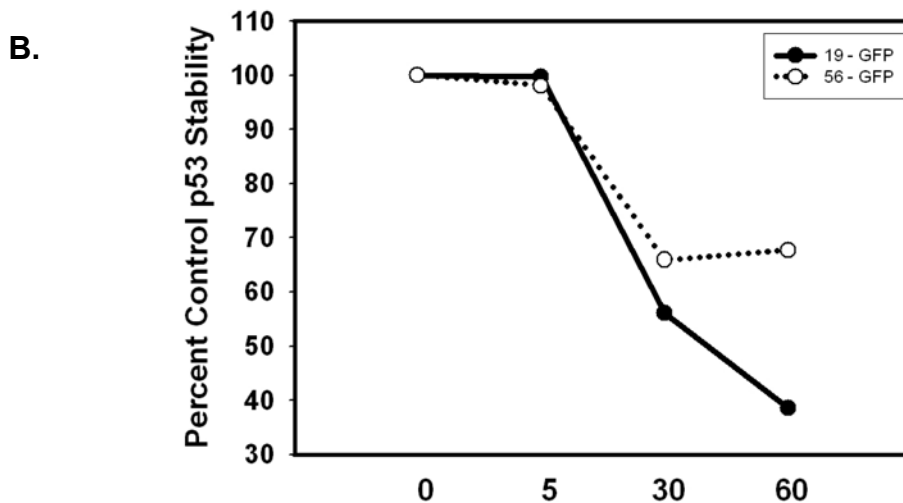
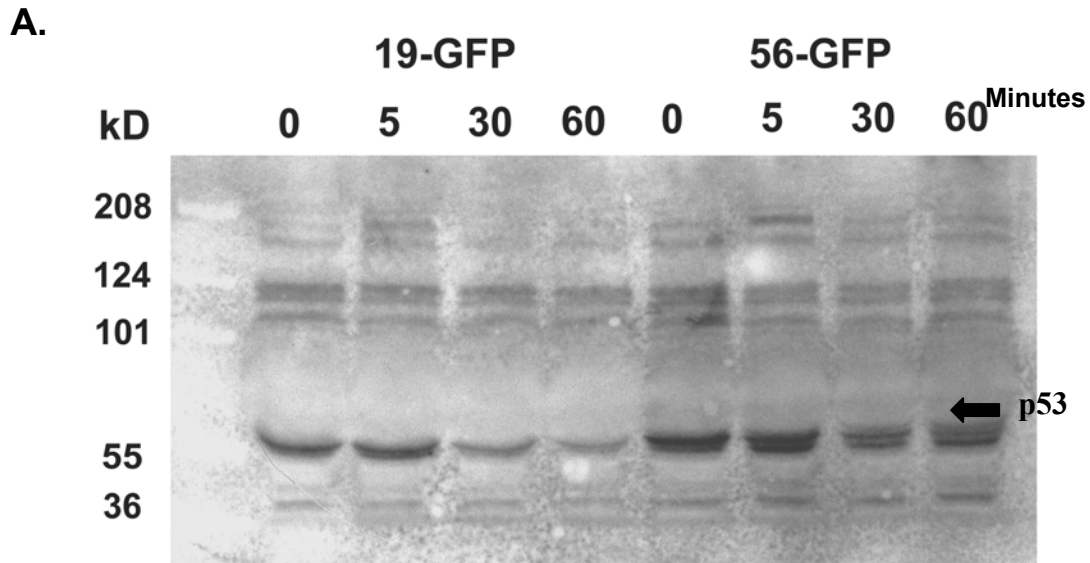


Figure 2. p53 expression is altered in neural cells that express polyglutamine expansion. Differentiated 19-GFP and 56-GFP cells were treated with 10 μ M cycloheximide. Following treatment, the cells were collected at the times indicated (**minutes, A**) and p53 expression examined in 50 μ g total protein by Western blot analysis (**A**). Note the multiple bands observed for 56-GFP cells (**arrow, A**). Bands were examined by dose densitometry and the percent control (**0 minutes**) of p53 expression for 19-GFP and 56-GFP calculated (**B**). The blot (**A**) and graph (**B**) are representative of 2 experiments.

Cycloheximide inhibits the translocase step of translation (Obrig et al., 1971), thereby halting the synthesis of new protein. Lysates of 19-GFP and 56-GFP cells collected at various times after treatment with cycloheximide were analyzed for p53 protein expression. The level of p53 decreased faster in 19-GFP (Figure 2A, left panel) expressing SY5Y cells than in 56-GFP cells (Figure 2A, right panel). Levels of p53 expression in both 19-GFP and 56-GFP cells were quantified by densitometry and plotted as percent control for each cell line (Figure 2B). At 60 minutes post-treatment, in 56-GFP cells there was 68% of the p53 level observed in control. However, in 19-GFP cells after 60 minutes cycloheximide treatment, there was 38 % of p53 observed in control cells. These data suggest that polyglutamine expansion somehow stabilizes p53 protein, and that this accounts the increased preservation of p53. In 56-GFP cells, a triple band was observed (arrow, Figure 2A). The lower bands are consistent with p53 that has been phosphorylated. Phosphorylation of p53 causes an electrophoretic shift, and data supplied by the manufacturer of an antibody to phosphorylated p53 that I used reported that phosphorylated p53 runs at 47kD. It was also noted that with increasing time after cycloheximide treatment, there appeared to be a redistribution of p53 amongst the different bands (Figure 2B). Specifically, the lowest band increased in intensity and the upper bands decreased in intensity. This could indicate that with increasing time following cycloheximide treatment, stable p53 is modified. Phosphorylation of p53 has been associated with increased stability of the protein, so this could explain the redistribution observed in Figure 2A (Shieh et al., 1997).

One protein that has a role in the normal turnover of p53 is Mdm2 (Alarcon-Vargas et al., 2002; Ashcroft et al., 1999; Kubbutat et al., 1998). Mdm2 functions as an E3 ubiquitin ligase and is known to mediate the proteolysis of p53. Given that p53 is stabilized in 56-GFP expressing cells compared to 19-GFP cells or wildtype, I conducted experiments to determine if Mdm2 plays a role in p53 stability. Mdm2 expression was increased in 56-GFP cells compared to 19-GFP cells (Figure 3). Increased Mdm2 typically leads to increased turnover of p53

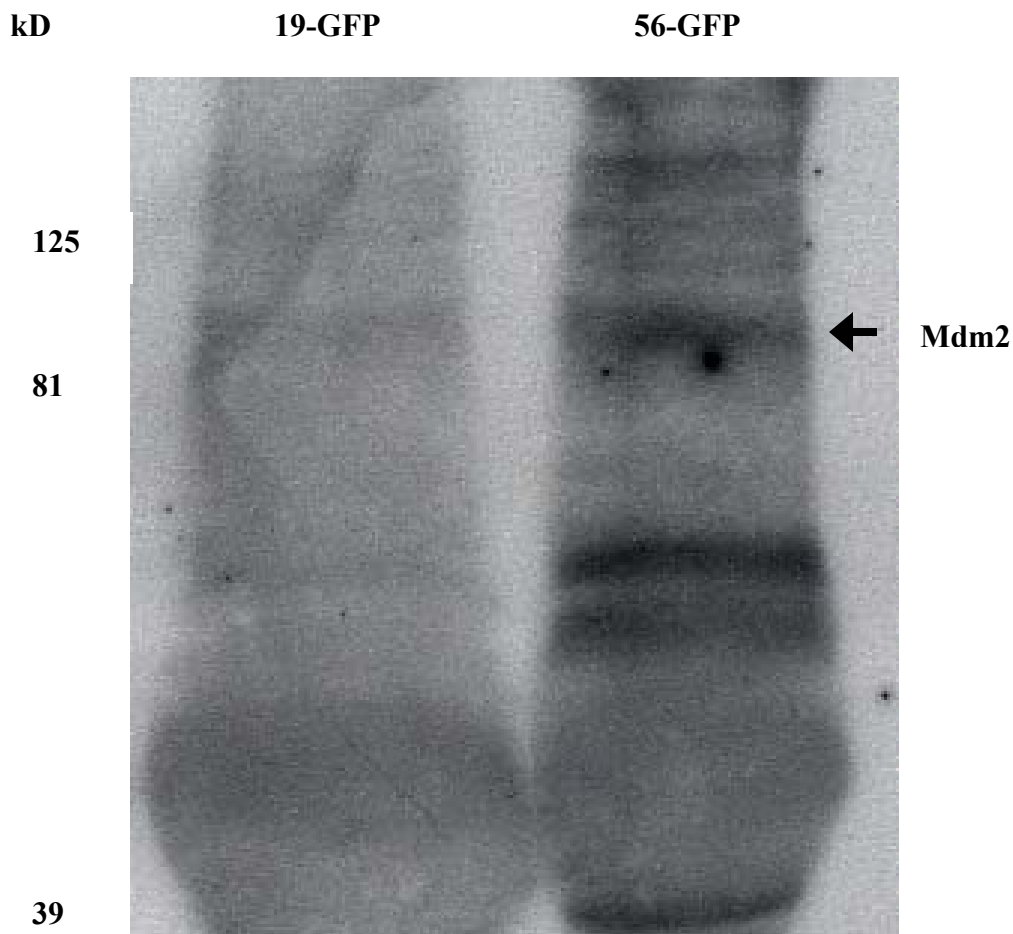


Figure 3. Mdm2 expression is altered in neural cells expressing polyglutamine expansion. Mdm2 expression was analyzed in a total of 50 μ g protein from differentiated 19-GFP or 56-GFP cells. Blot is representative of 2 similar experiments.

(Freedman et al., 1998; Kobet et al., 2000; Kubbutat et al., 1998). These data taken together with the data in Figure 2 suggest that p53 is stabilized in 56-GFP expressing cells in the presence of increased levels of MDM2 compared to 19-GFP expressing cells.

Nuclear localization is required for p53 transcriptional activity (Vousden, 2002). Additionally, Mdm2 localization in the nucleus has a role in p53 stability (Freedman et al., 1998; Kobet et al., 2000; Kubbutat et al., 1998). Because of these factors, I next analyzed the localization of p53 in human neural cells expressing 19-GFP or 56-GFP. In 19-GFP expressing cells, p53 was barely detectable, and localized throughout the cell (Figure 4A). In 56-GFP, there was much higher p53 expression, but p53 was chiefly localized to the nucleus (Figure 4B). The majority of Mdm2 was localized to the nucleus as well in 19-GFP cells (Figure 4C). There was consistently more p53 immunoreactivity in 56-GFP cells compared to 19-GFP cells. In 56-GFP cells, p53 was predominantly localized to the nucleus (Figure 4D). These data, taken with the data in Figure 3, suggest that p53 coexists with increased levels of Mdm2, even though Mdm2 normally mediates the degradation of p53.

To investigate the p53-Mdm2 interaction in 19-GFP and 56-GFP cells, I conducted immunoprecipitation (IP) experiments (Figure 5). In 19-GFP cells, there was more interaction between p53 and Mdm2 than in 56-GFP cells. Specifically, the intensity of the band representing Mdm2 that was precipitated with p53 antibody in 56-GFP cells was 3.8% of the total Mdm2 protein in 56-GFP cells. Mdm2 protein precipitated in 19-GFP cells was 7.9% of the total Mdm2 in 56-GFP cells. A comparison of Mdm2 precipitated in 19-GFP cells to total Mdm2 protein in 19-GFP cells was not

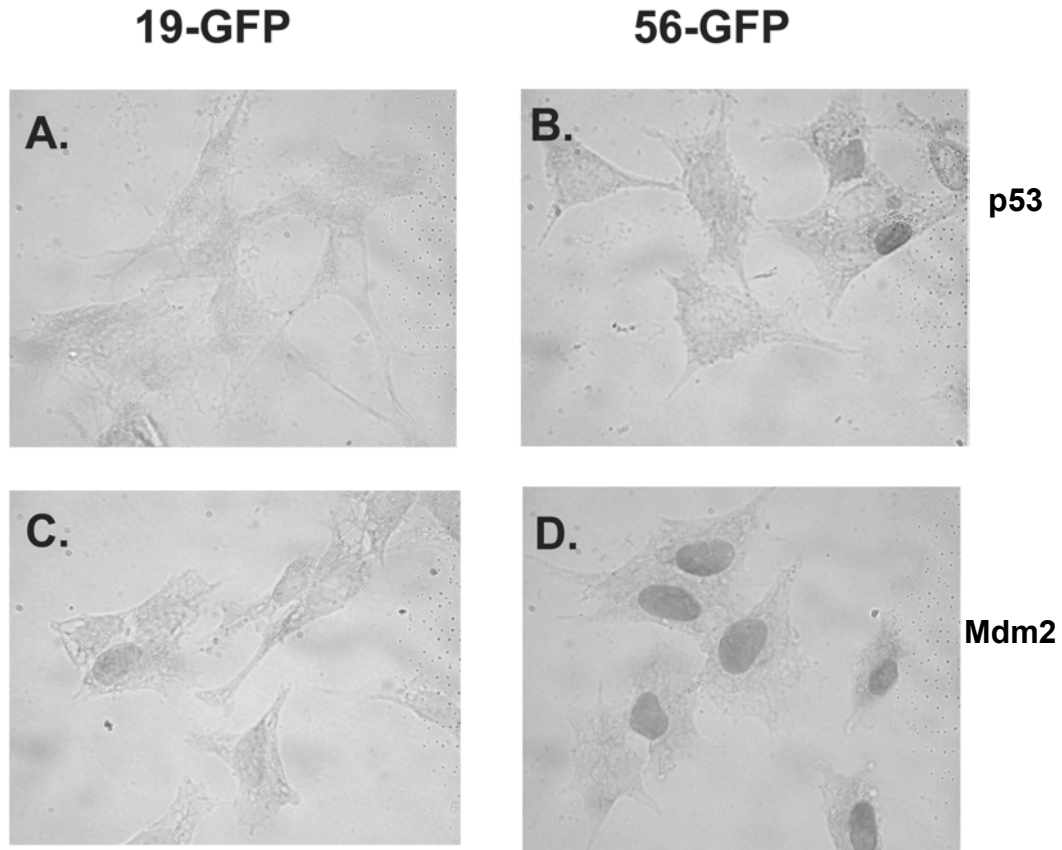


Figure 4. Polyglutamine expansion alters expression of p53 and Mdm2 in human neural cells. The expression of p53 (A, B) and Mdm2 (C,D) were analyzed by immunocytochemistry in undifferentiated 19-GFP (A,C) and 56-GFP (C,D) expressing SY5Y cells under basal conditions. Images are representative of results from at least 3 similar experiments. Total magnification was 400X.

made. Another measurement that would have been useful but was not conducted would be to determine the ratio of Mdm2 protein precipitated by p53 antibody to p53 protein precipitated by the same antibody. These measurements would have helped determine the fraction of Mdm2 interacting with p53 in 19-GFP and 56-GFP cells. Taken together, these data suggest that there is more Mdm2 expressed in 56-GFP cells, but there is also less interaction between Mdm2 and p53 in 56-GFP cells compared to 19-GFP cells. Less interaction between p53 and Mdm2 could indicate that

there is less Mdm2 mediated proteolysis of p53 in 56-GFP cells. This would be consistent with the data that indicated that p53 was more stable in 56-GFP cells than in 19-GFP cells (Figure 2). To evaluate the role of Mdm2 in polyglutamine toxicity, I utilized antisense oligonucleotides to reduce Mdm2 expression in 19-GFP and 56-GFP cells (Figure 6A). Treatment of 56-GFP cells with antisense oligonucleotide reduced Mdm2 protein expression by approximately 40% (Figure 6A). Treatment of cells with a scrambled oligonucleotide had no effect on Mdm2 expression (Figure 6A). Inhibition of

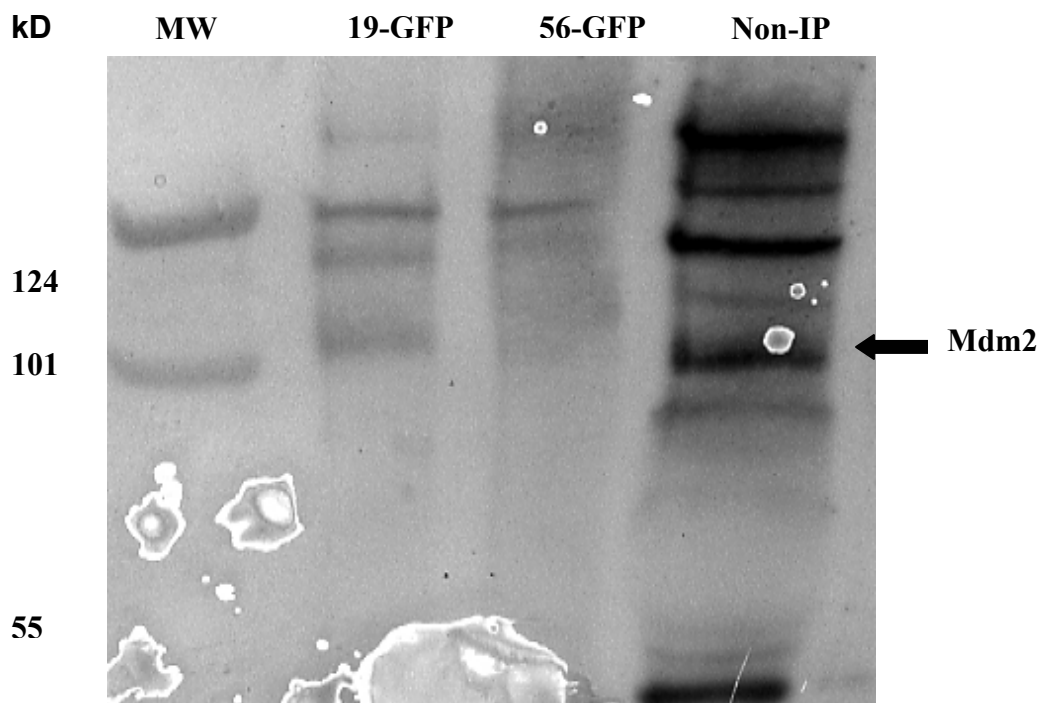


Figure 5. The interaction of p53 with Mdm2 is altered in polyglutamine expansion expressing cells. Lysates from 19-GFP and 56-GFP expressing cells (200 μ g total protein) were incubated with p53 antibody and precipitated with Protein G coated sepharose beads. Precipitated proteins were analyzed by Western blot to determine Mdm2 level. Molecular weight markers (MW) were used to identify Mdm2. To demonstrate normal expression of Mdm2, a total of 50 μ g protein from 56-GFP lysate (Non-IP) was also analyzed. Blot is representative of 2 experiments.

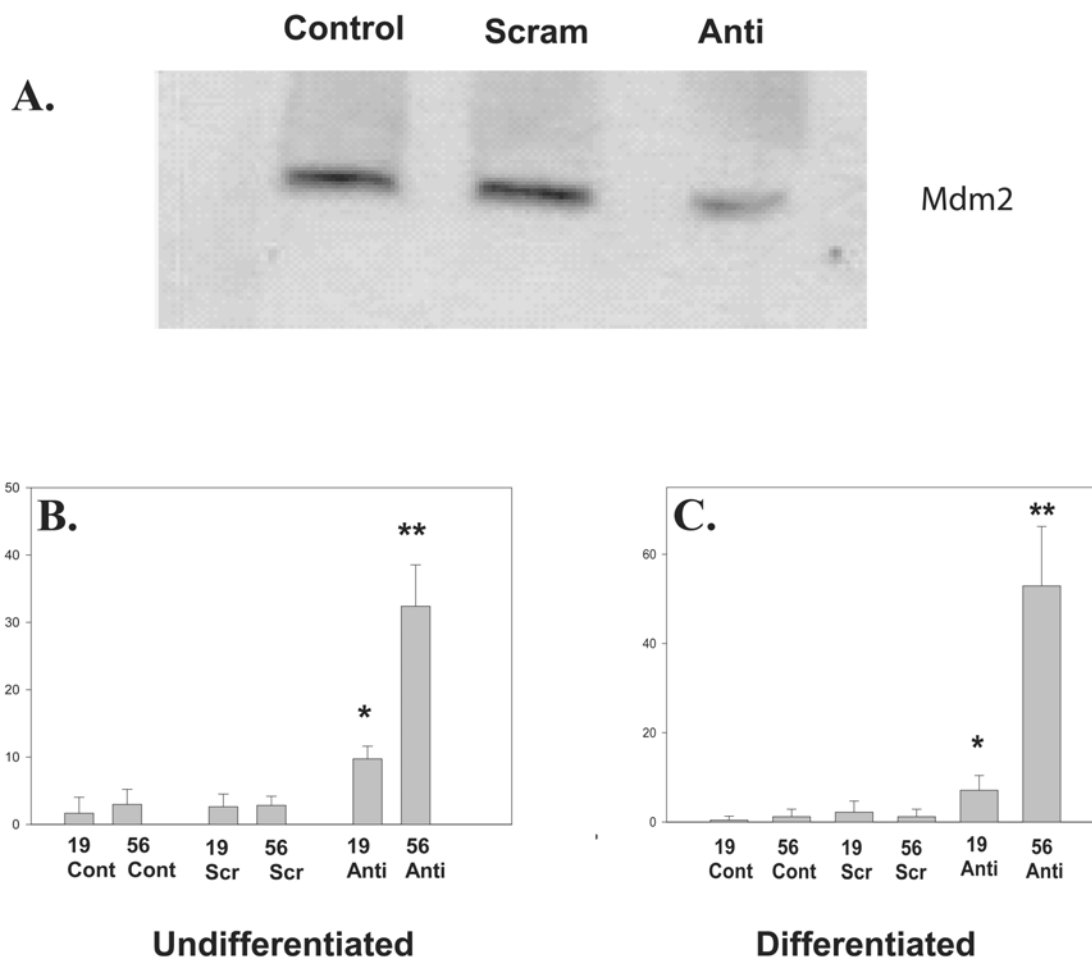


Figure 6. Decreased expression of MDM2 is sufficient to increase neural cell toxicity in differentiated and undifferentiated neural cells. (A) Cells expressing 56-GFP were analyzed for levels of MDM2 immunoreactivity 24 hours following treatment with vehicle (Cont), scrambled oligonucleotide (Scram), or MDM2 antisense (Anti) oligonucleotides. The levels of cell death were quantified in undifferentiated (B) and differentiated (C) 19-GFP (19) and 56-GFP (56) expressing cells 24 hours following the administration of vehicle (Cont), scrambled oligonucleotide (Scr), or MDM2 antisense (Anti). Data are the mean and SEM of results from at least 3 separate experiments.

* $p < 0.05$ compared with control values; ** $p < 0.05$ compared with 19-GFP expressing cells.

Mdm2 expression resulted in neural death in both differentiated and undifferentiated cells (Figure 6B, C), although the cell death was much greater in 56-GFP cells compared to 19-GFP cells. It did not appear that differentiation had an effect on sensitivity of antisense (Figure 6B, C), as 56-GFP cells with or without retinoic acid differentiation had similar amounts of death. This was also observed in 19-GFP cells (Figure 6B, C). Taken together, these data suggest that decreased Mdm2 is toxic for both 19-GFP and 56-GFP cells, but, much more toxic for 56-GFP cells. Taken together with the earlier data, this would suggest that Mdm2 plays a role in mitigating polyglutamine expansion toxicity.

p53 is modified in neural cells expressing polyglutamine expansion

Previous studies have suggested that post-translational modifications of p53 alter its interactions with other proteins, particularly Mdm2 (Ashcroft et al., 1999). Two of these modifications are phosphorylation and acetylation (Gu et al., 1997). Additionally, phosphorylation and acetylation of p53 have been suggested to alter p53 transcriptional activity (Gao et al., 1999; Gu et al., 1997; Shieh et al., 1997). To determine whether p53 was differentially acetylated or phosphorylated in the presence of polyglutamine expansion, lysates from 19-GFP and 56-GFP expressing cells were examined by Western blot analysis using antibodies that recognize either acetylated or phosphorylated p53. Acetylation of p53 on Lysine 320 was observed in 56-GFP cells (Figure 7A), but little or no acetylated p53 was observed in 19-GFP cells (Figure 7A). Phosphorylation of p53 on Serine 15 was also detected in 56-GFP cells, with little or none detected in 19-GFP cells (Figure 7B). However, there was also less p53 in 19-

GFP cells compared to 56-GFP cells (Figure 1). This could account for the decreased modified p53 observed in 19-GFP cells.

Genetic Inhibition of p53 activity attenuates polyglutamine neurotoxicity

To address whether polyglutamine expansion mediated changes to p53 activity alter neural homeostasis, I utilized another neural cell line (PC12) that stably expresses a p53 temperature sensitive mutant (*ts* p53 cells) that is deficient in DNA binding (Hughes et al., 2000). Mutant p53 is overexpressed at the restrictive temperature (39°C). PC12 cells expressing *ts* p53 were transiently transfected with 56-GFP DNA (Ding et al., 2002). As a control, cells that stably expressed only empty retrovector

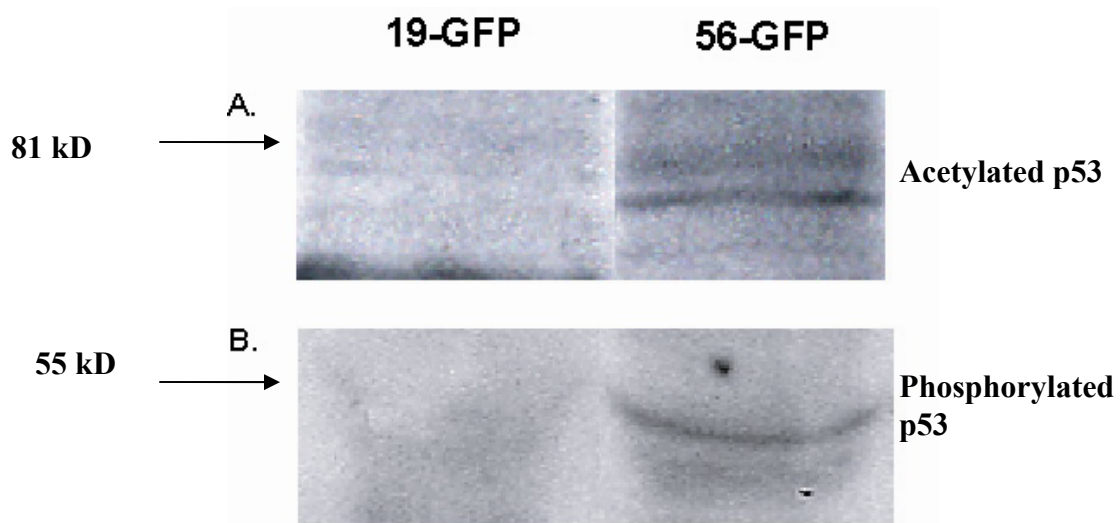


Figure 7. p53 is modified in cells expressing polyglutamine expansion. Lysates from differentiated 19-GFP and 56-GFP cells were analyzed by Western blot for expression of acetylated (**A**) and phosphorylated (**B**) p53. Acetylated p53 was detected with an antibody that recognizes p53 acetylated on lysine 320. Phosphorylated p53 was detected with an antibody that recognizes only p53 phosphorylated on serine 15. Blots are representative of results from at least 3 similar experiments.

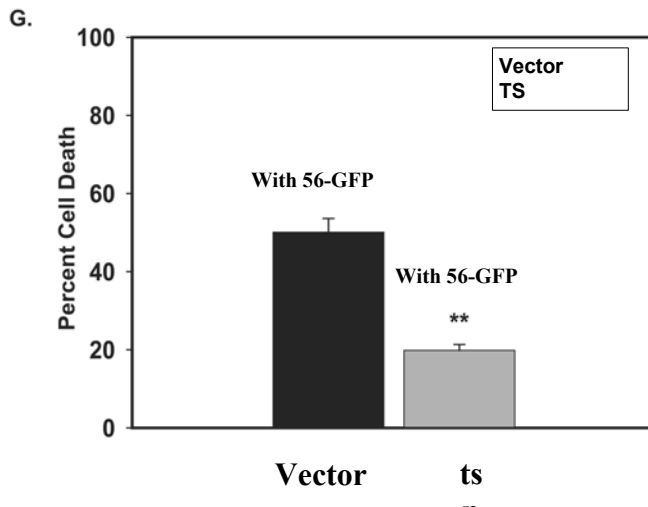
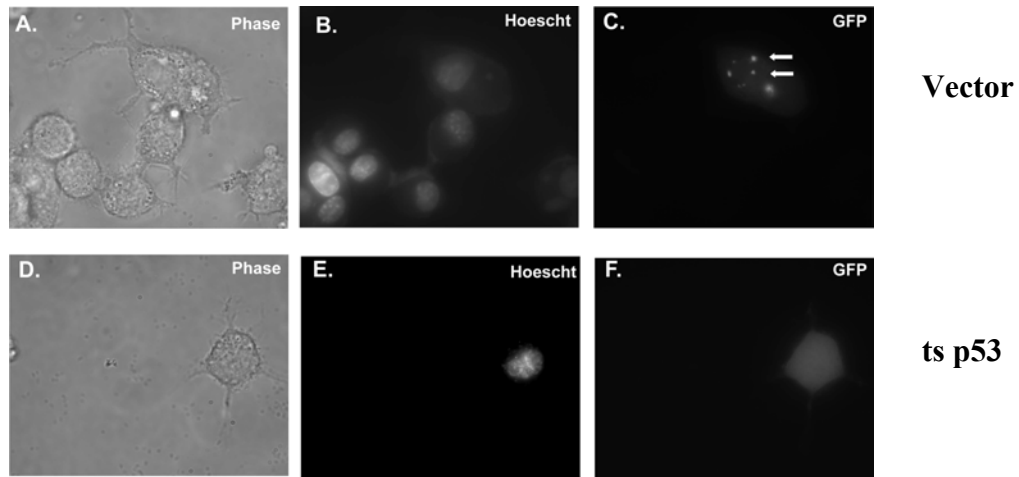


Figure 8. p53 DNA binding activity is required for polyglutamine expansion induced neural death. PC12 neural cells were stably transfected with either empty retrovector (**vector cells, A-C**) or a p53 temperature sensitive (ts p53) mutant deficient in DNA binding (**ts cells, D-E**). Both cell lines were transiently transfected with plasmid DNA encoding 56-GFP. After incubation at the restrictive temperature for 48 hours, cell morphology and death were examined. PC12 cells were visualized under phase contrast optics (**A,D**). Cell nuclei were visualized using Hoescht 33344, a dye that binds DNA. Expression of 56-GFP was visualized using fluorescence optics (**C,F**). Note the aggregates in vector cells (**C, arrow**). PC12 cells, stably expressing empty retrovector (●) or ts p53 (●), were transiently transfected with 56-GFP. Cell death was assessed by visualization of cell nuclear morphology with Hoescht stain (**G**). Data represents the percentage of cells with fragmented or condensed nuclei. Error bars represent the S.E.M. of 6 cultures. Data shown is representative of 3 similar experiments. Error bars represent S.E.M for at least 5 cultures. ** $p < 0.001$ compared to vector cells

(vector cells) were also used. Some cultures were also mock transfected. These cultures underwent the same transfection procedure without the addition of 56-GFP DNA. After 48 hours of incubation at the restrictive temperature, expression of 56-GFP was observed in both vector and *ts* p53 cell lines (Figure 8C and 8F). Protein aggregates containing 56-GFP were observed in both vector (Figure 8C) and *ts* p53 cells (data not shown). Diffuse expression of 56-GFP was also seen in vector cells (data not shown) and *ts* p53 cells (Figure 8F). At that time, the cells were assayed for death by visualization of their nuclei with Hoescht stain (Figure 8B and 8E). Cells were counted as dead if they had fragmented or condensed chromatin which are characteristics of apoptotic death (Zimmermann et al., 2001). Cells that contained the empty vector had increased nuclear condensation and/or fragmentation following transfection with 56-GFP DNA (Figure 8G). In contrast, cells that express the p53 mutant had much less cell death (Figure 8G). The amount of cell death seen in p53 mutant cells was comparable to that seen in cells that were mock transfected (data not shown). Neural death was alleviated in cells over expressing *ts* p53. Taken together, these data suggest a role for p53 transcriptional activity in polyglutamine neurotoxicity.

p53 in HD mice and human HD

The *in vitro* studies suggest that in neural cells p53 is altered in the presence of polyglutamine expansion, and that this has implications for neural homeostasis. When p53 is unable to bind to DNA, polyglutamine neurotoxicity is attenuated as observed in the *ts* p53 expressing PC12 cells. These studies were conducted in rodent cell lines. The constructs used were fusions of

polyglutamine and GFP. Polyglutamine fusions with innocuous proteins have been shown to induce characteristics similar to those seen in polyglutamine disorders (Marsh et al., 2000; Ordway et al., 1997). However, in order to examine alterations to p53 expression in the context of HD, I sought to compare and contrast p53 expression and modification *in vivo*. The expression of p53 was determined in an HD mouse model (R6/2) as well as in human HD brain. Additionally, acetylated and phosphorylated p53 were examined in HD mice and human HD brains. R6/2 mice are transgenic for an N-terminal fragment of human htt containing the region of polyglutamine expansion (Mangiarini et al., 1996). These mice are pre-symptomatic before approximately 8 weeks of age, after which they develop a progressive neurological phenotype (Mangiarini et al., 1996). These mice develop a resting tremor and seizure activity has been observed, evidence that there are neurophysiological abnormalities. When suspended by their tail, R6/2 mice clasp their back feet whereas a wild type mouse will splay his limbs to steady itself. They do not exhibit the neurodegeneration specific to the striatum as do human HD patients, but brain size is decreased approximately 20% compared to age-matched controls. Protein aggregates reminiscent of those seen in HD are also found in R6/2 brains (Davies et al., 1997). Late in life (17 weeks), R6/2 mice develop neurodegeneration of the cerebellum, cortex, and striatum. There is condensation of the cytoplasm and nucleus, but no DNA fragmentation, which would be characteristic of nucleosomal cleavage, a marker for apoptotic death (Hickey et al., 2003; Turmaine et al., 2000).

The expression of p53 and modified p53 were examined in R6/2 mice. There appeared to be decreased p53 expression in 4 week old HD mice compared to control

mice (Figure 9A, left panel). There also appeared to be less p53 protein in the brains of 10 week old HD mice compared to control (Figure 9A, right panel). There appeared to be more phosphorylated p53 in 4 week old control mice compared to HD mice (Figure 9B, left panel). There was also more phosphorylated p53 in 10 week old control mice than in 10 week old HD mice (Figure 9B, right panel). It was unclear if there was a change in phosphorylated p53 with age in either control or HD mice. In pre-symptomatic HD mice, there was slightly less acetylated p53 compared to control mice, though there was variability from animal to animal (Figure 9C, left panel). There was no difference in acetylated p53 levels between HD and control mice of either age (Figure 9C, right panel), and it appears that acetylated p53 is increased with age in both HD and wild type mice. Acetylated and phosphorylated p53 are associated with increased p53 transcriptional activity. It is unclear whether decreased level of phosphorylated p53 in HD mice is due to reduced p53 expression in these mice, or whether reduced p53 is due to reduced phosphorylation of p53. Taken together, these data suggest that p53 and phosphorylated p53 are more prevalent in control mice than in R6/2 mice, and that acetylated p53 is unchanged in symptomatic HD mice compared to control. However, expression levels were not normalized to an innocuous protein such as actin or B-tubulin to account for loading differences in each lane, so it is difficult to determine how much difference there is for each p53 species between HD and control mice.

The expression of p53 was also examined in human HD and control brains (Figure 10). The brains obtained from HD patients exhibited Grade 4 pathology, and the post-mortem interval was approximately 18 hours. Brains with this pathology exhibit

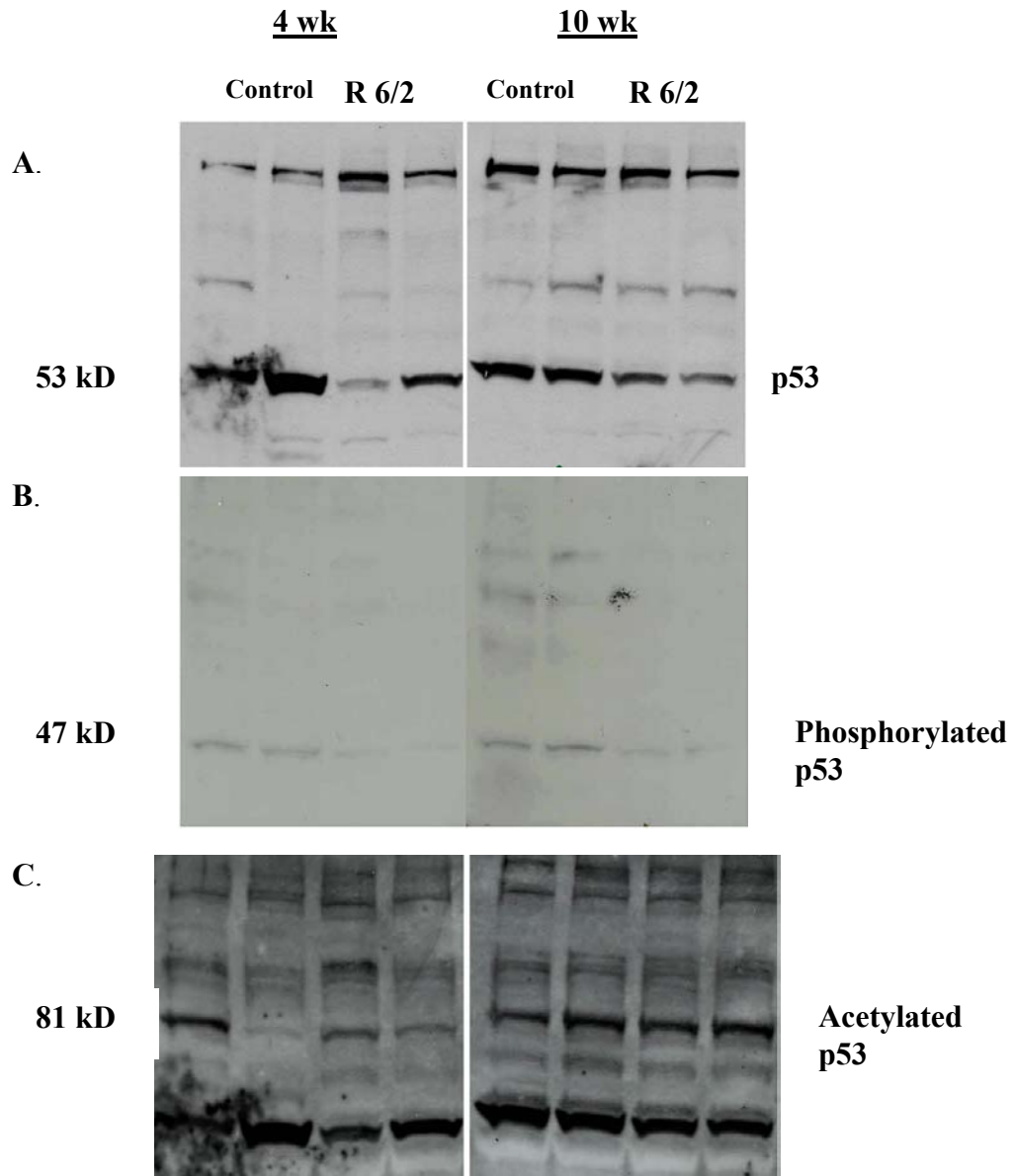


Figure 9. Expression of p53 and post-translational modifications to p53 are altered in HD mice. Lysates prepared from the cortices of either presymptomatic (4 week) or symptomatic (10 week) HD (R 6/2) or control mice were examined by Western blot analysis for **(A)** p53 **(B)** phosphorylated p53 and **(C)** acetylated p53. For **(C)**, the blot in A was stripped and then reprobbed with acetylated p53 antibody. Data shown represent 2 animals for each timepoint. Blot is representative of 2 experiments.

severe loss of neurons in the caudate and pronounced astrogliosis (Vonsattel et al., 1985). The expression of p53 protein was detected in control brain, though the level varied from patient to patient (Figure 10A, left panel). Little or no p53 was detected in HD brains (Figure 10A, right panel). Phosphorylated p53 was detected in control brains, though levels varied from patient to patient (Figure 10B, left panel). Brains from HD patients all expressed phosphorylated p53, though the level varied from patient to patient (Figure 10B, right panel). All brains examined expressed acetylated p53, though there was consistently less in HD patients compared to control (Figure 10C). The level of acetylated p53 varied from patient to patient in both control and HD patients. Taken together, these data indicate a different pattern of expression in human HD than the *in vitro* studies conducted using polyglutamine-GFP constructs. With increasing polyglutamine expansion (HD patients), there was less p53 expression compared to control brains. These data are in contrast to *in vitro* studies, where more p53 expression was observed in 56-GFP expressing cells than in 19-GFP expressing cells (Figure 1A and 1B)

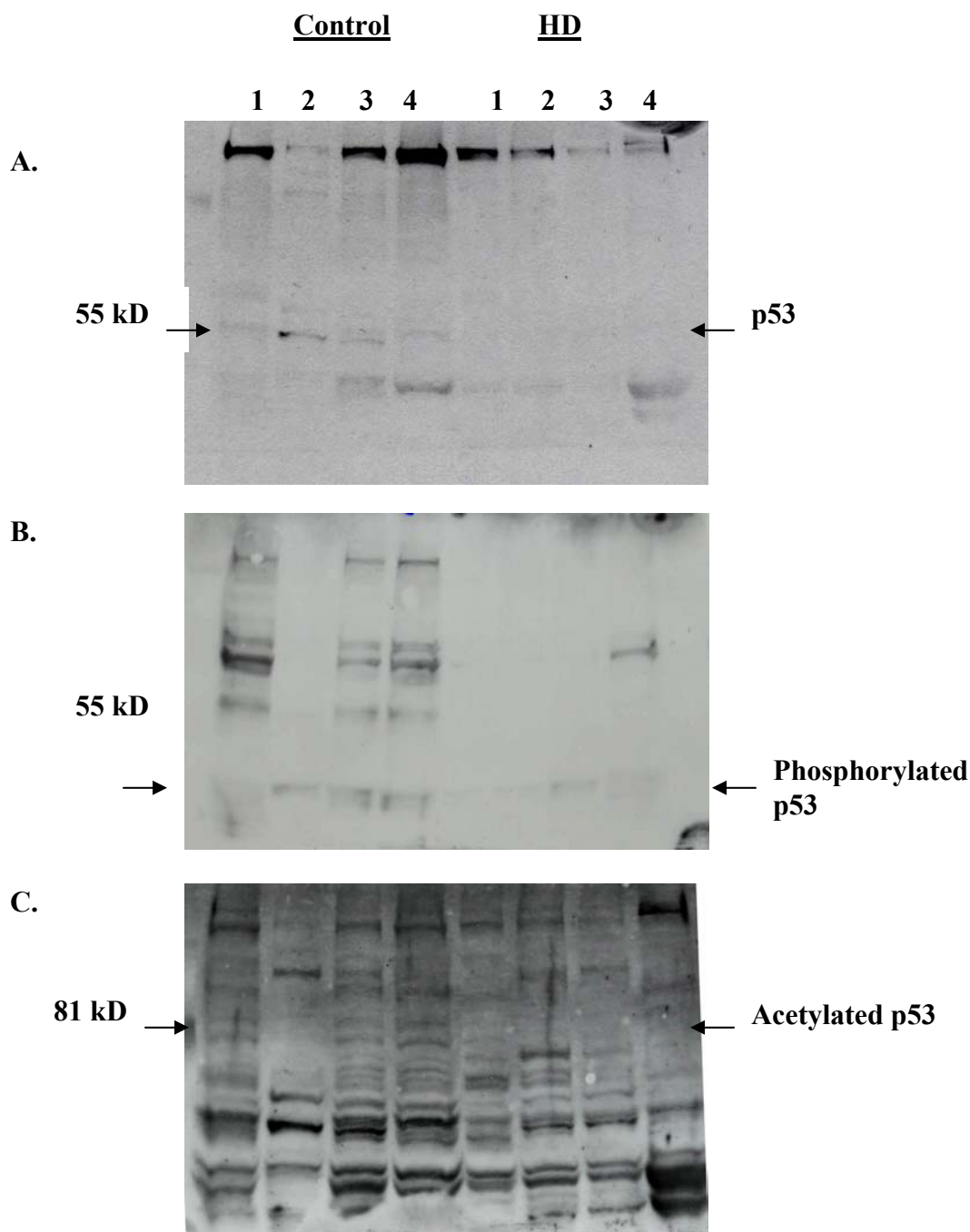


Figure 10. The expression of p53 is altered in HD Brain. Samples from the caudate of 4 control and 4 HD human brains were examined for p53 expression. Western blot analysis was used to determine levels of p53 (**A**), phosphorylated p53 (**B**), and acetylated p53 (**C**). The blot in C was stripped and reprobed with p53 antibody to give the blot seen in A. Blots are representative of 2 separate experiments.

Chapter Three

Oxidative Stress Mediated Alterations to p53 Expression and Transcriptional

Activity

Oxidative stress has been implicated in HD from several lines of evidence (Andreassen et al., 2001; Perez-Severiano et al., 2002; Polidori et al., 1999; Schilling et al., 2001). Whether oxidative stress is a causal factor in HD, or a secondary effect of the disease is unclear. Dysfunctions in energy metabolism and mitochondria have been suggested to lead to oxidative stress in HD (Browne et al., 1999). Oxidative stress has been noted in animal models of HD that express polyglutamine expansion (Perez-Severiano et al., 2000; Wyttenbach et al., 2002). Oxidative stress has also been implicated in animals that develop HD-like neurodegeneration following treatment with 3-nitropropionic acid and quinolinic acid (Kim et al., 2002; Takahashi, 1999). The hypothesis that oxidative stress plays a role in HD and chemical models of HD has been strengthened by studies showing that antioxidant therapy improves survival and motor skills in HD models (Beal, 2002; Ferrante et al., 2002).

In order to address the hypothesis that p53 expression and activity is altered following oxidative injury, I utilized neural cells, both primary cultures and human neural cell lines. I used primary rat embryonic neuronal cultures (embryonic day 18 or E18). These cultures are of a mixed nature, containing several types of cells, both neuronal and non-neuronal (Keller et al., 1998; Keller et al., 1999), and could perhaps better represent what is happening in the brain than immortal cell lines.

To induce oxidative stress, I treated primary cortical rat neurons with hydrogen peroxide (H_2O_2) (Figure 11). Exogenous H_2O_2 has been used to induce oxidative stress in a variety of cell types (Datta et al., 2002; Ouyang et al., 2002; Uberti et al., 1999), and causes p53 dependent apoptosis in glial cells (Datta et al., 2002). Cultures were treated with H_2O_2 for 24 hours and their nuclei visualized with Hoescht stain. Phase contrast visualization of the cells revealed dystrophic neurites in treated cells, a characteristic lacking in control cells (Figure 11A and 11C, respectively). Neurons were counted as dead if they had condensed (Figure 11D) or fragmented (Figure 11B) chromatin. Neurons were counted, and the percentage of dead neurons determined. H_2O_2 induced a dose-dependent increase in neural death (Figure 11E). There was some variability from experiment to experiment, but 10 μM and 50 μM concentrations consistently yielded significant increases in neural death (Figure 11E, 13).

To determine if p53 expression is altered in primary neurons treated with H_2O_2 , lysates of primary neuronal cultures were examined by Western blot analysis. There were time- and dose-related changes in p53 expression (Figure 12). In most experiments, there was a modest increase in p53 levels, even with 1 μM H_2O_2 (Figure 12A, B). Dose densitometry was used to measure the intensity of relevant bands. The percent of band density at each time point compared to control cultures (0 Hours) were calculated and plotted with respect to time (Figure 12B). Surprisingly, even with the more lethal doses, such as

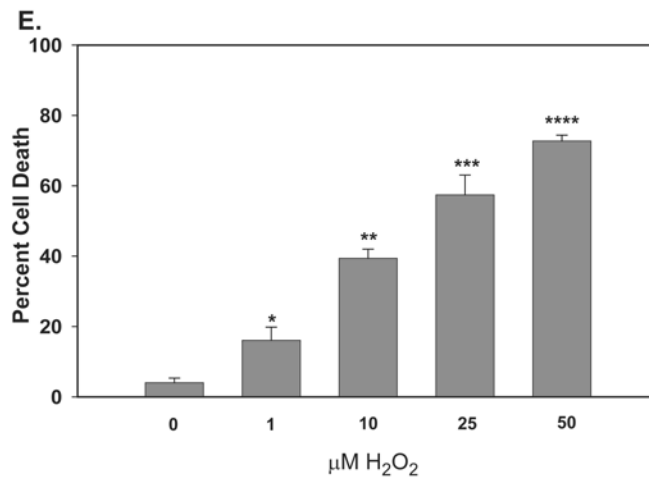
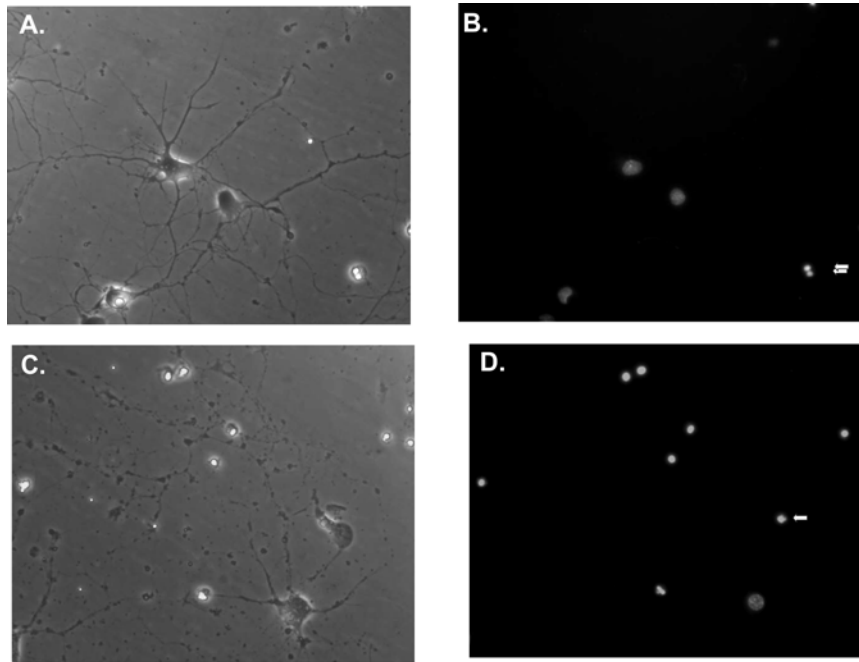


Figure 11. H₂O₂ induces dose dependent neural death. Cultured rat neurons were treated with the indicated concentrations of H₂O₂ for 24 hours. Control cells received no treatment (0 μM H₂O₂) (A,B). Control (A) and treated (C, 10 μM H₂O₂) cells were visualized under phase contrast optics to assess cell type and morphology, Cell death was assessed in control and treated cells by visualization of cell nuclear morphology with Hoescht stain (B, D). Data in (E) represents the percentage of neurons with condensed or fragmented (B, double arrow) nuclei. Error bars represent the S.E.M. of 6 cultures. Data shown is representative of 3 similar experiments. *p< 0.01 compared to control. ** p< 0.001 compared to 1μM H₂O₂. *** p<0.05 compared to 10 μM H₂O₂. **** p< 0.05 compared to 25 μM H₂O₂

50 μM H_2O_2 , there was often a return to basal p53 levels or even a reduction in p53 expression after 24 hours (Figure 12B). Overall, the data revealed that there were modest, transient increases in p53 expression in these cells, but never a very large increase, even with the higher doses.

Although p53 expression was not greatly altered, even in the presence of H_2O_2 induced cell death, p53 could still be involved with cell death if p53 transcriptional activity were increased. Transcriptional activity of p53 has been shown to be inhibited by the compound pifithrin- α (Komarov et al., 1999). In one study, pifithrin- α inhibited neuron loss following excitotoxic and genotoxic injury, as well as ischemic injury (Culmsee et al., 2001).

The mechanism for p53 inactivation by pifithrin is unclear, although nuclear accumulation of p53 is reduced by pifithrin in liver cells (Schafer et al., 2003), which presumably prevents p53 from interacting with nuclear DNA. Neurons were treated with H_2O_2 in the absence or presence of pifithrin. Pifithrin reduced the neural death induced by H_2O_2 at both concentrations tested (Figure 13). Taken together, these data suggest that oxidative stress induced by H_2O_2 induces dose dependent death in primary neurons. This is mediated, at least in part, by p53 transcriptional activity, as pharmacological inhibition of p53 attenuates neural death. It is unclear whether upregulation of p53 is responsible for neural death induced by H_2O_2 , as p53 levels were not greatly changed, or an increase in p53 activity is responsible.

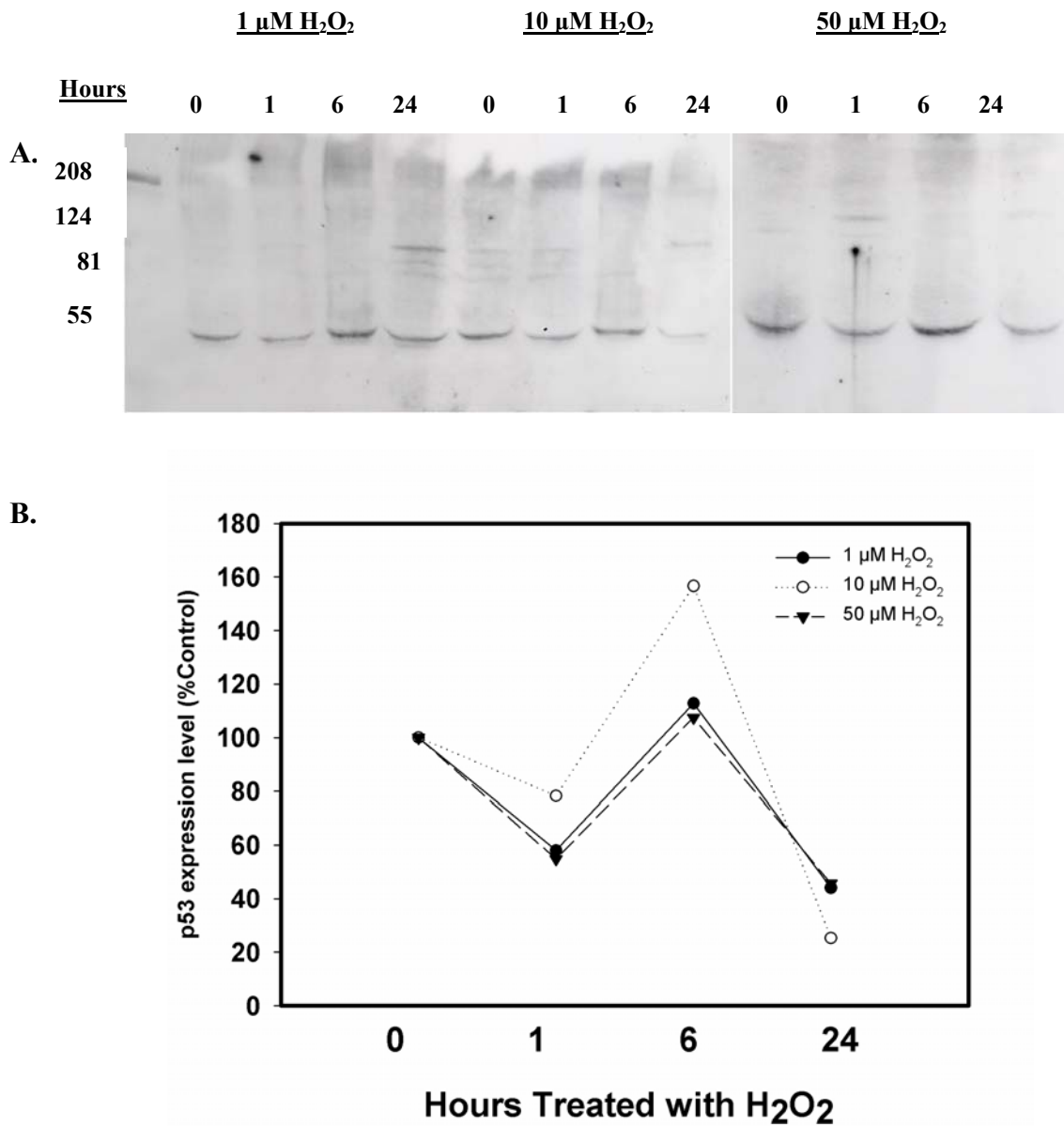


Figure 12. H_2O_2 alters p53 expression in rat cortical neurons. Rat cortical neurons were isolated from E18 embryos and cultured for 5-7 days. Cells were treated with 1 μM , 10 μM , or 50 μM of H_2O_2 . At the indicated times after treatment, cells were collected and lysed. Cell lysates of 50 μg total protein were examined for p53 expression by Western blot analysis (**A**). Densities of bands corresponding to p53 were analyzed, percent control calculated for each dose, and plotted against length of treatment (**B**). (**A**) and (**B**) are representative of 3 experiments.

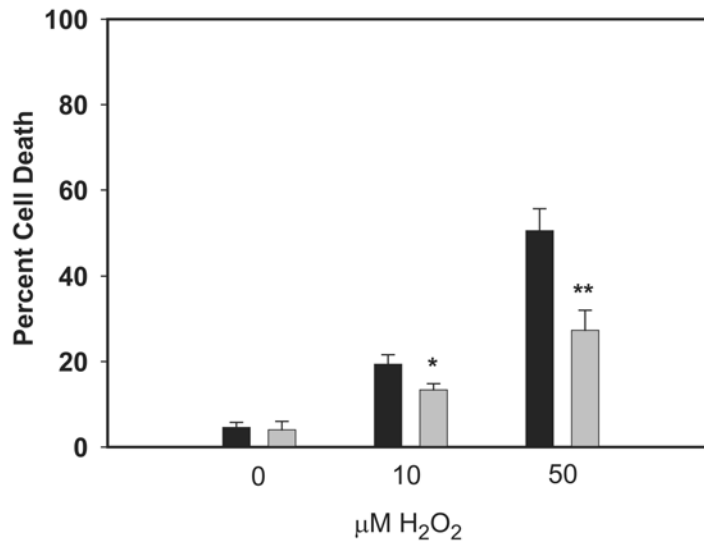


Figure 13. Pharmacological inhibition of p53 attenuates neural death induced by oxidative stress . Cultured rat neurons were treated with the indicated concentrations of H₂O₂ for 24 hours in the absence (●) or presence (●) of 1 μM pifithrin. Cell death was assessed by visualization of cell nuclear morphology with Hoescht stain. Data represent the percentage of cells with fragmented or condensed nuclei. Error bars represent S.E.M for at least 6 cultures. * p<0.05 compared to 10 μM H₂O₂. ** p < 0.001 compared to 50 μM H₂O₂. Graph is representative of 3 similar experiments.

Inhibition of p53 Attenuates H₂O₂ Mediated Death in Neural Cells

To further investigate the role of p53 activity in oxidative stress mediated neural death, a human neural cell line was utilized. Undifferentiated SY5Y cells were treated with H₂O₂ in the absence or presence of pifithrin. After 24 hours, cell death was analyzed by Hoechst staining. H₂O₂ induced a dose-dependent death (Figure 14), which was inhibited by pifithrin at both tested concentrations. Attenuation was incomplete, with less than a 20% decrease in neural death induced by 50 μM H₂O₂. There was approximately a 40% attenuation of neural death induced by 10 μM H₂O₂.

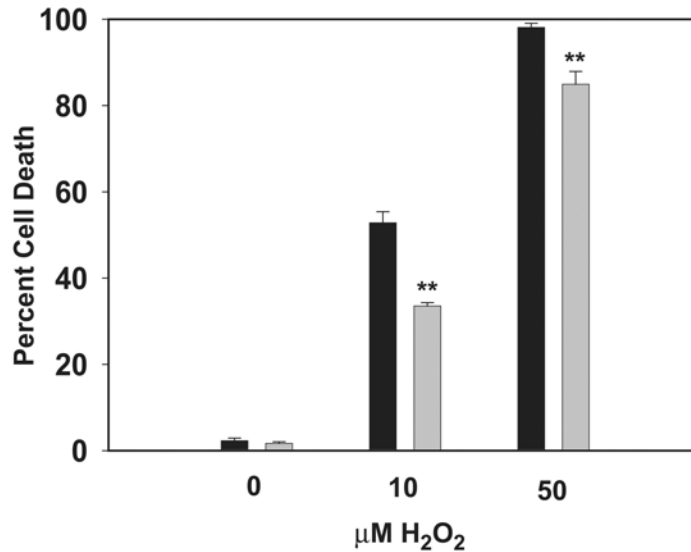


Figure 14. Pharmacological inhibition of p53 attenuates cell death induced by oxidative stress in human neural cells. Neural SH-SY5Y cells were treated with the indicated concentrations of H₂O₂ for 24 hours in the absence (●) or presence (●) of 1 μM pifithrin. Cell death was assessed by visualization of cell nuclear morphology with Hoescht stain. Error bars represent the S.E.M. of at least 6 cultures. ** p < 0.002 compared to H₂O₂. Graph is representative of 3 similar experiments.

Since primary neurons are post-mitotic cells, I utilized retinoic acid differentiated SY5Y cells in similar experiments. Similar to undifferentiated SY5Y cells and primary neurons, cell death was also attenuated by pifithrin (Figure 15). There was no significant difference between control and 10 μM H₂O₂ with pifithrin, unlike in undifferentiated cells. However, at the concentration tested, 10μM, there was approximately half of the cell death observed in undifferentiated cells (Figure 15). Taken together, these data indicate that H₂O₂ induces cell death in human neural cells as was observed in rat primary neurons. Pharmacological inhibition of p53 transcriptional activity attenuated H₂O₂ induced

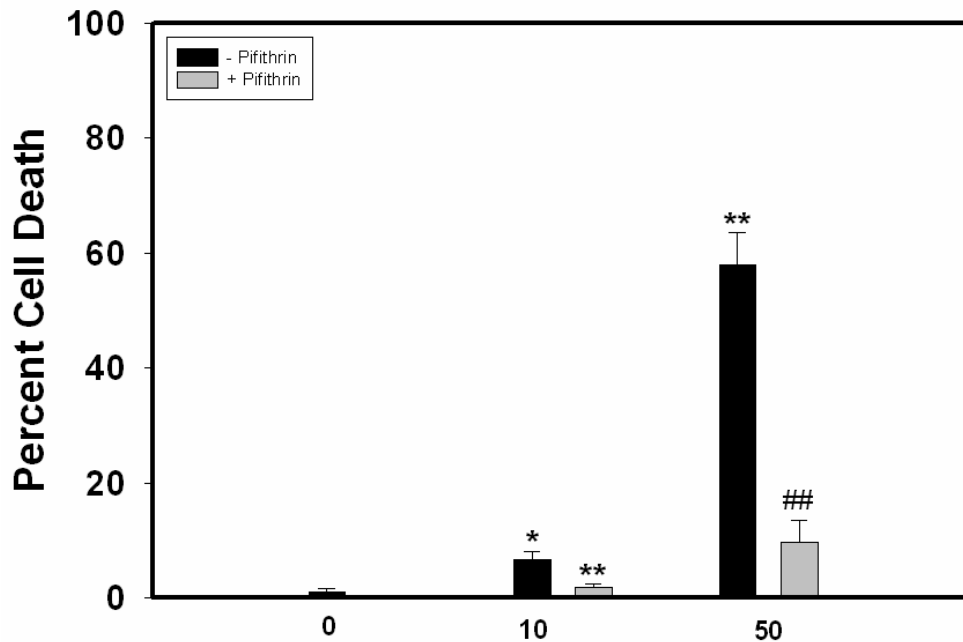


Figure 15. Pharmacological inhibition of p53 activity attenuates oxidative stress induced death of differentiated human neural cells. Differentiated SY5Y cells were treated with 10 μM or 50 μM H_2O_2 in the absence (●) or presence (●) of 1 μM pifithrin. Cell death was assessed by visualization of cell nuclear morphology with Hoechst stain. Data shown represent the percentage of cells with fragmented or condensed nuclei. Error bars represent S.E.M. of 6 cultures. * $p < 0.01$ compared to control. ** $p < 0.001$ compared to 10 μM H_2O_2 . ### $p < 0.001$ compared to 50 μM H_2O_2 without absence. Graph is representative of 2 similar experiments.

cell death. These data suggest that there is a role for p53 transcriptional activity in oxidative injury mediated neural death.

A Genetic Inhibitor of p53 Attenuates H_2O_2 Induced Death

A primary concern with using pharmacological inhibitors is that of specificity. To further corroborate the data implicating p53 in oxidative stress mediated neurotoxicity, I utilized cell lines defective in p53 DNA binding

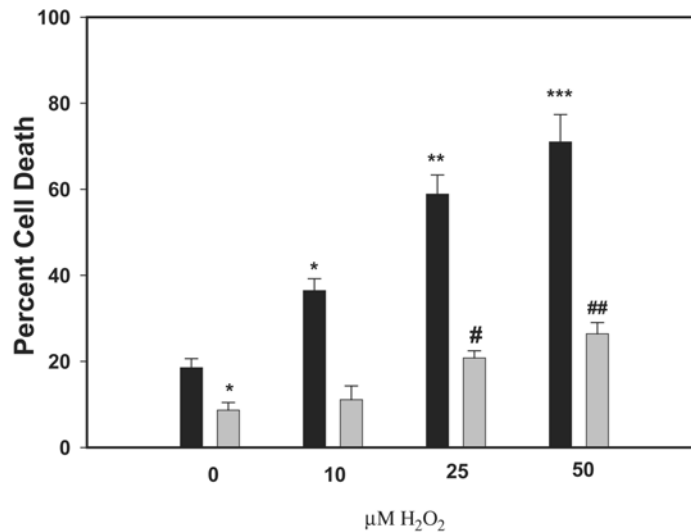


Figure 16. Loss of p53 DNA binding attenuates oxidative stress mediated neural death. PC12 cells stably transfected with either empty vector (■) or a p53 mutant deficient in DNA binding (▒), were treated with the indicated concentrations of H₂O₂ for 24 hours. Cell death was assessed by visualization of cell nuclear morphology with Hoescht stain. Data represents the percentage of cells with fragmented or condensed nuclei. Error bars represent S.E.M. for at least 6 cultures. * p < 0.01 compared to vector cells with no treatment. ** p < 0.01 compared to vector cells treated with 10 μM H₂O₂. *** p < 0.001 compared to vector cells treated with 10 μM H₂O₂. # p < 0.01 compared to mutant p53 expressing cells treated with no treatment. ## p < 0.001 compared to mutant p53 expressing cells treated with 10 μM H₂O₂.

(Hughes et al., 2000) . At restrictive temperature (39 °C), these cells over-express mutant p53, which effectively abrogates p53 mediated transcription. PC12 cells were treated with H₂O₂ for 24 hours and their nuclei visualized with Hoescht stain (Figure 16). PC12 cells that contained only the vector died in a dose dependent manner following treatment with H₂O₂. However, cell death was almost completely blocked in cells expressing mutant p53. To further corroborate these data, cultures treated in the same way were analyzed for cell survival by MTT analysis. The MTT assay measures the reduction of MTT [(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], which

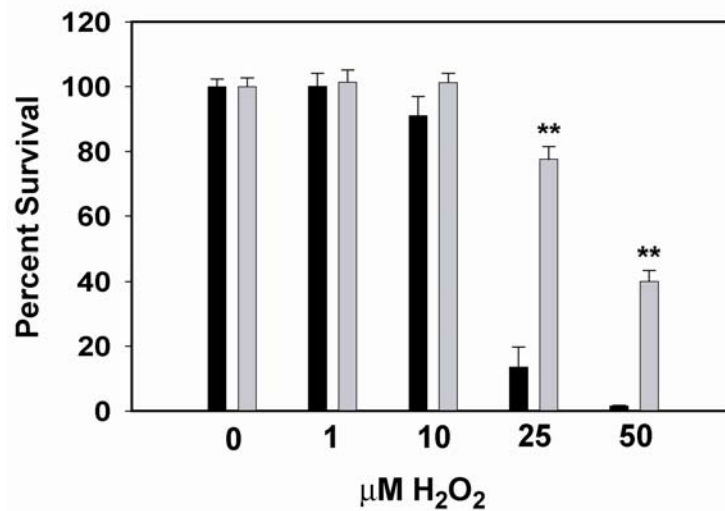


Figure 17. Genetic inhibition of p53 DNA binding attenuates neural death induced by H₂O₂. PC12 cells stably transfected with either empty retrovector (■), or a p53 mutant deficient in DNA binding (▒), were treated with the indicated concentrations of H₂O₂ for 24 hours. Cell survival was determined by the MTT assay. Percent cell survival compared to untreated cells was determined. Error bars represent S.E.M. of 8 cultures. **p < 0.001 compared to cells containing empty retrovector. Graph is representative of at least 3 similar experiments.

produces a blue-violet color. The ability of cells to reduce MTT is a measure of cell viability. MTT analysis of cell death indicated that neurotoxicity was attenuated greatly in cells that express *ts* p53 (Figure 17). Taken together, these data indicate that p53 transcriptional activity is a factor in oxidative stress induced neurotoxicity. These data taken with the pifithrin studies suggest a role for p53 transcriptional activity in mediating neural death following oxidative injury.

Chapter Four

Discussion and Conclusions

Expression and regulation of p53

The tumor suppressor protein p53 has a myriad of roles in the normal cell. For example, p53 has roles in the cell cycle, neuronal differentiation and apoptosis. Most of the literature on p53 has been in the context of its role in cancer. At least 50% of human cancers are linked to mutations in p53 (Vousden, 2002). Its role in neurodevelopment has been studied extensively as well (Miller et al., 2000). Some studies have implicated p53 in neural trauma or neurodegenerative disease, although its role has not been fully elucidated. The involvement of p53 in polyglutamine disorders has been suggested but not fully studied (Shahbazian et al., 2001; Steffan et al., 2000; Suhr et al., 2001; Trettel et al., 2000). Other transcription factors, however, including SP1 and CBP, have been studied in polyglutamine disorders. Soluble, but not aggregated, mutant Htt has been found to interact directly with SP1, and in doing so, repress SP1 mediated gene transcription (Li et al., 2002). The genes affected include the nerve growth factor mediated pathways, which are responsible for neuritic outgrowth responsible for retinoid signaling, important pathways for maintaining neurohomeostasis. Loss of these pathways leads to neural dysfunction and neurite retraction in a cell model of HD (Li et al., 2002; Wyttenbach et al., 2001). These events are also observed in human HD. At present, it is unclear how the loss of CBP and SP1 occur in HD.

Since p53 is a potent regulator of the cell cycle and apoptotic pathways, p53 expression and activity are tightly regulated by the cell (Alarcon-Vargas et al., 2002; Ashcroft et al., 1999). When p53 is mutated, uncontrollable cell growth can result, leading to cancer. If p53 is inappropriately active, then premature cell death can occur. In my studies, increased levels of p53 were present in the nucleus in cells expressing pathological lengths of polyglutamine (Figure 4B). When p53 is activated, or induced, it is localized to the nucleus, and nuclear export is minimized, befitting its role as a transcription factor (Vousden, 2002). This increase in p53 levels can be due to induction of the p53 gene, leading to production of p53 protein, or it can be due to stabilization of existing p53 protein. Under normal conditions, p53 concentrations are relatively low due to its short half-life. At the transcriptional level, p53 can be induced by a variety of insults, the best studied of which is genotoxic stress (Balint et al., 2001; Shieh et al., 1997; Shieh et al., 1999; Shieh et al., 2000; Vousden, 2002). The activity and stability of p53 is regulated by both post-translational modifications and interaction with other proteins. In my studies, I found that p53 expression and activity were altered *in vitro*.

In chapter 2 I described research aimed at investigating the hypothesis that p53 expression and activity is altered in the presence of polyglutamine expansion. In SY5Y neural cells, the p53 protein levels were greater in cells that expressed 56-GFP than in 19-GFP (Figure 2). Increases in p53 stabilization was not associated with increased mRNA abundance of p53 (Figure 1D). Two pieces of evidence support this. When protein synthesis was halted, p53 protein was observed to be more stable in 56-GFP cells than in 19-GFP or wild type cells (Figure 2). Additionally, levels of p53 mRNA were the same for 19-GFP and 56-GFP expressing cells (Figure 1B).

The stability of p53 is largely mediated through p53 interaction with Mdm2 (Alarcon-Vargas et al., 2002). Mdm2 has E3 ubiquitin ligase activity and ubiquitinates p53. This can lead to localization of p53 to the cytosol where it is degraded by the 26S proteasome (Freedman et al., 1999). Mdm2 is transcriptionally transactivated by p53 (Barak et al., 1993), thereby participating in a p53 mediated negative autoregulatory loop (Alarcon-Vargas et al., 2002; Ashcroft et al., 1999; Freedman et al., 1999). Mdm2 has also been found to be important for regulation of p53 activity in neuroblastoma derived cell lines, such as the SH-SY5Y cells used in my studies (Rodriguez-Lopez et al., 2001). In my studies, Mdm2 expression was found to be higher in 56-GFP cells than in 19-GFP (Figures 3, 4C, 4D). Mdm2 was localized chiefly in the nucleus, as revealed by immunocytochemistry (Figures 4C, D). Mdm2 immunoreactivity was more intense in the nuclei of 56-GFP cells, consistent with the immunoblot data (Figures 4D and 3, respectively). The expression of p53 was higher in 56-GFP cells and localized in the nucleus (Figure 4A), despite the increased expression of Mdm2 (Figure 3, 4B). While the immunoprecipitation data was not convincing on its own, there did appear to be more interaction of Mdm2 with p53 in 19-GFP cells compared to 56-GFP cells. More work needs to be done in the area of p53 protein-protein interaction, but it appears that there may be differences between the two cell lines. Taken together, these data indicate that p53 is stabilized in the presence of increased Mdm2 levels in 56-GFP expressing cells, and that this may involve there being less interaction between Mdm2 and p53 in 56-GFP expressing cells.

The mechanism behind this change in p53-Mdm2 interaction is unclear. Recent studies have linked other protein interactions with either p53 or Mdm2 and p53 stability.

For example, the HAT protein p300 has been shown to interact with both Mdm2 and p53 (Grossman et al., 1998), and participates in Mdm2 mediated degradation of p53, perhaps through its own ubiquitin ligase activity (Grossman et al., 2003). I did conduct experiments investigating the interaction between Mdm2 and p300, but the results were inconclusive (data not shown). There is no difference in viability of SY5Y cells stably expressing 19-GFP or 56-GFP (Ding et al., 2002). However, reduction of Mdm2 expression with Mdm2 antisense oligonucleotides did result in significantly more cell death in untreated 56-GFP cells than in 19-GFP cells (Figure 6). Treatment of cells with antisense oligonucleotides resulted in reduction of MDM2 expression of approximately 40% (Figure 6A). Concomitant with this decrease in Mdm2 expression, there was significant increase in cell death of 56-GFP cells compared to 19-GFP cells (Figure 6B, C). There was also a small, but significant increase in cell death in 19-GFP cells. However, the 56-GFP cells had approximately 5 times the cell death of 19-GFP cells. With regard to HD, this could mean that, in the presence of polyglutamine expansion, interactions between p53 and Mdm2 would be altered, possibly causing alterations to p53 stability. Loss of Mdm2 leads to greater death in 56-GFP, suggesting that Mdm2 mitigates polyglutamine toxicity. A mechanism for this could be that through some signaling pathway, p53 is stabilized in cells that express polyglutamine. The mechanism that stabilizes the protein by definition protects p53 from degradation, probably by masking or modifying residues that are important for Mdm2 mediated ubiquitination. One glaring example of this are the C-terminal lysines that are ubiquitinated. Acetylation of these sites could prevent the ubiquitination of these sites.

Mdm2 is a transcriptional target of p53; Mdm2 expression increases with increases in p53 activity (Li, 2002 #1420).

Mdm2 can also mediate p53 interaction with DNA. Mdm2 associates with the amino terminal of p53 in the same region that contains p53 DNA transactivation domains (Oliner et al., 1993; Wadgaonkar et al., 1999) . This interaction can also inhibit p53 interaction with other transcriptional coactivators such as the HAT proteins (Grossman et al., 1998; Wadgaonkar et al., 1999).

In my studies, I found that p53 underwent modifications *in vitro*. These included phosphorylation and acetylation in cells expressing pathological lengths of polyglutamine. Multiple serine and threonine residues of p53 can be phosphorylated in response to a variety of stimuli. (Ashcroft et al., 1999; Balint et al., 2001; Vousden, 2002; Woods et al., 2001; Xu, 2003). Phosphorylation of p53 has been associated with increased p53 transcriptional activity and increased p53 stability (Ashcroft et al., 1999; Li et al., 2002; Shieh et al., 1997). Phosphorylation at some sites may prevent or attenuate a protein-protein interaction. Serine 15 of p53 lies within the region bound by Mdm2 (Alarcon-Vargas et al., 2002). While the contribution of Serine 15 phosphorylation to the p53 –Mdm2 interaction is not completely understood, there is consensus that this and neighboring sites probably contribute to both p53 stability and localization (Ashcroft et al., 1999; Bargonetti et al., 2002; Shieh et al., 1997). It's thought that phosphorylation of this and/or nearby sites attenuates the p53-Mdm2 interaction (Shieh et al., 1997) . The p53 N-terminal nuclear export sequence (NES) also lies within this region (Zhang et al., 2001). So phosphorylation of Serine 15 and/or nearby sites may also serve to mask this site and promote p53 retention in the nucleus

(Zhang et al., 2001). In my studies, phosphorylation of p53 on Serine 15 was analyzed in human neural cells expressing 19-GFP or 56-GFP. Though these experiments were not normalized to some other protein such as actin, there was consistently more phosphorylation at this site in 56-GFP expressing cells than in 19-GFP cells (Figure 7B). Phosphorylation of p53 on Serine 15 occurs in response to UV radiation or other inducers of DNA damage (Shieh et al., 1997). Oxidative stress has been associated with DNA damage in HD (Bogdanov et al., 2001), and could play a role in the increase in p53, possibly through phosphorylation of p53.

Acetylation of p53 occurs on 6 different lysine residues including 320, 373, 381 and 382 (Gu et al., 1997) (Grossman, 2001; Liu et al., 1999). The first site is acetylated by P/CAF and the latter by p300/CBP. These HAT proteins typically are recruited to specific promoters to acetylate amino terminal regions of the core histone proteins. This results in decompression of the DNA from the nucleosome, allowing the transcriptional machinery access to target genes. HAT's also acetylate p53 and it is thought that this also serves to recruit other transcriptional coactivators (Barlev et al., 2001; Chan et al., 2001). Acetylation has been associated with increased transcriptional activity of p53, and with stabilization of the protein (Balint et al., 2001; Grossman, 2001; Li et al., 2002).

There was more acetylated p53 in 56-GFP cells than in 19-GFP cells (Figure 7A). It has been suggested that acetylation of p53 serves to stabilize p53 or increase its transcriptional activity. Acetylation of p53 occurred on lysine 320, which is mediated by P/CAF, as detected by an antibody raised against p53 acetylated only on that residue. P/CAF acetylation at lysine 320 was demonstrated to be inhibited by MDM2

association with P/CAF in one study (Jin et al., 2002). Data from other studies found similar relationships between p300 and Mdm2 (Ito et al., 2001). P/CAF is associated with p300 and CBP, and all 3 proteins promote p53 transcriptional activity.

It is interesting to note that histone deacetylase inhibitors, as well as over expression of CBP, have been shown to ameliorate some aspects of polyglutamine neurotoxicity (McCampbell et al., 2001; Steffan et al., 2001; Taylor et al., 2003). This is consistent with the hypothesis that neuronal dysfunction in HD is due to polyglutamine expansion mediated alterations to transcription. CBP is a major part of the transcriptional machinery, as it mediates acetylation of histones. CBP also acetylates p53 (Gu et al., 1997), and this could reveal a role for p53 in the dysregulation of transcription observed in HD. CBP contains a polyglutamine region and it has been suggested that this region is important for interaction with mutant Htt. CBP binds to phosphorylated CREB, which in turn binds to the cyclic AMP response element (CRE). CBP serves to acetylate histones, thereby decompressing the nucleosomes and allowing the transcriptional machinery to assemble at the appropriate promoter sites. CBP, along with p300 and P/CAF, serve as “bridges” to the Pol II enzyme (Chan et al., 2001). Alterations to CBP function are most likely to cause disruptions to normal transcription (Chan et al., 2001). Alterations to CREB and its regulators have been found to cause neurodegeneration reminiscent of HD (Mantamadiotis et al., 2002). Alterations of CRE- mediated transcription have been found in animal (Luthi-Carter et al., 2002) and cell models of HD (Wytttenbach et al., 2001), as well as models of SBMA, a polyglutamine disorder caused by expansion of the polyglutamine region of the androgen receptor (McCampbell et al., 2000). Changes in CRE-mediated transcription

can affect pathways involved in neural homeostasis. For example, Brain Derived Neurotrophic Factor (BDNF) is regulated by CRE- mediated transcription, and was found to be downregulated in human HD brain (Ferrer et al., 2000). BDNF has been found to be important for neural homeostasis, and is up regulated by wild type htt (Zuccato et al., 2001). Treatment of an inducible cell model with cAMP, the second messenger that induces CRE mediated transcription, ameliorated some polyglutamine dependent deficiencies in an HD cell model, including neurite outgrowth and cell death (Wytttenbach et al., 2001). Interactions between CBP and mutant Htt have been reported in several studies (Nucifora et al., 2001; Steffan et al., 2000; Yu et al., 2002). In one study, almost complete sequestration was reported in cell models, mouse models, and human brain (Nucifora et al., 2001). Soluble CBP was greatly reduced in the brains of HD patients. Reduced CBP reporter activity was also reported in cell models of HD and dentatorubral and pallidoluysian atrophy (DRPLA), another polyglutamine disorder caused by polyglutamine expansion of atrophin-1. CBP over expression rescued polyglutamine expansion cell death in cell models of both diseases. Deletion of CBP resulted in pathology in cells expressing nonpathological lengths of polyglutamine. In another study, CBP was also found to aggregate with mutant Htt in cell and mouse models of HD (Steffan et al., 2000). This occurred concomitant with p53 interaction with mutant Htt. The mechanism of mutant Htt induced alterations to transcription is somewhat controversial. Yu and colleagues found that even though CBP may be co localized with mutant Htt in the nucleus, it was not however depleted (Yu et al., 2002). This study suggested that CBP interacted with soluble mutant Htt,

comparable to what was seen with SP1, which is another transcription factor that contains a polyglutamine region.

Mutant Htt constructs have been shown to interact with p53 in one study (Steffan et al., 2000). In this study, p53 aggregated with mutant Htt in intracellular inclusions observed in cell models of HD. Mutant Htt co-immunoprecipitated with p53 as well as with CBP. Mutant Htt interaction with p53 was independent of the proline rich region of Htt in cells, although there was a requirement for the proline rich region *in vitro*. Additionally, p53 interaction with mutant Htt required the C terminal region of p53, a region that is highly regulated. For example, the NES domains and tetramerization domains of p53 lie in this region (McLure et al., 1998). Interaction of mutant Htt with p53 would alter p53 mediated transcription (Steffan et al., 2000). This same group found that there was aberrant histone acetylation in cells that expressed a polyglutamine expanded mutant Htt fragment. Treatment of animals transgenic for mutant htt with HDAC's alleviated the neurodegeneration observed. It was found that HDAC's also improved histone acetylation in these animals. These data gives pause when one considers the effects maintaining acetylated sites on p53. If acetylated p53 is active, and HDACs attenuate neurotoxicity, then active p53 in HD models may not be detrimental to cells. Active p53 could be beneficial or may play no significant role *in vivo*. This idea becomes more important when I discuss the *in vivo* studies I conducted.

Taken together, these data have lead researchers to investigate the use of HDAC inhibitors in potential clinical trials. HDAC inhibitors have been found to alleviate polyglutamine neurotoxicity in animal and cell models (Steffan et al., 2001). However, in light of our studies, it is likely that HDAC inhibitors may adversely affect p53 function.

HDAC inhibitors keep p53 acetylated and p53 mediated transcription active (Juan et al., 2000). It was also found that use of HDAC enzymes reduced p53 interaction with the BAX promoter. BAX has been implicated in neural death and mitochondrial dysfunction (Fortin et al., 2001). This data suggests that inhibition of HDAC would promote higher transcription of the BAX gene, which could be detrimental to neural homeostasis (Juan et al., 2000). However, there was increased acetylation in 56-GFP cells compared to 19-GFP cells, but this has no effect on cell viability (Ding, 2002 #403).

The expression, stability, and localization of p53 were altered in differentiated 56-GFP cells. Additionally, post-translational modifications were found to be increased in 56-GFP cells. Interactions between Mdm2 and p53 were found to be altered in 56-GFP cells. Taken together, these data appear to support the hypothesis that p53 expression, stability, and localization are altered in a cell model of HD. It remains to be seen what the mechanism of p53 stabilization in 56-GFP cells is, and what the ramifications of p53 stability may be.

Polyglutamine Expansion Alters p53 Expression and Activity in neural cells

Transactivation of genes by p53 is dependent upon sequence-specific p53 interaction with DNA (Oren, 2003), so this data implies that p53 transcriptional activity may be higher in 56-GFP cells compared to 19-GFP cells. However, this data only demonstrated increased interactions *in vitro*. To determine if p53 DNA binding activity had a role in polyglutamine mediated neurotoxicity, a cell line expressing a *ts* mutant of p53 mutant was used (Hughes et al., 2000). This mutation has been found to greatly inhibit p53 transcriptional activity, notably of p21^{Waf1/CIP1} protein, a protein responsible

for growth arrest following treatment of cells with Nerve Growth Factor (NGF) (Hughes et al., 2000). Following treatment with NGF, cells that express *ts* p53 do not undergo cell cycle arrest, but do differentiate into a neuronal phenotype (Hughes et al., 2000). PC12 cells transiently transfected with 56-GFP were found to undergo cell death. Cell death was greatly attenuated in cells that stably express *ts* p53 (Figure 9G). This data indicates that transient transfection of cells with 56-GFP induces cell death that is dependent on p53 transcriptional activity. The possibility that p53 could contribute to neural death in other ways besides transactivation of genes was considered. Recent studies have shown that a fraction of p53 localizes to the mitochondrion following ionizing radiation, a classic inducer of p53, and binds with anti-apoptotic proteins of the BCL-2 family of protein (Manfredi, 2003; Mihara et al., 2003). This in turn leads to perturbations of the membrane and cytochrome C release, an event which precedes apoptosis. This interaction also occurs through the DNA binding domain of p53, which is interesting because *ts* p53 is deficient in DNA binding (Mihara et al., 2003). It may prove useful to examine localization of p53 in the *ts* p53 cells. It might also prove useful to show that in vector cells transiently transfected with 56-GFP, p53 promotes the transactivation of pro-apoptotic genes such as BAX (Morrison et al., 2000) or APAF-1 (Fortin et al., 2001), and these p53 mediated actions are attenuated in the *ts* p53 cells.

The expression and localization of p53 were found to be altered in cells expressing pathological lengths of polyglutamine compared to cells expressing physiological lengths. The data indicate that polyglutamine expansion in this model serves to stabilize p53. Polyglutamine expansion induced neurotoxicity which was relieved by disabling p53 transcriptional activity (Figure 9). Taken together, the data

agree with the hypothesis that polyglutamine expansion alters p53 expression, localization, and activity. There was stabilized expression of p53 and higher levels of modified p53 in 56-GFP cells compared to 19-GFP cells. There were differences in p53-Mdm2 interactions and p53 localization in 56-GFP cells compared to 19-GFP cells. Mdm2 expression was highest in 56-GFP cells but Mdm2 interactions seemed to be lower than in 19-GFP cells. Reduction of Mdm2 expression resulted in cell death that was much greater in 56-GFP cells than in 19-GFP cells. It would appear from the data that a role for p53 in HD can be elucidated. Whether this role is mediation of neural death in HD remains to be seen. It could be that p53 is not directly involved with neural death in polyglutamine diseases. The data on this is very thin, but absence of p53 in a mouse model of SCA1, another polyglutamine disorder, demonstrated absence of p53 did not affect cell number or motor performance (Shahbazian et al., 2001). However, absence of p53 did reduce the amount of dendritic thinning seen in SCA1 mice. Dendritic thinning, also known as dearborization, is typical of SCA1. This may reflect a contribution of p53 to other pathways other than the well known apoptotic pathways.

p53 in the R 6/2 mouse

The experiments I've described served to investigate basic p53 biology in the presence of polyglutamine expansion *in vitro*. In order to investigate the role of p53 in HD *in vivo*, p53 and modified p53 expression were examined in R6/2 mice, a model of HD, as well as in human HD brains. R6/2 mice express an N-terminal fragment of exon 1 of the human HD gene containing a polyglutamine region of 145 residues in length (Mangiarini et al., 1996). This mouse develops a pathology at 8 weeks that is

somewhat reminiscent of human HD. Starting at approximately 7 weeks of age, intranuclear aggregates and inclusions within dystrophic neurites are observed in the brains of R6/2 mice (Davies et al., 1997). However, neurodegeneration is not specific to the striatum, but rather diffuse throughout the cortex and cerebellum as well. Changes to nuclear morphology, including indentations of the nuclear membrane and an increase in nuclear pore density have been observed (Davies et al., 1999). Similar changes to nuclear morphology have been observed in human postmortem HD brain (Davies et al., 1997). A later study found that there is non-apoptotic neurodegeneration that develops late in life (14 weeks) (Turmaine et al., 2000). R6/2 mice also develop a progressive neurological phenotype that includes clasping behavior (inability to splay when suspended by the tail), and a movement disorder like that seen in persons with HD. There is a loss of body weight as well as decreased brain size compared to wildtype littermates. Contrary to data in the SY5Y model, p53 expression was markedly less in the R6/2 mouse compared to wild type controls at 4 weeks and at 10 weeks of age (Figure 9A). There was a slight decline in p53 expression at 10 weeks in HD mice compared to 4 weeks (Figure 9A).

One explanation of the differences between the mouse and *in vitro* data is that there are differences in the polyglutamine constructs used and that this may influence interaction between p53 and polyglutamine expanded protein. In the SY5Y studies, the constructs used was a fusion of GFP and a polyglutamine fragment (Ding et al., 2002). In the R6/2 mouse, the construct is an N-terminal fragment of polyglutamine expanded human mutant Htt. Suhr and colleagues used similar fragments fused to GFP to demonstrate protein aggregation in human embryonic kidney 293 cells (HEK 293 cells)

transfected with htt constructs (Suhr et al., 2001). When aggregates and cell lysates were examined by Western blot, it was found that p53 aggregated with mutant Htt. Additionally, it was found that the relative amount of sequestered p53 was approximately 3 fold greater than soluble p53. The constructs were made preserving the polyproline region downstream of the polyglutamine region of the *htt* fragment. This region has been suggested to play a part in mutant Htt –p53 interaction *in vitro* (Steffan et al., 2000). However, in cell culture systems, there was no such dependence on the proline rich region. The proline rich region of p53 lies between amino acids 64 and 91. Repression of transcription by exogenous p53 of a reporter gene (multi-drug resistance gene or MDR-1, fused to luciferase) was examined by Steffan and colleagues in cells that lack endogenous p53. They found that p53 effectively repressed transcription of MDR-1. Interestingly, they found that mutant Htt fragments repressed MDR-1 transcription as effectively as p53. mSin3a has been shown to interact physically with the N-terminal region of p53 through amino acids 40-160. This region contains the transactivation domain and the proline rich region. Interaction of mSin3a with p53 has been shown to prevent MDM2 mediated proteolysis of p53, thereby stabilizing p53 (Zilfou et al., 2001). Presumably this allows p53 to remain attached to promoters with mSin3a for a longer period of time. The interaction of p53 and mSin3a required amino acids 61-75 of p53. This region lies within the proline rich region of p53, which is essential for p53 mediated apoptosis. However, mSin3a was also found by Steffan and colleagues to interact *in vitro* with mutant Htt in a polyglutamine length and Htt polyproline region dependent manner, although this was a weak interaction. This could explain the differences between the *in vitro* and mouse studies I've conducted. If

mSin3a interacts with mutant Htt *in vitro*, this could disrupt the mSin3a mediated protection of p53 from Mdm2 directed proteolysis. If mSin3a normally protects a fraction of p53 protein from proteolysis, then disruption of the association would be a likely avenue for decreased p53 in R6/2 mice. Mdm2 and p53 are both sequestered in cell models of HD, (Suhr et al., 2001), so examination of relative levels of p53 and Mdm2 would be useful in mice. Mdm2 was not examined in my analysis of R6/2 mice. The GFP constructs used in the *in vitro* studies do not contain a proline rich region. This could explain why there is more p53 in the 56-GFP cells, as these constructs lack the region of mSin3a interaction. Experiments could be done in cell models of HD expressing human htt N-terminal fragments. Experiments that might be informative would be immunoprecipitation experiments to show interactions between p53 and mSIN3a, p53 and Mdm2, and mSin3a and htt. Differences in cells expressing pathological and nonpathological polyglutamine expansion could be noted and contrasted to parallel experiments with GFP-polyglutamine fusions. Additional experiments could investigate mSIN3a's effects on p53 stability in cells expressing polyglutamine expansion similar to the mSin3a –p53 interaction study (Zilfou et al., 2001). If my proposed model is valid, then these data would show that p53 is less stable in cells expressing pathological htt compared to cells expressing nonpathological htt. This could help deduce why there is less p53 expression in HD mice than wild type mouse, and help elucidate the role of p53 in HD neurotoxicity.

Phosphorylation of p53 at serine 392 has been shown to stabilize p53 tetramer formation (Liang et al., 2001). Studies have indicated that p53 binds DNA as a tetramer (McLure et al., 1998), and stabilization of this complex may help in preventing nuclear

export of p53. In HD mice, p53 was phosphorylated on Serine 392 less than in wild type controls (Figure 9B). There did not appear to be an age dependent change in phosphorylation. Phosphorylation of p53 tended to correlate with p53 expression in HD and wild type mouse, suggesting a possible contributing factor for p53 stability in HD mice. In other words, when phosphorylation of p53 on Serine 392 is reduced, p53 is not maintained as a tetramer, and p53 stability goes down. Another possibility is that in the normal mouse brain, p53 levels are maintained at a low level that is sufficient to prevent uncontrolled growth. Acetylated p53 levels were similar in wild type and HD mice at 10 weeks. (Figure 9C). There was significant variability between the animals at 4 weeks, making a strict interpretation difficult.

Human HD

In human HD brains, p53 expression was notably less than in control brains (Figure 10A). This pattern was repeated for both phosphorylated and acetylated p53 as well (Figure 10B and 10C, respectively). At first observation, it would appear that p53 is down regulated in the caudate of HD brains. However, these brains were all of Grade 4 pathology. At this level of pathology, most of the neurons of the caudate have been lost concomitant with greater astrogliosis (Vonsattel et al., 1985). Most of the neural tissue is gone and there are approximately 30 % more astrocytes, which proliferate after or during neurodegeneration. In control brains, there were marked levels of p53 as well as modified p53 species. It must be noted that though p53 levels were barely detectable in HD brain, phosphorylated and acetylated p53 were also detected at very low levels compared to controls. This would imply that active, stable p53 is present in normal

human striatum. One of the primary functions of p53 is to maintain genomic stability and prevent aberrant growth (Oren, 2003). Interpreting the results in this experiment (Figure 10) is difficult. Control brains are so different from HD brains with regard to resident cells in the caudate, that p53 expression data may be irrelevant to the disease.

The data suggest that loss of p53 may bear responsibility for the neuropathology observed, and that in its normal function, p53 is beneficial for the brain, as well as the individual neuron. In vivo, p53 serves different roles depending upon cellular and temporal context. Blood cells such as lymphocytes are removed from the population through p53 dependent apoptotic death. During neurodevelopment, p53 mediated apoptosis removes up to 50 % of the neurons from the brain (Miller et al., 2000). Temporal and spatial distribution of p53 and p53 mediated pro-apoptotic genes are varied in neurodevelopment (van Lookeren Campagne et al., 1998). PC12 neural cells with Nerve Growth Factor (NGF) resulted in up regulation and nuclear localization of p53. This was followed by a cell cycle arrest and neurite outgrowth (Hughes et al., 2000). Using the *ts* p53 expressing PC12 cells used in my studies, they found that neuritic growth is not dependent upon p53 DNA transcriptional activity, but that cell cycle arrest is. This data suggests that one function of p53 is to prevent aberrant reentry into the cell cycle, and that a neuron could remain differentiated even though p53 was inactive. Inappropriate activation of some cell cycle components has been reported in neurodegenerative disorders. A recent paper found that there is neurogenesis in the HD brain, even in very pathological cases (Curtis et al., 2003). The cells detected were neural progenitor cells. These cells proliferate and differentiate into neurons. This has been observed in adult rat brains as well. If resident cells are

proliferating, then p53 function and activity presumably would be decreased compared to controls. In that case, given the low levels of p53 in the mature brain already (Figures 9 and 10), p53 expression may fall below detectable levels.

To determine the role of p53 in the neurodegeneration of HD it would be necessary analyze brains over the course of the disease. With the few brains analyzed in this study, and given the ethical constraints of analyzing human samples, it would be difficult to assess p53 levels in relevant portions of the brain during the course of HD. However, lymphoblasts from HD patients have been used to analyze the effects of mutant Htt during the course of the disease (Panov et al., 1999; Panov et al., 2002). One study investigated the role of Htt mediated defects in mitochondrial calcium homeostasis (Panov et al., 2002). These defects were seen in patients and mice early in disease progression. They found that there was a lower mitochondrial membrane potential in mitochondria isolated from lymphoblasts of HD patients and HD mice compared to controls. This was associated with greater mitochondrial depolarization which was inhibited by cyclosporine A that inhibits mitochondrial permeability transition (MPT). Cells from HD patients were more vulnerable to MPT dependent apoptosis. There was a direct interaction between htt and the mitochondrion (Panov et al., 1999). Perturbations of the mitochondrion are associated with oxidative stress, which is a key factor in HD. Additionally, recent data have shown that p53 is also localized to the mitochondrion, though the evidence for this is still emerging (Manfredi, 2003; Mihara et al., 2003). Localization of p53 to the mitochondria of lymphoblasts from HD patients might be useful to study. Regardless, the use of cells derived from human patients would be useful to study p53 biology in the context of human HD. Experiments in these

cells could include examining the role of p53 in oxidative stress in an HD background and comparing that to normal patients. It may be that differences would not be seen under basal conditions, but when the cells are under stress, i.e. staurosporine treatment observed in the Panov study. Also, it may be possible to study changes in p53 expression and activity through the course of the disease. One of the caveats to studying *in vitro* systems of HD is that most experiments last on the order of hours or days. HD takes many years to manifest, and extrapolation of data from tissue culture or even animal models may be difficult. One point made by Panov and colleagues (Panov et al., 2002), was that mitochondrial changes in membrane potential in HD patients were slight, but otherwise, mitochondria functioned normally in HD. A good example of this would be in my *in vitro* studies. The expression of p53 was elevated in 56-GFP cells, yet 56-GFP cells are as viable as wild type or 19-GFP cells (Ding et al., 2002). However, transient transfection of PC12 cells with 56-GFP led to cell death which was attenuated by overexpression of a p53 protein deficient in DNA binding. But if these changes are over the lifetime of a human, especially in a neuron that has to function for up to 100 years, then perhaps they could prove detrimental. Perhaps lymphoblasts from HD patients could be used to shed some light on p53 biology in HD in humans.

p53 and oxidative stress

Oxidative stress has been implicated in several neurodegenerative disease including HD (Bogdanov et al., 2001; Browne et al., 1999; Perez-Severiano et al.,

2002). Evidence for a role for oxidative stress in HD has been found in human patients (Browne et al., 1997; Polidori et al., 1999) , animal models for HD (Bogdanov et al., 2001; Perez-Severiano et al., 2000), cellular models of HD (Wytttenbach et al., 2002), and chemical models of HD (Kim et al., 2002; Tkac et al., 2001). Oxidative damage to proteins, lipids, and DNA has been observed in all of these models (Bogdanov et al., 2001; Matthews et al., 1998). Treatment of animals with the succinate dehydrogenase inhibitors 3-NP or malonate has been shown to produce energy depletion in animal and humans, as well as striatal lesions that are highly similar to those observed in HD. Humans that have accidentally ingested 3-NP develop neuropathology similar to that seen in HD. These toxins also lead to production of ROS. Hence 3-NP and malonate have been used as experimental models of HD (Grunewald et al., 1999) . As further evidence for the role of oxidative stress in HD, treatment of cellular (Wytttenbach et al., 2002) or animal models of HD (Ferrante et al., 2000; Ferrante et al., 2002) with antioxidants has been shown to ameliorate some of the pathology and neurophysiology of the disease. In one study, for example, mice that expressed an N-terminal fragment of polyglutamine expanded human htt (HD-N-171-82Q) were treated with the antioxidant coenzyme Q10 (CoQ10). CoQ10 is an essential cofactor of the electron transport chain and serves to neutralize ROS (Beal, 1999). Additionally, mice were treated with remacemide, an N-methyl-D-aspartate (NMDA) receptor antagonist, which was used because of its attenuation of the effects of glutamate excitotoxicity. Treated mice outperformed untreated mice in motor skills, and that they had greater body weight. There was no difference in survival between treated and untreated mice. Another study investigated the effects of CoQ10 and remacemide on motor

performance, survival, and brain weight in R6/2 mice. Motor performance was improved with COQ10 or remacemide, or both in combination. Brain size is decreased approximately 20% in R6/2 mice compared to controls (Davies et al., 1997). Brain size was significantly increased in treated animals versus untreated mice, probably due to reduced ventricle size (Ferrante et al., 2000). Enlarged ventricles are a characteristic of R6/3 symptomatic brain and result from degenerative neurons. Metabolic impairment has been linked to HD, and can result in the production of ROS (Browne et al., 1999). Treatment of animals with creatine or cyclocreatine, substrates for creatine kinase, an enzyme that regulates the ATP/ADP ratio in the brain, reduced metabolic dysfunction and ROS production in 3-NP and malonate treated animals (Andreassen et al., 2001). This was evidenced by lower levels of lactate production, reduced hydroxyl production, and reduced levels of 3-nitrotyrosine, a marker of peroxynitrite mediated protein oxidation.

HD is associated with increased oxidative stress (Browne et al., 1999; Grunewald et al., 1999). Oxidative stress is associated with increased p53 expression and p53 transcriptional activity in a variety of models of neural injury and disease (Bates et al., 1999; Mattson et al., 2001). Taken together, this suggests a possible role for p53 in HD neurotoxicity. In Chapter 3 I described research aimed at investigating the hypothesis that alterations to p53 expression and/or activity mediated neural death induced by oxidative injury. The expression of p53 was analyzed as well as the effects of genetic and pharmacological inhibitors of p53 transcriptional activity on neural death induced by H₂O₂. I did not investigate a direct role for oxidative stress neurotoxicity,

but I did investigate the hypothesis that alterations to p53 expression and activity mediated neural death induced by oxidative injury.

H₂O₂ induced dose dependent death in embryonic rat cortical neurons (Figure 11, 13). Analysis of p53 expression revealed elevated levels of p53 after 6 hours, with a decrease in expression after 24 hours (Figure 12). This increase was noted with all concentrations tested. There was some variation in the time course of p53 induction between experiments. Specifically, in some experiments treatment with 10 μM H₂O₂ sometimes induced elevated p53 at earlier times than 1 μM H₂O₂.

Since the magnitude of p53 induction was similar for all concentrations of H₂O₂, despite the dose dependent neural death, it is likely that neural death was not completely dependent upon p53. Transcriptional activity of p53 has been implicated in other models of oxidative stress (Duan et al., 2002). Inhibition of p53 expression with p53 oligonucleotides was shown to inhibit neural death following excitotoxic injury (Lakkaraju et al., 2001). An inhibitor of p53, pifithrin-α, has also been shown to ameliorate the effects of ischemia and excitotoxicity (Culmsee et al., 2001). Cotreatment of H₂O₂ treated neurons with pifithrin resulted in attenuation of neural death (Figure 13). There was not complete inhibition of cell death. This could be for various reasons, including insufficient concentration of pifithrin, or production of survival factors by other cells in the culture. There was no death attributed to pifithrin (Figures 13,14,15). The use of embryonic rat neuronal cultures was useful to study the induction of p53 expression and cell death, as well as the establishment that cell death was at least partially mediated by p53 transcriptional activity. However, there may be differences in the role of p53 in rat and human. Also, the primary cultures utilized were

of a mixed cell type nature, that is, they contained glial cells as well as neurons. Glial cells, specifically astrocytes, are mitotic cells in culture, and the ratio of astrocytes to neural cells was variable from experiment to experiment. And the induction of p53 in mitotic cells in response to oxidative injury is probably dissimilar to that of neurons. This is a possible source of variability in p53 expression data. To study the effects in a more relevant model to human conditions, human neural SY5Y cells were used. This cell line, derived from human neuroblastoma cells, are unique from many other types of tumor derived cell lines in that they express wildtype p53.

Comparable to studies in primary cultures, H₂O₂ induced neural death in SY5Y cells (Figures 15 and 16). Cell death was attenuated, but not abrogated by pifithrin in undifferentiated cells. Likewise, in differentiated SY5Y cells, H₂O₂ induced a dose dependent increase in cell death. Unlike differentiated cells, though, pifithrin blocked cell death induced by 10 μM H₂O₂ (Figure 16). There are two possible reasons for this. First, there could be differences in sensitivity of differentiated cells to pifithrin, compared to undifferentiated cells. This was not tested. Second, differentiated cells may have different pathways that are responsive to oxidative stress. There may be less of a role for p53 in oxidative stress in undifferentiated neural cells. Taken together the data in SY5Y cells demonstrated that p53 inhibition attenuates the cell death induced by application of H₂O₂.

To corroborate data obtained with pifithrin, genetic inhibition of p53 was utilized. In PC12 cells, the role of p53 transcriptional activity in response to oxidative stress was investigated. In control cells, treatment with pifithrin resulted in a dose dependent increase in cell death as determined by nuclear morphology and the MTT assay. Cells

that over expressed *ts* p53, which was deficient in DNA binding, were resistant to this cell death (Figures 16 and 17). This data suggests that p53 transcriptional activity has a role oxidative stress mediated neural death. This distinction is important because it shows that p53 was not acting in some other role besides as a transcriptional activator. There are studies that have investigated p53 dependent apoptosis in the absence of p53 transcriptional activity (Bates et al., 1999). For example, p53 interaction with anti-apoptotic members of the Bcl family has been shown to promote the release of cytochrome C from the mitochondrion (Mihara et al., 2003). There are also reports of p53 interacting with other proteins, such as DNA helicases, which are involved in DNA repair (Wang et al., 2001). It was found that p53 interacts with BLM, a DNA helicase that is implicated in Bloom syndrome, a disease characterized by cancer disposition. BLM interacted with the C-terminal region of p53. Disruption of this interaction results in alterations to BLM function and aberrant growth, whereas deletion of the p53 DNA binding domain did not. Hence, p53 but not p53 transcriptional activity was required for apoptosis. The *in vitro* studies I conducted don't support this type of interaction in p53 mediated apoptosis, as mutation of the DNA binding site attenuated cell death. If p53 mediated apoptosis was not dependent on transactivation of p53 dependent genes, but rather helicase –p53 interaction, then cell death would not have been reduced in *ts* p53 cells unless the helicase mediated event was dependent upon p53 interaction with DNA.

Taken together, the data presented in Chapter 3 suggests that p53 expression is altered following oxidative injury, and that p53 activity is increased. This latter point was determined indirectly, as no measurement of p53 mediated transcription, i.e. using p53

reporter assays, was made. However, in the presence of *ts* mutant p53 protein, cell death was largely abrogated. This data fits in well with other studies utilizing either pharmacological or antisense approaches to elucidation of the role of p53 in neural injury and death (Culmsee et al., 2001; Duan et al., 2002; Lakkaraju et al., 2001). In a chemical model of Parkinsonism, treatment of mice with the mitochondrial toxin 1,2,3,6-tetrahydropyridine (MPTP), results in the production of BAX, which is transcriptionally activated by p53, as well as mitochondrial dysfunction and oxidative stress (Bates et al., 1999; Duan et al., 2002; Morrison et al., 2000). BAX has been implicated in neuronal death in several studies. In mice pretreated with pifithrin, MPTP induced BAX production was decreased, and the mice had improved motor skills compared to mice treated with MPTP alone. This study clearly implicated a role for p53 in oxidative stress resulting from mitochondrial dysfunction (Duan et al., 2002). When treated with pifithrin, neurons were protected from apoptotic death induced by glutamate, amyloid-beta (a prime suspect in AD), and DNA damaging agents (Culmsee et al., 2001). Additionally, mitochondrial dysfunction in neurons was attenuated by pifithrin following treatment with these agents, and BAX production and p53 DNA binding activity were reduced. Taken together these data fit with the *in vitro* studies I conducted. Pifithrin attenuated cell death, suggesting that p53 was involved with H₂O₂ toxicity. This was true at all concentrations tested and it was also true for all models tested, in both primary neuronal cultures and human neural cell lines. These data were corroborated by the PC12 data where it was demonstrated that over expression of *ts* p53 attenuated cell death. Oxidative injury induced cell death and changes in p53 expression. These data support the hypothesis that p53 plays a direct role in mediating oxidative stress toxicity.

The details of p53 involvement remain to be worked out. Localization of p53 to the nucleus is almost assumed, due to the dependence of cell death on p53 transcriptional activity in primary neurons as well as in cell lines, but high resolution immunocytochemistry would be informative.

Cell death in both 56-GFP PC12 cells and H₂O₂ treated cells was almost completely blocked in cells expressing the mutant p53, indicating that neural death was mediated by p53 transcriptional activity.. Oxidative stress has been implicated in HD (Beal et al., 1994; Deckel et al., 2001; Schilling et al., 2001), and investigating the mechanism of death in 56-GFP cells would be appropriate. Taken together, the data demonstrates that p53 is altered in expression and activity in a model for oxidative stress. Neural death is attenuated by inhibition of p53 transcriptional activity. The possibility that a connection could be made between HD and p53 and oxidative stress is intriguing.

Constructing a model that describes all the interactions between p53, other proteins, and their effects on neural homeostasis would be a Herculean effort. However, a possible model of the interactions between polyglutamine expansion, oxidative stress, p53 and neural death associated with HD is presented in Figure 18. Oxidative stress can both induce and is induced by p53 activity. Huntingtin has been shown to interact with the mitochondrion. Mutant huntingtin has been shown to affect mitochondrial homeostasis, which over the lifetime of a neuron could make it more vulnerable to other stresses (Panov et al., 2002). Excitotoxic stress can also induce p53, and excitotoxicity has been suggested to have a role in HD

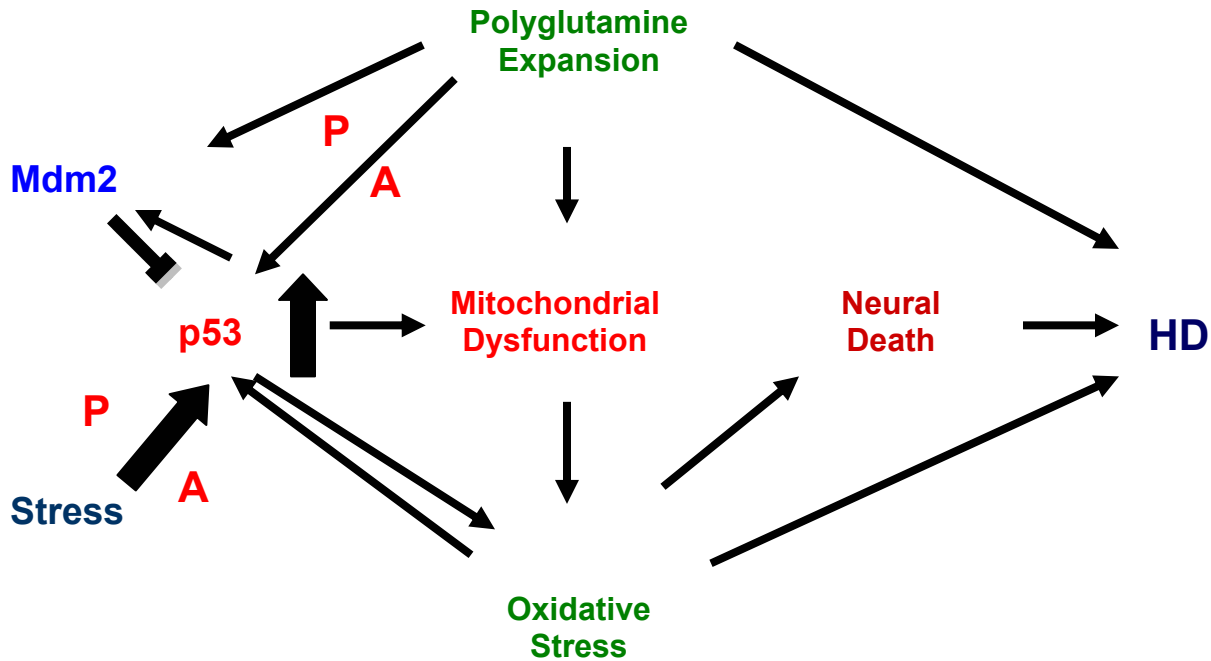


Figure 18. There are many possible routes to HD through p53.

Polyglutamine expansion, oxidative stress, and other stresses can activate p53 through upregulation or stabilization. Oxidative stress can occur by p53 dependent or independent means. p53 can be stabilized by inhibition of Mdm2. The activity of p53 can be increased through phosphorylation and/or acetylation of p53 (P,A). Mitochondrial dysfunction is associated with oxidative stress and is possibly induced by polyglutamine expansion. Oxidative stress eventually can lead to neural death.

(Sieradzan et al., 2001; Uberti et al., 1998). A direct role for p53 in HD is difficult to elucidate at this point. My studies have shown that p53 is stabilized in neural cells expressing polyglutamine-GFP fusion proteins. Levels of modified p53 were higher in cells expressing 56-GFP than in cells expressing 19-GFP. Mdm2 expression and nuclear localization was higher in 56-GFP cells, yet interaction with p53 was decreased compared to 19-GFP cells. Transient transfection of neural cells with 56-GFP induced neural death. This was attenuated by inhibition of p53 activity. Oxidative stress

mediated neurotoxicity was also attenuated by inhibition of p53 activity. These data together suggest a link between oxidative stress, p53, and polyglutamine neurotoxicity in an indirect way. Further investigations will need to be done to dissect these interactions so that a more complete model can be elucidated.

Future Directions

The role of p53 in polyglutamine disorders was not elucidated in these studies, though some qualified statements can be made about p53 biology in a variety of polyglutamine expansion models. Two hypotheses were tested in my studies. First, polyglutamine expansion induces alterations in p53 expression, stability, and activity. Second, p53 plays a direct role in mediating some aspects of oxidative stress, which is relevant to HD.

To investigate the first hypothesis, the expression of p53 and modified p53 was examined in 4 different models of HD, 2 *in vitro* and 2 *in vivo*. These were a human neural cell model utilizing different lengths of polyglutamine fused to GFP, a rodent neural cell model, the R6/2 mouse model, and human HD brain. The expression of p53 was altered in each of these models; however, the alterations investigated were different from model to model. Transcriptional activity of p53 was implicated in neural death induced by transient transfection of 56-GFP. Expression of p53 in 56-GFP cells was more stable than in 19-GFP cells, even in the absence of increased p53 mRNA in differentiated 56-GFP cells. In the absence of protein synthesis, p53 was more stable in 56-GFP cells, indicating that there could be less proteolysis of p53. Mdm2 has ubiquitin E3 ligase activity and is a potent negative regulator of p53. Mdm2-p53 interaction was

decreased in 56-GFP cells even though Mdm2 was up regulated and localized to the nucleus. Treatment of 19-GFP and 56-GFP cells with Mdm2 antisense oligonucleotides caused increased cell death in both 19-GFP and 56-GFP cells, but with much greater death in 56-GFP cells (Figure 6). I described differences between the polyglutamine-GFP constructs I used and those used in other investigations that implicated p53 in HD (Steffan et al., 2000; Suhr et al., 2001). Future investigations could look at modifications to p53 in an htt background and how mutant Htt may or may not affect p53 activity.

Future studies should investigate which pathways were induced in the PC12 cells by 56-GFP and mediated by p53. Assays for BAX or APAF-1 expression (mRNA or protein or both), both p53 mediated genes, could be utilized in the existing PC12 cells lines. In a chemical model of HD, p53 expression was found to be induced (Qin et al., 1999). To show that p53 mediated transcription was altered by oxidative stress, antioxidants could be used. If oxidative stress did induce p53, perhaps through DNA damage, then use of these reagents, such as coenzyme Q10, would attenuate production of these proteins. Another possibility would be to examine mitochondrial function in mutant p53 and control PC12 cells after transfection with 56-GFP. Mitochondrial membrane potential has been shown to be altered in lymphoblasts from HD patients (Panov et al., 2002), so it may be useful to tie dysregulation of mitochondria in with p53 in human HD lymphoblasts, or another cellular model of HD.

The expression of p53 protein was found to be altered in neural cells that were treated with H₂O₂. Whether this was due to induction of p53 or stabilization of p53 was

not determined. The role of Mdm2 was not investigated with regard to oxidative stress, but would need to be determined in future studies. Post-translational modifications would also be useful to study. These studies would reveal the regulatory elements of the p53 mediated response. As discussed for the 56-GFP cells and PC12 cells, H₂O₂ induced cell death could be studied in more detail with regard to p53. It is clear that p53 transcriptional activity had a role in H₂O₂ induced neural death. It would be useful to know the pathways .

Pharmacological inhibitors of p53 have been used in cancer research. The original study that described pifithrin found it to inhibit p53 dependent apoptosis following treatment with anti-cancer drugs or UV radiation (Komarov et al., 1999). Pifithrin has also been found to ameliorate some effects of neural injury and oxidative stress *in vitro* and *in vivo* (Culmsee et al., 2001; Duan et al., 2002). Quinolinic acid (QA), an NMDA receptor agonist, induces an HD like neuropathology, including striatal lesions in rats and man. QA has been shown to induce oxidative stress in animals and *in vitro* cell cultures that is alleviated by antioxidant therapy (Cabrera et al., 2000). QA was also found to induce p53 and p53 responsive genes in rats (Qin et al., 1999). Possible studies could investigate pifithrin mediated protection in QA treated rats. Pifithrin treatments could be given with and without antioxidant therapy. If some oxidative stress induced by QA is p53 independent, then there should be at least an additive effect with both treatments. Survival and behavioral studies could lead to estimations of the gross contribution of p53 to HD neurophysiology and neurophysiology. More detailed neuropathological examination could reveal other

facets of the role of p53 in this model of HD.. For example, there may be less neural death in animals treated with pifithrin.

Similar studies could be conducted using transgenic mice such as the R6/2. The data obtained in the present studies indicate less p53 protein expression in R 6/2 mice. However, this study did not address p53 activity *in vivo*. One way to do that would be the use of pifithrin. If p53 activity were important in disease pathology and/or physiology, then use of pifithrin may result in a decrease of neural atrophy observed in these animals. We have obtained very preliminary data in this area. Pifithrin treated R 6/2 mice did not survive as long as untreated R 6/2 mice. Interestingly, wild type mice treated with pifithrin lived and seemed unaffected. The mechanism of pifithrin mediated death in R 6/2 mice is unclear, but a recent paper by Komarova and colleagues demonstrated pifithrin mediated reductions in the heat shock response and glucocorticoid signaling in mice and *in vitro* (Komarova et al., 2003). They found that these reductions were p53 independent. Some connection has been made between the heat shock response, polyglutamine disorders, and glucocorticoid signaling have been made (Komarova et al., 2003). The data here is emerging, but perhaps with further investigation, we will be able to determine the cause of death in the R6/2 mice treated with pifithrin. An intriguing possibility is that pifithrin will help reveal beneficial activities of p53 in the HD mouse.

Chapter Five

Materials and Methods

Cell Culture

Cortical neurons were isolated from embryonic day 18 (E18) Sprague-Dawley rats as previously described (Keller et al., 1999). Briefly, brains were removed from the embryos, stripped of meninges, and rinsed with twice with minimal essential medium (MEM). Brains were incubated in 0.25% trypsin for 10 minutes at 37 °C. Dissociated brains were triturated with a 10 mL pipette, filtered through a 40 µm cell strainer, and brought to 50 mL with complete medium. Complete medium consisted of 60% medium A (Neurobasal medium supplemented with B-27 and 1% (v/v) penicillin/streptomycin) and 40% medium B (MEM supplemented 2% (v/v) fetal bovine serum (FBS), N2 supplement, and 1% (v/v) penicillin/streptomycin). Brains were then pelleted by centrifugation at 1000 x g for 5 minutes. Pelleted cells were then resuspended in complete medium at 1 brain/mL of medium. Cells were plated at appropriate density on 35 mm, 60 mm, or glass-bottom 35 mm dishes that had been previously coated with polyethyleneimine (PEI). After 2 hours, the medium was replaced with fresh complete medium. Experiments were conducted after 5 days incubation in a 5% CO₂ incubator (37 ° C) to allow neurons to achieve post-mitotic neuronal phenotype.

Neural SH-SY5Y cells were purchased from American Type Culture Collection (ATCC) and stably transfected with 19Q-GFP and 56Q-GFP constructs (Moulder et al., 1999), kindly provided by Dr. Eugene Johnson (Washington University), as previously described (Ding et al., 2002). Cells were maintained in a 5% CO₂ incubator (37 ° C) in MEM complete medium (Minimal Essential Medium, 10% (v/v) FBS, 1% (v/v)

penicillin/streptomycin, and 800 µg/mL G418). For wild-type cells, G418 was omitted from the medium. Cells were incubated for 5-7 days in retinoic acid, in order to differentiate into post-mitotic neuron phenotype. All cells utilized were of fewer than 15 passages. . For antisense treatment, cells were incubated with either MDM2 antisense (5'-GTT GGT ATT GCA CAT TTG CCT GCT C-3'; 50 nmol), or scrambled oligonucleotide (5'-TCT AAG TGT CGC TTA CTG GTC TGT C-3'; 50 nmol), and analyzed for MDM2 expression or cell death 24 hours following application.

PC12 cell lines were kindly provided by Dr. Kenneth E. Neet (Finch University of Health Sciences, Chicago Medical School). The cell line stably expressed a temperature sensitive (ts) mutant p53 engineered in the lab of Dr. Moshe Oren (Weizmann institute of Science, Rehovot, Israel) that is defective in DNA binding. The ts p53 gene was inserted into a retroviral vector under control of the cytomegalovirus promoter. The control cell line was stably transfected with retroviral vector without any insert (Barak et al., 1992; Hughes et al., 2000). At restrictive temperature, 39 (°C), mutant p53 is overexpressed, and acts a dominant negative, abolishing wildtype activity. Cells were maintained as described (Hughes et al., 2000). All experiments described were conducted at the restrictive temperature.

HD Mouse Model

The R6/2 transgenic mouse model was used. This mouse expresses mutant human huntingtin (htt) that contains ~1kb of human HD promoter region, exon 1 that carries the CAG-repeat expansion unit (Davies et al., 1997; Mangiarini et al., 1996). These mice have polyglutamine repeats of 141 to 157 repeats. They have a

progressive phenotype with disease onset at approximately eight weeks. Mice younger than this exhibit limited neuropathology, including decreased brain weight, and htt immunoreactivity in the nuclei of cortical brain and striatum. After age eight weeks, these mice develop the characteristic movement disorder of HD: resting tremor, abrupt shuddering movements, and seizure. They also develop a progressive neurophysiology and neuropathology including striatal nuclear inclusions, ubiquitin immunoreactivity, and nuclear membrane perturbances. However, this neuropathology is not as specific as in human HD. Degenerate, but not dead neurons, have been reported in both cortex and striatum (Turmaine et al., 2000). For experimentation, brains were removed from euthanized mice and immediately cooled on dry ice. Brains were stored at -80°C until use.

Human Tissue

Samples of caudate from HD brains and control brains (n=4) were obtained from the Harvard University Tissue Bank. Brains had a postmortem interval of 18-24 hours. Samples were stored at -80°C.

Analysis of Protein Expression

To study the stability of p53 in polyglutamine expressing cells, cells growing in 60mm dishes were treated with 10 µM cyclohexamide to halt protein synthesis. Cells were collected after 0, 5, 30, or 60 minutes, and p53 expression levels determined by Western blot analysis. Adherent cells were scraped and collected in ice-cold phosphate-buffered saline (PBS) - containing protease inhibitor mixture (Western blot

buffer), and phosphatase inhibitor as described previously (Ding et al., 2002). For analysis of brain tissue, a section was removed from the cortex (mouse) or caudate sample (human) and solubilized in Western blot buffer after being ground with a Dounce homogenizer. After determination of protein content, 50 µg of proteins were loaded onto a 7.5% polyacrylamide (50 µM Tris-HCl) gel and run at 100V for 60 minutes. Proteins were transferred to a nitrocellulose membrane at 100V for 60 minutes. After blocking at room temperature in 5 % nonfat milk in TBS –Tween -20, blots were incubated in primary antibody (1:1000) overnight at 4°C. Phosphorylated p53 was detected in SY5Y cells with an antibody that recognizes p53 phosphorylated on Serine 15.

Phosphorylated p53 was detected in lysates from mouse or human brain samples using an antibody that recognizes p53 phosphorylated on Serine 392. Acetylated p53 was detected in all models studied using an antibody that recognizes p53 acetylated on Lysine 320. Blots were washed in TBS-Tween-20 and then incubated in peroxidase conjugated secondary antibody (1:7500) for 1 hour. The blots were washed in TBS-Tween-20 and antibodies detected by Enhanced Chemiluminescence (ECL).

To determine relative expression levels, intensity of bands corresponding to the protein of interest were measured using Scion Image software. To determine percent control intensity, band intensities were divided by the intensity of the relevant control band (i.e. 0 hours) and that quotient multiplied by 100. To determine percent of Mdm2 interaction with p53, Mdm2 band intensities were divided by the intensity of the unprecipitated Mdm2 band (Non-IP from 56-GFP cells) and then divided by 4, as there was 4 times as much total protein that was immunoprecipitated as unprecipitated 56-GFP protein analyzed.

Immunoprecipitation

Neural SH-SY5Y, 19-GFP, or 56-GFP cells were collected in Western blot buffer. A total of 200 µg protein was suspended in 250 µL Western blot buffer and incubated for 2 hours with 3 µL precipitating antibody. A total of 25 µL of Fast Flow 4 Sepharose-Protein G beads was added to the suspension and then rocked at 4 °C overnight. All subsequent steps were conducted at 4°C. The beads were washed 3 times in Western blot buffer, and then resuspended in protein loading buffer. After boiling for 5 minutes, the beads were pelleted and proteins loaded on a 7.5% polyacrylamide gel. After electrophoresis, proteins were transferred to a nitrocellulose membrane and then blocked in 5 % nonfat milk (TBS- Tween-20) for 1 hour at room temperature. Blots were incubated overnight at 4°C with antibodies diluted 1:1000 in 5% nonfat milk (TBS- Tween-20). After washing 3 times in TBS-Tween-20, blots were incubated in secondary antibody (1:7500) for 1 hour at room temperature. After washing, proteins were detected by ECL.

Immunostaining

To analyze localization of the protein of interest, immunostaining was performed. Cells grown on glass bottom dishes were fixed with 4% paraformaldehyde in PBS (10 minutes), and permeabilized with TritonX - 100 (0.002%). After washing and blocking steps (2% Horse Serum (v/v) in TBS), cells were incubated with primary antibody (1:500) overnight. After further washing steps, were incubated with biotin-conjugated secondary antibody (1:500) for 1 hour. Following washing of the cells, and incubation

for 1 hour with ABC complex containing horseradish peroxidase, antibody was detected using DAB. Cells were visualized on a Zeiss Axiovert 200 microscope using bright-field and phase-contrast microscopy.

Analysis of Neural Death

To determine neural survival, nuclear morphology was assessed. In the last hour of treatment, cells were treated with 1 µg/mL Hoechst 33342 stain, and visualized fluorescently using a Zeiss Axiovert 200 microscope. Cells with fragmented or condensed nuclei were considered to be nonviable. At least 300 cells in at least 6 dishes were counted in each experiment and the percentage of nonviable cells determined. Cells with no treatment (i.e. empty vector or no treatment) served as control. Mean and standard error were determined for at least 6 cultures.

p53 binding activity and neurotoxicity

To analyze the role of p53 DNA binding activity in polyglutamine neurotoxicity, PC12 cells expressing a mutant p53 (*ts* p53) were utilized. Cells were plated on PEI coated glass-bottomed 35 mm dishes and maintained for 2 days in PC12 at 39 °C in a 5% CO₂ incubator. Cells were then transiently transfected with 6 µg of the 56-GFP plasmid as described previously (Ding et al., 2002). Briefly, plasmid DNA was suspended with Lipofectamine reagent in OptiMEM and incubated for 1.5 hours at room temperature. The medium was removed from the cells and replaced with OPTI-MEM prior to addition of plasmid DNA. The cells were incubated at 39°C for 4 hours and then

the medium replaced with DMEM complete medium. After 48 hours, cells were treated with Hoescht 33342 stain and their nuclei visualized using fluorescence microscopy. At least 300 cells in at least 6 dishes were counted in each experiment and the percentage of nonviable cells determined. Cells with mock treatment (i.e. incubated with OptiMEM alone) served as control.

Neural survival of PC12 cells was also analyzed by the MTT cell viability assay. The MTT assay measures the reduction of MTT [(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], which produces a blue-violet color. The ability of cells to reduce MTT is a measure of cell viability. Growing neural cells were incubated with 0.1 mg/mL MTT for 1 hour. After removal of medium, cells were lysed with dimethyl sulfoxide (DMSO), transferred to a 96 well plate and their absorbance read in duplicate at 595 nm on an absorbance plate reader. Cells with no treatment served as control and their mean absorbance set at 100% viability. Mean and standard error were determined for at least 6 cultures.

Statistical Analysis

For experiments where neural death or cell survival was quantified, the mean and standard error of the mean for each experimental group were calculated. Differences between experimental groups were considered significant when $p < 0.05$ as determined by the student's t-test.

References

- Alarcon-Vargas, D. and Ronai, Z. (2002). "p53-Mdm2--the affair that never ends." Carcinogenesis **23**(4): 541-547.
- Andreassen, O. A., Dedeoglu, A., Ferrante, R. J., Jenkins, B. G., Ferrante, K. L., Thomas, M., Friedlich, A., Browne, S. E., Schilling, G., Borchelt, D. R., Hersch, S. M., Ross, C. A. and Beal, M. F. (2001). "Creatine increase survival and delays motor symptoms in a transgenic animal model of Huntington's disease." Neurobiol Dis **8**(3): 479-491.
- Andreassen, O. A., Ferrante, R. J., Dedeoglu, A. and Beal, M. F. (2001). "Lipoic acid improves survival in transgenic mouse models of Huntington's disease." Neuroreport **12**(15): 3371-3373.
- Ashcroft, M. and Vousden, K. H. (1999). "Regulation of p53 stability." Oncogene **18**(53): 7637-7643.
- Ashcroft, M., Kubbutat, M. H. and Vousden, K. H. (1999). "Regulation of p53 function and stability by phosphorylation." Mol Cell Biol **19**(3): 1751-1758.
- Atlante, A., Calissano, P., Bobba, A., Giannattasio, S., Marra, E. and Passarella, S. (2001). "Glutamate neurotoxicity, oxidative stress and mitochondria." FEBS Letters **497**(1): 1-5.
- Balint, E. E. and Vousden, K. H. (2001). "Activation and activities of the p53 tumour suppressor protein." Br J Cancer **85**(12): 1813-1823.
- Barak, Y. and Oren, M. (1992). "Enhanced binding of a 95 kDa protein to p53 in cells undergoing p53-mediated growth arrest." Embo J **11**(6): 2115-2121.
- Barak, Y., Juven, T., Haffner, R. and Oren, M. (1993). "mdm2 expression is induced by wild type p53 activity." Embo J **12**(2): 461-468.
- Bargonetti, J. and Manfredi, J. J. (2002). "Multiple roles of the tumor suppressor p53." Curr Opin Oncol **14**(1): 86-91.
- Barlev, N. A., Liu, L., Chehab, N. H., Mansfield, K., Harris, K. G., Halazonetis, T. D. and Berger, S. L. (2001). "Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases." Mol Cell **8**(6): 1243-1254.
- Bates, S. and Vousden, K. H. (1999). "Mechanisms of p53-mediated apoptosis." Cell Mol Life Sci **55**(1): 28-37.

- Beal, M. F., Henshaw, D. R., Jenkins, B. G., Rosen, B. R. and Schulz, J. B. (1994). "Coenzyme Q10 and nicotinamide block striatal lesions produced by the mitochondrial toxin malonate." Ann Neurol **36**(6): 882-888.
- Beal, M. F. (1999). "Coenzyme Q10 administration and its potential for treatment of neurodegenerative diseases." Biofactors **9**(2-4): 261-266.
- Beal, M. F. (2002). "Coenzyme Q10 as a possible treatment for neurodegenerative diseases." Free Radic Res **36**(4): 455-460.
- Becher, M. W., Kotzok, J. A., Sharp, A. H., Davies, S. W., Bates, G. P., Price, D. L. and Ross, C. A. (1998). "Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidolusian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length." Neurobiol Dis **4**(6): 387-397.
- Blum, D., Wu, Y., Nissou, M. F., Arnaud, S., Alim Louis, B. and Verna, J. M. (1997). "p53 and Bax activation in 6-hydroxydopamine-induced apoptosis in PC12 cells." Brain Res **751**(1): 139-142.
- Bogdanov, M. B., Andreassen, O. A., Dedeoglu, A., Ferrante, R. J. and Beal, M. F. (2001). "Increased oxidative damage to DNA in a transgenic mouse model of Huntington's disease." J Neurochem **79**(6): 1246-1249.
- Brouillet, E., Conde, F., Beal, M. F. and Hantraye, P. (1999). "Replicating Huntington's disease phenotype in experimental animals." Prog Neurobiol **59**(5): 427-468.
- Browne, S. E., Bowling, A. C., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D. and Beal, M. F. (1997). "Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia." Ann Neurol **41**(5): 646-653.
- Browne, S. E., Ferrante, R. J. and Beal, M. F. (1999). "Oxidative stress in Huntington's disease." Brain Pathol **9**(1): 147-163.
- Butterfield, D. A., Castegna, A., Lauderback, C. M. and Drake, J. (2002). "Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death." Neurobiol Aging **23**(5): 655-664.
- Butterfield, D. A., Griffin, S., Munch, G. and Pasinetti, G. M. (2002). "Amyloid beta-peptide and amyloid pathology are central to the oxidative stress and inflammatory cascades under which Alzheimer's disease brain exists." J Alzheimers Dis **4**(3): 193-201.
- Cabrera, J., Reiter, R. J., Tan, D.-X., Qi, W., Sainz, R. M., Mayo, J. C., Garcia, J. J., Kim, S. J. and El-Sokkary, G. (2000). "Melatonin reduces oxidative neurotoxicity due to quinolinic acid: in vitro and in vivo findings." Neuropharmacology **39**(3): 507-514.

- Chan, H. M. and La Thangue, N. B. (2001). "p300/CBP proteins: HATs for transcriptional bridges and scaffolds." J Cell Sci **114**(Pt 13): 2363-2373.
- Cooper, J. K., Schilling, G., Peters, M. F., Herring, W. J., Sharp, A. H., Kaminsky, Z., Masone, J., Khan, F. A., Delanoy, M., Borchelt, D. R., Dawson, V. L., Dawson, T. M. and Ross, C. A. (1998). "Truncated N-terminal fragments of huntingtin with expanded glutamine repeats form nuclear and cytoplasmic aggregates in cell culture." Hum Mol Genet **7**(5): 783-790.
- Crawford, L. V., Pim, D. C., Gurney, E. G., Goodfellow, P. and Taylor-Papadimitriou, J. (1981). "Detection of a common feature in several human tumor cell lines--a 53,000-dalton protein." Proc Natl Acad Sci U S A **78**(1): 41-45.
- Culmsee, C., Zhu, X., Yu, Q. S., Chan, S. L., Camandola, S., Guo, Z., Greig, N. H. and Mattson, M. P. (2001). "A synthetic inhibitor of p53 protects neurons against death induced by ischemic and excitotoxic insults, and amyloid beta-peptide." J Neurochem **77**(1): 220-228.
- Cummings, C. J. and Zoghbi, H. Y. (2000). "Trinucleotide repeats: mechanisms and pathophysiology." Annu Rev Genomics Hum Genet **1**: 281-328.
- Curtis, M. A., Penney, E. B., Pearson, A. G., Van Roon-Mom, W. M., Butterworth, N. J., Dragunow, M., Connor, B. and Faull, R. L. (2003). "Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain." Proc Natl Acad Sci U S A.
- Datta, K., Babbar, P., Srivastava, T., Sinha, S. and Chattopadhyay, P. (2002). "p53 dependent apoptosis in glioma cell lines in response to hydrogen peroxide induced oxidative stress." Int J Biochem Cell Biol **34**(2): 148-157.
- Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L. and Bates, G. P. (1997). "Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation." Cell **90**(3): 537-548.
- Davies, S. W., Turmaine, M., Cozens, B. A., Raza, A. S., Mahal, A., Mangiarini, L. and Bates, G. P. (1999). "From neuronal inclusions to neurodegeneration: neuropathological investigation of a transgenic mouse model of Huntington's disease." Philos Trans R Soc Lond B Biol Sci **354**(1386): 981-989.
- de la Monte, S. M., Sohn, Y. K. and Wands, J. R. (1997). "Correlates of p53- and Fas (CD95)-mediated apoptosis in Alzheimer's disease." J Neurol Sci **152**(1): 73-83.
- de la Monte, S. M., Sohn, Y. K., Ganju, N. and Wands, J. R. (1998). "P53- and CD95-associated apoptosis in neurodegenerative diseases." Lab Invest **78**(4): 401-411.

- Deckel, A. W. (2001). "Nitric oxide and nitric oxide synthase in Huntington's disease." J Neurosci Res **64**(2): 99-107.
- Deckel, A. W., Gordinier, A., Nuttal, D., Tang, V., Kuwada, C., Freitas, R. and Gary, K. A. (2001). "Reduced activity and protein expression of NOS in R6/2 HD transgenic mice: effects of L-NAME on symptom progression." Brain Res **919**(1): 70-81.
- Deckel, A. W., Tang, V., Nuttal, D., Gary, K. and Elder, R. (2002). "Altered neuronal nitric oxide synthase expression contributes to disease progression in Huntington's disease transgenic mice." Brain Res **939**(1-2): 76-86.
- DeLeo, A. B., Jay, G., Appella, E., Dubois, G. C., Law, L. W. and Old, L. J. (1979). "Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse." Proc Natl Acad Sci U S A **76**(5): 2420-2424.
- DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P. and Aronin, N. (1997). "Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain." Science **277**(5334): 1990-1993.
- Ding, Q., Lewis, J. J., Strum, K. M., Dimayuga, E., Bruce-Keller, A. J., Dunn, J. C. and Keller, J. N. (2002). "Polyglutamine expansion, protein aggregation, proteasome activity, and neural survival." J Biol Chem **277**(16): 13935-13942.
- Duan, W., Zhu, X., Ladenheim, B., Yu, Q. S., Guo, Z., Oyler, J., Cutler, R. G., Cadet, J. L., Greig, N. H. and Mattson, M. P. (2002). "p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism." Ann Neurol **52**(5): 597-606.
- el-Deiry, W. S. (1998). "Regulation of p53 downstream genes." Semin Cancer Biol **8**(5): 345-357.
- Feigin, A. and Zgaljardic, D. (2002). "Recent advances in Huntington's disease: implications for experimental therapeutics." Curr Opin Neurol **15**(4): 483-489.
- Ferrante, R. J., Andreassen, O. A., Jenkins, B. G., Dedeoglu, A., Kuemmerle, S., Kubilus, J. K., Kaddurah-Daouk, R., Hersch, S. M. and Beal, M. F. (2000). "Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease." J Neurosci **20**(12): 4389-4397.
- Ferrante, R. J., Andreassen, O. A., Dedeoglu, A., Ferrante, K. L., Jenkins, B. G., Hersch, S. M. and Beal, M. F. (2002). "Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease." J Neurosci **22**(5): 1592-1599.
- Ferrer, I., Goutan, E., Marin, C., Rey, M. J. and Ribalta, T. (2000). "Brain-derived neurotrophic factor in Huntington disease." Brain Res **866**(1-2): 257-261.

- Flatt, P. M. and Pietsch, J. A. (2000). "Mechanisms of cell-cycle checkpoints: at the crossroads of carcinogenesis and drug discovery." Drug Metab Rev **32**(3-4): 283-305.
- Fleury, C., Mignotte, B. and Vayssiere, J. L. (2002). "Mitochondrial reactive oxygen species in cell death signaling." Biochimie **84**(2-3): 131-141.
- Fortin, A., Cregan, S. P., MacLaurin, J. G., Kushwaha, N., Hickman, E. S., Thompson, C. S., Hakim, A., Albert, P. R., Cecconi, F., Helin, K., Park, D. S. and Slack, R. S. (2001). "APAF1 is a key transcriptional target for p53 in the regulation of neuronal cell death." J Cell Biol **155**(2): 207-216.
- Freedman, D. A. and Levine, A. J. (1998). "Nuclear export is required for degradation of endogenous p53 by MDM2 and human papillomavirus E6." Mol Cell Biol **18**(12): 7288-7293.
- Freedman, D. A., Wu, L. and Levine, A. J. (1999). "Functions of the MDM2 oncoprotein." Cell Mol Life Sci **55**(1): 96-107.
- Freeland, K., Boxer, L. M. and Latchman, D. S. (2001). "The cyclic AMP response element in the Bcl-2 promoter confers inducibility by hypoxia in neuronal cells." Brain Res Mol Brain Res **92**(1-2): 98-106.
- Gao, C., Nakajima, T., Taya, Y. and Tsuchida, N. (1999). "Activation of p53 in MDM2-overexpressing cells through phosphorylation." Biochem Biophys Res Commun **264**(3): 860-864.
- Geller, H. M., Cheng, K. Y., Goldsmith, N. K., Romero, A. A., Zhang, A. L., Morris, E. J. and Grandison, L. (2001). "Oxidative stress mediates neuronal DNA damage and apoptosis in response to cytosine arabinoside." J Neurochem **78**(2): 265-275.
- Grossman, S. R., Perez, M., Kung, A. L., Joseph, M., Mansur, C., Xiao, Z. X., Kumar, S., Howley, P. M. and Livingston, D. M. (1998). "p300/MDM2 complexes participate in MDM2-mediated p53 degradation." Mol Cell **2**(4): 405-415.
- Grossman, S. R. (2001). "p300/CBP/p53 interaction and regulation of the p53 response." Eur J Biochem **268**(10): 2773-2778.
- Grossman, S. R., Deato, M. E., Brignone, C., Chan, H. M., Kung, A. L., Tagami, H., Nakatani, Y. and Livingston, D. M. (2003). "Polyubiquitination of p53 by a ubiquitin ligase activity of p300." Science **300**(5617): 342-344.
- Grunewald, T. and Beal, M. F. (1999). "Bioenergetics in Huntington's disease." Ann N Y Acad Sci **893**: 203-213.
- Gu, W. and Roeder, R. G. (1997). "Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain." Cell **90**(4): 595-606.

- Gutekunst, C. A., Li, S. H., Yi, H., Mulroy, J. S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R. J., Hersch, S. M. and Li, X. J. (1999). "Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology." J Neurosci **19**(7): 2522-2534.
- HDCRG (1993). "A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group." Cell **72**(6): 971-983.
- Heales, S. J. R. and Bolanos, J. P. (2002). "Impairment of brain mitochondrial function by reactive nitrogen species: the role of glutathione in dictating susceptibility." Neurochemistry International **40**(6): 469-474.
- Hickey, M. A. and Chesselet, M. F. (2003). "Apoptosis in Huntington's disease." Prog Neuropsychopharmacol Biol Psychiatry **27**(2): 255-265.
- Ho, L. W., Brown, R., Maxwell, M., Wytenbach, A. and Rubinsztein, D. C. (2001). "Wild type Huntingtin reduces the cellular toxicity of mutant Huntingtin in mammalian cell models of Huntington's disease." J Med Genet **38**(7): 450-452.
- Hodgson, J. G., Smith, D. J., McCutcheon, K., Koide, H. B., Nishiyama, K., Dinulos, M. B., Stevens, M. E., Bissada, N., Nasir, J., Kanazawa, I., Disteche, C. M., Rubin, E. M. and Hayden, M. R. (1996). "Human huntingtin derived from YAC transgenes compensates for loss of murine huntingtin by rescue of the embryonic lethal phenotype." Hum Mol Genet **5**(12): 1875-1885.
- Hodgson, J. G., Agopyan, N., Gutekunst, C. A., Leavitt, B. R., LePiane, F., Singaraja, R., Smith, D. J., Bissada, N., McCutcheon, K., Nasir, J., Jamot, L., Li, X. J., Stevens, M. E., Rosemond, E., Roder, J. C., Phillips, A. G., Rubin, E. M., Hersch, S. M. and Hayden, M. R. (1999). "A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration." Neuron **23**(1): 181-192.
- Hughes, A. L., Gollapudi, L., Sladek, T. L. and Neet, K. E. (2000). "Mediation of nerve growth factor-driven cell cycle arrest in PC12 cells by p53. Simultaneous differentiation and proliferation subsequent to p53 functional inactivation." J Biol Chem **275**(48): 37829-37837.
- Igarashi, S., Morita, H., Bennett, K. M., Tanaka, Y., Engelender, S., Peters, M. F., Cooper, J. K., Wood, J. D., Sawa, A. and Ross, C. A. (2003). "Inducible PC12 cell model of Huntington's disease shows toxicity and decreased histone acetylation." Neuroreport **14**(4): 565-568.
- Ito, A., Lai, C. H., Zhao, X., Saito, S., Hamilton, M. H., Appella, E. and Yao, T. P. (2001). "p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2." Embo J **20**(6): 1331-1340.

- Jana, N. R., Zemskov, E. A., Wang, G. and Nukina, N. (2001). "Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release." Hum Mol Genet **10**(10): 1049-1059.
- Jenner, P. (2003). "Oxidative stress in Parkinson's disease." Ann Neurol **53 Suppl 3**: S26-36; discussion S36-28.
- Jin, K., Mao, X. O., Simon, R. P. and Greenberg, D. A. (2001). "Cyclic AMP response element binding protein (CREB) and CREB binding protein (CBP) in global cerebral ischemia." J Mol Neurosci **16**(1): 49-56.
- Jin, Y., Zeng, S. X., Dai, M. S., Yang, X. J. and Lu, H. (2002). "MDM2 inhibits PCAF (p300/CREB-binding protein-associated factor)-mediated p53 acetylation." J Biol Chem **277**(34): 30838-30843.
- Juan, L. J., Shia, W. J., Chen, M. H., Yang, W. M., Seto, E., Lin, Y. S. and Wu, C. W. (2000). "Histone deacetylases specifically down-regulate p53-dependent gene activation." J Biol Chem **275**(27): 20436-20443.
- Kaya, S. S., Mahmood, A., Li, Y., Yavuz, E., Goksel, M. and Chopp, M. (1999). "Apoptosis and expression of p53 response proteins and cyclin D1 after cortical impact in rat brain." Brain Research **818**(1): 23-33.
- Keller, J. N., Kindy, M. S., Holtsberg, F. W., St Clair, D. K., Yen, H. C., Germeyer, A., Steiner, S. M., Bruce-Keller, A. J., Hutchins, J. B. and Mattson, M. P. (1998). "Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction." J Neurosci **18**(2): 687-697.
- Keller, J. N., Hanni, K. B., Mattson, M. P. and Markesbery, W. R. (1998). "Cyclic nucleotides attenuate lipid peroxidation-mediated neuron toxicity." Neuroreport **9**(16): 3731-3734.
- Keller, J. N., Hanni, K. B. and Markesbery, W. R. (1999). "4-hydroxynonenal increases neuronal susceptibility to oxidative stress." J Neurosci Res **58**(6): 823-830.
- Keller, J. N. and Glazner, G. W. (2001). "Mitochondrial Oxidative Stress and Metabolic Alterations in Neurodegenerative Disorders." Interorganellar Signaling in Age-Related Disease: 205-237.
- Kim, G. W. and Chan, P. H. (2002). "Involvement of superoxide in excitotoxicity and DNA fragmentation in striatal vulnerability in mice after treatment with the mitochondrial toxin, 3-nitropropionic acid." J Cereb Blood Flow Metab **22**(7): 798-809.

- Klein, J. A. and Ackerman, S. L. (2003). "Oxidative stress, cell cycle, and neurodegeneration." J. Clin. Invest. **111**(6): 785-793.
- Kobet, E., Zeng, X., Zhu, Y., Keller, D. and Lu, H. (2000). "MDM2 inhibits p300-mediated p53 acetylation and activation by forming a ternary complex with the two proteins." Proc Natl Acad Sci U S A **97**(23): 12547-12552.
- Komarov, P. G., Komarova, E. A., Kondratov, R. V., Christov-Tselkov, K., Coon, J. S., Chernov, M. V. and Gudkov, A. V. (1999). "A Chemical Inhibitor of p53 That Protects Mice from the Side Effects of Cancer Therapy." Science **285**(5434): 1733-1737.
- Komarova, E. A., Neznanov, N., Komarov, P. G., Chernov, M. V., Wang, K. and Gudkov, A. V. (2003). "p53 inhibitor pifithrin alpha can suppress heat shock and glucocorticoid signaling pathways." J Biol Chem **278**(18): 15465-15468.
- Koshy, B. T. and Zoghbi, H. Y. (1997). "The CAG/polyglutamine tract diseases: gene products and molecular pathogenesis." Brain Pathol **7**(3): 927-942.
- Kubbutat, M. H. and Vousden, K. H. (1998). "Keeping an old friend under control: regulation of p53 stability." Mol Med Today **4**(6): 250-256.
- Kubbutat, M. H., Ludwig, R. L., Ashcroft, M. and Vousden, K. H. (1998). "Regulation of Mdm2-directed degradation by the C terminus of p53." Mol Cell Biol **18**(10): 5690-5698.
- Lakkaraju, A., Dubinsky, J. M., Low, W. C. and Rahman, Y. E. (2001). "Neurons are protected from excitotoxic death by p53 antisense oligonucleotides delivered in anionic liposomes." J Biol Chem **276**(34): 32000-32007.
- Lane, D. P. and Crawford, L. V. (1979). "T antigen is bound to a host protein in SV40-transformed cells." Nature **278**(5701): 261-263.
- Leavitt, B. R., Guttman, J. A., Hodgson, J. G., Kimel, G. H., Singaraja, R., Vogl, A. W. and Hayden, M. R. (2001). "Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo." Am J Hum Genet **68**(2): 313-324.
- Li, H., Li, S. H., Yu, Z. X., Shelbourne, P. and Li, X. J. (2001). "Huntingtin aggregate-associated axonal degeneration is an early pathological event in Huntington's disease mice." J Neurosci **21**(21): 8473-8481.
- Li, M., Luo, J., Brooks, C. L. and Gu, W. (2002). "Acetylation of p53 inhibits its ubiquitination by Mdm2." J Biol Chem **277**(52): 50607-50611.
- Li, S. H., Cheng, A. L., Li, H. and Li, X. J. (1999). "Cellular defects and altered gene expression in PC12 cells stably expressing mutant huntingtin." J Neurosci **19**(13): 5159-5172.

- Li, S. H., Cheng, A. L., Zhou, H., Lam, S., Rao, M., Li, H. and Li, X. J. (2002). "Interaction of Huntington disease protein with transcriptional activator Sp1." Mol Cell Biol **22**(5): 1277-1287.
- Liang, S. H. and Clarke, M. F. (2001). "Regulation of p53 localization." Eur J Biochem **268**(10): 2779-2783.
- Liu, L., Scolnick, D. M., Trievel, R. C., Zhang, H. B., Marmorstein, R., Halazonetis, T. D. and Berger, S. L. (1999). "p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage." Mol Cell Biol **19**(2): 1202-1209.
- Luthi-Carter, R., Hanson, S. A., Strand, A. D., Bergstrom, D. A., Chun, W., Peters, N. L., Woods, A. M., Chan, E. Y., Kooperberg, C., Krainc, D., Young, A. B., Tapscott, S. J. and Olson, J. M. (2002). "Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain." Hum Mol Genet **11**(17): 1911-1926.
- Maacke, H., Kessler, A., Schmiegel, W., Roeder, C., Vogel, I., Deppert, W. and Kalthoff, H. (1997). "Overexpression of p53 protein during pancreatitis." Br J Cancer **75**(10): 1501-1504.
- Manfredi, J. J. (2003). "p53 and apoptosis: it's not just in the nucleus anymore." Mol Cell **11**(3): 552-554.
- Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trotter, Y., Leach, H., Davies, S. W. and Bates, G. P. (1996). "Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice." Cell **87**(3): 493-506.
- Mantamadiotis, T., Lemberger, T., Bleckmann, S. C., Kern, H., Kretz, O., Martin Villalba, A., Tronche, F., Kellendonk, C., Gau, D., Kapfhammer, J., Otto, C., Schmid, W. and Schutz, G. (2002). "Disruption of CREB function in brain leads to neurodegeneration." Nat Genet **31**(1): 47-54.
- Markesbery, W. R. (1997). "Oxidative stress hypothesis in Alzheimer's disease." Free Radic Biol Med **23**(1): 134-147.
- Marsh, J. L., Walker, H., Theisen, H., Zhu, Y. Z., Fielder, T., Purcell, J. and Thompson, L. M. (2000). "Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in Drosophila." Hum Mol Genet **9**(1): 13-25.
- Martin, L. J., Price, A. C., McClendon, K. B., Al-Abdulla, N. A., Subramaniam, J. R., Wong, P. C. and Liu, Z. (2003). "Early events of target deprivation/axotomy-induced neuronal apoptosis in vivo: oxidative stress, DNA damage, p53 phosphorylation and subcellular redistribution of death proteins." J Neurochem **85**(1): 234-247.

- Matthews, R. T., Yang, L., Jenkins, B. G., Ferrante, R. J., Rosen, B. R., Kaddurah-Daouk, R. and Beal, M. F. (1998). "Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease." J Neurosci **18**(1): 156-163.
- Mattson, M. P., Duan, W., Pedersen, W. A. and Culmsee, C. (2001). "Neurodegenerative disorders and ischemic brain diseases." Apoptosis **6**(1-2): 69-81.
- McCampbell, A., Taylor, J. P., Taye, A. A., Robitschek, J., Li, M., Walcott, J., Merry, D., Chai, Y., Paulson, H., Sobue, G. and Fischbeck, K. H. (2000). "CREB-binding protein sequestration by expanded polyglutamine." Hum Mol Genet **9**(14): 2197-2202.
- McCampbell, A., Taye, A. A., Whitty, L., Penney, E., Steffan, J. S. and Fischbeck, K. H. (2001). "Histone deacetylase inhibitors reduce polyglutamine toxicity." Proc Natl Acad Sci U S A **98**(26): 15179-15184.
- McLure, K. G. and Lee, P. W. (1998). "How p53 binds DNA as a tetramer." Embo J **17**(12): 3342-3350.
- Mecocci, P., Fano, G., Fulle, S., MacGarvey, U., Shinobu, L., Polidori, M. C., Cherubini, A., Vecchiet, J., Senin, U. and Beal, M. F. (1999). "Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle." Free Radic Biol Med **26**(3-4): 303-308.
- Michael, D. and Oren, M. (2003). "The p53-Mdm2 module and the ubiquitin system." Semin Cancer Biol **13**(1): 49-58.
- Mihara, M., Erster, S., Zaika, A., Petrenko, O., Chittenden, T., Pancoska, P. and Moll, U. M. (2003). "p53 has a direct apoptogenic role at the mitochondria." Mol Cell **11**(3): 577-590.
- Miller, F. D., Pozniak, C. D. and Walsh, G. S. (2000). "Neuronal life and death: an essential role for the p53 family." Cell Death Differ **7**(10): 880-888.
- Morrison, R. S. and Kinoshita, Y. (2000). "The role of p53 in neuronal cell death." Cell Death Differ **7**(10): 868-879.
- Moulder, K. L., Onodera, O., Burke, J. R., Strittmatter, W. J. and Johnson, E. M., Jr. (1999). "Generation of neuronal intranuclear inclusions by polyglutamine-GFP: analysis of inclusion clearance and toxicity as a function of polyglutamine length." J Neurosci **19**(2): 705-715.
- Naarding, P., Kremer, H. P. and Zitman, F. G. (2001). "Huntington's disease: a review of the literature on prevalence and treatment of neuropsychiatric phenomena." Eur Psychiatry **16**(8): 439-445.

- Napieralski, J. A., Raghupathi, R. and McIntosh, T. K. (1999). "The tumor-suppressor gene, p53, is induced in injured brain regions following experimental traumatic brain injury." Molecular Brain Research **71**(1): 78-86.
- Nucifora, F. C., Jr., Sasaki, M., Peters, M. F., Huang, H., Cooper, J. K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V. L., Dawson, T. M. and Ross, C. A. (2001). "Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity." Science **291**(5512): 2423-2428.
- Obrig, T. G., Culp, W. J., McKeehan, W. L. and Hardesty, B. (1971). "The mechanism by which cycloheximide and related glutarimide antibiotics inhibit peptide synthesis on reticulocyte ribosomes." J. Biol. Chem. **246**(1): 174-181.
- Oliner, J. D., Pietenpol, J. A., Thiagalingam, S., Gyuris, J., Kinzler, K. W. and Vogelstein, B. (1993). "Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53." Nature **362**(6423): 857-860.
- Ordway, J. M., Tallaksen-Greene, S., Gutekunst, C. A., Bernstein, E. M., Cearley, J. A., Wiener, H. W., Dure, L. S. t., Lindsey, R., Hersch, S. M., Jope, R. S., Albin, R. L. and Detloff, P. J. (1997). "Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse." Cell **91**(6): 753-763.
- Oren, M. and Rotter, V. (1999). "Introduction: p53--the first twenty years." Cell Mol Life Sci **55**(1): 9-11.
- Oren, M. (2003). "Decision making by p53: life, death and cancer." Cell Death Differ **10**(4): 431-442.
- Ouyang, Y. B., Carriedo, S. G. and Giffard, R. G. (2002). "Effect of Bcl-x(L) overexpression on reactive oxygen species, intracellular calcium, and mitochondrial membrane potential following injury in astrocytes." Free Radic Biol Med **33**(4): 544-551.
- Panov, A., Obertone, T., Bennett-Desmelik, J. and Greenamyre, J. T. (1999). "Ca(2+)-dependent permeability transition and complex I activity in lymphoblast mitochondria from normal individuals and patients with Huntington's or Alzheimer's disease." Ann N Y Acad Sci **893**: 365-368.
- Panov, A. V., Gutekunst, C. A., Leavitt, B. R., Hayden, M. R., Burke, J. R., Strittmatter, W. J. and Greenamyre, J. T. (2002). "Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines." Nat Neurosci **5**(8): 731-736.
- Panov, A. V., Burke, J. R., Strittmatter, W. J. and Greenamyre, J. T. (2003). "In vitro effects of polyglutamine tracts on Ca²⁺-dependent depolarization of rat and human mitochondria: relevance to Huntington's disease." Archives of Biochemistry and Biophysics **410**(1): 1-6.

- Perez-Severiano, F., Rios, C. and Segovia, J. (2000). "Striatal oxidative damage parallels the expression of a neurological phenotype in mice transgenic for the mutation of Huntington's disease." Brain Res **862**(1-2): 234-237.
- Perez-Severiano, F., Escalante, B., Vergara, P., Rios, C. and Segovia, J. (2002). "Age-dependent changes in nitric oxide synthase activity and protein expression in striata of mice transgenic for the Huntington's disease mutation." Brain Res **951**(1): 36-42.
- Polidori, M. C., Mecocci, P., Browne, S. E., Senin, U. and Beal, M. F. (1999). "Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex." Neurosci Lett **272**(1): 53-56.
- Prives, C. and Manley, J. L. (2001). "Why is p53 acetylated?" Cell **107**(7): 815-818.
- Qin, Z. H., Chen, R. W., Wang, Y., Nakai, M., Chuang, D. M. and Chase, T. N. (1999). "Nuclear factor kappaB nuclear translocation upregulates c-Myc and p53 expression during NMDA receptor-mediated apoptosis in rat striatum." J Neurosci **19**(10): 4023-4033.
- Reiter, R. J., Tan, D.-x. and Burkhardt, S. (2002). "Reactive oxygen and nitrogen species and cellular and organismal decline: amelioration with melatonin." Mechanisms of Ageing and Development **123**(8): 1007-1019.
- Rodriguez-Lopez, A. M., Xenaki, D., Eden, T. O., Hickman, J. A. and Chresta, C. M. (2001). "MDM2 mediated nuclear exclusion of p53 attenuates etoposide-induced apoptosis in neuroblastoma cells." Mol Pharmacol **59**(1): 135-143.
- Rubinsztein, D. C. (2002). "Lessons from animal models of Huntington's disease." Trends in Genetics **18**(4): 202-209.
- Schafer, T., Scheuer, C., Roemer, K., Menger, M. D. and Vollmar, B. (2003). "Inhibition of p53 protects liver tissue against endotoxin-induced apoptotic and necrotic cell death." Faseb J **17**(6): 660-667.
- Schilling, G., Coonfield, M. L., Ross, C. A. and Borchelt, D. R. (2001). "Coenzyme Q10 and remacemide hydrochloride ameliorate motor deficits in a Huntington's disease transgenic mouse model." Neurosci Lett **315**(3): 149-153.
- Scorrano, L. and Korsmeyer, S. J. (2003). "Mechanisms of cytochrome c release by proapoptotic BCL-2 family members." Biochem Biophys Res Commun **304**(3): 437-444.
- Shahbazian, M. D., Orr, H. T. and Zoghbi, H. Y. (2001). "Reduction of Purkinje cell pathology in SCA1 transgenic mice by p53 deletion." Neurobiol Dis **8**(6): 974-981.
- Shieh, S. Y., Ikeda, M., Taya, Y. and Prives, C. (1997). "DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2." Cell **91**(3): 325-334.

- Shieh, S. Y., Taya, Y. and Prives, C. (1999). "DNA damage-inducible phosphorylation of p53 at N-terminal sites including a novel site, Ser20, requires tetramerization." Embo J **18**(7): 1815-1823.
- Shieh, S. Y., Ahn, J., Tamai, K., Taya, Y. and Prives, C. (2000). "The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites." Genes Dev **14**(3): 289-300.
- Sieradzan, K. A. and Mann, D. M. (2001). "The selective vulnerability of nerve cells in Huntington's disease." Neuropathol Appl Neurobiol **27**(1): 1-21.
- Song, C., Perides, G. and Liu, Y. F. (2002). "Expression of full-length polyglutamine-expanded Huntingtin disrupts growth factor receptor signaling in rat pheochromocytoma (PC12) cells." J Biol Chem **277**(8): 6703-6707.
- Steffan, J. S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y. Z., Gohler, H., Wanker, E. E., Bates, G. P., Housman, D. E. and Thompson, L. M. (2000). "The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription." Proc Natl Acad Sci U S A **97**(12): 6763-6768.
- Steffan, J. S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M., Kurokawa, R., Housman, D. E., Jackson, G. R., Marsh, J. L. and Thompson, L. M. (2001). "Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*." Nature **413**(6857): 739-743.
- Suhr, S. T., Senut, M. C., Whitelegge, J. P., Faull, K. F., Cuizon, D. B. and Gage, F. H. (2001). "Identities of sequestered proteins in aggregates from cells with induced polyglutamine expression." J Cell Biol **153**(2): 283-294.
- Takahashi, H. (1999). "[Formation of neuronal inclusions and neurodegeneration: with special reference to dentatorubral-pallidolusian atrophy]." Rinsho Shinkeigaku **39**(12): 1279-1281.
- Taylor, J. P., Taye, A. A., Campbell, C., Kazemi-Esfarjani, P., Fischbeck, K. H. and Min, K. T. (2003). "Aberrant histone acetylation, altered transcription, and retinal degeneration in a *Drosophila* model of polyglutamine disease are rescued by CREB-binding protein." Genes Dev **17**(12): 1463-1468.
- Tkac, I., Keene, C. D., Pfeuffer, J., Low, W. C. and Gruetter, R. (2001). "Metabolic changes in quinolinic acid-lesioned rat striatum detected non-invasively by in vivo (1)H NMR spectroscopy." J Neurosci Res **66**(5): 891-898.
- Trettel, F., Rigamonti, D., Hilditch-Maguire, P., Wheeler, V. C., Sharp, A. H., Persichetti, F., Cattaneo, E. and MacDonald, M. E. (2000). "Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells." Hum Mol Genet **9**(19): 2799-2809.

- Tucholski, J., Lesort, M. and Johnson, G. V. (2001). "Tissue transglutaminase is essential for neurite outgrowth in human neuroblastoma SH-SY5Y cells." Neuroscience **102**(2): 481-491.
- Turmaine, M., Raza, A., Mahal, A., Mangiarini, L., Bates, G. P. and Davies, S. W. (2000). "Nonapoptotic neurodegeneration in a transgenic mouse model of Huntington's disease." Proc Natl Acad Sci U S A **97**(14): 8093-8097.
- Uberti, D., Belloni, M., Grilli, M., Spano, P. and Memo, M. (1998). "Induction of tumour-suppressor phosphoprotein p53 in the apoptosis of cultured rat cerebellar neurones triggered by excitatory amino acids." Eur J Neurosci **10**(1): 246-254.
- Uberti, D., Yavin, E., Gil, S., Ayasola, K. R., Goldfinger, N. and Rotter, V. (1999). "Hydrogen peroxide induces nuclear translocation of p53 and apoptosis in cells of oligodendroglia origin." Brain Res Mol Brain Res **65**(2): 167-175.
- Uberti, D., Grilli, M. and Memo, M. (2000). "Induction of p53 in the glutamate-induced cell death program." Amino Acids **19**(1): 253-261.
- van Lookeren Campagne, M. and Gill, R. (1998). "Tumor-suppressor p53 is expressed in proliferating and newly formed neurons of the embryonic and postnatal rat brain: comparison with expression of the cell cycle regulators p21Waf1/Cip1, p27Kip1, p57Kip2, p16Ink4a, cyclin G1, and the proto-oncogene Bax." J Comp Neurol **397**(2): 181-198.
- van Lookeren Campagne, M. and Gill, R. (1998). "Cell cycle-related gene expression in the adult rat brain: selective induction of cyclin G1 and p21WAF1/CIP1 in neurons following focal cerebral ischemia." Neuroscience **84**(4): 1097-1112.
- Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D. and Richardson, E. P., Jr. (1985). "Neuropathological classification of Huntington's disease." J Neuropathol Exp Neurol **44**(6): 559-577.
- Vousden, K. H. (2002). "Activation of the p53 tumor suppressor protein." Biochim Biophys Acta **1602**(1): 47-59.
- Wadgaonkar, R. and Collins, T. (1999). "Murine double minute (MDM2) blocks p53-coactivator interaction, a new mechanism for inhibition of p53-dependent gene expression." J Biol Chem **274**(20): 13760-13767.
- Wang, G. H., Mitsui, K., Kotliarova, S., Yamashita, A., Nagao, Y., Tokuhira, S., Iwatsubo, T., Kanazawa, I. and Nukina, N. (1999). "Caspase activation during apoptotic cell death induced by expanded polyglutamine in N2a cells." Neuroreport **10**(12): 2435-2438.
- Wang, X. W., Tseng, A., Ellis, N. A., Spillare, E. A., Linke, S. P., Robles, A. I., Seker, H., Yang, Q., Hu, P., Beresten, S., Bemmels, N. A., Garfield, S. and Harris, C. C. (2001).

- "Functional interaction of p53 and BLM DNA helicase in apoptosis." J Biol Chem **276**(35): 32948-32955.
- Wellington, C. L., Brinkman, R. R., O'Kusky, J. R. and Hayden, M. R. (1997). "Toward understanding the molecular pathology of Huntington's disease." Brain Pathol **7**(3): 979-1002.
- Winters, Z. E. (2002). "P53 pathways involving G2 checkpoint regulators and the role of their subcellular localisation." J R Coll Surg Edinb **47**(4): 591-598.
- Woods, D. B. and Vousden, K. H. (2001). "Regulation of p53 function." Exp Cell Res **264**(1): 56-66.
- Wytenbach, A., Swartz, J., Kita, H., Thykjaer, T., Carmichael, J., Bradley, J., Brown, R., Maxwell, M., Schapira, A., Orntoft, T. F., Kato, K. and Rubinsztein, D. C. (2001). "Polyglutamine expansions cause decreased CRE-mediated transcription and early gene expression changes prior to cell death in an inducible cell model of Huntington's disease." Hum Mol Genet **10**(17): 1829-1845.
- Wytenbach, A., Sauvageot, O., Carmichael, J., Diaz-Latoud, C., Arrigo, A. P. and Rubinsztein, D. C. (2002). "Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin." Hum Mol Genet **11**(9): 1137-1151.
- Xu, Y. (2003). "Regulation of p53 responses by post-translational modifications." Cell Death Differ **10**(4): 400-403.
- Yu, Z. X., Li, S. H., Nguyen, H. P. and Li, X. J. (2002). "Huntingtin inclusions do not deplete polyglutamine-containing transcription factors in HD mice." Hum Mol Genet **11**(8): 905-914.
- Zeitlin, S., Liu, J. P., Chapman, D. L., Papaioannou, V. E. and Efstratiadis, A. (1995). "Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue." Nat Genet **11**(2): 155-163.
- Zhang, Y. and Xiong, Y. (2001). "A p53 amino-terminal nuclear export signal inhibited by DNA damage-induced phosphorylation." Science **292**(5523): 1910-1915.
- Zhang, Y., McLaughlin, R., Goodyer, C. and LeBlanc, A. (2002). "Selective cytotoxicity of intracellular amyloid beta peptide1-42 through p53 and Bax in cultured primary human neurons." J Cell Biol **156**(3): 519-529.
- Zilfou, J. T., Hoffman, W. H., Sank, M., George, D. L. and Murphy, M. (2001). "The Corepressor mSin3a Interacts with the Proline-Rich Domain of p53 and Protects p53 from Proteasome-Mediated Degradation." Mol. Cell. Biol. **21**(12): 3974-3985.

Zimmermann, K. C., Bonzon, C. and Green, D. R. (2001). "The machinery of programmed cell death." Pharmacol Ther **92**(1): 57-70.

Zoghbi, H. Y. (1995). "Spinocerebellar ataxia type 1." Clin Neurosci **3**(1): 5-11.

Zoghbi, H. Y. and Orr, H. T. (2000). "Glutamine repeats and neurodegeneration." Annu Rev Neurosci **23**: 217-247.

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., Conti, L., MacDonald, M. E., Friedlander, R. M., Silani, V., Hayden, M. R., Timmusk, T., Sipione, S. and Cattaneo, E. (2001). "Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease." Science **293**(5529): 493-498.

Vita

Date of Birth

November 18, 1969

Place of Birth

Oakland, California, USA

Education

Bachelor's of Science, Biochemistry (1994)

University of Texas at Arlington

Tulane University, New Orleans, LA (1988)

Employment

Research Assistant (2002-2003)

Sanders-Brown Aging Center, University of Kentucky

Teaching Assistant (1998-2002)

Department of Biology, University of Kentucky

Analytical Chemist (1997-1998)

Swedish Match, North America, Owensboro, Kentucky

Science Teacher (1996-1997)

Owensboro Catholic High School, Owensboro, Kentucky

Research Technician (1994-1995)

University of Texas Southwestern Medical Center, Dallas, Texas

Publications

Ding, Q., Lewis, J. J., Strum, K. M., Dimayuga, E., Bruce-Keller, A. J., **Dunn, J. C.** and Keller, J. N. (2002). "Polyglutamine expansion, protein aggregation, proteasome activity, and neural survival." J Biol Chem **277**(16): 13935-13942.

Ding, Q., Reinacker, K., Dimayuga, E., Nukala, V., Drake, J., Butterfield, D. A., **Dunn, J. C.**, Martin, S., Bruce-Keller, A. J. and Keller, J. N. (2003). "Role of the proteasome in protein oxidation and neural viability following low-level oxidative stress." FEBS Lett **546**(2-3): 228-232.