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Genetic interaction between expanded murine *Hdh* alleles and *p53* reveal deleterious effects of p53 on Huntington's disease pathogenesis

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Huntingtin, the protein product of the Huntington's disease (HD) gene, is known to interact with the tumor suppressor p53. It has recently been shown that activation of p53 upregulates the level of huntingtin, both in vitro and in vivo, whereas p53 deficiency in HD-transgenic flies and mice has been found to be beneficial. To explore further the involvement of p53 in HD pathogenesis, we generated mice homozygous for a mutant allele of *Hdh* (*Hdh*^{Q140}) and with zero, one, or two functional alleles of p53. p53 deficiency resulted in a reduction of mutant huntingtin expression in brain and testis, an increase in proenkephalin mRNA expression and a significant increase in nuclear aggregate formation in the striatum. Because aggregation of mutant huntingtin is suggested to be a protective mechanism, both the increase in aggregate load and the restoration of proenkephalin expression suggest a functional rescue of at least several aspects of the HD phenotype by a deficiency in p53.

Keywords: Aging; Mouse model; Polyglutamine; Neurodegeneration; Proenkephalin; Transcription

Introduction

The sequence-specific transcription factor, p53, is a stress-induced regulator of cell function that has differing activities depending on p53 protein level, the inducing agent, and the cell type. p53 is also involved in lifespan regulation (Tyner et al., 2002; Maier et al., 2004), cell cycle control (Levine, 1997), DNA repair, and apoptosis (Vogelstein et al., 2000; Gomez-Lazaro et al., 2004). Because p53 is involved in cell death, it has the potential for playing a key role in the progression of degenerative diseases, and its role has been studied in some known disorders. In the neurodegenerative disorder spinal muscular atrophy (SMA), the normal interaction between SMN protein and p53 is reduced in the presence of *SMN1* gene mutations, and this results in enhanced disease severity (Young et al., 2002). Deletion of *p53* in a mouse model of the polyglutamine repeat disorder spinocerebellar ataxia 1 (SCA1) has been found to rescue Purkinje cell pathology and slow disease progression (Shahbazian et al., 2001). Most recently, p53 has been found to affect the phenotype of experimental models of the neurodegenerative disorder, Huntington's disease (HD) (Bae et al., 2005). The connection between p53 and HD is particularly striking in light of the fact that mice nullizygous for *p53* display variable expressivity of brain lesions very similar to those identified in *Hdh*-nullizygous mouse brain. Neurodegeneration of the basal ganglia comparable to that seen in *Hdh*$^{-/-}$ brain (Dragatsis et al., 2000) has been observed in approximately 25% of *p53*$^{-/-}$ mice (Amson et al., 2000).

HD is a progressive, autosomal dominant neurological disease that results from expansion of a polyglutamine (CAG) stretch located within the huntingtin protein (htt) encoded by the *HD* gene. Although htt exhibits widespread distribution in both the brain and peripheral tissues, the GABAergic medium spiny neurons of the striatum and the large pyramidal neurons in layers III, V, and VI of the cerebral cortex undergo preferential degeneration (Sharp et al., 1995; Ho et al., 2001; Sieradzan and Mann, 2001). The abundance of huntingtin protein does not appear to confer vulnerability on striatal neurons; rather, the expression of mutant htt in corticostriatal neurons seems to render them destructive to the striatal neurons that they innervate (Fusco et al., 1999). Both prior and subsequent to neuronal death, the indirect pathway of basal ganglia output is affected most severely, resulting in an increase in involuntary movements associated with loss of proenkephalin expression. On a molecular level, intraneuronal inclusions that contain an amino-terminal fragment of mutant htt have been identified in the brains of both human HD patients and HD animal models (DiFiglia et al., 1997; Ho et al., 2001). Inclusion formation does not directly correlate with increased neuronal vulnerability, as striatal neurons have been shown to form fewer inclusions than cortical neurons (Meade et al., 2002). However, within the striatum, vulnerable neuronal subtypes do form more inclusions than unaffected neuronal subtypes, and they do so at an earlier age (Meade et al., 2002). The consequences of inclusion formation have been unclear, with earlier reports suggesting a pathological function of inclusions in HD progression (Hackam et al., 1999). However, other groups have found that aggregate inhibition is detrimental to the cell (Saudou et al., 1998) and that formation of aggregates can actually predict improved survival and lead to decreased levels of mutant htt

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elsewhere in neurons (Arrasate et al., 2004). Furthermore, the "shortstop" mouse model of HD, which expresses an N-terminal huntingtin fragment containing ~120 CAG repeats, develops widespread inclusions but displays no evidence of neuronal dysfunction or degeneration (Slow et al., 2005). In fact, when inclusion formation in cellular models of HD and concomitantly prevents was amplified by pharmalogical means, huntingtin-mediated proteasome dysfunction was blocked (Bodner et al., 2006).

As described above, the involvement of p53 in the pathology of neurodegenerative diseases, including HD, has been established, and a deficiency in *p53* can result in neurological deficits and apoptotic brain lesions similar to those identified in *Hdh*-nullizygous mouse brain (Amson et al., 2000; Dragatsis et al., 2000). Together, this suggested to us that *p53* and *Hdh* might interact functionally, and that the modification of *p53* status on an HD background might result in a change of HD phenotype. This hypothesis is supported by recent work in which the activation of p53 in cultured cells was found to induce huntingtin transcription, and γ-irradiation of mice was found to upregulate the level of wild-type htt protein in a p53-dependent manner (Feng et al., 2006). Therefore, we examined the genetic interaction between *p53* and *Hdh* using tissues and mouse embryonic fibroblasts (MEFs) derived from a knock-in mouse model of HD expressing 140 glutamine repeats (Menalled et al., 2003) and with zero, one, or two functional copies of *p53*.

Materials and methods

Animals

All animal experiments were conducted in accordance with the ethical guidelines described in *Guide for the Care and Use of Laboratory Animals* by the National Research Council and reviewed and approved by the Animal Care and Use Committee of the University of Virginia. The $Hdh^{Q140/Q140}$ mice were generated using homologous recombination in ES cells and have been described previously (Menalled et al., 2003). Mice containing a floxed *Hdh* allele in the endogenous *Hdh* locus were generated using homologous recombination in ES cells and have also been described previously (Dragatsis et al., 2000). $p53^{-/-}$ mice (C57Bl/6) were obtained from Taconic Laboratories (Hudson, NY).

Generation of huntingtin reporter constructs

A mouse *Hdh* promoter fragment was obtained by digesting a targeting vector containing a 5′ genomic piece (4.5 kb) of mouse *Hdh* sequence (previously described in Zeitlin et al., 1995) with *Hin*dIII and *Alw*NI to yield an ~1500-bp fragment. The ends of the 1500-bp promoter fragment were filled in with Klenow, and then cloned into the *Sma*I site of the *p*GL3-Basic vector (Promega, Madison, WI), yielding *p*Hdhluc. Orientation was verified by analytical restriction enzyme digest. The generation of the human *p*HDluc reporter construct has been described previously (Ryan and Scrable, 2004).

Assay of huntingtin promoter activity

$p53^{+/+}$ and $p53^{-/-}$ mouse embryonic fibroblasts (MEFs) were prepared as described previously (Maier et al., 2004). MEFs were transiently transfected using Lipofectamine reagent (Invitrogen, Carlsbad, CA) with the *p*HDluc or *p*Hdhluc reporter construct, varying doses of p53 expression vector, and the *p*RL-TK vector (Promega, Madison, WI) to correct for transfection efficiency. Twenty-four hours following transfection, cells were treated with Dulbecco minimal essential medium (DMEM) (Gibco, Carlsbad, CA) supplemented with 1% fetal calf serum for 24 h, after which cells were lysed and assayed for luciferase activity using the Dual-Luciferase Reporter Assay System protocol (Promega, Madison, WI).

Analysis of huntingtin protein in transfected MEFs and in brain and peripheral tissues

$p53^{+/+}$ and $p53^{-/-}$ MEFs were transiently transfected using Lipofectamine reagent (Invitrogen, Carlsbad, CA) with varying doses of p53 expression vector. Twenty-four hours following transfection, cells were treated with DMEM (Gibco, Carlsbad, CA) supplemented with 1% fetal calf serum for 24 h, after which cells were serum deprived for 3 h in the presence of 0.5 μM adriamycin, 0.5 μM Trichostatin A, and 5 mM nicotinamide. Protein extracts were prepared with RIPA lysis buffer supplemented with protease inhibitors (Complete Mini protease inhibitor cocktail tablets, Roche, Indianapolis, IN). $Hdh^{Q140/Q140}$, $Hdh^{Q140/+}$, and $Hdh^{+/+}$ animals were generated having zero, one, or two p53 alleles. Tissues were dissected, and protein extracts were prepared with RIPA lysis buffer supplemented with protease inhibitors (Roche, Indianapolis, IN). Western blots were generated using standard procedures. Monoclonal anti-huntingtin antibody 2166 (Chemicon, Temecula, CA) was used at a 1:1000 dilution to detect total huntingtin protein. Antigen–antibody complexes were further reacted with HRP-conjugated secondary antibody (Jackson Immuno-Research Laboratories, West Grove, PA) and detected by chemiluminescence (Super-Signal Pico reagent, Pierce, Rockford, IL). The blots were stripped and re-reacted with monoclonal anti-GAPDH antibody (1:5000 dilution, Ambion, Austin, TX) or monoclonal anti-actin antibody (1:10000 dilution, MP Biomedicals Inc., Solon, OH) to normalize the signals. Signals were quantified by laser densitometry on a Molecular Dynamics (Sunnyvale, CA) instrument running Image-Quant software (GE Healthcare, Pittsburgh, PA).

Immunohistochemistry

Whole brains were fresh frozen in methylbutane pre-chilled on dry ice, and mounted in OCT for cryosectioning. Brains were sectioned into 25 μm slices, fixed for 15 min in 4% paraformaldehyde, washed three times in PBS containing 0.05% TritonX-100 (PBST), and blocked in PBST containing 10% heat-inactivated donkey serum for 30 min at room temperature. Monoclonal anti-huntingtin antibody EM48 (#MAB5374, Chemicon, Temecula, CA) was used at a 1:100 dilution to detect aggregated huntingtin protein. Primary antibody was diluted in blocking solution, and sections were incubated overnight at 4°C. Sections were washed 3 times in PBST containing 1% heat-inactivated donkey serum, and incubated with donkey anti-mouse Alexa Fluor 568 secondary antibody (diluted 1:300, Molecular Probes, Carlsbad, CA) for 1 h at room temperature. After 3 washes in PBST, sections were counterstained with DAPI, treated with Autofluorescence Eliminator Reagent per manufacturer's protocol (#2160, Chemicon, Temecula, CA), and mounted. Sections were visualized using a Zeiss Axiophot fluorescence microscope. Fluorescence images were captured using a Sony DSC-S7 digital camera and processed using Adobe Photoshop (Adobe Systems Inc., San Jose, CA).

Northern blot analysis

Brain tissue was harvested from animals of the desired genotypes, and whole-brain RNA extracted using Tri-Reagent (Molecular Research Center, Cincinnati, OH). Northern blotting and hybridizations were performed as described previously (Cronin et al., 2001).

Results

The level of huntingtin protein is lower in mice deficient in p53

The phenocopy of $Hdh^{-/-}$ brain lesions in a significant fraction of $p53^{-/-}$ mice suggested to us that p53 and htt might lie in a shared pathway that protects neuronal lifespan, and that loss of one or the other could lead to neurodegeneration. The fact that neurodegeneration is 100% penetrant in $Hdh^{-/-}$ mice but only ~25% penetrant in $p53^{-/-}$ mice, furthermore, suggested that p53 lies upstream of htt in this pathway. According to this model, when loss of p53 results in loss of htt, neurodegeneration occurs. To determine if p53 controls the level of htt, we compared htt levels in mice with normal or expanded (Q140) alleles of *Hdh* having zero, one, or two functional p53 alleles. Testis, liver, and total brain tissues were harvested, and the cortex and the core region of the striatum were dissected from the total brain. The level of htt protein in each sample was determined using Western blot analysis. As shown in Fig. 1, increasing the dose of p53 *in vivo* leads to a pronounced increase in the level of both mutant and wild-type htt protein in the total brain (Fig. 1A), as well as in isolated cortex (Fig. 1B) and striatum (Fig. 1C), and the testis (Fig. 1D). Testis and brain are reported to have the highest level of htt expression (Sharp et al., 1995), and the brain is the most affected tissue in HD. In liver, an unaffected tissue where htt expression is lower, this trend was not apparent (Fig. 1E).

The absence of p53 cannot rescue $Hdh^{-/-}$ embryonic lethality

If p53 is an upstream regulator of htt, then loss of p53 should have no effect on the phenotype of mice deficient in *Hdh*. Mice deficient in *Hdh* display embryonic lethality (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995), and this trait is fully penetrant. To verify that loss of p53 could not rescue embryonic lethality of $Hdh^{-/-}$ embryos, we intercrossed animals heterozygous for *Hdh* and nullizygous for *p53* and genotyped the progeny. Of the 29 progeny born to these crosses, 12 were $Hdh^{+/+}p53^{-/-}$, 17 were $Hdh^{+/-}p53^{-/-}$, and 0 were $Hdh^{-/-}p53^{-/-}$.

The murine and human huntingtin promoters contain putative p53 consensus sequences

Because p53 is a sequence-specific transcription factor, one mechanism by which p53 could modify htt protein level is by transactivating the huntingtin promoter. To identify potential p53 response elements in the huntingtin promoter, we analyzed the proximal 1500 bp region of the murine *Hdh* promoter region using Promolign (Zhao et al., 2004). A half consensus sequence corresponding to the p53 response element was identified ~1285 bp upstream from translation start (Fig. 2A; consensus sequence shaded in grey). Likewise, we analyzed the proximal 800 bp region of the human *HD* promoter region using Promolign, and a half consensus sequence corresponding to the p53 response element was identified ~630 bp upstream of translation start (Fig. 2C; consensus sequence shaded in grey). We also found an additional half-consensus sequence ~1200 bp upstream of translation start (not shown).

p53 modulates HD promoter activity

To determine if the murine and human htt promoters are transactivated by p53, we generated reporter constructs in which luciferase is driven by the ~1500-bp *Hdh* promoter (*Hdh*luc; depicted in Fig. 2B) or the ~800-bp *HD* promoter (*HD*luc; depicted in Fig. 2D). These promoter fragments were chosen due to the availability of restriction enzyme sites, and because they contained the p53-consensus sequences that were of interest (shaded in gray in Figs. 2A and C). The *HD*luc and *Hdh*luc reporter constructs were co-transfected with increasing doses of p53 expression vector into $p53^{+/+}$ and $p53^{-/-}$ mouse embryonic fibroblasts (MEFs) and htt promoter activation was determined by assaying luciferase activity. As shown in Fig. 2, the level of *Hdh*luc and *HD*luc activity in $p53^{-/-}$ MEFs is less then that in $p53^{+/+}$ MEFs. Transfection of additional p53 into $p53^{+/+}$ MEFs results in an enhancement of *Hdh*luc and *HD*luc activity (Fig. 2F), whereas transfection of p53 into $p53^{-/-}$ MEFs increased activity to levels close to that seen in $p53^{+/+}$ MEFs (Fig. 2E).

p53 modulates the level of huntingtin protein

To determine if modification of *Hdh* transactivation by p53 results in an alteration of htt protein level, we transfected $p53^{+/+}$ and $p53^{-/-}$ MEFs with 0, 1.0, 1.5, or 2.0 μg of p53 and determined the level of huntingtin protein using western blot analysis. As shown in Fig. 2G, the level of htt protein in $p53^{-/-}$ MEFs is less then that in $p53^{+/+}$ MEFs. Transfection of additional p53 into $p53^{+/+}$ MEFs results in an increase in htt protein level, whereas transfection of p53 into $p53^{-/-}$ MEFs increases htt protein level to levels close to that seen in $p53^{+/+}$ MEFs.

$Hdh^{Q140/Q140}p53^{-/-}$ mice are viable and are born with a normal Mendelian distribution

The fact that p53 had the same effect on mutant and normal huntingtin protein levels meant that p53 could affect the phenotype of mice with mutant *Hdh* alleles in either the positive or the negative direction. In other neurodegenerative diseases, p53 has been shown to be beneficial (such as in SMA) or deleterious (such as in SCA1). To determine how p53 might contribute to HD pathogenesis, we compared the phenotype of mice with two normal *Hdh* alleles to that of mice in which one or both had been replaced by the mutant allele with an expanded polyglutamine tract containing 140 repeats (Hdh^{Q140}). This mouse model of HD recreates the human disease in an accurate and physiologically relevant way (Menalled et al., 2003). If the role of p53 was to promote HD pathogenesis, then deletion of p53 would rescue aspects of the HD phenotype. However, if p53 was protective and delayed the progression of HD pathogenesis, then deletion of p53 would exacerbate the HD phenotype. To distinguish between these possibilities, we performed two different crosses to generate $Hdh^{Q140/Q140}$ animals that were nullizygous for *p53*. In the first cross, we generated 24 progeny by intercrossing animals heterozygous for mutant *Hdh* and nullizygous for *p53*. We predicted that, if they followed a normal Mendelian distribution, 6 would be $Hdh^{Q140/Q140}p53^{-/-}$. The actual distribution of progeny was 5 $Hdh^{+/+}p53^{-/-}$ pups, 14 $Hdh^{Q140/+}p53^{-/-}$ pups, and

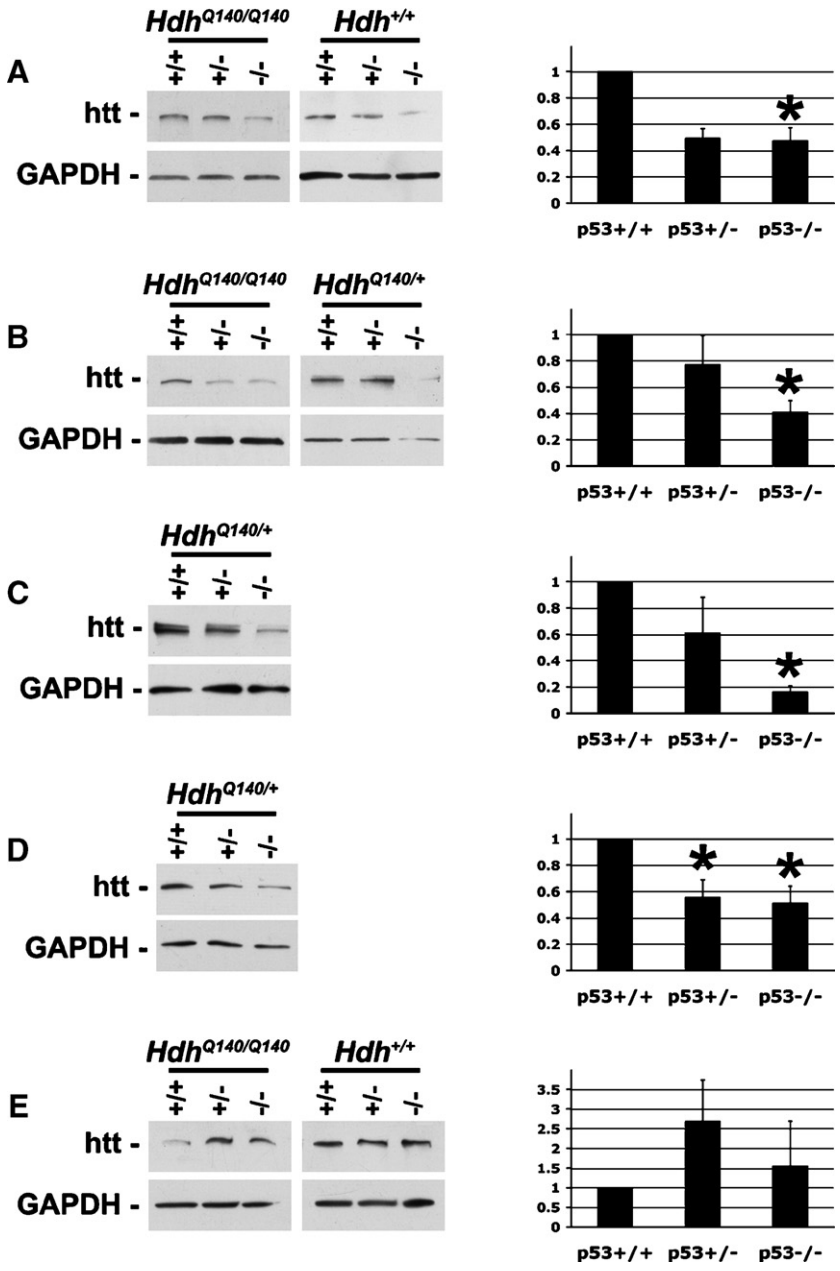

Fig. 1. The level of huntingtin protein is lower in mice deficient in p53. Western blot of htt protein in total brain (A), striatum (B), cortex (C), testis (D), and liver (E) dissected from animals ranging from 2 to 7 months of age. Blots were stripped and reanalyzed with anti-GAPDH or anti-actin antibody, and densitometry was performed. One representative western blot for each tissue is shown. Densitometry values derived from at least three independent experiments were averaged, and the results are graphed in the histograms in the right column. As the same dose-responsive trend was apparent in $Hdh^{Q140/Q140}$, $Hdh^{Q140/+}$, and $Hdh^{+/+}$ animals, densitometry values were pooled within each p53 genotype; for example $Hdh^{Q140/Q140}p53^{-/-}$, $Hdh^{Q140/+}p53^{-/-}$, and $Hdh^{+/+}p53^{-/-}$ were pooled under the $p53^{-/-}$ category in the histograms. Error bars = SEM. *Statistically different from $p53^{+/+}$ at $p < 0.05$.

5 $Hdh^{Q140/Q140}p53^{-/-}$ pups. In the second cross, we used $p53^{-/-}$ mice and mated animals heterozygous for mutant *Hdh* with animals homozygous for mutant *Hdh*. 49 progeny were born, of which 25 were $Hdh^{Q140/+}p53^{-/-}$ pups and 24 were $Hdh^{Q140/Q140}p53^{-/-}$ pups.

Loss of p53 partially rescues low proenkephalin mRNA levels in Hdh $^{Q140/Q140}$ mice

If p53 promotes HD pathogenesis, we would predict that animals nullizygous for p53 would present with a less severe HD phenotype. To test this, we examined the levels of the messenger RNA encoding the neurotransmitter proenkephalin. Analysis has shown that proenkephalin mRNA levels are reduced even in early stage HD patients and presymptomatic carriers (Albin et al., 1991), and this reduction is also seen in many HD animal models (Albin et al., 1991; Menalled et al., 2000). To determine if altering p53 status would rescue the levels of proenkephalin mRNA in our model of HD, we performed northern blot analysis on brain tissue taken from control, $p53^{-/-}$, $Hdh^{Q140/Q140}$, and $Hdh^{Q140/Q140}p53^{-/-}$ mice. As shown in Fig. 3A, whereas the levels of proenkephalin

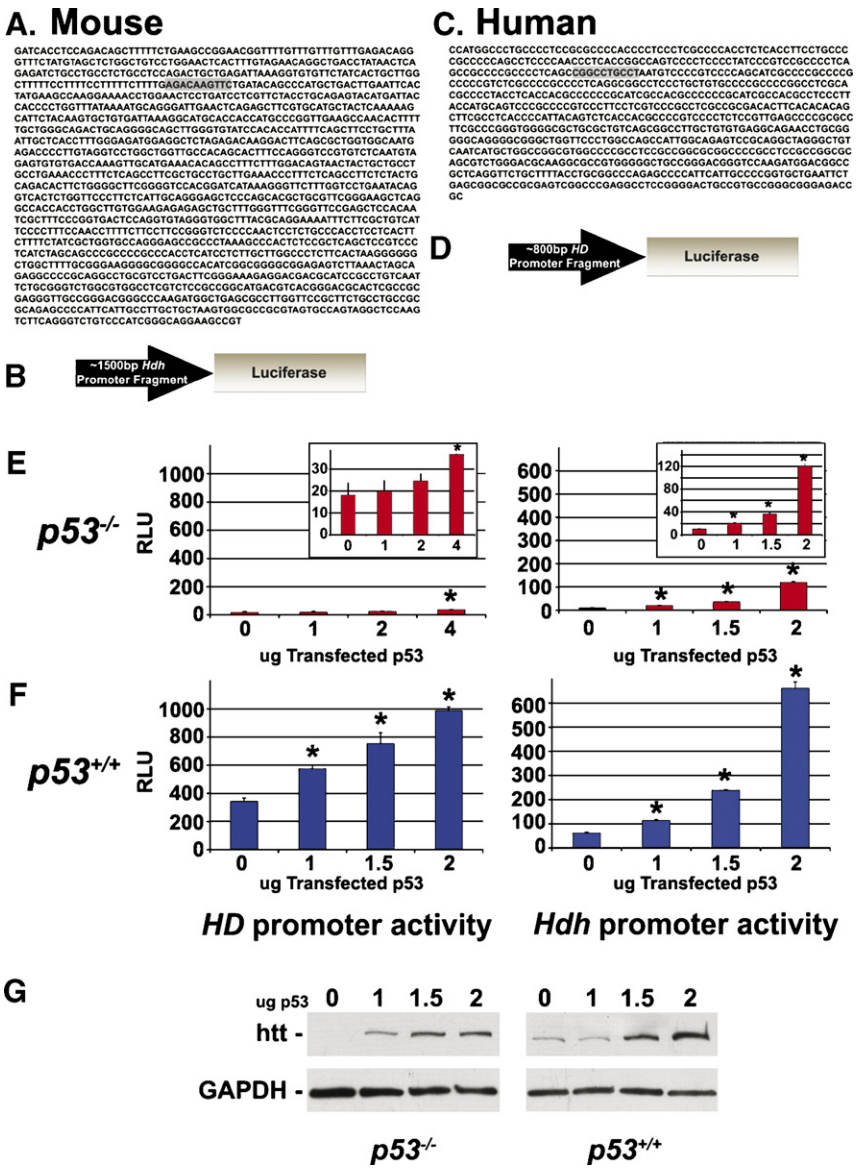

Fig. 2. p53 modulates huntingtin promoter activity and the level of huntingtin protein. (A) Mouse *Hdh* proximal promoter region. A half consensus sequence corresponding to the p53 response element (shaded in gray) is located ~1285 bp upstream from translation start. (B) *Hdh*luc reporter gene. An ~1500-bp fragment of murine *Hdh* promoter was cloned in front of the luciferase gene to generate an *Hdh* reporter gene. This promoter fragment contains the single half-response element for p53 highlighted in panel A above. (C) Human *HD* proximal promoter region. A half consensus sequence corresponding to the p53 response element (shaded in gray) is located ~630 bp upstream of translation start. (D) *HD*luc reporter gene. An ~800-bp fragment of the human *HD* promoter was cloned in front of the luciferase gene to generate an *HD* reporter gene. This promoter fragment contains the single half-response element for p53 highlighted in panel C above. (E) The *HD*luc or *Hdh*luc reporter construct (left and right panel, respectively) was co-transfected into $p53^{-/-}$ MEFs with increasing doses of p53 expression vector. Promoter activation in response to varying doses of p53 was determined by assaying luciferase activity. Individual groups were analyzed by two-tailed Student's *t* test. RLU = Relative Light Units; Error bars = SEM; *statistically different from 0 μg at $p < 0.005$. (F) The *HD*luc or *Hdh*luc reporter construct (left and right panel, respectively) was co-transfected into $p53^{+/+}$ MEFs with increasing doses of p53 expression vector. Promoter activation in response to varying doses of p53 was determined by assaying luciferase activity. Individual groups were analyzed by two-tailed Student's *t* test. RLU = relative light units; error bars = SEM; *Statistically different from 0 μg at $p < 0.005$. (G) $p53^{+/+}$ MEFs (left panel) and $p53^{-/-}$ MEFs (right panel) were transfected with 0, 1.0, 1.5, or 2.0 μg of p53, and the level of huntingtin protein was determined using western blot analysis. Blots were stripped and reanalyzed with anti-GAPDH antibody.

mRNA in $p53^{-/-}$ brain and in control brain are not significantly different from each other, the level of proenkephalin mRNA in $Hdh^{Q140/Q140}$ brain is significantly lower at all ages examined. Deletion of *p53*, however, brings the level of proenkephalin mRNA back to levels close to that in control brain. Quantitation of mRNA levels (Fig. 3A histogram) indicates that proenkephalin mRNA levels in mice with mutant *Hdh* alleles are restored from 60% of control to 80% in the absence of p53. In this mouse model of HD, there has never been any evidence of neuronal loss in the striatum, especially at the age we are investigating (Menalled et al., 2003). In fact, neuronal loss is rarely seen in any of the knock-in mouse models of HD that have been generated (Hickey and Chesselet, 2003; Menalled, 2005). For this reason, the decrease in proenkephalin mRNA seen in the striatum of $Hdh^{Q140/Q140}$ mice is

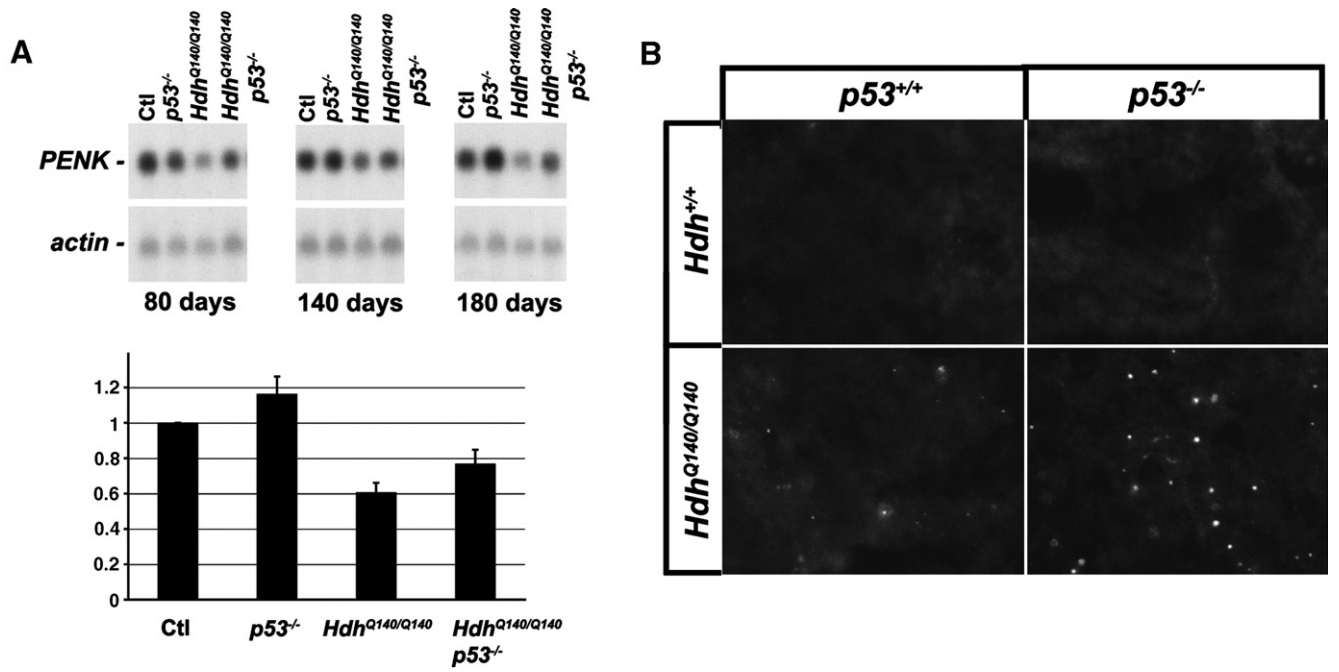

Fig. 3. Loss of p53 partially rescues aspects of the HD phenotype. (A) The level of proenkephalin mRNA was determined by northern blot in the total brain of male mice at 80 (left panel), 140 (middle panel), or 180 days (right panel) of age. Blots were stripped and reprobed for actin mRNA, and densitometry was performed. Striatum from at least six animals in each genotype (ranging in age from 80 to 180 days) were analyzed. Graphed in the histogram is the average ratio of proenkephalin to actin for each genotype. (B) Whole brains of the indicated genotypes were fresh frozen, sectioned, and immunolabeled for aggregated huntingtin using monoclonal antibody EM48.

likely due to a decrease in proenkephalin transcription within HD neurons, rather than the death of HD neurons that express enkephalin (Menalled et al., 2000). In situ hybridization studies performed on brain tissue from patients with presymptomatic Huntington's disease support this idea (Albin et al., 1991).

Loss of p53 increases aggregate formation $Hdh^{Q140/Q140}$ striatum

Another aspect of the HD phenotype that could be modified by deletion of p53 is the formation of intranuclear htt aggregates. To test this, we examined aggregate formation in the striata of 6-month-old male $Hdh^{+/+}p53^{+/+}$, $Hdh^{+/+}p53^{-/-}$, $Hdh^{Q140/Q140}p53^{+/+}$, and $Hdh^{Q140/Q140}p53^{-/-}$ mice. Although the striatum from mice with normal *Hdh* alleles($Hdh^{+/+}p53^{+/+}$ and $Hdh^{+/+}p53^{-/-}$; top two panels of Fig. 3B) did not contain any detectable inclusions, both striata from mice with mutant *Hdh* alleles contained numerous inclusions. However, there was also a dramatic difference between mice with or without p53. The striatum from the $Hdh^{Q140/Q140}$ mouse with *p53* (Fig. 3B, bottom left panel) displayed moderate inclusion formation, whereas the striatum from the mutant mouse without *p53* (Fig. 3B, bottom right panel) had substantially more inclusions. Blood vessels are also stained by EM48 in brain sections from all genotypes analyzed. However, this background staining is identical and easily distinguishable on morphological grounds from the specific staining shown in Fig. 3A, which is unique to brains from animals with expanded *Hdh* alleles.

Discussion

The present results indicate that p53 can transactivate the human and murine *HD*/*Hdh* promoters and modify the level of wild-type

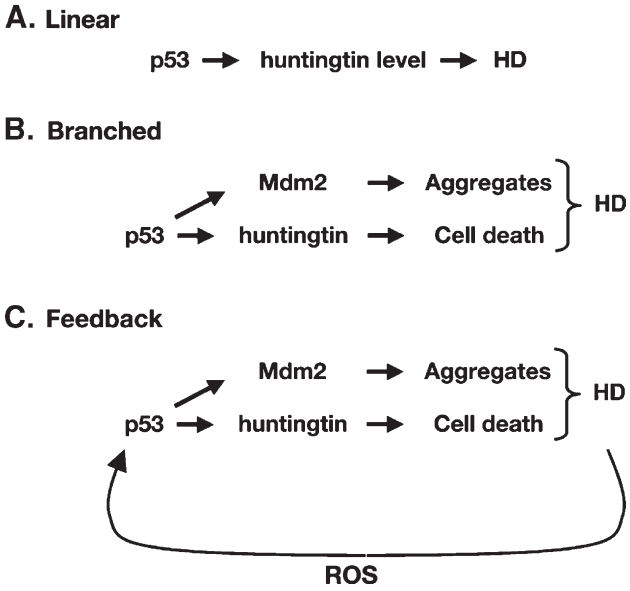

Fig. 4. Model of *Hdh* and p53 interaction. (A) *Linear model of interaction*: p53 resides upstream of *Hdh* and modifies the HD phenotype through the regulation of huntingtin level. (B) *Branched model of interaction*: p53 resides upstream of *Hdh* and modifies the HD phenotype both through the regulation of huntingtin level, and through the transcriptional regulation of additional genes, such as Mdm2. (C) *Feedback model of interaction:* p53 resides upstream of *Hdh*, thereby regulating the HD phenotype, and mutant huntingtin modifies p53 activity, possibly through the regulation of p53 level.

and mutant htt protein, both *in vivo* and *in vitro*. Successive loss of p53 alleles results in a dose-dependent reduction in the level of mutant htt in areas most affected in HD. This reduction in mutant htt is accompanied by a parallel increase in proenkephalin mRNA expression in the brain, and a significant increase in nuclear aggregate formation in the striatum. Because aggregation of mutant htt has been suggested to be a protective mechanism whereby toxic soluble mutant htt is sequestered, both the increase in aggregate load and the restoration of enkephalin expression point to a functional rescue of these HD phenotypes by a deficiency in p53. These results suggest that p53 plays a role in the pathology of Huntington's disease, and that the normal function of p53 may actually promote the disease process. A model for the role of p53 in HD is presented in Fig. 4 and described in more detail below.

The ability of p53 to modify *Hdh* promoter activity, thereby modulating the level of htt protein, suggests that p53 is upstream of htt, which can be depicted by a simple linear model (Fig. 4A). This is supported by the inability of p53 deletion to rescue the lethality caused by complete loss of *Hdh*. This model of interaction between htt and p53 predicts that deletion of p53 would reduce levels of mutant htt, thereby alleviating the HD phenotype. The partial rescue of proenkephalin mRNA levels in $Hdh^{Q140/Q140}$ brain through the elimination of p53 supports this model of interaction and suggests that loss of proenkephalin in patients with HD might be a direct effect of the mutant protein.

The normal distribution of p53-deficient progeny carrying mutant *Hdh* alleles demonstrates that removal of p53 has no deleterious effects on the phenotype of Hdh^{Q140} embryos. Thus, p53 does not appear to play a protective role by suppressing potentially lethal effects of mutant *Hdh* alleles on viability. However, elimination of p53 does increase the number of EM48-positive aggregates in $Hdh^{Q140/Q140}$ striatum, suggesting that the normal p53 response might inhibit aggregate formation in HD. Because aggregation of mutant htt has been suggested to be a protective mechanism whereby toxic soluble mutant htt is sequestered, this increase in aggregate load would suggest that a deficiency in *p53* might rescue the onset of behavioral aspects of HD arising from loss of function in those areas in which aggregate formation is especially prominent. For example, the onset or severity of motor deficits, such as chorea, might be significantly improved. However, it also demonstrates that a simple linear model in which p53 activity is upstream of htt cannot fully explain the *p53–Hdh* interaction. Instead, the role of p53 in HD pathogenesis must involve at least two pathways, one of which acts directly through htt and affects such things as proenkephalin expression and the other that acts through an as yet unidentified mediator of aggregate formation (Fig. 4B). In this branched model, additional targets of p53 activity are responsible for different aspects of HD phenotype. For example, the transactivation of a p53-target gene by p53 may dictate the degree of aggregate formation found in HD striatum.

To understand how the complex interaction between p53 and htt modulates the HD phenotype, it is critical to consider what the disease process itself might do to the activity of p53. p53 is activated as a transcription factor in response to cellular stressors such as DNA damage and oncogenic transformation. Once active, p53 regulates the expression of genes that dictate whether a cell enters senescence, apoptosis, or is repaired and re-enters the cell cycle. Our results demonstrate that p53 can modify the level of htt through transactivation of the htt promoter, and this is supported by work done by Levine's group (Feng et al., 2006). This raises the question of whether htt itself can function as a stress response protein. Previous studies suggest that wild-type htt has an anti-apoptotic function, and that overexpression of htt confers protection against neurodegeneration (Zeitlin et al., 1995; Zhang et al., 2003). If, in fact, htt is upregulated by p53 in response to stress, this would have dire implications in terms of HD pathogenesis. In this paradigm, p53-mediated upregulation of htt promoter activity would lead to an increase in the level of mutant htt protein, which would then exacerbate the disease process. In this way, a normal cellular response to stress – namely, p53-mediated upregulation of the anti-apoptotic activity of wild-type htt – would *increase* cellular stress and dysfunction.

The ability of p53 to modulate *Hdh* level may also lead to alterations in the function of p53 itself. We have found that p53 protein levels are upregulated in $Hdh^{Q140/Q140}$ mice and MEFs (data not shown), and this is supported by recent work by Sawa's group (Bae et al., 2005). This suggests that, in addition to the affect of p53 on the level of htt, mutant htt might alter the activity of p53. Therefore, the branched model presented in Fig. 4B may also not fully represent the interaction of htt and p53 in HD. Rather, we propose a model of HD pathogenesis in which a downstream effector of HD is capable of feedback on to p53 activity (Fig. 4C). This effector, for example, might be oxidative stress, which has been proposed as an initiating and underlying factor in HD pathogenesis, and is known to activate p53 and initiate p53-mediated apoptotic pathways. If mutant htt can alter the activity of p53, one would predict that cells expressing mutant htt might have numerous changes in their transcriptional profile. In fact, a number of transcriptional alterations have been identified by gene expression profile, RT-PCR, and Northern blot in response to mutant htt (Li et al., 1999; Cha, 2000; Luthi-Carter et al., 2000; Luthi-Carter et al., 2002; Sipione et al., 2002; Kotliarova et al., 2005; Zucker et al., 2005).

Our results suggest that a complex interaction exists between htt and p53, in which p53 modifies htt level, and downstream effectors of mutant htt may feedback on p53. Because p53 is such an integral molecule that is involved in numerous processes, alterations in p53 activity would have drastic repercussions at both the cellular and organismal level. Clearly, more work needs to be done to determine how the function of wild-type htt is affected by p53 and to identify the p53-regulated processes that are altered in Huntington's disease pathogenesis.

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