Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo

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Huntington disease (HD) results from polyglutamine expansion in the huntingtin protein (htt). Despite the widespread tissue expression pattern of htt, neuronal loss is highly selective to medium spiny neurons of the striatum. Huntingtin is phosphorylated on serine-421 (S421) by the pro-survival signaling protein kinase Akt (PKB) and this has been previously shown to be protective against the toxicity of polyglutamine-expanded htt in cell culture. Using an antibody specific for htt phosphorylated on S421, we now demonstrate that htt phosphorylation is present at significant levels under normal physiological conditions in human and mouse brain. Furthermore, htt phosphorylation shows a regional distribution with the highest levels in the cerebellum, less in the cortex, and least in the striatum. In cell cultures and in YAC transgenic mice, the endogenous phosphorylation of polyglutamine-expanded htt is significantly reduced relative to wild-type htt. The presence and pattern of significant htt phosphorylation in the brain indicates that this dynamic post-translational modification is important for the regulation of htt and may contribute to the selective neurodegeneration seen in HD.

INTRODUCTION

The neurodegeneration in Huntington disease (HD) is caused by a dominantly inherited mutation that results in an expanded polyglutamine tract in the huntingtin (htt) protein. The expanded polyglutamine tract confers cytotoxic properties to htt (1–3) and compromises some of its endogenous functions (4–8). Although the mutant htt protein is expressed at similar levels in all brain regions (9–11), there is regional selectivity and a temporal pattern to the neuropathology in HD. The molecular basis underlying this specificity is poorly understood. Striatal neurons are the first to degenerate and show the most severe neurodegeneration (12), whereas cortical neurons are often also affected at later stages of the disease (13). Currently, no therapeutic strategies are available to effectively slow the neurodegeneration in HD and a major challenge is to identify factors in the aging brain that create a local environment permissive to this neurodegeneration.

Several recent reports have demonstrated that htt undergoes numerous post-translational modifications such as phosphorylation (14,15), ubiquitination (16), sumoylation (17), palmitoylation (18) and proteolysis (19–23). Although it still remains to be definitively demonstrated, these processes likely play an important role in the pathogenesis of HD, either by modulating polyglutamine-expanded htt toxicity, endogenous function of htt or removal of polyglutamine-expanded htt from the neuron. Phosphorylation by Akt (also known as PKB) plays a central role in the pathology of another polyglutamine expansion disorder, spinocerebellar ataxia type 1 (SCA1) (24,25), and for this reason we wished to explore the Akt phosphorylation of htt in vitro and in vivo and its relationship to the pathogenesis of HD.

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It has been demonstrated in vitro and in cultured cells that htt can be phosphorylated on serine-421 (S421) by Akt and the closely related serum- and glucocorticoid-inducible kinase (SGK) (14,15). In primary striatal neurons, the toxicity of a transfected N-terminal fragment of polyglutamine-expanded htt could be attenuated by the co-transfection of Akt or SGK. This attenuation was dependant on S421 phosphorylation, as there was no protection if htt was genetically modified at S421 to render it non-phosphorylatable. In addition, a modification that mimics constitutive phosphorylation on S421 of this polyglutamine-expanded htt fragment also rendered it non-toxic (14).

Although the phosphorylation of huntingtin by Akt is protective and can prevent polyglutamine-expanded htt toxicity in cell culture, its role in the pathogenesis of HD remains unclear. Several lines of indirect evidence suggest that htt phosphorylation might be altered in the disease state. Given the profound effect of phosphorylation of expanded htt on its toxicity in cell culture, we sought to determine whether phosphorylation on S421 is altered by the polyglutamine expansion in vivo. We report here that htt is phosphorylated on S421 under normal physiological conditions in brain tissues, that the striatum has a low level of endogenous htt phosphorylation and that phosphorylation of polyglutamine-expanded htt is reduced in HD.

RESULTS

Generation of the huntingtin phosphorylation-specific antibody

Using the peptide sequence [RSG(pS)IVELIAGC] corresponding to phosphorylated S421 of human htt, we generated a phosphorylation-specific polyclonal antibody. We first validated the specificity of this phospho-antibody using lysates from HeLa cells transfected with truncated constructs containing amino acids 1–1212 of human htt with 15 polyglutamines. From these lysates, htt was immunoprecipitated and then immunoblotted using the phospho-htt antibody (Fig. 1). Interestingly, the phospho-htt antibody detected the truncated htt fragment in resting, untreated cells indicating that htt is normally phosphorylated to significant levels. As expected, the intensity of the pS421-htt signal increased when the immunoprecipitate was pre-incubated with purified active Akt. Conversely, the pS421-htt signal was completely removed when the immunoprecipitate was pre-incubated with recombinant bacteriophage protein phosphatase.

We also confirmed that the phospho-specific antibody does not detect htt with a serine to alanine (S421A) mutation that prevents its phosphorylation. To control for protein loading, the immunomembrane was reprobed for total htt. We conclude that the pS421-htt antibody specifically detects phosphorylation on S421 and that a portion of htt is phosphorylated under normal conditions in HeLa cells.

Huntingtin is phosphorylated at S421 under normal physiological conditions in brain tissues

We adapted our immunoprecipitation–immunoblot protocol described earlier to determine whether endogenous full-length htt is normally phosphorylated on S421 in brain tissues. Our initial attempts at detecting phosphorylation of htt in normal human brain samples proved difficult and we speculated that perhaps the modification was labile and especially sensitive to post-mortem interval (PMI) (26). To favor our ability to detect phosphorylation, we obtained a human frontal cortex sample with the shortest PMI available (3.5 h). Endogenous phosphorylation could be detected in extracts derived from this tissue with extended exposures of the immunoblot to film (Fig. 2A, lane 1). Incubation of the immunopurified htt from this sample with Akt increased phosphorylation of htt (lane 2).

Our analysis was extended to mouse brain samples in order to directly investigate the relationship between PMI and htt phosphorylation. A mouse was sacrificed and then given a controlled PMI of 2 h before harvesting of brain tissue from the skull cavity. Similar to findings from the human brain sample, phosphorylation on endogenous htt could be detected upon long exposures of the pS421 immunoblot to film, and this phosphorylation was increased by the addition of purified Akt (lanes 3 and 4). In fresh (zero PMI) mouse tissue, the signal was stronger but could still be increased by the addition of purified Akt to the sample. In all samples, huntingtin phosphorylation could be removed completely upon incubation of mouse brain extract with recombinant protein phosphatase 2A (Fig. 2B). In our experiments, an equivalent amount of total htt was always confirmed by reprobing the membrane with mAb 2166.

Our data indicate that htt is phosphorylated on S421 under normal physiological conditions, although this phosphorylation does not appear to be maximal. Furthermore, our data indicate that phosphorylation at this site is labile and is rapidly removed after death in vivo, presumably by endogenous phosphatases.
Huntingtin interacts with Akt

The htt amino acid sequence flanking serine-421 matches the Akt substrate motif RXRXX(S/T)p (27,28). A multiple sequence alignment of vertebrate htt orthologs demonstrates that the phosphorylated serine and two key arginine residues of the Akt motif are present in human htt and conserved in species as evolutionarily distant as zebrafish (Fig. 3A).

To assess whether Akt and htt interact in vivo, we performed co-immunoprecipitation experiments using whole mouse brain from wild-type (FVB) or YAC transgenic mice expressing human htt with a polyglutamine tract of 18 or 128 repeats (Fig. 3B). Lysates were immunoprecipitated with antibodies against Akt, and then immunoblotted for bound htt. These experiments demonstrate that Akt and htt co-immunoprecipitate in wild-type, YAC 18 and YAC 128 mouse-brains and is consistent with the phosphorylation of htt by Akt in vivo. The reverse immunoprecipitation experiment (immunoprecipitating htt and blotting for Akt) was inconclusive because of non-specific binding of htt to the beads (data not shown). Interestingly, htt and Akt appear to have co-immunoprecipitated to a similar degree from YAC 18 and YAC 128 mice, indicating that the protein–protein interaction between htt and Akt is not greatly disrupted by polyglutamine expansion.

Levels of huntingtin phosphorylation is lowest in regions that degenerate in HD

The striatum is the region of primary degeneration in HD, whereas the cerebellum remains relatively unaffected (12). To explore whether the degree of htt phosphorylation might be related to this regional specificity, we next determined if there were differences in the amount of htt phosphorylation in different areas of the normal brain. Huntingtin was immunoprecipitated from extracts of striatal, cortical and cerebellar tissues derived from 13 wild-type mouse brains at 3 months of age. The samples were immunoblotted with the pS421 antibody and then reprobed with mAb 2166 to derive phospho-htt/total htt ratios in each region (Fig. 4A). The amount of specific htt phosphorylation was 45% less in the striatum relative to the cerebellum (ANOVA $P < 0.001$, Tukey’s post hoc striatum versus cerebellum $P < 0.001$). Relative to the striatum, cortical pathology in HD is delayed and more variable in HD patients (12,29) and accordingly, we found the level of htt phosphorylation in the cortex was intermediate and variable in mouse brain.

To determine why there may be regional differences in the phospho-htt/htt ratio, we next assessed regions of the brain for differences in the activation of Akt. Samples from wild-type mouse brain ($n = 9$) were immunoblotted using an antibody for residue S473 of Akt that has been shown to be an effective marker for Akt activation state (30). Densitometry of pAkt and Akt immunoblot signals was used to compute the activated (pAkt)/total Akt ratio (Fig. 4B). The regional differences in the Akt ratios resemble regional differences in phospho-htt ratios: the ratio was significantly least in the striatum, slightly higher in the cortex and cerebellum had the highest Akt activation state (ANOVA $P < 0.0001$). Our data indicate that the regional variability in phospho-htt parallels regional differences in Akt activation status and this pattern inversely correlates with regions most affected by neurodegeneration in HD.

Polyglutamine expansion in htt decreases phosphorylation

Having confirmed the interaction between Akt and htt, and determined that htt phosphorylation is present in human and mouse brain, we sought to determine whether the phosphorylation of htt was altered as a result of the HD mutation. Phosphorylation has been shown to decrease polyglutamine-expanded htt toxicity (14), but we wanted to...
address whether the expansion also affected its ability to become phosphorylated. HEK293 cells were transiently transfected with constructs that express the N-terminal region of htt (1–1212) containing either 15Q or 128Q. Huntingtin was immunoprecipitated and immunoblotted with the pS421 antibody and then reprobed for total htt. Phosphorylation of htt containing 128Q was consistently lower than 15Q htt (Fig. 5A). Densitometry indicates the phospho-htt/htt ratio was reduced by 50% on htt 128Q when compared with htt 15Q ($t$-test $P = 0.005$).

We next wanted to determine whether differences in Akt activity might underlie the decreased phosphorylation of polyglutamine-expanded htt. We analyzed the pAkt/Akt ratio in the same cell lysates and observed no significant differences between cells expressing either the 15Q or the 128Q N-terminal htt fragment (Fig. 5B). As such, the reduced phosphorylation does not appear to be caused by a generalized decrease in Akt activity in cells expressing expanded htt.

To assess whether the phosphorylation of expanded htt was also reduced in vivo, we used whole brain lysates derived from 6- to 8-month-old YAC transgenic mice expressing either 18Q or 128Q full-length htt. To avoid the confounding effects of the endogenous mouse htt, human htt expressed from the transgene was specifically immunoprecipitated using a human-specific htt antibody (HD650). Phosphorylation of immunoprecipitated htt was then detected using the pS421-htt antibody. Consistent with the cell culture experiments, the phosphorylation of 128Q htt was reduced compared with 18Q full-length htt (Fig. 6A). Our quantitative analysis of nine mice per genotype demonstrated a 50% reduction of specific phosphorylation of 128Q compared with 18Q htt ($t$-test $P = 0.016$).

Consistent with the experiments in transfected cells, there was no difference in the Akt activation state of these YAC 18Q and 128Q mice based on immunoblot analysis (Fig. 6B). We further assessed the Akt activity in the R6/2 HD mouse model, which overexpresses the N-terminal fragment (exon 1) of the huntingtin protein containing an expanded polyglutamine tract, but lacking the S421 site. In agreement with the results obtained from the YAC model, the pAkt/Akt ratio was unaltered in pre-symptomatic (4-week-old) (Fig. 6C) and symptomatic (10-week-old) (data not shown) R6/2 mice.
DISCUSSION

In the present study, we have shown that the phosphorylation of htt on S421 is present in brain and altered as a result of the HD mutation. Using a novel phospho (S421)-specific antibody, we find that a portion of huntingtin is phosphorylated under normal physiological conditions in mouse and human brain. This phosphorylation is very labile and htt is rapidly dephosphorylated in brain tissue during a PMI. We find that some, but not all, of the cellular htt in brain is phosphorylated on S421, indicating that this is a dynamic means of regulating the huntingtin protein.

The amount of phospho-S421 htt varied between major regions of the wild-type mouse brain and negatively correlates with the pattern of degeneration seen in HD. The ratio of phospho-htt/total htt was least in the striatum, intermediate and variable in the cortex and greatest in the cerebellum. In part, the regional differences in the phosphorylation state appear to be due to regional differences in the activation of Akt as it had a similar regional specificity. Phosphorylation of polyglutamine-expanded huntingtin has been shown to reduce its toxicity (14), indicating that the low level of striatal phosphorylation may predispose it to degeneration and may provide part of the answer to the long standing question as to why this disease has a regional neuropathology despite widespread expression of htt in the CNS.

Phosphorylation by Akt is known to play an important role in the pathogenesis of another polyglutamine expansion disorder, SCA1. SCA1 results from polyglutamine expansion in the ataxin-1 protein, causing upper motor neuron and cerebellar degeneration in humans. Phosphorylation of ataxin-1 by Akt is required in the pathogenesis of SCA1 (25) and preventing the phosphorylation of polyglutamine-expanded ataxin-1 reduces its toxicity in a mouse model of SCA1 (24). The profound effect of Akt phosphorylation in ataxin-1 and the previous demonstration that genetic modifications that mimic phosphorylation of S421 of mutant htt can prevent toxicity in striatal cell cultures (14) provide a strong

Figure 4. Huntingtin phosphorylation is significantly less in the striatum than the cerebellum in normal mice. (A) Striatal (St), cortical (Cx) and cerebellar (Cb) samples from 3-month-old wild-type mice were blotted with the pS421-htt antibody and then reprobed for total huntingtin to obtain a relative ratio of phosphorylated huntingtin. A representative immunoblot from two mice is shown on the left. The treatment of the sample with lambda phosphatase (PH) is a control to confirm the specificity of the phospho-antibody (lane 1). The phospho-huntingtin ratio was obtained by quantifying the band intensity of the pS421 signal divided by the mAb2166 (total huntingtin) intensity on the film. The chart on the right is the mean ratio (normalized to striatum) and standard deviation of 13 wild-type mice. The ratio of pS421-htt/htt is lowest in the striatum, the primary region of degeneration of HD, and highest in the cerebellum, a brain region spared from pathology in the disease (ANOVA P < 0.001, Tukey’s post hoc St versus Cx P < 0.05, St versus Cb P < 0.001, Cx versus Cb P = 0.21). (B) Regional differences in the activation of Akt reflect the regional differences in pS421. Nine wild-type mice were blotted for activated (P-Ser 472/473/474) pan-Akt and total pan-Akt. The activated Akt/Akt levels were significantly least in the striatum, highest in the cerebellum and intermediate in the cortex (ANOVA P < 0.0001, Tukey’s post hoc St versus Cx P < 0.05, St versus Cb P < 0.001, Cx versus Cb P < 0.001).
impetus for the further study of Akt phosphorylation in HD. Consistent with previous reports that a transfected Akt construct is capable of phosphorylating htt in cell cultures (14), we find that these two proteins stably interact in brain tissues, indicating that the regulation of huntingtin by Akt is a real and important phenomenon in vivo.

In addition to Akt, it has been shown recently that SGK is capable of phosphorylating htt on S421 (15). Transfection of SGK into cell cultures results in increased phosphorylation of htt and protects against expanded-polyglutamine toxicity. Akt and SGK are both activated by PDK1, have homologous catalytic domains, share a similar substrate consensus sequence (31) and are believed to share many cellular functions (15). SGK has been implicated in stress signaling following brain injury (32) and cell volume changes (33) and is likely increased in HD patients as a response to degenerative processes already underway. The stress-induced regulation of SGK argues that it is a response to injury and not part of the pathogenesis of the disease. For this reason, we chose to focus on the Akt pathway and, more specifically, the phosphorylation of htt itself.

There are many conflicting lines of indirect evidence to suggest that the phosphorylation of htt on S421 may be altered in HD. Some data indicate that Akt is proteolytically processed in grades 3 and 4 HD patient brain tissues (14), presumably leading to its inactivation and decreased levels of phosphorylated huntingtin. Others have found increased expression of SGK in late stage HD patient brains (15), suggesting increased huntingtin phosphorylation. Similarly, Akt activity is constitutively elevated in the Q111/Q111 murine knock-in model of HD (34). Considering that the increased phosphorylation of polyglutamine-expanded huntingtin reduces its toxicity (14), we ultimately sought to determine whether expanded polyglutamines alters phospho-S421 directly.

**Figure 5**. Phosphorylation of polyglutamine-expanded huntingtin is reduced in-vitro. (A) HEK293 cells overexpressing 1–1212 amino acids huntingtin with 15Q or 128Q were immunoprobbed with the pS421-htt antibody against phospho-huntingtin and then with mAb2166 for total huntingtin. Densitometry was performed and the ratio of phosphorylated huntingtin/total huntingtin calculated and graphed. Phosphorylation of 128Q huntingtin was reduced by at least 50% relative to 15Q huntingtin in these cells (n = 4, t-test P < 0.005). (B) In these same HEK293 cells expressing 15Q or 128Q, the ratio of activated Akt/total Akt was not altered. Samples were first probed for pAkt and then reprobed for total pan-Akt and actin (n = 4).

**Figure 6**. Phosphorylation of polyglutamine-expanded huntingtin is reduced in vivo and not due to altered Akt activation. (A) Phosphorylation of expanded huntingtin is reduced by 50% compared with wild-type huntingtin in YAC transgenic mice. Lysates from whole brain of YAC 18 versus YAC 128 mice (n = 9 mice) were immunoprecipitated with the human specific HD650 antibody to separate the human transgene from the endogenous mouse huntingtin homolog. Lysates from wild-type mice were used to demonstrate the specificity of the human huntingtin antibody (wt control, lane 1). The immunoprecipitates were separated on an SDS–PAGE gel, probed with the pS421-htt antibody and then reprobed with the antibody to total huntingtin. Similar to the cell culture experiments, the ratio of the phospho huntingtin/total huntingtin is significantly reduced in lysates from the YAC 128 mice versus YAC 18 mice (t-test, P< 0.016). (B) The activation of Akt does not differ between YAC 18 and YAC 128 mice. Lysates from YAC 18 and 128 were probed for pAkt and then reprobed for total pan Akt. The lysate in the first lane (+PH) was pre-treated with lambda phosphatase as a control to remove phosphorylation. Quantification of samples from nine mice shows no differences in the Akt activation state between YAC 18 and 128. (C) Akt activation is not altered in R6/2 mice relative to wild-type (WT) littermates. On the basis of immunoblot analysis, the ratio of pAkt/total pan Akt is similar in WT and R6/2 mice at 4 weeks of age (n = 3).
We find that the S421 phosphorylation of polyglutamine-expanded htt is reduced in cell culture and a mouse model of HD relative to non-expanded htt. In both cells overexpressing expanded htt and in the brains of 6-month-old YAC mice expressing expanded human htt, the ratio of phosphorylated htt/total htt was reduced by 50%. Unfortunately, because huntingtin is rapidly dephosphorylated during the PMI, a comparative examination of phosphorylation status in human brain tissue is not feasible at this time. However, the data from the YAC mice argues that the reduction in phosphorylation occurs early in the disease. At 6–8 months of age, YAC 128 demonstrate behavioral dysfunction just prior to overt striatal degeneration (35). This significant and early reduction in phosphorylation may contribute to the neuronal dysfunction that precedes selective neuronal degeneration in HD.

Interestingly, decreased phosphorylation of mutant htt was independent of changes in Akt activity as demonstrated using brain extracts of YAC HD mice and transfected cells. These data indicate that polyQ expansion in htt interferes with its ability to be phosphorylated by Akt. However, we also find that the co-immunoprecipitation of htt and Akt from YAC 18 and YAC 128 mice was similar, indicating that mutant htt interacts with Akt generally to the same degree as wild-type htt. Therefore, the inhibitory effect of polyQ expansion on htt phosphorylation appears to involve aberrant protein conformation as opposed to altered kinase-substrate interaction.

The normal levels of Akt activity in the YAC128 HD mice are interesting in contrast to the Q111/Q111 HD knock-in mouse model, where there is increased Akt activity in primary neuron cultures and brain tissues from 2 to 18 months of age (34). We also examined the R6/2 mouse, which overexpresses the exon 1 fragment of the HD gene (and therefore lacks the S421 site) containing expanded polyglutamines (36). Similar to the YAC model, Akt levels in R6/2 were unaltered at 4 weeks (pre-symptomatic) and 10 weeks (symptomatic) of age when compared with wild-type littermates. Although the knock-in model of HD is a genetically accurate model of HD (37), it does not result in overt striatal degeneration or decreased lifespan, and clearly has the least severe phenotype of these three mouse models. The increased Akt activity in the knock-in may protect against the degenerative processes (34) through the phosphorylation of the polyglutamine-expanded htt.

Both the full-length YAC transgenic model, which exhibits specific degeneration of the striatum and an HD-like phenotype, and the truncated htt R6/2 model, which has brain atrophy and a significantly decreased lifespan, lack elevated Akt activation and htt phosphorylation and thus are susceptible to the effects of expanded htt. Comparison of these mouse models suggests that increasing Akt activation and htt phosphorylation may be an effective means of preventing striatal degeneration.

In the current study, we find that htt phosphorylation is present in vivo and altered early in the pathogenesis of HD. Huntingtin is phosphorylated on S241 in mouse and human brain under normal physiological conditions. This phosphorylation could play an important regulatory role for the htt protein both in its wild-type and in its polyglutamine-expanded forms. The endogenous pattern of htt phosphorylation is lowest in the striatum relative to the cortex and cerebellum of wild-type mice and may predispose the striatum to degeneration.

Most importantly, the S421 phosphorylation is reduced in cell culture and brains of YAC transgenic mice when htt is polyglutamine expanded and as such, the decreased phosphorylation would be predicted to increase the toxicity of mutant htt in dysfunctional but metabolically active neurons. The decreased phosphorylation is seen early in the disease, prior to overt neuropathology in the YAC mice, and may therefore contribute to pathogenesis. Given that decreased phosphorylation of polyglutamine-expanded huntingtin is linked to increased toxicity in vitro, restoring or increasing the phosphorylation of expanded huntingtin may be an attractive therapeutic strategy for HD.

MATERIALS AND METHODS

Antibodies and reagents

Immunoblotting was performed with antibodies against htt (mAb 2166 Chemicon), human specific htt (HD650) (35), anti-Akt (BD Pharmingen), anti-pAkt rabbit polyclonal (P-SER472/473/474, BD Pharmingen) and Actin (Sigma). Experiments with the BD Pharmingen antibodies were repeated with pAkt and total Akt antibodies from cell signaling and produced equivalent data. Purified Akt1/PKAlphka (Upstate) was used for in vitro phosphorylation and lambda protein phosphatase (gamma-Phos, New England Biolabs) or PP2A (Upstate) was used to dephosphorylate htt. The 1212 amino acid pRecMV-3949-15Q htt constructs have been described previously (21). Serine-421 substitution was created using a site-directed mutagenesis approach as previously described (38) and confirmed by sequencing. The mutagenic primers used were: S421A-F: GAAGCCGTAG TGGGGCTATTGTGGAAC; S421A-R: GTTCCACAATAG CCCACTACGGCTTC. The cloning primers used were: Xho-F: CAAAGTTACAGCTCGAGCTTAAAG; Km-R: CAAAATCTGTTGTGCTGCTACGTCTAAG.

The mutations were first generated in the htt fragments containing 3840 nucleotides (1–1212 amino acids) and were subsequently subcloned to generate the serine mutation in full-length htt.

Generation of anti-pS421 htt antibody

Rabbits were immunized with peptide RSG(pS)IVELIAGC conjugated to KLH (complete Freund’s adjuvant) followed by repeated boosts (incomplete Freund’s adjuvant). Serum was collected 49 days after the initial immunization and screened in ELISA assays. Serum displaying strong immune response was used for double affinity purification. Serum was passed over a column containing the peptide RSGSIVELIAGC and the flow-through was affinity-purified on a column containing RSG(pS)IVELIAGC phospho-peptide.

Cell culture and tissue lysis

HeLa and HEK293 cells were grown at 37°C in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10%
FBS (GIBCO). Transient transfection of the cells was performed with FuGene (Roche) following the manufacturer’s standard instructions. Briefly, cells were transfected in a six-well dish (1 μg DNA + 3 μl FuGene reagent) and then harvested 24 h later.

To harvest the cultured cells, SDP + lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% Igepal, 40 mM NaF, 1 mM phe- nylmethylsulfonyl fluoride (PMSF), 2 mM sodium vanadate, 1× protease inhibitors (Roche)] was added to the plates and left on ice for 5 min. The cells were scraped, sonicated briefly and centrifuged at 14 000 r.p.m. in an Eppendorf 5417c centrifuge for 15 min at 4°C. To generate the brain lysates, FVB wild-type or YAC transgenic mice were sacrificed with CO2 and the brain was isolated and homogenized with a Dounce homogenizer in SDP + lysis buffer. Samples were left on ice for 10 min, sonicated briefly and centrifuged at 10 000 r.c.f. for 15 min at 4°C. Protein concentration was assessed with the Bradford assay (BioRad). Human samples were from the frontal cortex of a 69-year-old male. The YAC 18Q (line 212) (39) and YAC 128Q (line 53) (35) mouse models contain the full-length genomic human HD gene with its endogenous promoter elements, providing appropriate developmental and tissue specific expression of the htt protein (35).

R6/2 mice, which express a truncated N-terminal fragment of huntingtin (36) were sacrificed with sodium pentobarbital. The tissue was extracted, snap frozen in liquid nitrogen and stored in the −80 freezer until it was processed. The tissue was homogenized using a motorized Teflon pestle (Sigma) in 0.32 M sucrose with Complete Mini (Roche) protease inhibitors and PMSF.

Immunopurification of htt was performed on Sepharose G beads pre-bound with mAb2166 or HD650. Lysates were incubated with 0.5 U of purified Akt at 30°C for 1 h. For co-immunoprecipitation, brains from 6-month-old R6/2 mice, which express a truncated N-terminal fragment of huntingtin (36) were sacrificed with sodium pentobarbital. The tissue was extracted, snap frozen in liquid nitrogen and stored in the −80 freezer until it was processed. The tissue was homogenized using a motorized Teflon pestle (Sigma) in 0.32 M sucrose with Complete Mini (Roche) protease inhibitors and PMSF.

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Conflict of Interest statement. None declared.

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