Phosphorylation of Arfaptin 2 at Ser²⁶₀ by Akt Inhibits PolyQ-
huntingtin-induced Toxicity by Rescuing Proteasome Impairment*

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Huntington disease (HD) is caused by an abnormal expanded polyglutamine repeat in the huntingtin protein. Insulin-like growth factor-1 is of particular interest in HD because it strongly inhibits polyQ-huntingtin-induced neurotoxicity. This neuroprotective effect involves the phosphorylation of huntingtin at Ser²⁴¹ by the prosurvival kinase Akt (Humbert, S., Bryson, E. A., Cordelieres, F. P., Connors, N. C., Datta, S. R., Finkbeiner, S., Greenberg, M. E., and Saudou, F. (2002) Dev. Cell 2, 831–837). Here, we report that Akt inhibits polyQ-huntingtin-induced toxicity in the absence of phosphorylation of huntingtin at Ser²⁴¹, suggesting that Akt also acts on other downstream effectors to prevent neuronal death in HD. We show that this survival effect involves the ADP-ribosylation factor-interacting protein arfaptin 2, the levels of which are increased in HD patients. Akt phosphorylated arfaptin 2 at Ser²⁶₀. Lack of phosphorylation of arfaptin 2 at this site substantially modified its subcellular distribution and increased neuronal death and intranuclear inclusions caused by polyQ-huntingtin. In contrast, arfaptin 2 had a neuroprotective effect on striatal neurons when phosphorylated by Akt. This effect is mediated through the proteasome, as phosphorylated arfaptin 2 inhibited the blockade of the proteasome induced by polyQ-huntingtin. This study points out a new mechanism by which Akt promotes neuroprotection in HD, emphasizing the potential therapeutic interest of this pathway in the disease.

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1 The abbreviations used are: HD, Huntington disease; IGF-1, insulin-like growth factor-1; GST, glutathione S-transferase; Akt-ca, constitutively activated Akt; SGK, serum- and glucocorticoid-induced kinase; GFP, green fluorescent protein; NLS, neuronal intranuclear inclusions; MT, microtubule; UPS, ubiquitin/proteasome system; ANOVA, analysis of variance.
neuronal death and inclusion formation that are induced by an N-terminal fragment of huntingtin that does not contain Ser421. We demonstrate that this Ser421 huntingtin-independent mechanism involves the ADP-ribosylation factor-interacting protein arfaptin 2. Phosphorylation of arfaptin 2 at Ser260 by Akt promoted neuronal survival and decreased intranuclear inclusion formation in a neuronal model of HD. This demonstrates that arfaptin 2 is neuroprotective in HD. Finally, we show that phosphorylated arfaptin 2 inhibits the polyQ-huntingtin-induced blockade of the proteasome.

EXPERIMENTAL PROCEDURES

Constructs—The vectors encoding β-galactosidase, hemagglutinin-tagged huntingtin N-terminal fragments 171-171 and 171-68, (exon 1 of huntingtin with 17 glutamines (exon 1-17) and 68 glutamines (exon 1-68), glutathione S-transferase (GST)-fused human huntingtin-(384-467), constitutively activated Akt (Akt-ca), inactivated Akt, and hemagglutinin-tagged Akt have been described previously (2, 11, 12). A bacterial expression plasmid encoding GST-fused arfaptin 2 (13) was used as a template to generate plasmid encoding the C-terminal fragment of arfaptin 2. A mammalian expression plasmid (pcDNA3.1/GS) encoding full-length human arfaptin 2 was purchased from Invitrogen (GeneStorm). The arfaptin 2 mutants for Akt phosphorylation sites were generated by QuikChange site-directed mutagenesis (Stratagene) using the mammalian or bacterial plasmid of arfaptin 2 as a non-mutated parent template and complementary oligonucleotides containing the desired mutation for S260A arfaptin 2 (5′-CGTCGACTTGAGGCTGCCAGGCCACTTTC-3′) and for S260D arfaptin 2 (5′-CGTCGACTTGAGGCTGCCAGGCCACTTTC-3′). The flow-through was collected, concentrated, and applied to the phosphopeptide column. Elution was performed with 100 mM sodium inorganic pyrophosphate, 2 mM dithiothreitol, 1 mM sodium vanadate, and 100 μM phosphoethanolamine fluoride) for 20 min at 4 °C. Soluble and insoluble Nonidet P-40 fractions were separated after centrifugation at 10,000 rpm for 15 min at 4 °C.

Human tissues were from the Harvard Brain Tissue Resource Center (Belmont, MA). Brain samples were homogenized in Nonidet P-40 lysis buffer and cleared by centrifugation at 6000 × g for 15 min at 4 °C. 50 μg of homogenates were subjected to Western blot analysis. Samples 1–5 correspond to brain numbers 4741, 4744, 4751, 4797, and 4740, respectively, as numbered by the Harvard Brain Tissue Resource Center. Quantification of Western blots was performed and is expressed relative to actin levels. Human biopsies were procured following the guidelines recommended by the National Institutes of Health. Proteins were loaded onto 10% SDS-PAGE; transferred to polyvinylidene difluoride membrane; and immunoblotted with anti-arfaptin 2 (1:1000) (13), anti-arfaptin 2/2POR1 (1:100; N19, Santa Cruz Biotechnology, Inc.), and anti-His tag antibody (1:5000; Amersham Biosciences). Immunofluorescence—Transfected cells were grown on laminin- and/or poly-d-lysine-coated glass coverslip, fixed with 4% paraformaldehyde for 20 min, and incubated with the following primary antibodies: anti-arfaptin 2 (1:200) (13), anti-His tag (1:500; Cell Signaling Technology), anti-GM130 (1:100, BD Biosciences), and fluorescein isothiocyanate-conjugated anti-α-tubulin (1:100, Sigma). Pictures of fixed cells were captured with a three-dimensional deconvolution imaging system.

Analysis of Arfaptin 2 Effect on Proteasome Activity in Cells—GFP-pu-1 cells (16) were transfected with constructs of interest. DsRed2-C1 DNA (Clontech) was included in the transfection mixture at a 1:5 ratio. After 72 h, cells were trypsinized and analyzed in a BD Biosciences FACScan-Calibur flow cytometer. A minimum of 10^4 Dicocosoma sp. red fluorescing cells were analyzed for green fluorescent protein (GFP) fluorescence under each condition using CellQuest software. The levels of fluorescence were standardized to the exon 1-17 condition. The results from three independent experiments are presented as median values of green fluorescence histogram plots.

Measurement of Neuronal Survival and Intranuclear Inclusions—Four days post-plating, primary cultures of striatal neurons were transfected with constructs of interest. Neurons were analyzed for the presence of ubiquitin-positive intranuclear inclusions (anti-ubiquitin antibody) in a blinded manner 16 and 36 h post-transfection. Cell death occurring within the GFP-positive cells was determined as the difference in the number of surviving neurons between the two time points and is expressed as a percentage of cell survival. For intranuclear inclusion scoring, striatal neurons were transfected with vectors of interest and a plasmid encoding β-galactosidase (10:1 ratio). Neurons were fixed 5 days post-transfection, immunostained, and analyzed for the presence of ubiquitin-positive intranuclear inclusions (anti-β-galactosidase antibody (1:300), 5 Prime → 3 Prime, Inc.; and anti-ubiquitin antibody (1:100), Dako Corp.). Each graph represents at least two to three independent experiments performed in duplicate or triplicate. Each bar in a given graph corresponds to the scoring of ~2000 neurons in neuronal survival experiments and to 500 neurons for inclusion scoring. Data were submitted to complete statistical analyses (see figure legends).

RESULTS

Akt Exerts a Neuroprotective Effect Independently of Ser421 Phosphorylation in Huntingtin—We tested whether Akt could promote neuroprotection independently of Ser421 phosphorylation. We used an in vitro neuronal model of HD to determine whether Akt could block the neuronal toxicity induced by an N-terminal fragment of huntingtin not containing Ser421. In primary cultures of striatal neurons, fragments of huntingtin containing the abnormal polyglutamine expansion induce neuronal death and the formation of neuronal intranuclear inclu-
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**Fig. 1.** Akt inhibits polyQ-huntingtin-induced toxicity in striatal neurons in a Ser421 phosphorylation-independent manner. Wild-type huntingtin (construct 171-17) or polyQ-huntingtin (construct 171-68) was cotransfected with Akt-ca (Akt c.a.) or the corresponding empty vector in striatal neurons. A, data were from two independent experiments (analysis of variance [ANOVA]; F(3,10) = 13.69; p < 0.0007). Death induced by construct 171-68 was significantly different from all other cases. *, p < 0.01 (post hoc Fisher’s analysis). B, data from two independent transfections (ANOVA; F(1,15) = 3.99; p < 0.001) revealed that the percentage of striatal neurons exhibiting NIHs was significantly reduced by Akt-ca. *, p < 0.05 (post hoc Fisher’s analysis).

**Fig. 2.** Arfaptin 2 is up-regulated in HD brains. A, protein extracts were prepared from post-mortem striatal samples, resolved by SDS-PAGE, and immunoblotted with anti-arfaptin 2/POR1 antibody (upper panel) or anti-β-actin antibody (lower panel). The levels of arfaptin 2 in human striatal samples from control (CT; samples 1–3), grade 3 HD (HD3; sample 4), and grade 4 HD (HD4; sample 5) patients were analyzed. PMI, post-mortem interval (hours). B, quantification of the Western blots shows a statistically significant increase in the protein level of arfaptin 2 in HD samples compared with control samples. ***, p < 0.01 (Student’s t test; t(5) = 9.98).

**Fig. 3.** Akt phosphorylates arfaptin 2 at Ser260 in vitro and in cells. A, the Akt consensus site in arfaptin 2 is defined by RGRRLS with phosphorylation at Ser260. Full-length arfaptin 2 and a C-terminally truncated form of arfaptin 2 (Arfaptin 2 Cter) are shown. B, kinase assays were performed using Akt-ca (Akt c.a.) or inactivated (kinase-null) Akt (Akt k.n.) and GST-fused proteins as substrates. The reaction products were resolved by SDS-PAGE; the gel was stained with Coomassie blue (left panel); and the 32P-labeled proteins were visualized by autoradiography (right panel). Akt-ca phosphorylated full-length arfaptin 2 and its C-terminal portion. Phosphorylation was abolished when Ser260 was mutated to Ala in the C-terminal portion of arfaptin 2 (S260A). After in vitro phosphorylation by Akt and trypsin digestion, the resulting phosphopeptides of wild-type and S260A arfaptin 2 were resolved by two-dimensional migration. The major dot observed with wild-type arfaptin 2 (see arrows) was lost when Ser260 could not be phosphorylated. D, human embryonic kidney 293 cells were transfected with wild-type or S260A arfaptin 2 and with Akt-ca or the corresponding empty vector and serum-starved for 24 h. Protein extracts were analyzed with antibody against arfaptin 2 phosphorylated at Ser260 (P-Arfa-S260; upper panel) and anti-arfaptin 2/POR1 antibody (lower panel). E, kinase assays were performed using recombinant SGK and GST-fused human huntingtin-(384–467) (GST-hu-htt) as used as a positive control for SGK phosphorylation. SGK phosphorylated GST-fused human huntingtin-(384–467), but did not phosphorylate GST-fused arfaptin 2. CB, Coomassie Blue.

We have previously demonstrated that part of the neuroprotective effect of IGF-1 is mediated through the phosphorylation of huntingtin at Ser421 (2). We show here that Akt protected striatal neurons from polyQ-huntingtin-induced toxicity not only by directly phosphorylating huntingtin, but also by phosphorylating other substrates that in turn modulate polyQ-huntingtin-induced toxicity. This indirect effect of Akt led to inhibition of both the death of striatal neurons and the formation of fragment 171-68 was phosphorylated by Akt-ca (data not shown). This suggests that the protective effects of Akt are probably not due to phosphorylation between amino acids 1 and 171 of huntingtin.
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intranuclear inclusions (Fig. 1). Therefore, Akt may act on a substrate that directly controls polyQ-huntingtin-induced toxicity. To identify such an Akt substrate, we screened data bases for proteins containing an Akt consensus phosphorylation site (17). We found, among many others, arfaptin 2, which has been shown to regulate polyQ-huntingtin aggregation (13).

Arfaptin 2 Is Up-regulated in HD—The striatum is the most affected region in HD. To determine whether arfaptin 2 is modified in the pathological situation, we analyzed the levels of arfaptin 2 in human striatal samples from control (CT), grade 3 HD (HD3), and grade 4 HD (HD4) patients (Fig. 2A). Brain extracts were analyzed using anti-arfaptin 2/POR1 antibody (upper panel) and anti-β-actin antibody as a control for protein levels (lower panel). Arfaptin 2 levels were higher in brain extracts from HD patients than in those from control individuals (Fig. 2B). This agrees with previous work on HD transgenic mouse brains showing up-regulation of arfaptin 2 (13) and further suggests its involvement in HD.

Arfaptin 2 Is a Substrate of Akt—Arfaptin 2 has a putative Akt phosphorylation site at Ser260 (Fig. 3A). To test whether Akt phosphorylates arfaptin 2 in vitro, we incubated GST-fused arfaptin 2 with Akt-ca or inactivated Akt in the presence of [γ-32P]ATP. Akt-ca phosphorylated arfaptin 2, whereas inactivated Akt did not (Fig. 3B). To demonstrate that Ser260 is phosphorylated by Akt-ca, we generated a GST-fused C-terminal fragment of arfaptin 2 (amino acids 249–341) with either Ser260 or a Ser-to-Ala mutation at this position (S260A). The C-terminal fragment of arfaptin 2 was no longer phosphorylated by Akt-ca when Ser260 was replaced with Ala (Fig. 3B), even though the amount of the GST-fused protein was similar. We carried out two-dimensional phosphopeptide mapping of full-length wild-type and S260A arfaptin 2 to confirm Ser260 as a site of phosphorylation of arfaptin 2 (Fig. 3C). The major phosphopeptide observed in wild-type arfaptin 2 (see arrows) was not seen when Ser260 was mutated to Ala, suggesting Ser260 as the main site of phosphorylation of arfaptin 2 by Akt.

To unequivocally identify Ser260 as an Akt phosphorylation site, we raised a polyclonal antibody that specifically recognizes arfaptin 2 phosphorylated at Ser260. As shown in Fig. 3D, the antibody against arfaptin 2 phosphorylated at Ser260 recognized arfaptin 2 only when coexpressed with Akt-ca, but failed to recognize the same protein with the S260A mutation. These results show that Akt phosphorylates arfaptin 2 at Ser260 in cells and that arfaptin 2 is a substrate of Akt.

We investigated whether SGK phosphorylates arfaptin 2 because this kinase has a protective effect by phosphorylating huntingtin at Ser421 after IGF-1 activation (14). Unlike huntingtin, arfaptin 2 was not phosphorylated by SGK, indicating that Akt specifically phosphorylates arfaptin 2 at Ser260 (Fig. 3E).

Phosphorylation of Arfaptin 2 Modifies Its Cellular Distribution—Arfaptin 2 is located mainly in the perinuclear region of Chinese hamster ovary cells, in particular around the microtubule (MT)-organizing center and in cytoplasmic vesicular structures (13). To investigate whether phosphorylation alters the distribution of arfaptin 2, we transfected NG108-15 neuroblastoma cells with His-tagged wild-type or S260A arfaptin 2. We used immunofluorescence to compare the distribution of arfap-
tin 2 and the Golgi marker protein GM130 (Fig. 4A). The transfected wild-type and endogenous arfaptin 2 proteins appeared in the cytoplasm as diffuse punctate staining and partially co-localized with GM130 in the perinuclear region. However, the distribution of S260A arfaptin 2 was very different. The protein was redistributed to bundle structures in the cytoplasm and was no longer detected in Golgi bodies or vesicles. To ensure that this effect was due to loss of phosphorylation at Ser260, we assessed the effect of constitutive phosphorylation of Ser260 by replacing it with Asp (S260D). S260D arfaptin 2 had the same subcellular localization as arfaptin 2 in neuroblastoma cells (Fig. 4A) and in striatal neurons (data not shown). S260A arfaptin 2 also re-localized into bundle structures in neuritic extensions of striatal neurons (Fig. 4B). Quantification of bundle formation in neuroblastoma cells and neurons demonstrated that it was specifically associated with loss of phosphorylation at Ser260, as wild-type and S260D arfaptin 2 never formed bundles. In contrast, bundles were observed in 35–45% of NG108-15 neuroblastoma cells and neurons expressing S260A arfaptin 2 (Fig. 4C).

Arfaptin 2 Subcellular Localization Depends on the MT Network—Proteins that associate with MTs often induce bundles when overexpressed in cells (18). Bundle formation induced by loss of phosphorylation of arfaptin 2 at Ser260 together with the localization of arfaptin 2 around the MT-organizing center led us to investigate the effect of phosphorylation on the distribution of arfaptin 2 in relation to the MT network (Fig. 5A). Unfortunately, the paraformaldehyde fixation step, which is essential for the detection of the anti-histidine antibody that localizes transfected arfaptin 2, slightly disrupted the MT network. Nevertheless, wild-type arfaptin 2 was located mainly around the MT-organizing center in a punctate staining pattern as described previously (13), whereas in cells expressing S260A arfaptin 2, the MT network was disrupted (Fig. 5A). To ensure that the localization of arfaptin 2 and its regulation by phosphorylation depend on MTs, we treated cells with nocodazole, an MT-depolymerizing agent. We found that both wild-type and S260A arfaptin 2 distributions were disrupted, with the proteins being concentrated in dense clusters throughout the cytoplasm (Fig. 5B). These results show that arfaptin 2 localization depends on the integrity of the MT network and that loss of phosphorylation of arfaptin 2 leads to an alteration of the MT organization.

We next studied the effect of phosphorylation on arfaptin 2 distribution in cells. We analyzed arfaptin 2 in whole cell extracts and in soluble and insoluble Nonidet P-40 fractions from COS-7 cells transfected with various arfaptin 2 constructs (Fig. 5C). Wild-type and S260D arfaptin 2 were concentrated in the soluble Nonidet P-40 protein fraction, whereas S260A arfaptin 2 was strongly enriched in the insoluble Nonidet P-40 protein fraction.

Thus, we have demonstrated by immunostaining and biochemical experiments that loss of phosphorylation of arfaptin 2 at Ser260 dramatically alters the cellular distribution of arfaptin 2. This results in its accumulation into insoluble bundle structures and to the partial disruption of the MT network.

Arfaptin 2 Modulates PolyQ-huntingtin-induced Toxicity: Effect of Phosphorylation by Akt—We studied the physiological consequences of arfaptin 2 expression and phosphorylation of Ser260 on polyQ-huntingtin-induced toxicity in primary cultures of striatal neurons (Fig. 6A). We cotransfected striatal neurons with construct 171-17 or 171-68 and various constructs of arfaptin 2 and analyzed cell survival. Wild-type and S260D arfaptin 2 partially rescued neurons from construct 171-68-induced neuronal death, whereas S260A arfaptin 2 enhanced neuronal death. We then analyzed striatal neurons transfected with the same constructs for the presence of NIIs (Fig. 6B). S260D arfaptin 2 decreased the percentage of neurons containing nuclear inclusions, whereas wild-type arfaptin 2 did not. In contrast, S260A arfaptin 2 significantly increased this percentage. These results show that arfaptin 2 phosphorylated at Ser260 has a neuroprotective effect on polyQ-huntingtin-induced toxicity by reducing both neuronal death and nuclear inclusions.

We cotransfected primary cultures of striatal neurons with huntingtin, arfaptin 2, and Akt-ca to see whether the Ser260-dependent toxicity of arfaptin 2 is related to phosphorylation by Akt at this site (Fig. 6, C and D). As we have shown (Fig. 1A), Akt-ca inhibited neuronal death induced by construct 171-68. In the presence of wild-type arfaptin 2, Akt still exerted a neuroprotective effect and blocked polyQ-huntingtin-induced neuronal death (Fig. 6C). However, when S260A arfaptin 2 was expressed in neurons, Akt-ca only partially rescued polyQ-huntingtin-induced neuronal death (Fig. 6C). Thus, the neuroprotective effect of Akt is at least partly mediated through phosphorylation of arfaptin 2. When construct 171-68, Akt, and wild-type arfaptin 2 were coexpressed in striatal neurons, a decrease in formation of NIIs was observed (Fig. 6D).
effect is related to phosphorylation of Ser260 by Akt-ca, as this was not observed in the absence of Akt (Fig. 6B) and was abolished when Ser260 was mutated to Ala (Fig. 6D). Therefore, phosphorylated arfaptin 2 exerts a protective effect in HD that is mediated via phosphorylation of Ser260 by Akt.

**Arfaptin 2 Phosphorylation Rescues PolyQ-huntingtin-induced Proteasome Impairment**—The accumulation of S260A arfaptin 2 in cells and the toxicity of this protein with increased formation of NIIs led us to test whether phosphorylation of arfaptin 2 could modulate proteasome function (Fig. 7A). Although the function of arfaptin is not fully understood, arfaptin 2 has been shown to regulate proteasome activity (13). We used GFPu-1 cells stably producing a short degron (CL1) fused to GFP such that the fusion protein is targeted for proteasomal degradation (16). Proteasome activity was monitored by the fluorescence in GFPu-1 cells: an increase in fluorescence indicates inhibition of protein degradation. We cotransfected GFPu-1 cells with wild-type or expanded polyglutamine N-terminal fragments corresponding to exon 1 of huntingtin (exon 1-17 or 1-68, respectively) and wild-type or S260A arfaptin 2. The levels of GFP fluorescence were monitored by fluorescence-activated cell sorting analysis. Cells expressing exon 1-68 were more fluorescent than cells expressing exon 1-17 (Fig. 7A), indicating that abnormal polyglutamine expansion in huntingtin impairs the proteasome. The fluorescence intensity was significantly lower in cells transfected with wild-type arfaptin 2 and exon 1-68. This shows that arfaptin 2 inhibits the polyglutamine-induced proteasome blockade, thereby facilitating protein degradation. This beneficial property is lost in the absence of Ser260 phosphorylation.

**DISCUSSION**

We have shown that arfaptin 2 is neuroprotective in a neuronal model that has characteristics that are observed in HD patients, such as intranuclear inclusions of polyQ-huntingtin.
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and neuronal death. We have demonstrated that arfaptin 2 is a substrate of Akt and that phosphorylation of arfaptin 2 by Akt at Ser260 promotes survival of striatal neurons. This shows that Akt regulates ubiquitination and degradation of proteins. For example, Akt stabilizes Mdm2 and XIAP (X-linked inhibitor of apoptosis protein) (20, 21). This mechanism involves the phosphorylation of Mdm2 and XIAP by Akt, which leads to the inhibition of their ubiquitination and thus inhibits degradation by the proteasome. The prosurvival effect of Akt can also be mediated by the enhanced degradation of negative regulators of cell growth and survival. Akt also promotes degradation of tuberin and a FOXO family transcription factor, FOXO3a, by a phosphorylation-dependent ubiquitination mechanism (22).

The ubiquitin/proteasome system (UPS) is involved in several neurodegenerative disorders, including HD and other polyglutamine disorders (23). In polyglutamine disorders, aggregates may allow the temporary storage of misfolded toxic proteins prior to their degradation by the proteasome (12). In agreement with this hypothesis, to clear the misfolded and aggregated proteins, the proteasome must show efficient ubiquitination and degradation activity (12, 24–26).

Several studies have revealed that the UPS is impaired during disease progression. Misfolded and/or aggregated proteins inhibit the UPS and lead to the accumulation of ubiquitin conjugates in cells (16, 19). In addition, polyglutamine-containing proteins are not efficiently degraded by the UPS. This results in the further accumulation of expanded polyglutamine-containing short peptides. These are then prone to aggregation and thereby compromise the function of the UPS (27). Finally, the age-dependent decrease in proteasome activity also contributes to the accumulation of short fragments of polyQ-huntingtin (28). Therefore, aggregates are probably both inhibitors of the UPS and the consequence of UPS inhibition (16, 29). Thus, the identification of molecules or pathways that regulate the UPS is of potential therapeutic value.

Our study shows that phosphorylated arfaptin 2 leads to recovery from the proteasome impairment induced by polyQ-huntingtin. How does arfaptin 2 regulate proteasome function? Although arfaptin 2 interacts with Arf6 and Rac1, its function remains unknown (30–32). The nature of the proteins that
interact with arfaptin 2 and its subcellular localization (13) suggest that arfaptin 2 may regulate cytoskeletal remodeling involving the MT network. This is supported by our finding that loss of phosphorylation of arfaptin 2 at Ser260 disrupts the MT network in neuroblastoma cells and neurons. As for aggregates (33), an intact MT network is required for aggregate formation. This indicates that active transport along MTs participates in the sequestration of aggregates and in their subsequent processing by the proteasome (34). Arfaptin 2 may be a positive regulator of this process. As a defective proteasome is involved in several neurodegenerative disorders, it will be of interest to address this mechanism in future works.

We have previously shown that, upon IGF-1 activation, SGK phosphorylates polyQ-huntingtin at Ser421 and abrogates its toxicity (14). This kinase does not phosphorylate arfaptin 2. These data further emphasize the importance of Akt in HD because Akt targets different proteins to protect neurons against polyQ-huntingtin (Fig. 7B). Akt abolishes the toxicity of full-length polyQ-huntingtin, which contains Ser421. The phosphorylation of specific substrates by Akt may be a complementary process during the progression of the disease, further suggesting that the Akt pathway is of therapeutic value in HD.

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