Activation of MLK2-mediated Signaling Cascades by Polyglutamine-expanded Huntingtin*

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We previously reported that expression of polyglutamine-expanded huntingtin induces apoptosis via c-Jun amino-terminal kinase (JNK) activation in HN33 cells (Liu, Y. F. (1998) J. Biol. Chem. 273, 28873–28882). Extending this study, we now demonstrate a role of mixed-lineage kinase 2 (MLK2), a JNK activator, in polyglutamine-expanded huntingtin-mediated neuronal toxicity. We find that normal huntingtin interacts with MLK2, whereas the polyglutamine expansion interferes with this interaction. Similar to the expression of polyglutamine-expanded huntingtin, expression of MLK2 also induces JNK activation and apoptosis in HN33 cells. Co-expression of dominant negative MLK2 significantly attenuates neuronal apoptosis induced by the mutated huntingtin. Furthermore, over-expression of the N terminus of normal huntingtin partially rescues the neuronal toxicity induced by MLK2. Our results suggest that activation of MLK2-mediated signaling cascades may be partially involved in neuronal death induced by polyglutamine-expanded huntingtin.

Huntington’s disease (HD) is a neurodegenerative disorder with dominant inheritance (2). The disease is characterized by choreiform movement, mental impairment, and cognitive symptoms (3, 4). The HD gene encodes a 350-kDa protein designated as huntingtin (2), which is ubiquitously expressed with the highest levels being found in the brain, lung, and testes (5, 6). Immunocytochemistry reveals that in neurons huntingtin is a cytoplasmic protein found in cell bodies, dendrites, and also in nerve terminals, where huntingtin is associated with synaptic vesicles and microtubule complexes (6, 7). The defect in the HD gene causes an expansion of a polyglutamine stretch near the N terminus of huntingtin, and the length of the polyglutamine repeat is correlated with the age of onset and the severity of the disease (8). To date, the normal function of huntingtin remains to be determined, and the molecular mechanism underlying neuronal death in HD is poorly understood.

In previous studies, we found that expression of polyglutamine-expanded huntingtin caused neuronal apoptosis via activation of JNKS in HN33 cells, a hippocampal neuron-derived cell line (1). The aim of the present study was to investigate the molecular mechanism by which polyglutamine-expanded huntingtin activates JNKs and induces neuronal apoptosis. Huntingtin contains multiple proline-rich motifs that may bind to both SH3 and WW domain-containing proteins (9). Interestingly, the N-terminal proline-rich region, which is about 40 amino acids long, is adjacent to the polyglutamine stretch. This proline-rich region has been shown to bind to both SH3 and WW domain-containing proteins (10–12). MLK2 is a member of the mixed-lineage kinase family whose kinase domain shows structural features of both tyrosine-specific and serine/threonine-specific protein kinases (13). MLK2 possesses an SH3 domain that is homologous to the SH3 domains of Grb2 (13). MLK2 is predominately expressed in the brain (13), and it has been reported that MLK2 can directly bind and mediate activation of MKK7 and SEK1, which in turn induces JNK activation (14–19). At moderate expression levels, MLK2 appears to selectively activate JNKS and has little effect on other mitogen-activated protein kinases (14–16). Thus, MLK2 is a potential candidate for the involvement in JNK activation and neuronal toxicity induced by polyglutamine-expanded huntingtin. The current study was undertaken to investigate the role of MLK2 in mutated huntingtin-mediated neuronal toxicity. Our results suggest that huntingtin binds to the SH3 domain of MLK2 and the polyglutamine expansion interferes with its binding to the kinase. Activation of MLK2-mediated signal transduction pathways may be involved in initiating neuronal death in HD.

MATERIALS AND METHODS

Cell Culture and Transient Transfection—HN33 cells, an immortalized rat hippocampal neuronal cell line (1), and 293T cells, human embryonic kidney cells expressing SV40 large T antigen, were maintained in Dulbecco’s modified Eagle’s medium/F12 medium supplemented with 10% fetal bovine serum on 10-cm plates. HN33 or 293T cells at 50 to 60% confluence were washed once with serum-free medium prior to transfection. Transfection was performed using Lipofectin (Life Technologies, Inc.) according to manufacturer instructions. 10–50 µg of plasmid with 10–20 µl of Lipofectin/10-cm plate was used in transfection experiments.

Western Blotting and Immunoprecipitation—48–72 h after transfection, 293T cells were harvested and lysed in 1% Nonidet P-40 lysis buffer, and co-immunoprecipitation experiments were conducted as described previously using an anti-huntingtin’s N terminus antibody 437 (10) or anti-c-Myc-tagged antibody 9E10 (Santa Cruz). Human brain tissues were obtained from Dr. J.-P. Vonsattel or Human Brain Bank at McLean Hospital, Boston, MA with institutional review board approval. Post-mortem time was between 10–12 h. The diagnosis of HD was confirmed with neuropathological and genetic phenotype analysis. Human cortex tissues from normal subjects or HD patients were ho-
mogenized in detergent-free lysis buffer, and Nonidet P-40 was added to a final concentration of 1%. The mixture was then incubated at 4 °C with constant shaking for 1–2 h, and insoluble fractions were removed by centrifugation.

**Purification of GST Fusion Proteins and in Vitro Binding Assay—**

Huntingtin N-terminal GST fusion protein constructs were generated by subcloning of a cDNA fragment encoding the first three exons of the HD gene containing 16 or 56 CAG repeats into pGEX2T. Construct for huntingtin's N terminus with 16 CAG repeats, a cDNA fragment was excised from pFL16HD with EcoRI and subcloned into pcDNA 1.1 (Invitrogen). The full-length c-Myc-tagged MLK2 was a gift from Dr. Alan Hall (14). The MLK2 SH3 domain cDNA fragment was amplified by reverse transcription-PCR and inserted into pGEX4T1. To generate an expression vector for huntingtin’s N terminus with 16 CAG repeats, a cDNA fragment was excised from pFL16HD with NotI and SphI and subcloned into pcDNA 1.1 (Invitrogen).

**Activation of MLK2 by Mutated Huntingtin**

JNK and TUNEL Assays—16 h after transfection, HN33 cells were lysed with 1% Triton X-100 lysis buffer (1). JNK was assayed as described previously (1). For TUNEL assay, HN33 cells were plated on a slide culture chamber. After transfection, cells were fixed at the time indicated in the figures, and TUNEL staining was performed as described previously (1). Most apoptotic HN33 cells were detached from the slides, and TUNEL stain was performed on remaining attached cells. TUNEL stain-negative cells (living cells) were counted in the 20× power field in four different locations on the slides and ~600–800 cells were counted in the control (1).

**RESULTS**

293T cells, which are rich in huntingtin (10), were utilized to study the interaction of huntingtin with MLK2. c-Myc-tagged MLK2 was transiently expressed in 293T cells, and cell lysates were immunoprecipitated with 437, an anti-huntingtin N-terminal antibody (10), and the resulting blot was probed with 9E10, an anti-c-Myc-tagged monoclonal antibody. MLK2 was detected in 437 and 9E10 immunoprecipitates of lysates transfected with c-Myc-tagged MLK2 but not in the negative controls, 9E10 or 437 immunoprecipitates of wild-type or vector-transfected 293T cell lysates (Fig. 1A). Conversely, we also found that huntingtin protein was present in 9E10 immunoprecipitates of c-Myc-tagged MLK2-transfected 293T cell lysates but not wild-type or vector-transfected lysates (Fig. 1B).
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These data show that MLK2 is associated with huntingtin in intact cells.

Next, we examined the interaction of MLK2 with huntingtin in a hippocampal neuronal cell line, HN33 cells in which we found that expression of polyglutamine-expanded huntingtin induced apoptosis (1). MLK2 is richly expressed in HN33 cells, and the amount of MLK2 proteins in the cell is similar to that of 293T cells over-expressing c-Myc-tagged MLK2 (Fig. 1C). HN33 cell lysates were immunoprecipitated with 437 or 4C8, a well characterized anti-huntingtin monoclonal antibody (5), and the resulting blot was probed with a specific anti-MLK2 antibody that has been characterized previously (20). As shown in Fig. 2B, both 437 and 4C8 were able to precipitate MLK2 from HN33 cell lysates, whereas the peptide-antigen pre-absorbed 437 failed to do so. These data provide further evidence that normal huntingtin is associated with MLK2 in neuronal cells.

To determine whether the SH3 domain of MLK2 mediates its interaction with huntingtin, we investigated the binding of huntingtin to a GST MLK2-SH3 domain fusion protein, whereas a GST MLK2 C terminus fusion protein, which lacks the SH3 domain, served as a negative control. As shown in Fig. 1D, huntingtin binds to the MLK2 SH3 domain but not GST alone or the MLK2 C terminus. To verify whether the SH3 domain of MLK2 mediates its interaction with huntingtin, we generated a MLK2-SH3 domain-deficient GST fusion protein by substitution of the first tryptophan of the highly conserved tryptophan doublet of the MLK2 SH3 domain to lysine. Mutation of the tryptophan doublet of the SH3 domain is known to eliminate its ability to bind to proline-rich ligands (10). Huntingtin failed to bind to MLK2-SH3 domain-deficient GST fusion proteins (Fig. 1D). These data show that the SH3 domain of MLK2 mediates its interaction with normal huntingtin.

We next determined whether expansion of the polyglutamine repeat in huntingtin would alter its interaction with MLK2. The N-terminal proline-rich region adjacent to the polyglutamine repeat has been reported to bind to SH3 domain-containing proteins (10–11). Thus, we examined the binding of MLK2 to huntingtin’s N terminus containing either a normal or expanded polyglutamine stretch. GST fusion proteins of huntingtin’s N terminus containing 16 or 56 polyglutamine repeats were generated and purified. These GST fusion proteins were utilized as a template to examine whether huntingtin’s N terminus is responsible for its interaction with MLK2 and how expansion of the polyglutamine repeat affects this interaction. As shown in Fig. 2A, MLK2 binds to huntingtin’s N terminus containing 16 polyglutamine repeats. Because the N-terminal proline region is the only potential SH3 domain binding site in this small N-terminal segment of huntingtin and others have shown that this region mediates huntingtin binding to SH3 domain (11), these data suggest that the N-terminal proline region is involved in huntingtin interaction with MLK2. The amount of c-Myc-tagged MLK2 bound to huntingtin’s N terminus with 56 polyglutamine repeats was significantly reduced, about 70% less than that associated with the N terminus of normal huntingtin (Fig. 2A). This data indicates that expansion of the polyglutamine repeat may inhibit the ability of huntingtin’s N terminus to interact with the SH3 domain of MLK2.

Next, we examined the interaction of MLK2 with huntingtin in the human brain. Because our MLK2 antibody cannot be used for immunoprecipitation, a MLK2 SH3 domain GST fusion protein was used to test the ability of normal and polyglutamine-expanded huntingtin proteins from human brain tissues to bind to MLK2. Lysates of human brain cortex tissues from a normal subject and a mid-age onset HD patient were prepared. Wild-type or mutated MLK2 SH3 domain GST fusion protein was incubated with human brain lysates, and the resulting blot was probed with 437. As shown in Fig. 2B, normal huntingtin protein from normal or HD human brain tissues bound to the wild-type MLK2 SH3 domain GST fusion protein but not to GST alone or to the MLK2 SH3 domain-deficient GST fusion protein. In contrast, polyglutamine-expanded huntingtin protein from the HD patient brain only weakly bound to the SH3 domain of MLK2 (Fig. 2B). These data further support our findings that normal huntingtin binds to the SH3 domain of MLK2 and that the polyglutamine expansion interferes with its ability to interact with the SH3 domain of the kinase.

MLK2 is known to induce JNK activation in Cos-1 cells (14). Therefore, we tested whether expression of MLK2 activates JNKs in HN33 cells. The MLK2 expression vector (pR5K) or vector alone was transiently transfected into HN33 cells. JNKs were precipitated using a GST-c-Jun protein, and an in vitro JNK assay was performed. An equal amount of JNK proteins were precipitated in each JNK assay (data not shown). As observed in other neuronal cells (21), a basal level of JNK activity was found in HN33 cells (Fig. 3A). MLK2 induced constitutive activation of JNKs in HN33 cells. As shown in Fig. 3A, the level of the JNK activity was increased by 8-fold (Fig. 3, A and B). Because MLK2 mediates JNK activation via phosphorylation and activation of both MKK7 and SEK1 (14–16), we determined whether co-expression of dominant negative
MKK7 and SEK1 could block MLK2-mediated JNK activation. As shown in Fig. 3A, MLK2-mediated JNK activation was significantly attenuated by co-expression of dominant negative MKK7 and SEK1 but not by co-expression of wild-type MKK7 and SEK1, which had little effect on the JNK activity induced by MLK2. Increase of JNK activity was determined by analyzing the blots with a densitometer. The values depicted represent the fold stimulation of JNK activity of HN33 cells transfected with different plasmids as indicated in the figure over the activity of HN33 cells transfected with vector alone. Data are the average of three independent experiments. C, expression of MLK2 in HN33 cells induced apoptotic cell death. HN33 cells were transfected with MLK2 expression vector. Following transfection, cells were fixed at the times indicated in the figure, and TUNEL staining was performed as described under “Materials and Methods.” Most apoptotic HN33 cells were detached from slides, and TUNEL staining was performed on the remaining cells. Cells showing the retraction of neurites and positive stain in the nucleus were recognized as apoptotic. TUNEL-negative cells (living cells) were counted, and the number of TUNEL-negative cells in the control (transfected with pRK5 + pEBG + pcDNA1) was designated as 100%. Data are the average of three independent experiments. D, co-expression of dominant negative MKK7 and SEK1 significantly inhibited MLK2-mediated apoptosis in HN33 cells. HN33 cells were co-transfected with MLK2 expression vector and wild-type or dominant negative SEK1 or MKK7. TUNEL staining was conducted, and TUNEL-negative cells were counted.

Fig. 3. Expression of MLK2 in HN33 cells induces JNK activation and neuronal apoptosis. A, expression of MLK2-mediated JNK activation in HN33 cells; HN33 cells were transiently transfected with different plasmids as indicated in the figure. 16 h after transfection, HN33 cells were lysed, and JNK activity was measured as described previously (24). S, Sek1; 7, MKK7, MS, dominant negative Sek1 (24); M7, dominant negative MKK7; vectors, pRK5 and pEBG. B, the fold of the JNK activity induced by MLK2. Increase of JNK activity was determined by analyzing the blots with a densitometer. The values depicted represent the fold stimulation of JNK activity of HN33 cells transfected with different plasmids as indicated in the figure over the activity of HN33 cells transfected with vector alone. Data are the average of three independent experiments. C, expression of MLK2 in HN33 cells induced apoptotic cell death. HN33 cells were transfected with MLK2 expression vector. Following transfection, cells were fixed at the times indicated in the figure, and TUNEL staining was performed as described under “Materials and Methods.” Most apoptotic HN33 cells were detached from slides, and TUNEL staining was performed on the remaining cells. Cells showing the retraction of neurites and positive stain in the nucleus were recognized as apoptotic. TUNEL-negative cells (living cells) were counted, and the number of TUNEL-negative cells in the control (transfected with pRK5 + pEBG + pcDNA1) was designated as 100%. Data are the average of three independent experiments. D, co-expression of dominant negative MKK7 and SEK1 significantly inhibited MLK2-mediated apoptosis in HN33 cells. HN33 cells were co-transfected with MLK2 expression vector and wild-type or dominant negative SEK1 or MKK7. TUNEL staining was conducted, and TUNEL-negative cells were counted.
generate any cell toxicity (Fig. 3D), whereas co-expression of
dominant negative SEK1 and MKK7 significantly attenuated
neuronal toxicity induced by MLK2. As shown in Fig. 3D,
~75% of HN33 cells were rescued upon co-expression of the
dominant negative mutant form of MKK7 and SEK1. These
data indicate that JNK-mediated neuronal toxicity is induced
by MLK2 in HN33 cells. Co-expression of dominant negative
MKK7 and SEK1 without MLK2 caused rapid apoptosis in
HN33 cells (Fig. 3D). Inhibition of basal JNK activity may
account for this cell toxicity, because co-expression of dominant
negative MKK7 and SEK1 decreased basal JNK activity in
HN33 cells (Fig. 3, A and B), whereas when these two mutated
kinases were co-expressed with MLK2 in HN33 cells, JNK
activity was double the basal level (Fig. 3, A and B), and under
this condition HN33 cells were viable (Fig. 3D). These results
suggest that a certain basal level of JNK activity appears to be
essential for the survival of HN33 cells, and either over-activa-
tion or inhibition of basal JNK activity triggers apoptosis.

To further investigate whether MLK2-mediated signaling
cascades are involved in neuronal death induced by polyglu-
tamine-expanded huntingtin, a dominant negative (kinase-
dead) form of MLK2, which is known to competitively inhibit
the endogenous kinase, was generated. Different full-length
huntingtin expression vectors containing 16, 48, or 89 polyglu-
tamine repeats were separately co-transfected with wild-type
or the dominant negative form of MLK2 into HN33 cells. As
shown in Fig. 4A, co-expression of dominant negative MLK2
significantly inhibited neuronal toxicity mediated by polyglu-
tamine-expanded huntingtin in HN33 cells. At 48 h post-trans-
fection of the huntingtin construct containing 48 or 89 CAG
repeats, over 75% of HN33 cells remained viable (i.e. trypan
blue stain-negative) when dominant negative MLK2 is co-ex-
pressed, compared with 70–80% of apoptotic cells when the
mutated huntingtin was expressed alone (Fig. 4A). These data
further support a role for MLK2 in the mediation of neuronal
toxicity induced by polyglutamine-expanded huntingtin.

Our data show that the N-terminal proline-rich region of
huntingtin interacts with MLK2, and the polyglutamine ex-
pansion interferes with this interaction. These results suggest
that the polyglutamine expansion may lead to an increase in
free MLK2 proteins, which are constitutively active and cell
toxic (14). If this hypothesis is true, over-expression of the N
terminal of normal huntingtin, which binds to free MLK2
proteins, should be able to overcome the neuronal toxicity
induced by MLK2 and polyglutamine-expanded huntingtin. We
prepared a construct encoding a small region of the normal
huntingtin N terminus, containing a 16 polyglutamine repeat
and the proline-rich region. As shown in Fig. 4B, co-expression
of this N-terminal fragment of normal huntingtin significantly
attenuated neuronal toxicity mediated by MLK2 and by the
mutated huntingtin with 48 polyglutamine repeats. Over 50%
of neurons remained viable at 48 h post-transfection, compared
with less than 20% of viable cells when the N-terminal frag-
ment was not co-expressed (Fig. 4B). These studies support
our hypothesis that the polyglutamine expansion in huntingtin
may interfere with its interaction with MLK2 thereby leading to
an increase of free MLK2 proteins that in turn mediate JNK
activation and neuronal apoptosis.

**DISCUSSION**

In the present study, we demonstrate that MLK2, an up-
stream activator of the JNK pathway, is involved in JNK
activation and neuronal apoptosis mediated by polyglutamine-
expanded huntingtin (1). The polyglutamine expansion de-
creases the association of huntingtin with MLK2 leading to an
increase in unregulated MLK2 proteins that, being constitu-
tively active, cause JNK activation and neuronal toxicity. Co-
expression of dominant negative MKK7 and SEK1, the down-
stream effectors of MLK2, blocks JNK activation and neuronal
apoptosis induced by MLK2 and by polyglutamine-expanded
huntingtin. Additionally, co-expression of dominant negative
MLK2 significantly attenuated JNK activation and neuronal
toxicity mediated by the polyglutamine expanded huntingtin.
Finally, over-expression of a normal huntingtin N-terminal
protein significantly attenuated neuronal toxicity induced by
both MLK2 and polyglutamine-expanded huntingtin. These
results show that MLK2-mediated cellular signaling cascades
may play a significant role in neuronal death induced by poly-
 glutamine-expanded huntingtin in HN33 cells.

Because huntingtin is a ubiquitously expressed protein,
whereas the pathology of HD is restricted to the brain, it is
likely that huntingtin binds to proteins that are largely found
in the brain, and the polyglutamine expansion alters hunting-
tin’s ability to interact with these proteins thereby resulting in
activation of neurotoxic pathways. MLK2 being almost exclu-

**FIG. 4.** MLK2 may be partially involved in polyglutamine-ex-

dended huntingtin-mediated neuronal apoptosis. HN33 cells
were transiently transfected with different plasmids as indicated in
the figure. Cells were fixed at 48 h post-transfection, and TUNEL
staining was carried out. TUNEL-negative cells were counted, and the number of
TUNEL-negative cells co-transfected with vector (pCDNA1 + pRK5) was
designated as 100%. Data are the average of three independent
experiments. A, co-expression of dominant negative MLK2 significantly at-

tenuated neuronal toxicity mediated by polyglutamine-expanded hun-
gtingtin. Full-length huntingtin constructs containing 16, 48, or 89 CAG
repeats (1) were transfected alone or co-transfected with wild-type or
dominant negative MLK2 (MLK2/E) followed by TUNEL staining. B, over-
expression of the N terminus of normal huntingtin overcame neu-
ronal toxicity induced by MLK2 and by polyglutamine-expanded hun-
gtingtin. The expression vector for the N terminus of normal huntingtin
with 16 CAG repeats (pNT16HD) was co-transfected with MLK2 or
full-length huntingtin with 48 CAG repeats as indicated in the figure.
TUNEL-negative cells were counted as described above.
sively expressed in the brain could provide a partial explanation for why the polyglutamine-expanded huntingtin is selectively toxic to neurons. MLK2 is a strong activator of the JNK pathway that is now known to couple a variety of cell-toxic stimuli, leading to neuronal apoptosis (21, 22). Because MLK2 is a constitutively active kinase, and free MLK2 is the active form (14), any alteration of the amount of bound MLK2 is likely to lead to activation of MLK2-mediated signaling cascades and neuronal toxicity. We show that normal huntingtin proteins from normal or HD human cortex tissues specifically bind to wild-type but not the mutated SH3 domain of MLK2, and under the same conditions, the mutated huntingtin proteins from HD patient cortex tissues only weakly bound to the SH3 domain of MLK2. Moreover, over-expression of the N terminus of normal huntingtin, which binds and decreases free MLK2 proteins in HN33 cells, can significantly inhibit neuronal toxicity induced by MLK2 and by the mutated huntingtin, further supporting the notion that huntingtin’s N terminus interacts with the SH3 domain of MLK2, and the ability to bind to the kinase may be impaired upon polyglutamine expansion in huntingtin.

The polyglutamine expansion apparently alters the physical properties of huntingtin. The mobility of the mutated huntingtin on SDS-polyacrylamide gel electrophoresis is clearly decreased (5), and huntingtin’s N terminus protein carrying an expanded polyglutamine stretch forms amyloid-like protein aggregates both in vitro and in vivo (23, 24). Because the N-terminal proline-rich region is adjacent to the polyglutamine stretch, it is possible that the polyglutamine expansion may alter the binding properties of this proline-rich region. Our group previously found that normal huntingtin is associated with epidermal growth factor receptor signaling complexes through binding to the SH3 domains of Grb2 and RasGAP, and this association is regulated by activation of the epidermal growth factor receptor (10). Recently, other groups have also reported that huntingtin binds to Grb2-like SH3 domain-containing proteins, and the N-terminal proline-rich region mediates these interactions (10–11). Our results from the current study are consistent with these reports (10–11). In addition, we show that the ability of this proline-rich region to bind to the SH3 domain of MLK2 is impaired upon expansion of the polyglutamine stretch. The interaction of proline-rich motifs with SH3 domains is not a highly selective event (9). Thus, if the polyglutamine expansion in huntingtin interferes with its interaction with the SH3 domain of MLK2, it may inhibit its association with other SH3 domain-containing proteins. Perhaps the normal function of huntingtin is the modulation of the cellular signaling network by sequestering these SH3 domain-containing signaling proteins. When the ability of huntingtin to interact with SH3 domains is impaired, polyglutamine-expanded huntingtin may be disassociated from the microtubule complex where most SH3 domain-containing proteins are found, leading to the re-arrangement of SH3 domain-containing protein-associated signaling complexes, which may subsequently result in an imbalance of cellular signaling networks and neuronal death. Incidentally, huntingtin’s N terminus with an expanded length of polyglutamine repeat forms nuclear inclusions in the brains of HD patients or in cultured cells (25), indicating that some mutated huntingtin proteins are translocated and no longer co-present with MLK2 or other SH3 domain-containing proteins in the cytoplasm.

It is clear now that striatal medium-spiny neurons, which die first in HD, lack endogenous huntingtin. Thus, dif fusible neurotoxic factors may play an important role in early neuronal loss in HD. Our previous studies and current results are consistent with this notion; expression of the mutated huntingtin or MLK2 induces not only apoptotic cell death of transfected HN33 cells but also non-transfected cells. Because JNK activation has been reported to mediate free radical production (26), it is possible that polyglutamine-expanded huntingtin may mediate free radical production via activation of the MLK2-JNK pathway. In summary, our current studies show that activation of MLK2-mediated signaling cascades may be partially responsible for neuronal loss in HD, and an inhibitor of MLK2 may be useful for the prevention of neuronal loss in HD.

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