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Expression of Expanded Polyglutamine Proteins Suppresses the Activation of Transcription Factor NFκB*

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A major pathological hallmark of the polyglutamine diseases is the formation of neuronal intranuclear inclusions of the disease proteins that are ubiquitinated and often associated with various transcription factors, chaperones, and proteasome components. However, how the expanded polyglutamine proteins or their aggregates elicit complex pathogenic responses in the neuronal cells is not fully understood. Here, we have demonstrated that the expression of expanded polyglutamine proteins down-regulated the NFκB-dependent transcriptional activity. The expression of expanded polyglutamine proteins increased the stability and the levels of IκB-α and its phosphorylated derivatives. We have also found that various NFκB subunits and IκB-α aberrantly interacted with the expanded polyglutamine proteins and associated with their aggregates. Finally, we have shown that several NFκB-dependent genes are down-regulated in the expanded polyglutamine protein-expressing cells and down-regulation of NFκB activity enhances expanded polyglutamine protein-induced cell death. Because the NFκB pathway plays a very important role in cell survival, altered regulation of this pathway in expanded polyglutamine protein-expressing cells might be linked with the disease pathogenesis.

A common pathological feature of most age-related neurodegenerative disorders, including polyglutamine diseases, is the accumulation of intracellular protein deposits as inclusion bodies. Polyglutamine diseases are a group of familial neurodegenerative disorders that are caused by an abnormal expansion of CAG triplet repeats in the coding region of the target gene. These include Huntington disease (HD),[3] dentatorubral pallidoluysian atrophy, *X*-linked spinal bulbar muscular atrophy, and several spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7 and SCA17). All of these disorders are dominantly inherited (except *X*-linked spinal bulbar muscular atrophy), progressive, usually begin in midlife, and result in severe neuronal dysfunction and neuronal cell death in the selective region of the brain (1–4).

Polyglutamine aggregates or their soluble oligomeric forms can associate with several factors and disrupt the cellular function in many ways, including transcriptional dysregulation (5–11) and impairment of proteasome function (12–16). TATA box-binding protein, cAMP-response element-binding protein (CREB)-binding protein, Sp1, and other transcription factors have been reported to associate with the polyglutamine aggregates, and this may result in their sequestration and loss of function (5–11). Polyglutamine aggregates also have been found to associate with various components of the ubiquitin-proteasome system (UPS), and the impairment of UPS function has been observed in mutant huntingtin-expressing cells (12–20). But how UPS dysfunction is involved in the pathogenesis of polyglutamine diseases is unknown.

NFκB is a ubiquitous transcription factor that regulates the transcription of many genes involved in immune and inflammatory responses as well as cell survival and cell death (21–23). NFκB is localized in the cytoplasm in an inactive form in association with a family of inhibitory proteins (IκBs). In response to multiple activating signals, IκB is phosphorylated and subsequently degraded by the UPS. The rapid degradation of IκB proteins unmasks the nuclear localization signals of NFκB, which then translocate to the nucleus and activate the transcription of multiple genes. The activation of NFκB seems to stimulate some pathways that promote cell death, whereas other pathways promote cell survival (22, 23).

Several neurotrophic factors, cytokines, and excitotoxic neurotransmitters have been found to activate the NFκB pathway, and in most cases, activation leads to neuroprotection (22, 24–26). The NFκB activation in neurons is also observed in acute neurodegenerative conditions such as stroke and ischemic brain injury (22, 27, 28) and in chronic neurodegenerative disorders such as Alzheimer disease, HD, and amyotrophic lateral sclerosis (29–33). The increased NFκB activity in the affected neurons might be an early protective response to ongoing oxidative stress or mitochondrial dysfunction (34). This is supported by the fact that the mice lacking the p50 subunit of NFκB shows increased damage of striatal neurons and motor disturbances after administration of mitochondrial toxin 3-nitropropionic acid (31).

We had reported earlier the impairment of UPS function in the expanded polyglutamine protein-expressing cells, which might be linked with cell death. But how the UPS dysfunction leads to the death of expanded polyglutamine protein-express-

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[3] The abbreviations used are: HD, Huntington disease; SCA, spinocerebellar ataxia; tNhtt, truncated N-terminal huntingtin; UPS, ubiquitin proteasome system; GFP, green fluorescent protein; EGFP, enhanced green fluorescent protein; iNOS, inducible isoform of nitric-oxide synthase.

ing cells is not well understood. Because the NFκB activation is essential for cell survival and is highly regulated by the UPS (35), we investigated the possible role of the NFκB pathway in the expanded polyglutamine protein-induced cell death. Here, we report that the expression of expanded polyglutamine proteins causes down-regulation of NFκB activity and promotes expanded polyglutamine protein-induced cell death.

EXPERIMENTAL PROCEDURES

Materials—MG132, curcumin, cycloheximide, lipopolysaccharide, proteasome substrate, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, and all cell culture reagents were obtained from Sigma. Lipofectamine 2000, zeocin, G418, and ponasterone A were purchased from Invitrogen. SN50 (peptide inhibitor of NFκB) was purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-IκB-α, anti-p-IκB-α, anti-NFκB p65 and p50 subunit, anti-ubiquitin, anti-iNOS, mouse monoclonal anti-β-tubulin, and goat polyclonal anti-Cox2 were from Santa Cruz Biotechnology, and mouse monoclonal anti-GFP was from Roche Diagnostics. Goat anti-rabbit IgG-rhodamine was purchased from Vector Laboratories and horseradish peroxidase-conjugated anti-mouse and -rabbit IgG were from Amersham Biosciences. The source of ataxin-3 antibody has been described elsewhere (36).

Expression Plasmids and Stable Cell Lines—The enhanced green fluorescent protein (EGFP) and truncated N-terminal huntingtin (tNhtt) expression constructs pIND-tNhtt-EGFP-16Q and pIND-tNhtt-EGFP-150Q and the generation of the stable cell lines of these constructs have been described previously (37). The generation of the stable neuro-2a cell line expressing retinoic acid *X* receptor (*RXR*) also has been described earlier (37). The construction of plasmids pEGFP-N1-MJD(t)-20CAG, pEGFP-N1-MJD(t)-130CAG, pcDNA-MJD(t)-20CAG, and pcDNA-MJD(t)-130CAG have been described elsewhere (36). The plasmid NFκB-luciferase (containing multiple copies of NFκB response elements) was obtained from Clontech. The plasmid PRL-SV40 and the dual luciferase reporter assay system were obtained from Promega.

Cell Culture, Transfection, Cell Viability, and Reporter Gene Assay—The wild-type mouse neuro-2a and COS-1 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum and antibiotics penicillin/streptomycin. The stable neuro-2a cell lines HD 16Q and HD 150Q were maintained in the same medium containing 0.4 mg/ml zeocin and 0.4 mg/ml G418. One day prior to transfection, the cells were plated into 6-well tissue-cultured plates at a subconfluent density. For the reporter gene assay, cells were transiently transfected with NFκB-luciferase and PRL-SV40 plasmids together using Lipofectamine 2000 reagent according to the manufacturer's instructions. Transfection efficiency was ~80–90%. The cells were left untreated or induced with ponasterone A (1 μM) for different time periods and then processed for luciferase assay. In some experiments, cells were induced for 2 days, treated with different NFκB-modulating agents for 8 h, and then processed for luciferase assay. In another experiment, the neuro-2a cells were first transfected with pEGFP-N1-MJD(t)-20CAG and pEGFP-N1-MJD(t)-130CAG plasmids followed by NFκB-luciferase and PRL-SV40 plasmids. Luciferase activity was measured using the dual luciferase reporter assay system according to the manufacturer's instructions. PRL-SV40 was used for co-transfection to normalize the data and transfected at a very low concentration (150-fold lower than NFκB luciferase plasmid). Data were represented as relative luciferase activity (the ratio of firefly to *Renilla* values). For the cell viability assay, HD 16Q and HD 150Q cells were plated into 96-well plates (5×10^3 cells/well). The cells were then induced with 1 μM ponasterone A for 2 days and then treated with various agents for 8 h. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described previously (37). The proteasome assay was performed as described earlier (38, 39). Statistical analysis was performed using Student's *t* test, and $p < 0.05$ was considered to indicate statistical significance.

Co-immunoprecipitation and Immunoblotting Experiment—After 24 or 48 h of induction, cells were washed with cold phosphate-buffered saline, scraped, pelleted by centrifugation, and lysed on ice for 30 min with Nonidet P-40 lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, complete protease inhibitor mixture). Cell lysates were briefly sonicated, centrifuged for 10 min at 15,000 × *g* at 4 °C, and the supernatants (total soluble extract) were used for immunoprecipitation as described earlier (38, 39). For each immunoprecipitation experiment, 200 μg of protein in 0.2 ml of Nonidet P-40 lysis buffer was incubated either with 5 μl (2 μg) of GFP antibody or 4 μl (2 μg) of normal mouse IgG. Bound proteins were eluted from the beads with SDS (1×) sample buffer, vortexed, boiled for 5 min, and analyzed by immunoblotting according to the procedure described earlier (38, 39). Blot detection was carried out with enhanced chemiluminescence reagent. All primary antibodies were used at 1:1000 dilutions for immunoblotting.

Immunofluorescence Techniques—Cells grown in chamber slides were induced with ponasterone A. Forty-eight hours after induction, the cells were washed twice with phosphate-buffered saline, fixed with 4% paraformaldehyde in phosphate-buffered saline for 20 min, permeabilized with 0.5% Triton X-100 in phosphate-buffered saline for 5 min, washed extensively, and then blocked with 5% nonfat dried milk in TBST (Tris-buffered saline with Tween 20) for 1 h. Primary antibody (anti-NFκB and anti-IκB-α, 1:500 dilutions; anti-ataxin-3, 1:1000 dilutions) incubation was carried out overnight at 4 °C. After several washings with TBST, the cells were incubated with rhodamine-conjugated secondary antibody (1:500 dilutions) for 1 h, washed several times, and mounted in anti-fade solution. The samples were observed using a confocal microscope, and digital images were assembled using Adobe Photoshop.

Degradation Assay—HD 16Q and HD 150Q cells were plated in a 6-well tissue-cultured plate and induced with ponasterone A for 48 h. The cells were chased with 10 μg/ml of cycloheximide for different time periods. The cells collected at each time point were then processed for immunoblotting using antibodies against IκB-α and NFκB p65 and p50 subunits.

RESULTS

The Levels of IκB-α and Its Phosphorylated Derivatives Is Increased in Expanded Polyglutamine Protein-expressing Cells—We developed several stable neuro-2a cell lines in an inducible

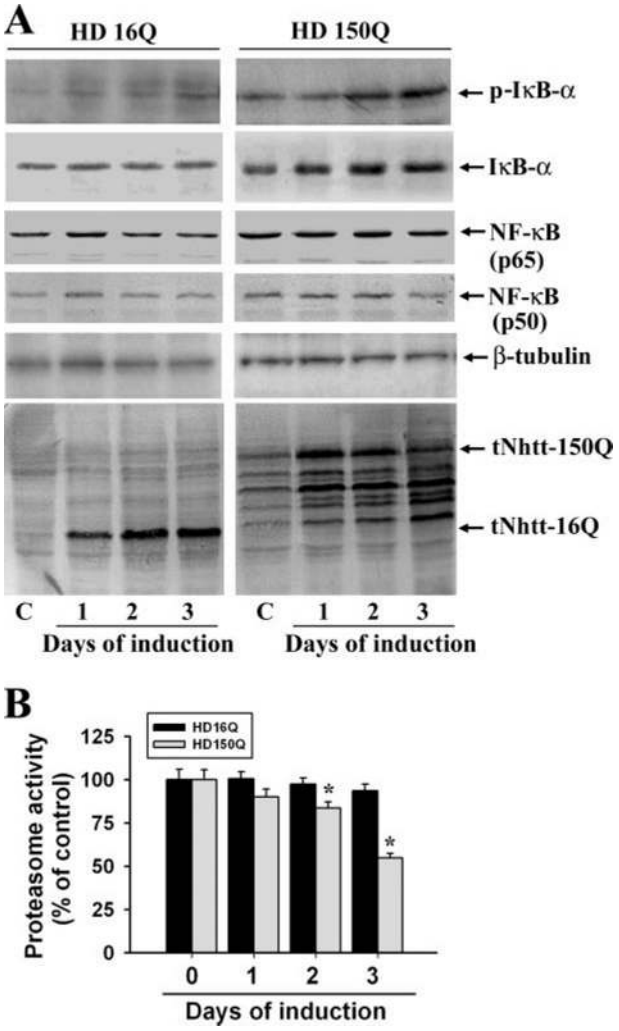

FIGURE 1. **Expression of expanded polyglutamine protein increases the levels of IκB-α and its phosphorylated derivatives.** The HD 16Q and HD 150Q cells were plated into 6-well tissue-cultured plates. On the following day, the cells were left untreated or induced with 1 μM ponasterone A for different time periods as indicated in the figure. Cells collected at each time point were then processed for either immunoblotting using antibodies against IκB-α, p-IκB-α, NFκB p65, and p50 subunits and β-tubulin (*A*) or proteasome activity (chymotrypsin-like) assay (*B*).

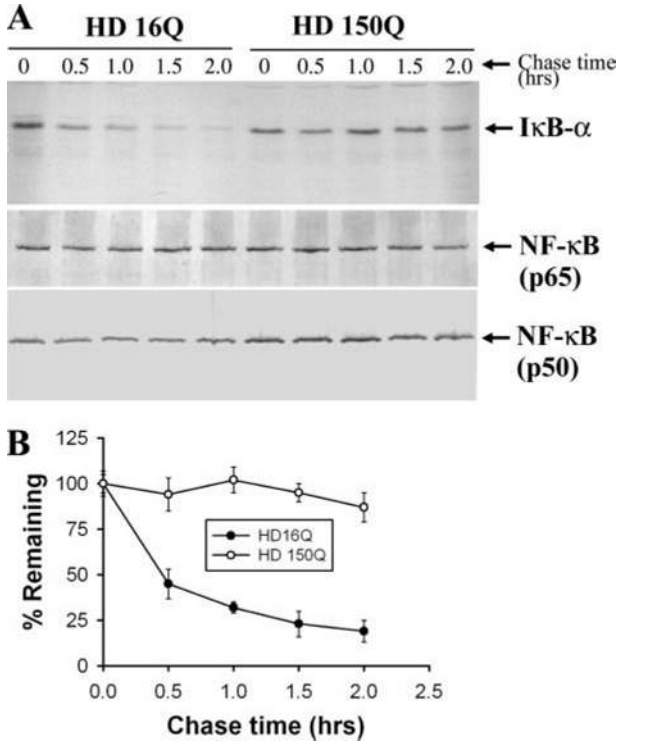

FIGURE 2. **Increased half-life of IκB-α in the expanded polyglutamine protein-expressing cells.** *A*, HD 16Q and HD 150Q cells were induced with ponasterone A for 48 h in a similar way as described in the legend to Fig. 1. Cells were chased in the presence of 10 μg/ml cycloheximide for different time period as indicated in the figure. Cells were then collected and processed for immunoblotting using anti-IκB-α and anti-NFκB p65 and p50. *B*, quantitation of the band intensities of the IκB-α blots collected from three independent experiments was performed using NIH image analysis software. Values are means ± S.D.

system that expresses tNhtt with normal (16Q) and expanded polyglutamine (150Q) proteins (37). These cell lines were named HD 16Q and HD 150Q, and their corresponding expressed proteins were named tNhtt-16Q and tNhtt-150Q. The cell lines were induced for different time periods with ponasterone A (1 μM) and then processed for immunoblotting using antibodies against IκB-α, p-IκB-α, NFκB p65 and p50 subunits, and β-tubulin. As shown in Fig. 1*A*, expression of tNhtt-150Q protein time-dependently increased the levels of IκB-α and p-IκB-α. There was no change of the levels of IκB-α and slight increase in p-IκB-α in the HD 16Q cells upon expression of tNhtt-16Q protein. The levels of IκB-α and its phosphorylated derivatives in the uninduced HD 150Q cells were much higher compared with uninduced HD 16Q cells. This is most likely because of the low levels of expression and aggregation of tNhtt-150Q protein without any induction (37). The levels of NFκB p65 and p50 subunit were not changed in both HD 16Q and HD 150Q cells upon different days of induction.

We had reported earlier the proteasomal dysfunction in the HD 150Q cells (15). Therefore, we presumed that the increased accumulations of IκB-α and its phosphorylated derivatives might be due to the altered proteasomal function in these cells. To confirm this hypothesis, we determined the proteasome activity in HD 16Q and HD 150Q cells upon different days of induction. As shown in Fig. 1*B*, the expression of expanded polyglutamine proteins time-dependently reduced the proteasome activity in HD 150Q cells. This inhibition of proteasome activity correlated well with the increased accumulation of IκB-α and its phosphorylated derivatives. The slight increase in p-IκB-α in the HD 16Q cells also might be due to the proteasomal dysfunction at higher levels of expression of tNhtt-16Q proteins. The HD 150 cells also show changes in morphology upon induction compared with HD 16Q cells. The cells exhibit neurite outgrowth and look bipolar. This again might be due to proteasomal dysfunction, because proteasome inhibitors are shown to induce neurite outgrowth.

Expression of Expanded Polyglutamine Proteins Increases the Stability of IκB-α—Because the levels of IκB-α and its phosphorylated forms are increased in HD 150Q cells upon induction, we assumed altered degradation of IκB-α in these cells. Therefore, we checked the half-life of IκB-α in both HD 16Q and HD 150Q cells after induction with ponasterone A for 2 days fol-

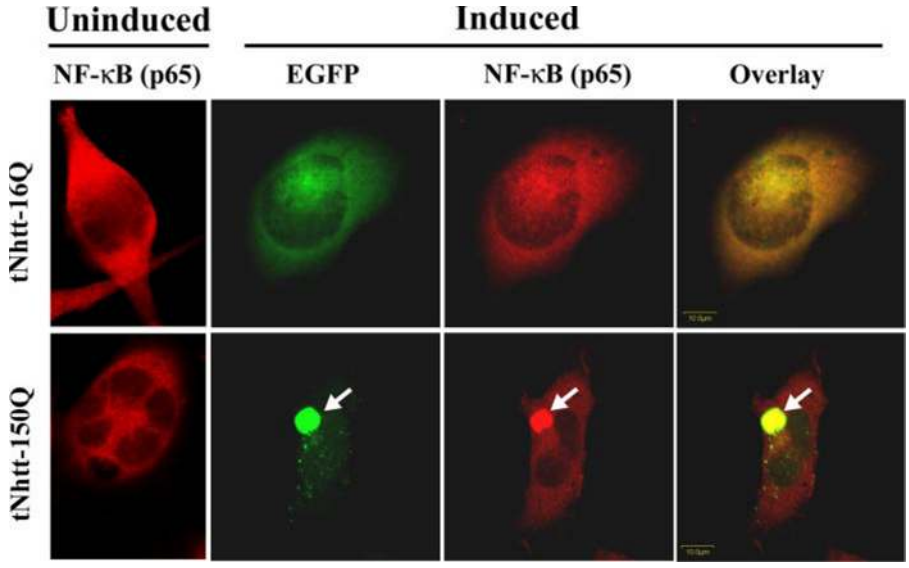

FIGURE 3. **Association of NFκB p65 protein with the polyglutamine-GFP aggregates.** The HD 16Q and HD 150Q cells were plated into a 2-well chamber slide. Cells were induced with ponasterone A for 48 h and processed for immunofluorescence staining using NFκB p65 antibody. Rhodamine-conjugated secondary antibody was used to stain the NFκB p65. *Arrows* indicate the recruitment of NFκB p65 to the mutant huntingtin aggregates.

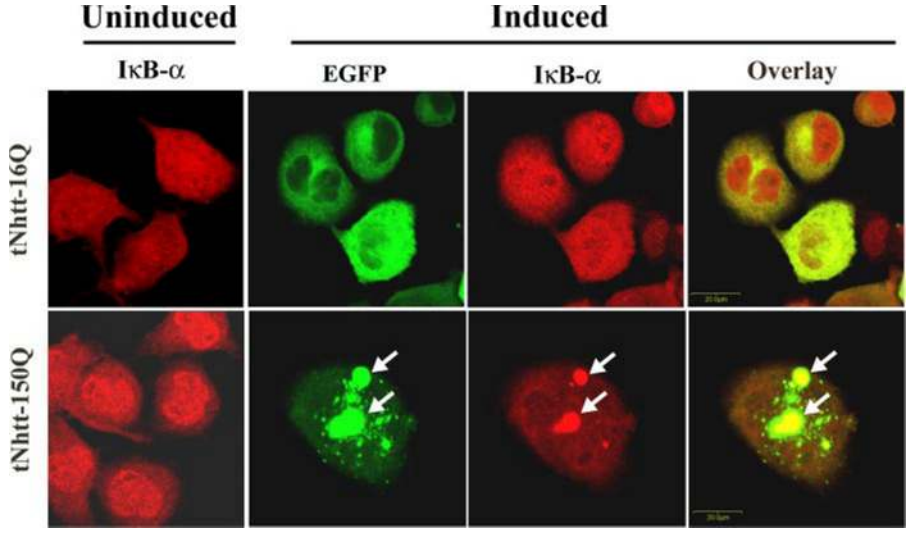

FIGURE 4. **Recruitment of IκB-α with the polyglutamine-GFP aggregates.** The HD 16Q and HD 150Q cells were plated and induced in a similar way as described in the legend to Fig. 3. Cells were subjected to immunofluorescence staining using antibody against IκB-α. Rhodamine-conjugated secondary antibody was used to visualize the IκB-α. *Arrows* indicate the association of IκB-α to the mutant huntingtin aggregates.

lowed by cycloheximide chase. More than 50% of HD 150Q cells form aggregates at 2 days of induction. Fig. 2 shows that the half-life of IκB-α dramatically increased in the HD 150Q cells in comparison with HD 16Q cells. The half-life of NFκB p65 and p50 subunits were not affected in both HD 16Q and HD 150Q cells when they were induced for 2 days and performed chase experiments.

Association of IκB-α and NFκB p65 with Polyglutamine Aggregates—Next, we searched for clues of increased half-life of IκB-α and their consequences in the HD 150Q cells. One of the reasons for increased half-life of IκB-α in the HD 150Q cells could be due to UPS dysfunction, because we have reported the inhibition of UPS function in these cells after 2 days of induction (15). The increased accumulation of IκB-α might lead to the inhibition of NFκB activity by preventing their nuclear translocation. To confirm this fact, we first performed immunofluorescence staining of the NFκB p65 subunit in the wild-type neuro-2a cells as well as in the HD 16Q and HD 150Q cells after 2 days of induction. The NFκB p65 protein was mostly localized in the cytoplasm with weak nuclear staining in the wild-type neuro-2a cells (data not shown). The expression of tNhtt-16Q in HD 16Q cells did not alter the distribution profile of NFκB p65 protein, in comparison with wild-type neuro-2a cells (Fig. 3). NFκB p65 was mostly localized in the cytoplasm in HD 16Q cells. However, to our surprise, we noticed that the expression of tNhtt-150Q protein in HD 150Q cells caused recruitment of NFκB p65 protein to the mutant huntingtin aggregates. We also observed reduction of the diffuse nuclear staining of NFκB p65 protein in these cells. Next, we performed immunofluorescence staining of IκB-α in the wild-type neuro-2a, HD 16Q, and HD 150Q cells. The HD 16Q and HD 150Q cells were similarly induced for 2 days. The IκB-α was normally distributed in both the cytoplasmic and nuclear compartments, with predominant nuclear localization in the wild-type neuro-2a and uninduced HD 16Q cells. The expression of tNhtt-16Q protein in HD 16Q cells did not alter the distribution pattern of IκB-α, as compared with uninduced HD 16Q cells (Fig. 4). The expression of tNhtt-150Q in HD 150Q cells caused recruitment of IκB-α to the mutant huntingtin aggregates, similar to NFκB p65 protein (Fig. 4). It seems that the whole NFκB complex is sequestered around the aggregates. Next, we performed co-immunoprecipitation experiments to confirm the interaction of soluble expanded polyglutamine proteins with the NFκB p65 and IκB-α. The HD 16Q and HD 150Q cell lines were induced for 1 day with ponasterone A (1 μM) and then processed for immunoprecipitation by anti-GFP. In other experiments, we transfected the truncated ataxin-3 constructs (pEGFP-N1-MJD(t)-20CAG and pEGFP-N1-MJD(t)-130CAG) to the COS-1 cell, and after 1 day, the cells were collected and processed for immunoprecipitation by the GFP antibody. Blots were probed with anti-IκB-α and anti-NFκB p65. Fig. 5 shows that both IκB-α and NFκB p65 are specifically interacted with polyglu-

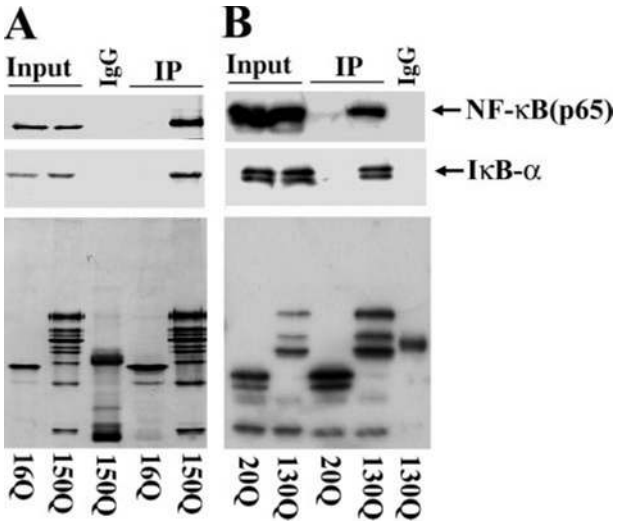

FIGURE 5. **Aberrant interaction of IκB-α and NFκB p65 with the expanded polyglutamine proteins.** *A*, the HD 16Q and HD 150Q cells were plated in a similar manner as described in the legend to Fig. 1. Twenty-four hours after induction, cells were collected and processed for immunoprecipitation (*IP*) by anti-GFP. Blots were probed with anti-NFκB p65 (*top blot*), anti-IκB-α (*middle blot*), and anti-GFP (*bottom blot*). *B*, the COS-1 cells were transiently transfected with the truncated ataxin-3-EGFP fusion constructs (1 μg each/well) containing 20Q and 130Q. Twenty-four hours after the transfection of ataxin-3 constructs, cell lysates were made and subjected to immunoprecipitation as described for *A*. The blots were sequentially probed with anti-NFκB p65 (*top blot*), anti-IκB-α (*middle blot*), and anti-GFP (*bottom blot*).

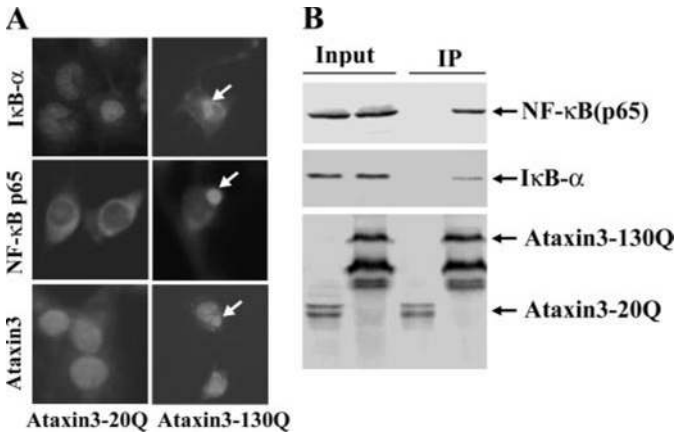

FIGURE 6. **Association of IκB-α and NFκB p65 with the expanded polyglutamine proteins.** Neuro-2a cells were transiently transfected with truncated ataxin-3 constructs. Forty-eight hours later, the cells were processed for either immunofluorescence staining using IκB-α, NFκB p65, and ataxin-3 antibody (*A*) or immunoprecipitation (*IP*) experiments using ataxin-3 antibody. Blots were probed sequentially with IκB-α, NFκB p65, and ataxin-3 antibodies (*B*).

tamine-expanded huntingtin or ataxin-3. The tNhtt-150Q or ataxin-3(t)-130Q appeared as multiple bands because of the instability of the CAG repeats.

To further confirm the association of the NFκB complex with expanded polyglutamine proteins or their aggregates and to exclude any interference of GFP, we transiently transfected truncated ataxin-3 constructs without the GFP tag. At 48 h of post-transfection, the cells were processed for either immunofluorescence or immunoblotting experiments using IκB-α, NFκB p65, and ataxin-3 antibodies. As expected, both IκB-α and NFκB p65 proteins were interacted and associated with the polyglutamine-expanded ataxin-3 (Fig. 6).

Down-regulation of NFκB-dependent Transcriptional Activity in the Expanded Polyglutamine Protein-expressing Cells—Because the half-life of IκB-α is increased in HD 150Q cells and the NFκB complex sequestered around the aggregates, we assumed that there might be down-regulation of NFκB activity in the HD 150Q cells. As expected, the expression of tNhtt-150Q protein in HD 150Q cells time-dependently decreased the NFκB-dependent transcriptional activity (Fig. 7*A*). The expression of tNhtt-16Q protein in HD 16Q cells did not have any effect on the NFκB-dependent transcriptional activity. The NFκB-dependent transcriptional activity was significantly lower in the uninduced HD 150Q cells as compared with HD 16Q cells most likely because of the leaky expression and aggregation of tNhtt-150Q proteins. Next, we determined the NFκB-dependent transcriptional activity in both HD 16Q and HD 150Q cells after treating with various modulators of the NFκB pathway. We used lipopolysaccharide as a stimulator and curcumin and MG132 as inhibitors of NFκB activation. Fig. 7*B* shows that treatment of lipopolysaccharide increased NFκB activity in HD 16Q cells while preventing the NFκB activation in HD 150Q cells. The curcumin and MG132 both enhanced the down-regulation of NFκB activity in the HD 150Q cells. We further checked the NFκB activity in the neuro-2a cells after transiently expressing the ataxin-3(t)-20Q and ataxin-3(t)-130Q proteins. In this case, we observed activation of NFκB in polyglutamine-expanded ataxin-3-expressing cells at 24 h of post-transfection, whereas we observed inhibition after 72 h of post-transfection (Fig. 7*C*). Possibly, the soluble expanded polyglutamine proteins are initially activating the NFκB, whereas later polyglutamine aggregates inhibit the NFκB activity.

We next checked the expression levels of several NFκB-dependent genes in the HD 16Q and HD 150Q cells after different days of induction. As shown in figure 8, the expression of tNhtt-150Q proteins in the HD 150Q cells results in down-regulation of Cox2 and iNOS expression. The expression levels of Cox2 and iNOS were very low in the uninduced HD 150Q cells. This was expected, because the uninduced HD 150Q cells showed a significantly low level of NFκB activity as compared with HD 16Q cell.

Inhibition of NFκB Enhances the Expanded Polyglutamine Protein-induced Cell Death—Because the NFκB activity was inhibited in the expanded polyglutamine protein-expressing cells, we next checked the impact of this down-regulation on cell viability. We induced the HD 16Q and HD 150Q cells for 2 days with ponasterone A and then treated them with varying doses of SN50 (a peptide inhibitor of NFκB that blocks nuclear import of the NFκB p50 subunit protein) or curcumin. Curcumin is well known to inhibit NFκB activity (40). We induced HD 150Q cells for 2 days, because there was significant reduction of NFκB activity, and these cells start dying from 2 days onward (15). Fig. 9 shows that treatment of both SN50 and curcumin induced HD 16Q and HD 150Q cell death. However, the percentage of HD 150Q cell death was significantly higher in comparison with HD 16Q cells. Results suggest that the NFκB inhibitors promote expanded polyglutamine protein-induced cell death.

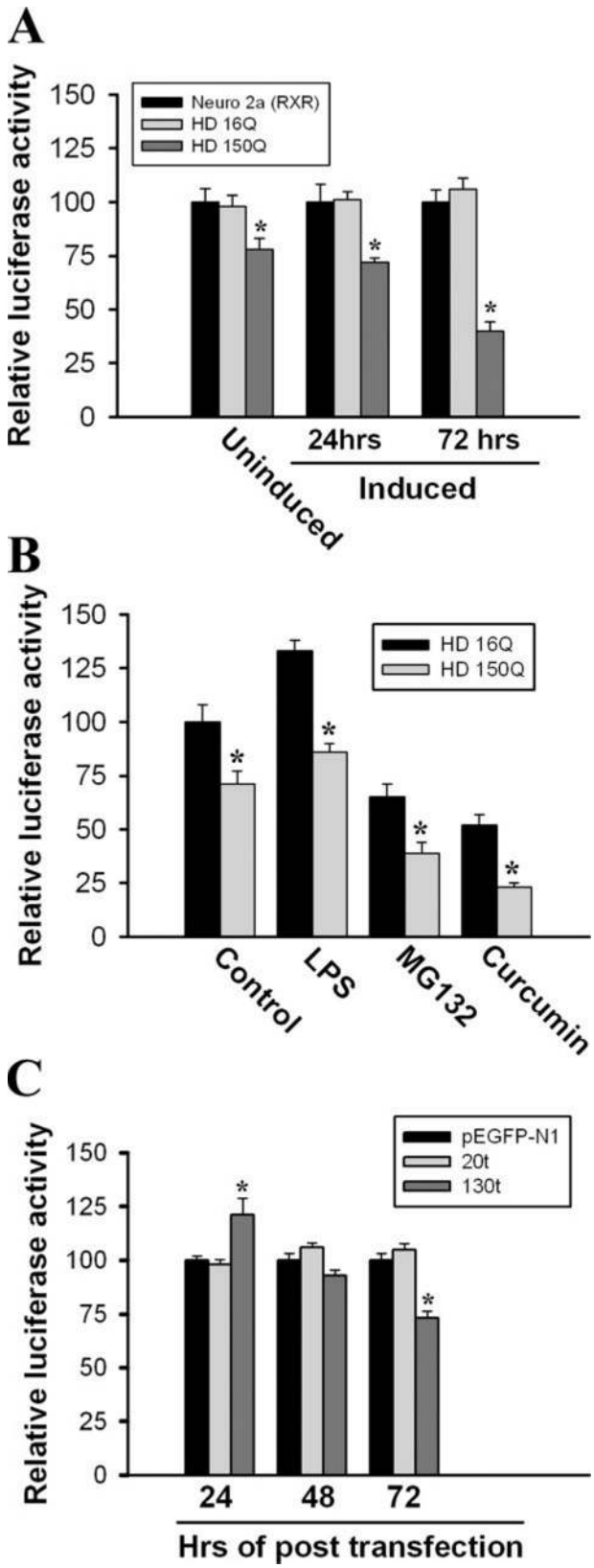

FIGURE 7. **Inhibition of NFκB activity in the expanded polyglutamine protein-expressing cells.** *A*, neuro-2a (RXR), HD 16Q, and HD 150Q cells were transiently transfected NFκB luciferase and PRL-SV40 plasmids as described under "Experimental Procedures." The cells were left uninduced or induced with 1 μM ponasterone A for different time periods as indicated. The cells were then collected and processed for dual luciferase reporter gene assay. Results are means ± S.D. of three independent experiments, each performed in triplicate. *, $p < 0.01$ as compared with neuro-2a (RXR) or HD 16Q cells. *B*, HD 16Q and HD 150Q cells were transiently transfected with NFκB luciferase and PRL-SV40 plasmids and simultaneously induced with ponasterone A for 2 days in a similar manner as described above. The cells were then treated with lipopolysaccharide (*LPS*, 100 ng/ml), MG132 (10 μM), and curcumin (10 μM) for 6 h, and the collected cells were processed for dual luciferase reporter gene assay. Results are means ± S.D. of two independent experiments, each performed in triplicate. *, $p < 0.01$ as compared with HD 16Q cells. *C*, neuro-2a cells were transiently transfected with pEGFP-N1 and the truncated ataxin-3-EGFP fusion constructs (1 μg each/well) containing 20Q and 130Q. Six hours later, the cells were again transfected with NFκB luciferase and PRL-SV40 plasmids. Cells were then collected at different time points, as indicated in the figure, and processed for dual luciferase reporter gene assay. Results are means ± S.D. of two independent experiments, each performed in triplicate. *, $p < 0.01$ as compared with pEGFP-N1 and ataxin-3 with 20Q-expressing cells.

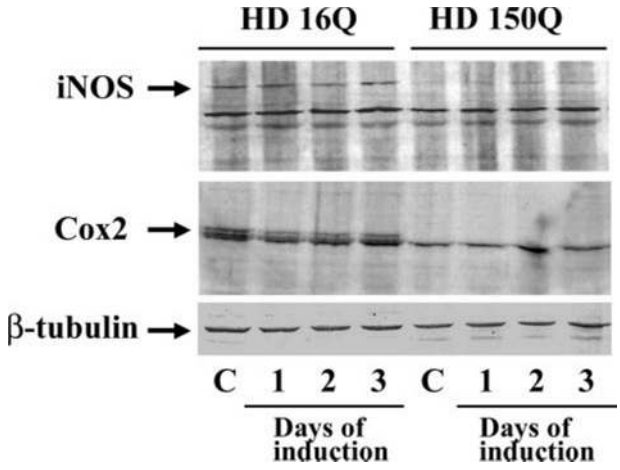

FIGURE 8. **Decreased expression of Cox2 and iNOS in the HD 150Q cells.** HD 16Q and HD 150Q cell lines were left untreated or treated with 1 μM ponasterone A for different time periods. Cells were then collected and subjected to immunoblotting using Cox2, iNOS, and β-tubulin antibody.

DISCUSSION

Activation of NFκB plays a very important role in controlling the pathways related to cell survival and apoptosis. Activation of this pathway is also implicated in various acute and chronic neurodegenerative conditions. In the present investigation, we have found that the expression of expanded polyglutamine proteins time-dependently down-regulates the NFκB activity.

First, we have shown that the expression of expanded polyglutamine protein results in an increased accumulation of IκB-α and its phosphorylated derivatives, and this increased accumulation is because of the slower rate of degradation of IκB-α. Second, we found that the IκB-α and NFκB subunits aberrantly interact with the expanded polyglutamine proteins and sequester around the aggregates. Therefore, most likely the association of IκB-α and NFκB complexes with the polyglutamine aggregates, as well as altered degradation of IκB-α, might be responsible for the down-regulation of NFκB activity.

Polyglutamine aggregates have been reported to associate with the various components of UPS (15–20), and continuous expression and aggregation of expanded polyglutamine proteins in neuronal cells lead to the impairment of UPS function (12–15). Therefore, the altered degradation of IκB-α could be due to the proteasomal dysfunction in the expanded polyglutamine protein-expressing cells. Aberrant association of IκB-α

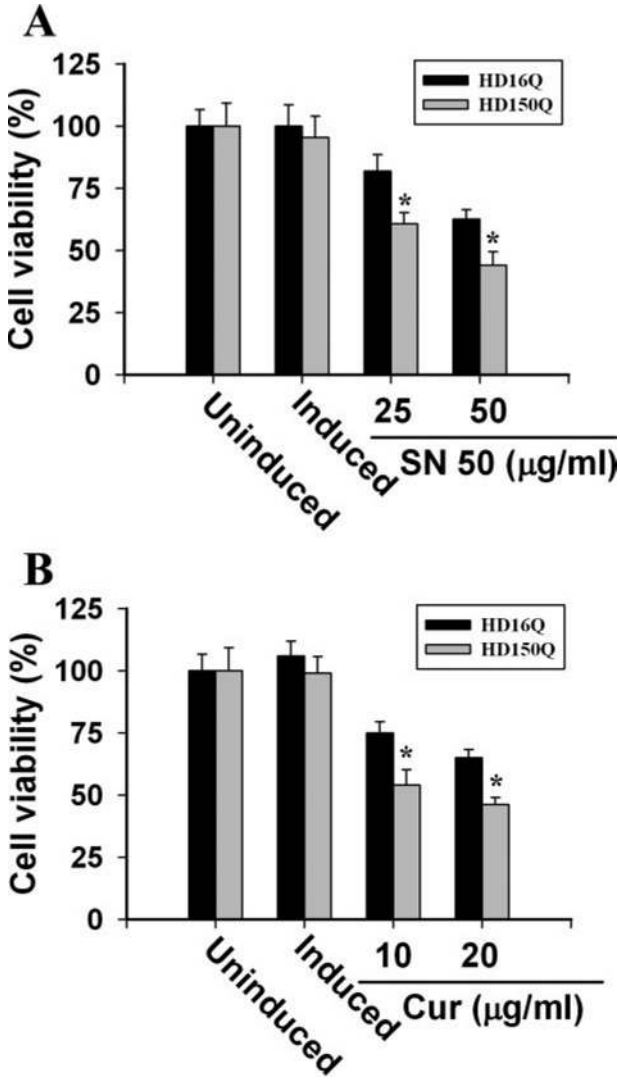

FIGURE 9. **Inhibition of NFκB activity enhances the expanded polyglutamine protein-induced cell death.** HD 16Q and HD 150Q cells were induced with 1 μM ponasterone A for 2 days. The cells were then treated with different concentrations of SN50 for 24 h (*A*) or curcumin for 8 h (*B*). Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Results are means ± S.D. of two independent experiments each performed in triplicate. *, $p < 0.01$ as compared with HD 16Q cells.

with the polyglutamine aggregates could also increase its half-life.

Expanded polyglutamine proteins are very unstable and quickly form aggregates (41), and during this process, they co-aggregate with many other proteins essential for normal cellular function. Among them, several transcription factors, such as TATA box-binding protein, CREB-binding protein, Sp1, and p53 have been found to associate with aggregates and result in their transcriptional deregulation, which ultimately leads to cellular dysfunction and cell death (5–11). Here, we found transcriptional deregulation of NFκB in the expanded polyglutamine protein-expressing cells, which might be due to the UPS dysfunction as well as sequestration of various components of NFκB complex and could be a common feature among all polyglutamine diseases. Finally, we have also shown that some of the NFκB-regulated genes, such as Cox2 and iNOS, are down-regulated in the expanded polyglutamine protein-expressing cells.

What could be the consequence of the down-regulation of NFκB activity in the expanded polyglutamine protein-expressing cells? NFκB activation has been reported to protect as well as to promote cell death, depending on the cell type and/or inducing signal (22–24). But in most cases, its activation leads to the protection of cell death, particularly in the neuronal cells (22). NFκB activity was reported to be increased in the striatal neurons of 3-nitropropionic acid-treated mice (an animal model for HD). However, the mice lacking the p50 subunit of NFκB show decreased activity and increased damage of striatal neurons (31). This suggests that down-regulation of NFκB activity might lead to neurodegeneration. However, a recent paper shows activation of NFκB in the cellular and transgenic mice model of HD, and this activation leads to neurodegeneration (32). We never detected an increase in NFκB activity in HD 150Q stable cells most likely because of a low level of expression and aggregation of polyglutamine-expanded huntingtin without any induction. However, we have found a significant increase in NFκB activity at 24 h of post-transfection and a decrease in NFκB activity at 72 h of post-transfection of the polyglutamine-expanded ataxin-3. In both cases, we have observed direct correlation of decrease in NFκB activity and induction of cell death. It seems that expanded polyglutamine protein could possibly have a dual effect on NFκB, an initial phase of activation and a later stage of inhibition. Initially, the expanded polyglutamine protein-expressing cells might be trying to protect themselves by activating NFκB, and later aggregates prevent the activation by promoting UPS dysfunction. Similarly, the amyloid β-peptide at low concentration has been found to activate NFκB that leads to neuroprotection, whereas at a higher concentration, it produces the opposite effect (29). We have observed down-regulation of NFκB activity promoting expanded polyglutamine protein-induced cell death, which suggests that inhibition of NFκB activity might be responsible for the expanded polyglutamine protein-induced cell death. Further work is necessary to confirm this.

Altogether, our results demonstrate the down-regulation of NFκB activity in the expanded polyglutamine protein-expressing cell, and this inhibition of NFκB activity might be linked with the expanded polyglutamine protein-induced cell death.

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