The Deubiquitinating Enzyme USP11 Controls an IκB Kinase α (IKKα)-p53 Signaling Pathway in Response to Tumor Necrosis Factor α (TNFα)*

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Tomoko Yamaguchi, Junko Kimura, Yoshio Miki†, and Kiyotugu Yoshida‡

From the Department of Molecular Genetics, Medical Research Institute, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8510, Japan

Post-translational modification and degradation of proteins by the ubiquitin-proteasome system are key regulatory events in cellular responses to various stimuli. The NF-κB signaling pathway is controlled by the ubiquitin-mediated proteolysis. Although mechanisms of ubiquitination in the NF-κB pathway have been extensively studied, deubiquitination-mediated regulation of the NF-κB signaling remains poorly understood. The present studies show that a deubiquitinating enzyme, USP11, specifically regulates IκB kinase α (IKKα) among the NF-κB signaling molecules. Knocking down USP11 attenuates expression of IKKα in the transcriptional, but not the post-translational, level. However, down-regulation of USP11 dramatically enhances NF-κB activity in response to tumor necrosis factor-α, indicating that IKKα does not require activation of NF-κB. Instead, knock down of USP11 or IKKα is associated with abrogation of p53 expression upon exposure to tumor necrosis factor-α. In concert with these results, silencing of USP11 is associated with transcriptional attenuation of the p53-responsive genes, such as p21 or Bax. Importantly, the ectopic expression of IKKα into cells silenced for USP11 restores p53 expression, demonstrating that USP11 functions as an upstream regulator of an IKKα-p53 signaling pathway.

These post-translational modifications are critical for “on-off” switch in the signaling cascade. For example, one member of IκB, designated as IκBα, is phosphorylated in response to TNFα or interleukin-1 and is thereby subjected to ubiquitination and degradation by the 26 S proteasome (5). The NF-κB essential modifier (NEMO, also known as IKKγ) is a key to activating IκB kinase (IKK) complex and is polyubiquitinated by TRAF6 (6). In contrast, deubiquitinating mechanisms that target to ubiquitinated NF-κB signaling molecules are largely unclear. Certain insights have been derived from the finding that CYLD was identified by its association with NEMO and through systematic screening for deubiquitinating enzymes that impede NF-κB signaling (7–9). Overexpression of CYLD represses NF-κB activation in response to various stimuli, including TNFα. Conversely, inactivation of CYLD by RNA interference increases inducible NF-κB activity. These findings thus suggest that CYLD acts as a negative regulator of the NF-κB signaling pathway. Little is otherwise known about direct deubiquitinating enzymes targeting to ubiquitinating molecules that are involved in NF-κB signaling.

A recent study mapped a protein interaction network for TNFα/NF-κB pathway components by means of an integrated approach, including tandem affinity purification, liquid chromatography tandem mass spectrometry, network analysis, and directed functional perturbation studies using RNA interference (10). This study identified numerous previously unknown interactors, including a deubiquitinating enzyme, USP11. However, the role for USP11 in the NF-κB signaling pathway remains obscure.

Our recent studies demonstrated that IKKα, but not IKKβ, is activated and translocates into the nucleus in response to oxidative stress (11). Upon exposure to oxidative stress, IKKα activation does not contribute to NF-κB activation; instead, nuclear IKKα regulates the transcription activity of the p53 tumor suppressor, indicating that the IKKα → p53 signaling pathway is associated with the cellular response to oxidative stress. In this study, we show that USP11 regulates IKKα expression in a transcriptional level. Importantly, down-regulation of IKKα does not contribute to NF-κB activation in response to TNFα. Instead, USP11-mediated regulation of IKKα functions to control p53 transcription activity. These findings support a novel mechanism in which the deubiquitinating enzyme USP11 contributes to TNFα-induced IKKα → p53 signaling pathway.
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EXPERIMENTAL PROCEDURES

Cell Culture—Human MOLT-4 and HL-60 leukemia cells were cultured in RPMI 1640 medium supplemented with human U2OS osteosarcoma cells, and MCF-7 mammary gland carcinoma cells were grown in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM). Cells were treated with 50 ng/ml TNFα (PeproTech EC) or 5 μM MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal; Nacalai Tesque).

Plasmids—Constructs of IKKα and IKKβ are described elsewhere (12). IKKγ cDNA was amplified by PCR from a human fetal brain cDNA library and cloned into the pcDNA3-FLAG vector as described elsewhere (13). USP11 cDNA (14) was cloned into the pcDNA3-FLAG vector. An RNA interference-resistant form of USP11 was constructed by introducing silent mutations using PCR-based site-directed mutagenesis. The sequences of oligonucleotide primers are as follows: 5′-CATACCGATTCAATaATaGGgCTAGTATTGCGC-3′ and 5′-GCCCAATACTAGcCcAtTGAATCGGTTAG-3′. Lowercase letters represent silent mutations. Mutations were confirmed by sequencing. HA-ubiquitin plasmid has been reported previously.

siRNA Transfections—siRNA duplexes (siRNAs) were synthesized and purified by Invitrogen (Stealth Select RNA interference). Transfection of siRNAs was performed using Lipofectamine RNAiMAX (Invitrogen).

Immunoprecipitation and Immunoblot Analysis—Cell lysates were prepared as described elsewhere (18, 19) and cleared by centrifugation at 12,000 × g for 15 min. Soluble proteins were incubated with anti-FLAG M2 affinity gel (Sigma-Aldrich) for 2 h at 4 °C. The immune complexes were washed three times with lysis buffer. Cell lysates or immunoprecipitates were separated by SDS-PAGE and transferred to nitrocellulose filters, which were then incubated with anti-FLAG, anti-HA (Roche Applied Science), anti-USP11 (Santa Cruz Biotechnology), anti-IKKα (MBL International Corp.), IKKβ (Cell Signaling Technology), anti-IKKγ (Santa Cruz Biotechnology), anti-RelA/p65 (Santa Cruz Biotechnology), anti-RelB (Santa Cruz Biotechnology), anti-p100 (Santa Cruz Biotechnology), anti-IκBα (Santa Cruz Biotechnology), anti-Ets-1 (Santa Cruz Biotechnology), anti-PCNA (Santa Cruz Biotechnology), or anti-tubulin (Sigma-Aldrich). The antigen-antibody complexes were visualized by chemiluminescence (PerkinElmer Life Sciences).

RT-PCR Analysis for Gene Expression—Total cellular RNA was extracted using the RNeasy kit (Qiagen). First-stand cDNA synthesis and the following PCR reactions were performed with 500 ng of total RNA using SuperScript one-step RT-PCR system (Invitrogen) according to the manufacturer’s protocol. The reaction products were resolved on a 2% agarose gel.

Reporter Gene Assays—293 cells stably transfected with pNF-κB-luc and pTK-hyg (Panomics) were transfected with a variety of siRNAs followed by the treatment with TNFα. The luciferase activity was determined with a Bright-Glo luciferase assay system (Promega) according to the manufacturer’s protocol.

Chromatin Immunoprecipitation Assays—Cells were harvested and washed with chilled phosphate-buffered saline once followed by incubation in 1% formaldehyde for 15 min at room temperature for chromatin cross-linking. The cells were then collected and washed with chilled phosphate-buffered saline again. After centrifugation, the cell pellets were resuspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0, 1 mM dithiothreitol, 1 mM phenylmethylsulfonil fluoride, 1 mM Na3VO4, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A), and the lysates were sonicated to obtain DNA fragments 200–500 bp in length. After centrifugation, 50 μl of the supernatant was used as an input, and the remainder was diluted 2–2.5-fold in washing buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1% Nonidet P-40, and protease inhibitors as described above). This diluted fraction was subjected to immunoprecipitation with 2 μg of indicated antibodies for 2 h to overnight at 4 °C with rotation. The immunocomplexes were collected with 30 μl of protein A-Sepharose beads (Santa Cruz Biotechnology) for 1–2 h at 4 °C with rotation. The beads were then pelleted by centrifugation and washed sequentially with 300 μl of the following buffers: wash buffer I (500 mM NaCl, 0.1% SDS, 2 mM EDTA, and 20 mM Tris-HCl, pH 8.0), wash buffer II (250 mM LiCl, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0, and 1% deoxycholate), and then twice with Tris-EDTA buffer. Precipitated chromatin complexes were removed from the beads by shaking with 150 μl of elution buffer (1% SDS and 0.1 M NaHCO3) for 15 min, and this step was repeated. All the eluates were collected, and then the cross-linking was reversed by adding NaCl to a final concentration of 200 mM. The mixture was allowed to stand overnight at 65 °C. The remaining proteins were digested with the extraction buffer (50 mM Tris-HCl, pH 6.8, 10 mM EDTA, and 40 μg/ml proteinase K) for 1 h at 45 °C. DNA was recovered by phenol/chloroform/isoamyl alcohol (25/24/1) extraction and precipitated with 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol. PCR amplification was performed in chromatin immunoprecipitated fragments using oligonucleotide pairs as described elsewhere.

Subcellular Fractionation—Subcellular fractionation was performed as described previously (21–23). Purity of the fractions was monitored by immunoblot analysis with anti-PCNA and anti-tubulin.

RESULTS AND DISCUSSION

USP11 Is Associated with IKKα Expression—Previous studies have implicated a number of deubiquitinating enzymes in several signaling cascades, including the NF-κB pathway. In particular, a recent study suggested a potential involvement of USP11 in NF-κB signaling. To determine whether USP11 affects the expression of NF-κB signalosome, U2OS cells were transfected with a scramble siRNA or a siRNA that targets USP11. We examined the expression of seven gene products that are directly involved in the NF-κB signaling pathway (Fig. 1A). Among these products, the expression of IKKα was significantly reduced in cells silenced for USP11. To exclude the possibility that knocking down USP11 is associated with off-target effects, siRNAs that target different USP11 sequences (USP11...
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To further define the USP11-independent regulation of Ets-1, we performed chromatin immunoprecipitation assays with anti-Ets-1 antibody. As reported previously (20), Ets-1 occupied IKKα promoter, and this occupancy was increased after TNFα stimulation in U2OS cells (Fig. 2C). Importantly, the finding that Ets-1 occupancy on the IKKα promoter in cells silenced for USP11 was comparable with that in control cells indicates that Ets-1 transcriptional activity is regulated in an USP11-independent manner (Fig. 2C). Similar results were obtained in MCF-7 cells (data not shown). Taken together,

showed that IKKα is positively regulated by a transcription factor, Ets-1 (20). Thus, it is plausible that USP11 affects Ets-1 by the ubiquitin-deubiquitin mechanism to control IKKα expression. To examine this possibility, U2OS cells were transfected with the scramble siRNA or USP11 siRNA followed by treatment with or without TNFα. The expression level of Ets-1 remained unchanged even in USP11-silenced cells, clearly indicating that Ets-1 is not targeted by USP11, at least post-translational level (Fig. 2B).

To further define the USP11-independent regulation of Ets-1, we performed chromatin immunoprecipitation assays with anti-Ets-1 antibody. As reported previously (20), Ets-1 occupied IKKα promoter, and this occupancy was increased after TNFα stimulation in U2OS cells (Fig. 2C). Importantly, the finding that Ets-1 occupancy on the IKKα promoter in cells silenced for USP11 was comparable with that in control cells indicates that Ets-1 transcriptional activity is regulated in an USP11-independent manner (Fig. 2C). Similar results were obtained in MCF-7 cells (data not shown). Taken together,

Transfection of USP11 siRNA-a or siRNA-b resulted in similar down-regulation of USP11 specifically attenuated IKKα expression, indicating that USP11 specifically down-regulates IKKα expression (see Fig. 4A). To confirm the regulation of IKKα expression by USP11, U2OS cells were transfected with the USP11 siRNA followed by the transfection of the FLAG-USP11 mutant that is resistant for the USP11 siRNA by introducing silent mutations. The results demonstrated that IKKα expression was restored by forced expression of USP11 into U2OS cells silenced for USP11 (Fig. 1B). To investigate whether suppression of IKKα by USP11 silencing is regulated by the ubiquitin-proteasome pathway, FLAG-tagged IKK families were transfected into U2OS cells together with HA-tagged ubiquitin. Previous studies demonstrated that IKKγ is degraded by the ubiquitin-proteasome system (4). In concert with these results, overexpression of IKKγ was sufficient for substantial ubiquitination, regardless of TNFα treatment (Fig. 1C). By contrast, there was little if any ubiquitination on IKKα or IKKβ, indicating that IKKα and IKKβ function independently of ubiquitination-mediated regulation. These results thus suggest that USP11 regulates IKKα by a ubiquitin-independent manner. Another possibility is that regulation of IKKα by USP11 is mediated by undefined molecules that are controlled by the ubiquitin-deubiquitin mechanism. To further define USP11-mediated regulation of NF-κB signalosome, total RNA from U2OS cells in the presence or absence of USP11 was subjected to RT-PCR analysis. Knockdown of USP11 specifically attenuated IKKα expression, suggesting that regulation of IKKα by USP11 occurs at the transcriptional level (Fig. 2A). In this context, a previous study confirmed the regulation of IKKα ubiquitination on IKKα expression by USP11, U2OS cells transfected with the USP11 siRNA followed by the transfection of the FLAG-USP11 mutant that is resistant for the USP11 siRNA by introducing silent mutations. The results demonstrated that IKKα expression was restored by forced expression of USP11 into U2OS cells silenced for USP11 (Fig. 1B). To investigate whether suppression of IKKα by USP11 silencing is regulated by the ubiquitin-proteasome pathway, FLAG-tagged IKK families were transfected into U2OS cells together with HA-tagged ubiquitin. Previous studies demonstrated that IKKγ is degraded by the ubiquitin-proteasome system (4). In concert with these results, overexpression of IKKγ was sufficient for substantial ubiquitination, regardless of TNFα treatment (Fig. 1C). By contrast, there was little if any ubiquitination on IKKα or IKKβ, indicating that IKKα and IKKβ function independently of ubiquitination-mediated regulation. These results thus suggest that USP11 regulates IKKα by a ubiquitin-independent manner. Another possibility is that regulation of IKKα by USP11 is mediated by undefined molecules that are controlled by the ubiquitin-deubiquitin mechanism. To further define USP11-mediated regulation of NF-κB signalosome, total RNA from U2OS cells in the presence or absence of USP11 was subjected to RT-PCR analysis. Knockdown of USP11 specifically attenuated IKKα expression, suggesting that regulation of IKKα by USP11 occurs at the transcriptional level (Fig. 2A). In this context, a previous study confirmed the regulation of IKKα ubiquitination on IKKα expression by USP11, U2OS cells transfected with the USP11 siRNA followed by the transfection of the FLAG-USP11 mutant that is resistant for the USP11 siRNA by introducing silent mutations. The results demonstrated that IKKα expression was restored by forced expression of USP11 into U2OS cells silenced for USP11 (Fig. 1B). To investigate whether suppression of IKKα by USP11 silencing is regulated by the ubiquitin-proteasome pathway, FLAG-tagged IKK families were transfected into U2OS cells together with HA-tagged ubiquitin. Previous studies demonstrated that IKKγ is degraded by the ubiquitin-proteasome system (4). In concert with these results, overexpression of IKKγ was sufficient for substantial ubiquitination, regardless of TNFα treatment (Fig. 1C). By contrast, there was little if any ubiquitination on IKKα or IKKβ, indicating that IKKα and IKKβ function independently of ubiquitination-mediated regulation. These results thus suggest that USP11 regulates IKKα by a ubiquitin-independent manner. Another possibility is that regulation of IKKα by USP11 is mediated by undefined molecules that are controlled by the ubiquitin-deubiquitin mechanism. To further define USP11-mediated regulation of NF-κB signalosome, total RNA from U2OS cells in the presence or absence of USP11 was subjected to RT-PCR analysis. Knockdown of USP11 specifically attenuated IKKα expression, suggesting that regulation of IKKα by USP11 occurs at the transcriptional level (Fig. 2A). In this context, a previous study
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Further define the involvement of USP11 in NF-κB activation, U2OS cells were transfected with the scramble siRNA or USP11 siRNA followed by TNFα treatment. Immunoblot analysis of whole cell lysates demonstrated substantial attenuation of resynthesized IkBα in the absence of USP11 (Fig. 3B). In accordance with this result, immunoblotting of nuclear lysates with anti-RelA/p65 revealed that at 2 h after TNFα treatment, 73% of nuclear RelA/p65 was exported from the nucleus in cells transfected with the scramble siRNA (Fig. 3B). In contrast, 83% of RelA/p65 remained in the nucleus in cells silenced for USP11 at the same time point (Fig. 3B), indicating that nuclear export of RelA/p65 was abrogated in cells silenced for USP11 (Fig. 3B). These findings provide a potential model in which, in USP11-silencing cells, RelA/p65 remains in the nucleus because of attenuation of resynthesized IkBα, resulting in sustained activation of NF-κB. In addition, given the data from reporter assays that imply enhancement of NF-κB activity in the absence of USP11, the confirmed results that IKKα expression is downregulated by silencing USP11 further suggested that NF-κB activation occurs independently of IKKα under our experimental conditions (Fig. 3B). Taken together, these findings indicate that USP11 negatively regulates NF-κB activity in response to TNFα by a yet undefined mechanism.

USP11 Controls the IKKα → p53 Signaling in Response to TNFα—Our recent study showed that the protein kinase C δ → IKKα signaling pathway stabilizes and activates p53 in response to oxidative stress (11). These finding led us to examine whether USP11 controls p53 through IKKα upon exposure to TNFα. As shown previously, knocking down USP11 in U2OS cells was associated with attenuation of IKKα expression (Fig. 4A). More importantly, steady-state levels of p53 expression were also down-regulated in the absence of USP11 (Fig. 4A). Although U2OS cells express p53 at low but detectable levels (13), p53 expression is little if any in unstressed MCF-7 cells. In this regard, we examined the effect of TNFα on p53 expression by MCF-7 cells. In concert with accumulating studies (24–27), p53 was stabilized after treatment of cells with TNFα for 8 h, and its expression was sustained at least 24 h following TNFα treatment (Fig. 4B). In these experimental conditions, TNFα-induced p53 expression was completely abrogated in cells silenced for USP11 (Fig. 4C). In concert with this result, mRNA expression of p53-responsive genes, such as Bax and p21, were also attenuated in the absence of USP11 (Fig. 4C). By contrast, mRNA of p53 was constant regardless of USP11 expression, suggesting that USP11 regulates p53 via a post-translational, not a transcriptional, mechanism (Fig. 4C). To further define whether regulation of p53 by USP11 is associated with ubiquitin-proteasome system, MCF-7 cells transfected with scramble siRNA or USP11 siRNA were pretreated with proteasome inhibitor, MG132, followed by treatment with TNFα. Immunoblot analysis with anti-p53 revealed that, even in MG132-treated cells, knocking down USP11 reduced the expression levels of p53, suggesting that regulation of p53 by USP11 occurs independently of ubiquitin-proteasome proteolysis (Fig. 4D). To determine whether USP11 regulation of p53 involves IKKα, MCF-7 cells were transfected with scramble siRNA, USP11 siRNA, or IKKα siRNA and then treated with or without TNFα. The results demonstrated that p53 expression was substantially

these findings support an essential role for USP11 in the regulation of IKKα expression at a transcriptional level.

Knocking Down USP11 Enhances TNFα-induced NF-κB Activation—To assess the effects of USP11 on the activity of NF-κB, 293 cells stably transfected with the luciferase-reporter vector containing NF-κB response elements were silenced for USP11 or RelA/p65. As shown previously, silencing of RelA/p65 was associated with pronounced inhibition of NF-κB activity in response to TNFα (Fig. 3A). By contrast, knocking down USP11 enhanced TNFα-induced NF-κB activation (Fig. 3B). To

FIGURE 3. USP11-mediated IKKα regulation is independent of NF-κB activation in response to TNFα. A, 293 cells expressing pNF-κB-luc were transfected with scramble siRNA or USP11 siRNA followed by the treatment with or without TNFα for 6 h. Luciferase activity was measured 48 h after transfection (left panel). The results are represented as mean ± S.D. obtained from three independent experiments, each performed in triplicate. Cell lysates were analyzed by immunoblotting (IB) with the indicated antibodies (right panel). RLU, relative light units. B, U2OS cells transfected with scramble siRNA or USP11 siRNA were treated with TNFα. Whole cell lysates or nuclear lysates were subjected to immunoblot analysis with the indicated antibodies. In nuclear lysates, a percentage of nuclear RelA/p65 remained in the nucleus because of attenuation of resynthesized IkBα, resulting in sustained activation of NF-κB. In addition, given the data from reporter assays that imply enhancement of NF-κB activity in the absence of USP11, the confirmed results that IKKα expression is downregulated by silencing USP11 further suggested that NF-κB activation occurs independently of IKKα under our experimental conditions (Fig. 3B). Taken together, these findings indicate that USP11 negatively regulates NF-κB activity in response to TNFα by a yet undefined mechanism.
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Regulation of IKKα → p53 Signaling Pathway by USP11—Previous work showed that protein kinase Cδ activates IKKα in response to oxidative stress (11). Such activation was associated with increased p53 expression, indicating that the protein kinase Cδ → IKKα signaling pathway functions as a positive regulator of p53. In this context, it should be clarified whether the protein kinase Cδ → IKKα signaling is also involved in TNFα-induced p53 expression. Nevertheless, the present study identified another regulator of IKKα, USP11. Interestingly, despite the fact that USP11 is a deubiquitinating enzyme, USP11 regulation of IKKα was independent of ubiquitin-deubiquitination status. Instead, USP11 controlled IKKα at the transcriptional level by unknown mechanism. Importantly, USP11 also controlled the stabilization and activation of p53 through IKKα regulation at a steady-state level and in response to TNFα. In this regard, there is indeed another possibility that USP11 directly deubiquitinates p53 to stabilize its expression. However, other work demonstrated that overexpression of USP11 has no obvious effect on the levels of p53 ubiquitination or stabilization of p53 (33). The present findings that ectopic expression of IKKα in USP11-depleted MCF-7 cells markedly increased p53 expression also support an indirect role for USP11 in the regulation of p53. Although the precise mechanism by which IKKα controls p53 remains unclear, available

attenuated in USP11- or IKKα-deficient cells (Fig. 4E). Importantly, the ectopic expression of IKKα in USP11-silenced cells restored p53 expression (Fig. 4E), suggesting the possibility that effect of USP11 on p53 expression is regulated exclusively through IKKα. In this regard, recent studies have demonstrated that IKKα specifically translocates into the nucleus and phosphorylates histone H3 (28, 29). Moreover, IKKα forms a complex with transcription coactivators such as AIB1/SRC-3 to modulate gene expression (30). Other studies have suggested that IKKα is recruited to the chromatin to derepress the silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) (31, 32). Taken together, these findings indicate a pivotal role for nuclear IKKα as a regulator of transcription factors in response to TNFα. Given the previous finding that IKKα also stabilizes p53 by Ser-20 phosphorylation (11), these results thus support a mechanism by which USP11 regulates the expression of IKKα, which in turn controls p53 in response to TNFα.

FIGURE 4. USP11 controls IKKα → p53 signaling in response to TNFα. A, U2OS cells transfected with various siRNAs were analyzed by immunoblotting (IB) with the indicated antibodies. B, MCF-7 cells were treated with TNFα for the indicated times. Cell lysates were analyzed by immunoblotting with the indicated antibodies. C, MCF-7 cells transfected with scramble siRNA or USP11 siRNA were treated with TNFα for the indicated times and then analyzed by Western blotting and RT-PCR. D, MCF-7 cells transfected with scramble siRNA (S) or USP11 siRNA (U) were pretreated with MG132 followed by treatment with TNFα for 8 h. Cell lysates were analyzed by immunoblotting with the indicated antibodies. E, MCF-7 cells transfected as indicated were left untreated or treated with TNFα for 8 h. Cell lysates were analyzed by immunoblotting with the indicated antibodies.
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FIGURE 5. A proposed model of the USP11 \(\rightarrow\) IKK\(\alpha\) \(\rightarrow\) p53 signaling cascade in response to TNF\(\alpha\). USP11 positively regulates expression of IKK\(\alpha\). On the other hand, USP11 negatively controls NF-\(\kappa\)B activity by an unknown mechanism. Exposure to TNF\(\alpha\), IKK\(\alpha\) is activated and moves into the nucleus. The involvement of protein kinase C (PKC\(\delta\)) in TNF\(\alpha\)-induced IKK\(\alpha\) activation remains unclear. Nuclear IKK\(\alpha\) in turn induces stabilization and activation of p53.

Evidence indicated that IKK\(\alpha\) phosphorylates p53 for its stabilization and activation in response to oxidative stress. In this context, activation of p53 by TNF\(\alpha\) may be, at least in part, involved in IKK\(\alpha\)-mediated phosphorylation.

A recent study has demonstrated that p53 expression in IKK\(\alpha\)^{-/-} knock-out mouse embryonic fibroblast is comparable with that in wild type mouse embryonic fibroblast (34). The result also demonstrated that p53 expression was substantially high even in the steady-state level, and no increase was observed after TNF\(\alpha\) exposure. In this regard, given the present study demonstrating that p53 expression was attenuated in IKK\(\alpha\) (Fig. 4E), there is indeed an apparent discrepancy to be solved in the future work. However, regulation of expression for human p53 is considerably different from that for mouse p53 (35), suggesting the possibility that human IKK\(\alpha\) is specifically associated with the regulation of human p53 expression. Obviously, further studies are needed to clarify this issue.

In summary, the present studies demonstrate that USP11 controls IKK\(\alpha\) by a deubiquitination-independent mechanism. Regulation of IKK\(\alpha\) by USP11 is associated with p53 stabilization and activation upon exposure to TNF\(\alpha\) (Fig. 5). These findings indicate that USP11 controls the IKK\(\alpha\) \(\rightarrow\) p53 signaling pathway in response to TNF\(\alpha\).

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