Published OnlineFirst September 29, 2009; DOI: 10.1158/0008-5472.CAN-09-2148

Priority Report

Phosphorylation-Dependent Lys^{63}-Linked Polyubiquitination of Daxx Is Essential for Sustained TNF-α–Induced ASK1 Activation

Yayoi Fukuyo,[1] Tetsuya Kitamura,[1,2] Masahiro Inoue,[3] Nobuko T. Horikoshi,[1] Ryuji Higashikubo,[1] Clayton R. Hunt,[1] Anny Usheva,[4] and Nobuo Horikoshi[1]

[1]Department of Radiation Oncology, Washington University School of Medicine, St. Louis, Missouri; [2]Department of Oral Pathology, Hokkaido University, Sapporo, Japan; [3]Department of Parasitology, Kurume University Medical School, Kurume, Japan; and [4]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

Abstract

Apoptosis signal–regulating kinase 1 (ASK1) is a key regulatory kinase in the proapoptotic response to various stresses. ASK1 phosphorylation of Daxx, an ASK1 activator protein, increases Daxx accumulation in cells and further enhances ASK1 activity through a positive feedback mechanism. Here, we show that ASK1-dependent phosphorylation of Daxx induces Lys^{63} (K63)-linked polyubiquitination on Lys^{122} of Daxx. Polyubiquitination is dispensable for Daxx accumulation or Daxx interaction with ASK1 because mutant Daxx deficient in polyubiquitin still exhibits ASK1-dependent accumulation and interaction with cellular ASK1. However, K63-linked Daxx polyubiquitination is required for tumor necrosis factor-α (TNF-α)–induced activation of ASK1. Therefore, K63-linked polyubiquitination of Daxx functions as a molecular switch to initiate and amplify the stress kinase response in the TNF-α signaling pathway. [Cancer Res 2009;69(19):7512–7]

Introduction

Apoptosis signal–regulating kinase 1 (ASK1) is a mitogen-activated protein kinase kinase kinase that plays an essential role in the apoptotic response induced by tumor necrosis factor-α (TNF-α), Fas ligand, and oxidative stress (1, 2). Activated ASK1 phosphorylates MKK4/MKK6 mitogen-activated protein kinase kinases, leading to c-Jun NH_2-terminal kinase (JNK)/p38 mitogen-activated protein kinase activation. ASK1 can also be activated through direct interaction with Daxx, a Fas death domain–binding protein (3). Moreover, a positive feedback mechanism links Daxx and ASK1 (4), as ASK1 phosphorylation of two Daxx serine residues (Ser^{176} and Ser^{184}) results in Daxx stabilization and accumulation, further enhancing ASK1 activation. In contrast, tumorigenic mutant p53 directly interacts with Daxx and inhibits Daxx accumulation and ASK1 activation, which prevents JNK activation and apoptosis (4, 5). These findings suggest that the Daxx-ASK1 positive feedback loop reinforces the amplification of downstream kinase activations, including JNK and p38, and that breaking this circuit is part of the mechanism by which p53 gain-of-function mutations contribute to tumorigenesis.

Protein phosphorylation can induce polyubiquitination that results in proteasome-dependent protein degradation, a mechanism well documented for transcription factor NF-κB activation. When TNF-associated factor 2 is activated, the NF-κB inhibitor IκB is phosphorylated by IKKα/β kinase and subsequently undergoes polyubiquitination-mediated degradation to release active NF-κB, which then localizes to the nucleus (6). Target proteins destined for degradation through 26S proteasome-dependent mechanisms are generally conjugated with an identifying polyubiquitin chain(s) linked through internal ubiquitin Lys^{48} (K48) residues (7). However, recent studies have identified chain elongations that occur via alternative ubiquitin lysine residues and that have proteasome-independent functions. One such example is Lys^{63} (K63)-mediated polyubiquitination, which can mediate cellular signaling, DNA repair, protein localization, and endocytosis (8–10). The ubiquitin ligases TNF-associated factors 2 and 6, which harbor intact RING finger domains, mediate K63-linked polyubiquitination of themselves as well as stress-responsive kinases in signaling pathways activating JNK, p38, and IKK (11). Therefore, it is likely that the mode of ubiquitin chain linkage is closely related to their biological functions.

We report here that, following Daxx phosphorylation by ASK1, Daxx undergoes K63-linked polyubiquitination that, although not required for either Daxx interaction with ASK1 or Daxx stabilization, plays a critical role in Daxx-mediated ASK1 activation.

Materials and Methods

Plasmids construction. Specific Daxx substitution mutants were generated by site-directed mutagenesis. Daxx 75K, 122K, 135K, 140-2K, 208K, K0, and K122R were constructed by PCR with the primers:

K75R/F (5′-CTTGAACTTTGTAGGATGCAGACAGCAGAC-3′) and
K75R/R (5′-GTCTGCTGTCTGCATCCTACAAAGTTCAAG-3′),
K122R/F (5′-CGGAGCCGGCCAGCGCGACTCTATGTCTAC-3′) and
K122R/R (5′-GTAGACATAGAGTCGCGCTGGCCGGCTCCG-3′),
K135R/F (5′-CTCTGCACTGTTCTCAGGGCCCACTCAGCC-3′) and
K135R/R (5′-GGCTGAGTGGGCCCTGAGAACAGTGCAGAG-3′),
K140-2R/F (5′-GCCCACTCAGCCAGACGTCGACTGAACTTGGCC-3′) and
K140-2R/R (5′-GGCCAAGTTCAGTCGACGTCTGGCTGAGTGGGC-3′), and
K208R/F (5′-CGGCGGCTGCAGGAACGCGAGTTGGATCTC-3′) and
K208R/R (5′-GAGATCCAACTCGCGTTCCTGCAGCCGCCG-3′).

Ub lysine mutants were constructed based on pCMV-Myc-His-Ub wild-type.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Current address for Y. Fukuyo and N. Horikoshi: Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215.

Requests for reprints: Nobuo Horikoshi, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 3 Blackfan Circle, CLS-728, Boston, MA 02215. Phone: 617-735-3308; Fax: 617-735-3327; E-Mail: nhorikos@bidmc.harvard.edu.

doi:10.1158/0008-5472.CAN-09-2148

Published OnlineFirst September 29, 2009; DOI: 10.1158/0008-5472.CAN-09-2148

Cell culture and transfection. Cells were cultivated in DMEM (HyClone) supplemented with 10% FCS. For transfection with plasmid DNA, 293 cells were transfected by using FuGene 6 (Roche) as recommended by the manufacturer. Transfections into either HeLa or MCF cells were done by electroporation with Nucleofector (Amaxa Biosystems) and OMNI kit (Magus Reagents) according to the manufacturer's directions. For ubiquitination assays, 2 days after transfection, cells were treated with 10 μmol/L proteasome inhibitor MG115 for 12 h and harvested for protein analyses. For TNF-α stimulation, either HeLa or MCF7 cells were treated with TNF-α (Peprotech) for 18 h.

Immunoprecipitation and immunoblot analysis. Procedures for preparation of cell lysates, immunoprecipitation, and immunoblot are described previously (5). The primary antibodies were anti–green fluorescent protein (B-2; Santa Cruz Biotechnology), anti-FLAG (M5; Sigma), anti-HA (3F10; Roche), anti-Daxx (Sigma), anti-tubulin (Islet Cell Antibody), and anti–poly(ADP-ribose) polymerase (Affinity Bioreagents).

Results and Discussion

ASK1 kinase stabilizes Daxx and induces Daxx ubiquitination. Gene transfection studies indicate that expression of wild-type, but not kinase-defective (K709M), ASK1 induces Daxx accumulation (Fig. 1*A*). Stabilization was also observed with a truncated enhanced green fluorescent protein (EGFP)-Daxx (70-216), indicating that the domain(s) required for ASK1-dependent stabilization should be localized within Daxx amino acids 70 to 216, which also contain the ASK1 phosphorylation sites Ser^{176} and Ser^{184} (4). A similar Daxx accumulation was also observed in TNF-α–treated cells without ASK1 transfection (Fig. 1*A*).

It has been well documented that phosphorylation of IκB, cyclin-dependent kinase, or CDC25A triggers polyubiquitination of the proteins (12, 13). Because Daxx undergoes proteasome-mediated degradation (14, 15), we asked whether ASK1-dependent Daxx phosphorylation affected its ubiquitination status. Immunoprecipitation experiments revealed that active, but not kinase-defective, ASK1 induced Daxx polyubiquitination of both ectopically expressed Daxx (Fig. 1*B*) and endogenous Daxx (Fig. 1*C*). As expected from the previous results, the truncated form of FLAG-Daxx (amino acids 1-216) also showed ASK1-dependent polyubiquitination as well (Supplementary Fig. S1).

Phosphorylation of Daxx by ASK1 induces K63-linked polyubiquitination on Daxx Lys^{122}. ASK1-dependent Daxx

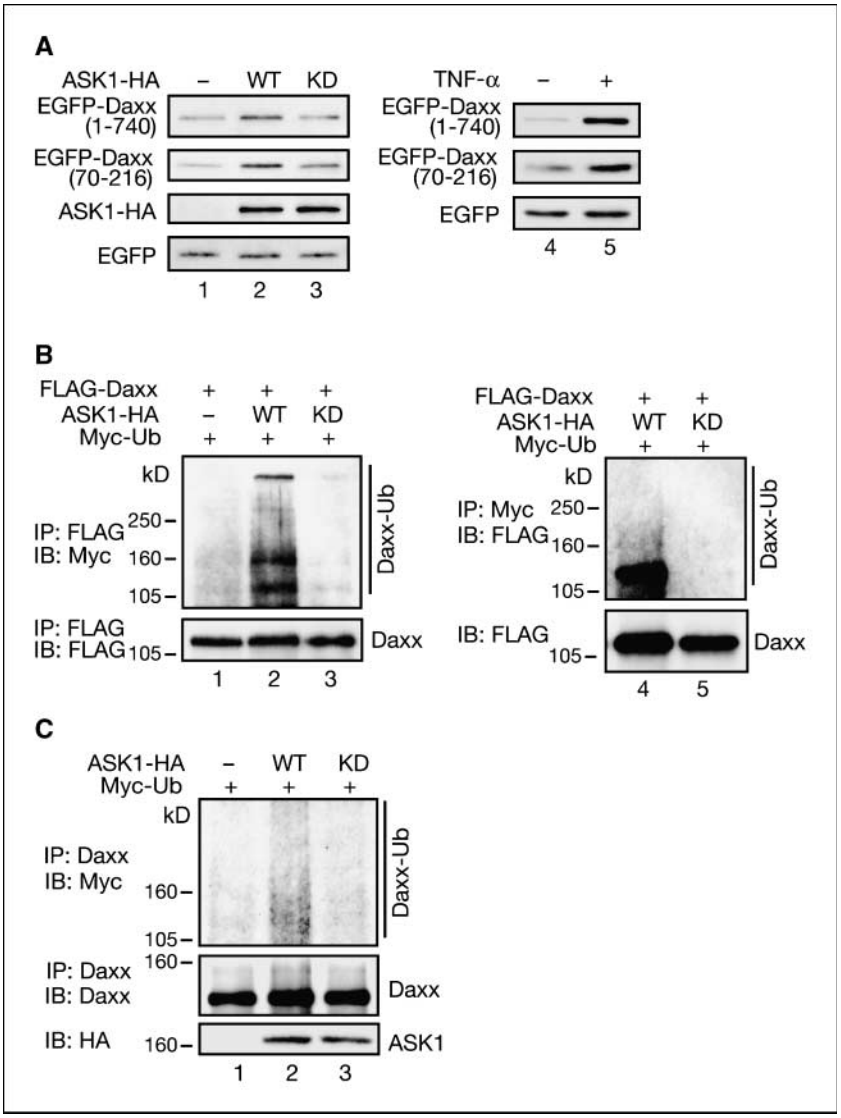

Figure 1. ASK1 stress kinase expression induces Daxx polyubiquitination. *A,* ASK1 and TNF-α stabilize Daxx. EGFP-Daxx or EGFP-Daxx (70-216) was expressed in 293 cells in the presence of ASK1-HA wild-type (*WT*) or a kinase-dead (*KD*; K709M) mutant or 18 h TNF-α treatment. EGFP served as an expression control. *B,* ASK1 increases polyubiquitination of Daxx. 293 cells were transfected with expression plasmids for ASK1-HA wild-type or kinase-defective together with FLAG-Daxx and Myc-Ub and cultured for 48 h. *Left,* Daxx was immunoprecipitated with anti-FLAG antibody and the precipitated materials were analyzed by immunoblotting with an antibody against Myc or FLAG. *IP,* immunoprecipitation; *IB,* immunoblotting. *Right,* reciprocal detection of ubiquitinated Daxx. Ubiquitinated Daxx products are indicated as Daxx-Ub. *C,* ASK1 induces the polyubiquitination of endogenous Daxx. 293 cells expressing Myc-Ub together with ASK1-HA wild-type or kinase-defective mutant were subjected to immunoprecipitation with anti-Daxx antibody followed by immunoblotting with anti-Myc or anti-Daxx antibodies.

Published OnlineFirst September 29, 2009; DOI: 10.1158/0008-5472.CAN-09-2148

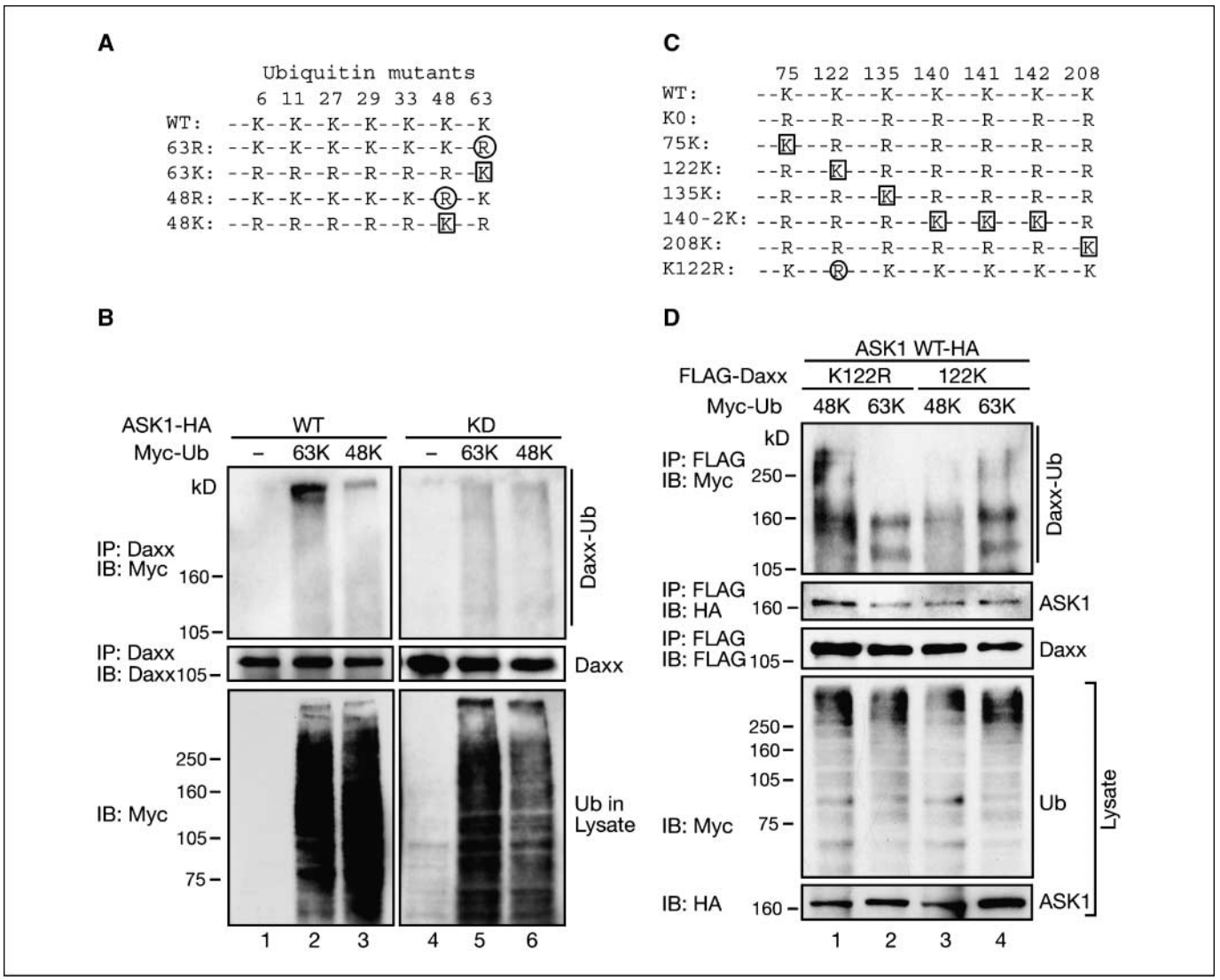

Figure 2. ASK1 induces K63-linked polyubiquitination of Daxx at K122. *A,* schematic diagrams of wild-type and mutant ubiquitin where arginine (*R*) amino acids are substituted for lysine (*K*). *B,* ASK1 induces K63-linked ubiquitination on endogenous Daxx. Cells were transfected with the indicated plasmids and cultured for 48 h. Daxx was immunoprecipitated with anti-Daxx antibody and the precipitated materials were subjected to immunoblotting. *C,* schematic diagram of wild-type and lysine mutants of Daxx. *D,* FLAG-Daxx mutants (K122R and 122K) were expressed in 293 cells together with ASK1-HA wild-type and Myc-Ub (48K or 63K).

phosphorylation followed by Daxx ubiquitination does not cause Daxx degradation. Instead, phosphorylation results in Daxx accumulation. The ubiquitin molecule contains seven lysines, which are potential conjugation residues for ubiquitin polymerization (ref. 16; Fig. 2*A*). To determine the mode of ASK1-induced ubiquitin conjugation on Daxx, we tested ubiquitin mutants with a single arginine-substituted mutation at position 63 (63R) or 48 (48R) as well as mutants containing only one lysine at position 63 (63K) or 48 (48K) for their incorporation in polyubiquitin chain(s) (Supplementary Fig. S2). The polyubiquitination of endogenous Daxx was induced by active ASK1 with primarily K63-linked but not K48-linked form (Fig. 2*B*). With kinase-defective ASK1, significantly less K63-conjugated Daxx was detected, confirming the requirement for ASK1 phosphorylation of Daxx for K63-linked ubiquitination. To identify the primary Daxx K63-linked ubiquitination site, a series of Daxx mutants with lysine point mutations in the NH_2-terminal region were constructed (Fig. 2*C*). Among mutants, only the 122K revertant Daxx mutant showed preferential K63-linked polyubiquitination over K48-linked polyubiquitination when expressed with ASK1 (Fig. 2*D*). Analysis using truncated Daxx (1-216) yielded similar results (Supplementary Fig. S3). Furthermore, the K122R mutant, which is deficient in ubiquitination on Lys^{122} of Daxx, subsequently showed a preferential K48-linked ubiquitin conjugation (Fig. 2*D*; Supplementary Fig. S3). Immunoprecipitation experiments revealed that all constructed Daxx mutants, including K0 and K122R, showed similar level of ASK1-Daxx interaction (Fig. 2*D*; Supplementary Fig. S3). Therefore, K63-linked polyubiquitination of Lys^{122} in the Daxx molecule is not required for interaction with ASK1.

The Lys^{122} ubiquitination site and surrounding amino acid sequence is evolutionally well conserved as are the ASK1 target serines, Ser^{176} and Ser^{184}. This suggests that ASK1-dependent K63-linked polyubiquitination on Lys^{122} of Daxx could be an important regulatory mechanism for Daxx function throughout many species.

Published OnlineFirst September 29, 2009; DOI: 10.1158/0008-5472.CAN-09-2148

ASK1-dependent specific Daxx phosphorylation is required for K63-linked ubiquitination of Daxx. Because ASK1 kinase activity is required for the K63-linked ubiquitination of Daxx, phosphorylation on Ser^{176} and Ser^{184} of Daxx is likely to play a critical role for its ubiquitination. The K63-linked ubiquitination of Daxx was induced by ASK1 transfection, whereas the phosphorylation-deficient mutant Daxx S176/184A had drastically reduced K63-linked ubiquitination levels in ASK1-transfected cells (Fig. 3*A*). Kinase-defective ASK1 failed to induce ubiquitination of Daxx. Thus, the K63-linked ubiquitination of Daxx depends on phosphorylation of Daxx on Ser^{176} and Ser^{184} by ASK1.

Next, we analyzed whether phosphorylated Daxx was simultaneously conjugated with K63 ubiquitin (Fig. 3*B*). Phosphorylated Daxx was immunoprecipitated with anti-phosphorylated Daxx–specific antibody (4), which recognizes phosphorylated Daxx Ser^{176} and Ser^{184}; then, the ubiquitination status of the immunoprecipitated Daxx was analyzed. Phosphorylated FLAG-Daxx was preferentially conjugated with K63-linked polyubiquitination rather than K48-linked polyubiquitination (Fig. 3*B*). These results show that ASK1-phosphorylated Daxx is preferentially K63-linked polyubiquitinated.

K63-linked polyubiquitination of Daxx is required for TNF-α–induced sustained JNK activation and apoptosis. We next determined the role of K63-linked Daxx ubiquitination in TNF-α signaling. Sustained JNK activation by full-length Daxx was observed only when active ASK1 was expressed (Fig. 4*A*), indicating that Daxx-dependent JNK activation is mediated through ASK1. When the K63 ubiquitination-deficient Daxx mutant K122R was expressed in cells, TNF-α–induced JNK activation was strongly inhibited (Fig. 4*B*). In addition, TNF-α–dependent apoptosis, as measured by poly(ADP-ribose) polymerase cleavage, was also prevented when the K122R mutant was expressed (Fig. 4*C*). The antiapoptotic effect of K122R mutant Daxx on TNF-α–dependent apoptosis was also shown by the additional method of measuring sub-G_1 fraction by fluorescence-activated cell sorting analysis (Supplementary Fig. S4). In Daxx wild-type transfected cells, the sub-G_1 fraction increased from 27.8% to 40.2% following TNF-α treatment, whereas transfection with the K122R mutant Daxx had essentially no effect on TNF-α–induced apoptosis (from 24.2% to 23.1% by TNF-α treatment). These results indicate that K63-linked polyubiquitination of Daxx on K122 is essential for TNF-α–dependent ASK1 activation, although K122 is dispensable for ASK1-induced Daxx accumulation. Because the K122 mutant Daxx still binds to ASK1 (Fig. 2*D*), K63-linked Daxx polyubiquitination is more likely involved in a post-interaction ASK1 activation step. This is also supported by the observation that the K122R Daxx mutant inhibited TNF-α–induced JNK activation and apoptosis, thereby functioning as dominant-negative fashion (Fig. 4*B* and *C*).

The NH_2-terminal domain of ASK1 inhibits the endogenous kinase activity (17), suggesting that the Daxx K63-linked polyubiquitin tail may induce an ASK1 conformational change resulting in kinase activation. Moreover, this NH_2-terminal domain is also the binding site for the ASK1 inhibitor thioredoxin, a reduction/oxidation-regulatory protein (17). Thus, the polyubiquitin tail may also prevent thioredoxin from interacting with ASK1. Alternatively, K63-linked polyubiquitin may recruit an activating cofactor(s), such as TNF-associated factor 2 (18), critical for sustained ASK1 kinase

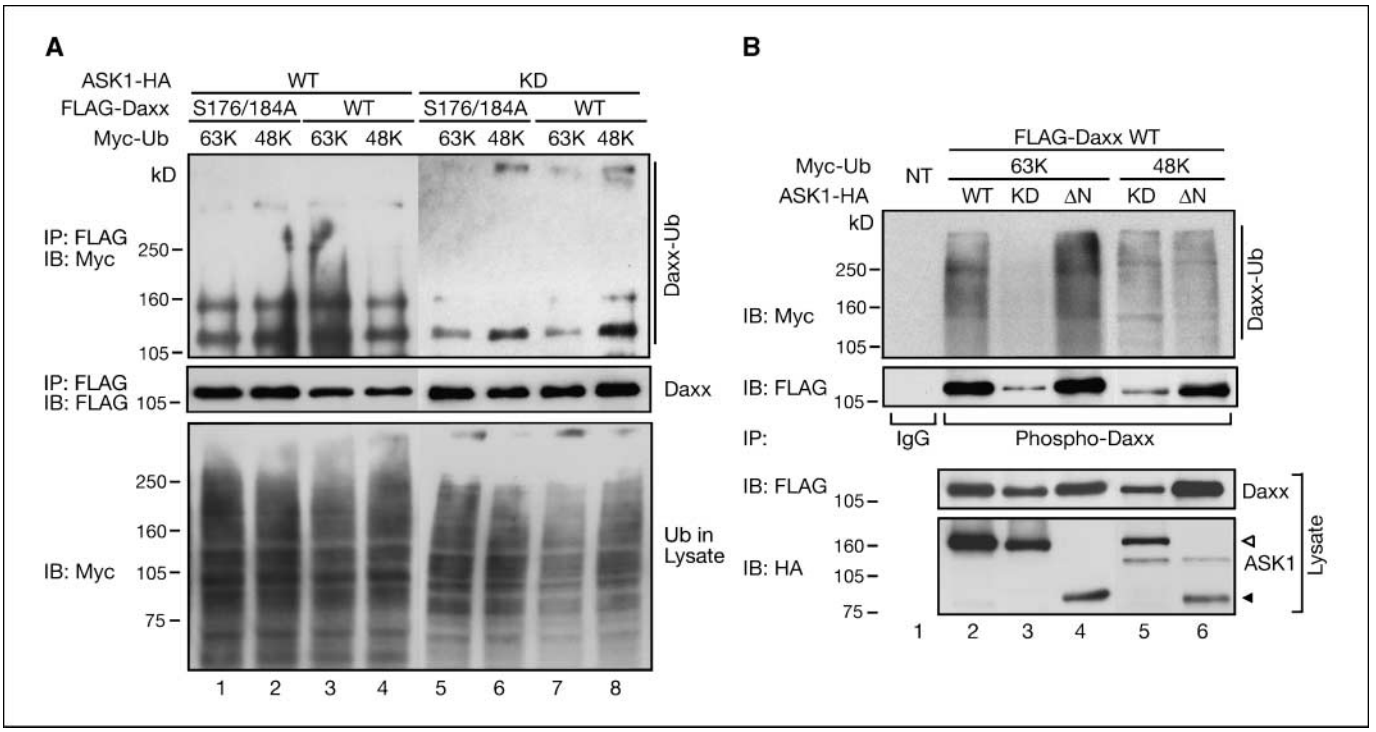

Figure 3. ASK1-dependent Daxx phosphorylation is required for K63-linked ubiquitin conjugation to Daxx. *A,* ASK1-dependent phosphorylation is required for Daxx K63-linked ubiquitination. Daxx was immunoprecipitated with anti-FLAG antibody and the precipitated materials were subjected to immunoblotting with anti-Myc or anti-FLAG antibodies. *B,* phosphorylated Daxx is K63-linked polyubiquitinated. FLAG-Daxx was coexpressed with Myc-Ub (63K or 48K) and ASK1-HA (wild-type, kinase-defective, or its constitutive active mutant ΔN; ref. 17). Cell lysates were subjected to immunoprecipitation with anti-phosphorylated Daxx antibody followed by immunoblotting with anti-Myc and anti-FLAG antibodies.

Published OnlineFirst September 29, 2009; DOI: 10.1158/0008-5472.CAN-09-2148

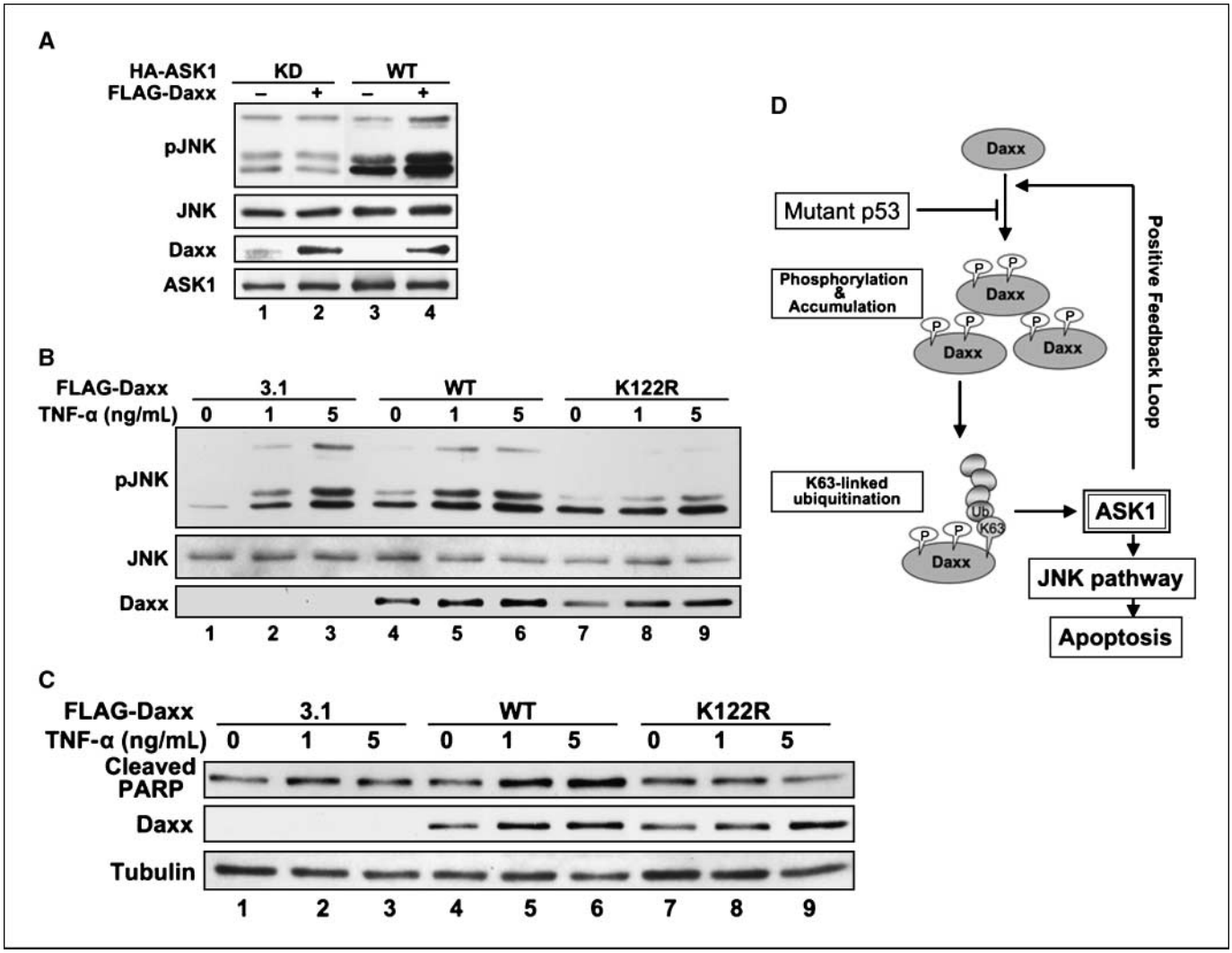

Figure 4. TNF-α–dependent JNK activation and apoptosis require K63-linked polyubiquitination of Daxx. *A,* Daxx-dependent sustained JNK activation is ASK1-dependent. HeLa cell extracts were prepared and analyzed for the expression levels of phosphorylated JNK, total JNK, Daxx, and HA-ASK1 by immunoblotting. *B,* K122 in Daxx is critical for TNF-α–induced sustained JNK activation. Two days posttransfection, HeLa cells were incubated with TNF-α (0, 1, or 5 ng/mL) for 18 h. *C,* Daxx K122 is essential for TNF-α–induced apoptosis. After 48 h of transfection, MCF7 cells were treated with TNF-α. The expression levels of cleaved poly(ADP-ribose) polymerase (*PARP*), which is an indicator of apoptosis, FLAG-Daxx, and tubulin were determined by immunoblotting. *D,* role of K63-linked polyubiquitination of Daxx in TNF-α signaling pathway. See text for details.

activity. The precise molecular mechanism for how Daxx K63-linked ubiquitination activates ASK1 remains to be elucidated.

TNF-α signaling involves TNF-associated factor 2 phosphorylation followed by K63-linked ubiquitination (19). Here, we show that phosphorylation induced K63-linked ubiquitination of Daxx plays a critical role in the Daxx-ASK1 positive feedback loop (Fig. 4*D*). Depletion of Daxx in cells produces resistance to cell death induced by UV irradiation and oxidative stress and an impaired JNK activation (20), suggesting that Daxx plays a critical role on JNK-induced apoptosis.

Daxx is essential for the sustained activation of JNK and TNF-α–induced apoptosis in cells (4). ASK1 is required for sustained activation of JNK/p38 and apoptosis (21). When ASK1 is activated by TNF receptor signaling, it phosphorylates Daxx at Ser^{176} and Ser^{184}. This phosphorylation stabilizes Daxx due to a reduced K48-linked polyubiquitination content causing cellular Daxx levels increase. Phosphorylated Daxx is then modified by K63-linked polyubiquitination at Lys^{122}. Polyubiquitination is not required for interaction with ASK1 but is essential for ASK1 activation. These findings establish a physiological significance to the network of Daxx-ASK1-JNK signaling in TNF-α–dependent cell death in which the K63-linked polyubiquitin tail on Daxx functions as a molecular switch for ASK1 activation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 6/16/09; revised 8/10/09; accepted 8/19/09; published OnlineFirst 9/29/09.

Grant support: NIH grant R01CA98666 (N. Horikoshi).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Drs. Helen Piwnica-Worms and Zhijian J. Chen for Ub constructs.

Published OnlineFirst September 29, 2009; DOI: 10.1158/0008-5472.CAN-09-2148

References

1. Ichijo H, Nishida E, Irie K, et al. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science 1997;275:90–4.
2. Kyriakis JM, Avruch J. Protein kinase cascades activated by stress and inflammatory cytokines. Bioessays 1996;18:567–77.
3. Yang X, Khosravi-Far R, Chang HY, Baltimore D. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell 1997;89:1067–76.
4. Kitamura T, Fukuyo Y, Inoue M, et al. Mutant p53 disrupts the stress MAPK activation circuit induced by ASK1-dependent stabilization of Daxx. Cancer Res 2009; 69:7681–8.
5. Ohiro Y, Usheva A, Kobayashi S, et al. Inhibition of stress-inducible kinase pathways by tumorigenic mutant p53. Mol Cell Biol 2003;23:322–34.
6. Miyamoto S, Seufzer BJ, Shumway SD. Novel IκBα proteolytic pathway in WEHI231 immature B cells. Mol Cell Biol 1998;18:19–29.
7. Pickart CM. Targeting of substrates to the 26S proteasome. FASEB J 1997;11:1055–66.
8. Habelhah H, Takahashi S, Cho SG, Kadoya T, Watanabe T, Ronai Z. Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF-κB. EMBO J 2004;23:322–32.
9. Clague MJ, Urbe S. Endocytosis: the DUB version. Trends Cell Biol 2006;16:551–9.
10. Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 2001;412:346–51.
11. Laine A, Ronai Z. Ubiquitin chains in the ladder of MAPK signaling. Sci STKE 2005;2005:re5.
12. Watanabe N, Arai H, Iwasaki J, et al. Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways. Proc Natl Acad Sci U S A 2005;102:11663–8.
13. Ang XL, Wade Harper J. SCF-mediated protein degradation and cell cycle control. Oncogene 2005;24: 2860–70.
14. Kitagawa D, Kajiho H, Negishi T, et al. Release of RASSF1C from the nucleus by Daxx degradation links DNA damage and SAPK/JNK activation. EMBO J 2006; 25:3286–97.
15. Ryo A, Hirai A, Nishi M, et al. A suppressive role of the prolyl isomerase Pin1 in cellular apoptosis mediated by the death-associated protein Daxx. J Biol Chem 2007; 282:36671–81.
16. Ikeda F, Dikic I. Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series. EMBO Rep 2008;9: 536–42.
17. Saitoh M, Nishitoh H, Fujii M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 1998;17: 2596–606.
18. Nishitoh H, Saitoh M, Mochida Y, et al. ASK1 is essential for JNK/SAPK activation by TRAF2. Mol Cell 1998;2:389–95.
19. Li S, Wang L, Dorf ME. PKC phosphorylation of TRAF2 mediates IKKα/β recruitment and K63-linked polyubiquitination. Mol Cell 2009;33:30–42.
20. Khelifi AF, D'Alcontres MS, Salomoni P. Daxx is required for stress-induced cell death and JNK activation. Cell Death Differ 2005;12:724–33.
21. Tobiume K, Matsuzawa A, Takahashi T, et al. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep 2001;2:222–8.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Phosphorylation-Dependent Lys^{63}-Linked Polyubiquitination of Daxx Is Essential for Sustained TNF- α–Induced ASK1 Activation

Yayoi Fukuyo, Tetsuya Kitamura, Masahiro Inoue, et al.

Cancer Res 2009;69:7512-7517. Published OnlineFirst September 29, 2009.