TRAF7 Potentiates MEKK3-induced AP1 and CHOP Activation and Induces Apoptosis*

Liang-Guo Xu‡, Lian-Yun Li§, and Hong-Bing Shu‡§¶

From the ‡Integrated Department of Immunology, National Jewish Medical and Research Center, University of Colorado Health Sciences Center, Denver, Colorado 80260 and the §Department of Cell Biology and Genetics, College of Life Sciences, Peking University, Beijing 100871, China

The tumor necrosis factor receptor-associated factor (TRAF) protein family members are critically involved in activation of NF-κB, JNK, and p38 activation triggered by tumor necrosis factor (TNF) receptor family members and toll/interleukin-1 receptor (TIR)-containing receptors. TRAF proteins (except for TRAF1) contain an N-terminal RING finger domain that is essential for their functions. In this report, we identified a protein designated as TRAF7, which contains a RING finger domain and a zinc finger domain that are mostly conserved with those of TRAFs. TRAF7 also contains seven WD40 repeats at its C terminus. TRAF7 specifically interacted with MEKK3 and potentiated MEKK3-mediated AP1 and CHOP activation. Depletion of TRAF7 by antisense RNA inhibited MEKK3-mediated AP1 and CHOP activation. Moreover, overexpression of TRAF7 induced caspase-dependent apoptosis. Domain mapping experiments indicated that TRAF7 potentiated MEKK3-mediated AP1 and CHOP activation and induced apoptosis through distinct domains. Our studies identified a novel TRAF family member that is involved in MEKK3 signaling and apoptosis.

EXPERIMENTAL PROCEDURES

Reagents—Monoclonal antibodies against FLAG and HA epitopes and β-tubulin (Sigma) and the caspase inhibitor BD-fmk (Enzyme Systems) were purchased from the indicated manufactures.

Constructs—CHOP luciferase reporter system was purchased from Stratagene. AP1-luciferase reporter construct and mammalian expression plasmid for MEKK1 (Dr. Gary Johnson), and mammalian expression plasmids for FLAG-tagged RIP and NIK (Dr. David Goeddel) were provided by the indicated investigators.

Mammalian expression plasmids for FLAG-tagged human MEKK2 and MEKK3 and HA-tagged human TRAF7 and its mutants were constructed by PCR amplification of the corresponding cDNA fragments and subsequently cloning into a CMV promoter-based vector containing an N-terminal HA or FLAG tag.

Northern Blot Hybridization—Human multiple tissue mRNA blot was purchased from Clontech. The blot was hybridized with [32P]dCTP-labeled cDNA probe corresponding to human TRAF7 coding sequence. Hybridization was performed in the Rapid Hybridization Buffer (Clontech) under high stringency conditions.

Co-immunoprecipitation, in Vitro Kinase Assays, and Western Blot Analysis—These experiments were performed as previously described (14).

Cell Transfection and Reporter Gene Assays—Transfection of 293 cells and reporter gene assays were performed as described (14). Within the same experiment, each transfection was performed in triplicate, and where necessary, empty control plasmid was added to ensure that each transfection receives the same amount of total DNA. To normalize for transfection efficiency, 0.2 µg of RSV-β-galactosidase luciferase reporter plasmid was added to each transfection. Luciferase activities were normalized on the basis of β-galactosidase activities. Data shown were averages from one representative experiment. The standard deviations were less than 10% of the average values for all samples.

Cell Death and DNA Fragmentation Assays—β-Galactosidase cotransfection assays for determination of cell death and DNA fragmentation assays were performed as described previously (5, 14).
RESULTS

Identification of TRAF7—We searched the GenBank™ expressed sequence tag data bases for proteins containing a TRAF-like RING finger domain. This search identified a novel protein designated as TRAF7. TRAF7 has two alternative splice forms. The longer form encodes a 670-amino acid protein (Fig. 1A). The shorter form encodes a protein lacking the first 66 amino acids of the longer form. The long form is referred as TRAF7, while the short form is referred as TRAF7s. TRAF7 contains a RING finger domain at aa 125–160 and an adjacent zinc finger domain at aa 221–287 (Fig. 1A). Blast searches of the GenBank™ data bases indicate that the RING and zinc finger domains of TRAF7 are mostly conserved with those of TRAF proteins. The C terminus of TRAF7 contains seven WD40 repeats, which are mostly conserved with β-transducin-like protein Het-e and Het-d, F-box, and WD40 domain-containing proteins FBXW6, FBXW7, and FBX30 (Fig. 1A).

TRAF7 is an evolutionarily highly conserved protein. Human TRAF7 shares 97 and 92% sequence identity with its orthologs in mouse and Xenopus respectively (Fig. 1A and data not shown).

Tissue Distribution of TRAF7—To examine tissue distribution of TRAF7 expression, we performed Northern blot analy-
The results suggest that human TRAF7 mRNA is expressed in all examined tissues as two transcripts of 2.6 and 4.0 kb, respectively (Fig. 1B). Expression of TRAF7 is relatively higher in skeletal muscle, heart, colon, spleen, kidney, liver, and placenta than in brain, thymus, small intestine, lung, and peripheral blood leukocytes (Fig. 1B).

**TRAF7 Interacts with MEKK3**—Previously, it has been shown that the RING finger domains of TRAF proteins are critically involved in activation of MAP3K family members involved in NF-κB and JNK activation and apoptosis (7, 8) and TAK1 (9–13). Since TRAF7 contains a TRAF-like RING finger domain, we determined whether TRAF7 can physically and functionally interact with MAP3K family members. We transfected 293 cells with expression plasmids for HA-TRAF7 and FLAG-tagged NIK, RIP, MEKK2, or MEKK3. Cell lysates were immunoprecipitated (IP) with anti-HA or control IgG. The immunoprecipitates were analyzed by Western blot (WB) with anti-FLAG antibody. The two lower panels are Western blot analysis of protein levels in the lysates with anti-FLAG and anti-HA antibodies, respectively. B, the WD40 repeats of TRAF7 were responsible for interaction with MEKK3. 293 cells were transfected with mammalian expression plasmids for FLAG-MEKK3 and the indicated HA-tagged TRAF7 mutants. Co-immunoprecipitation and Western blot analysis performed as in A. C and D, TRAF7 specifically potentiates the kinase activity of MEKK3. 293 cells were transfected with the indicated plasmids. Cell lysates were immunoprecipitated with anti-FLAG antibody, and the immunoprecipitates were subjected to in vitro kinase assays (C, upper panel). Cell lysates were also analyzed by Western blot with anti-FLAG antibody (C, lower panel). The kinase activities were also quantitated by phosphorimaging (D).

**TRAF7 Potentiates MEKK3-induced AP1 and CHOP Activation**—Previously, it has been shown that MEKK3 activates AP1 and CHOP. We transfected 293 cells with expression plasmids for HA-TRAF7 and FLAG-MEKK3, FLAG-MEKK2, or FLAG-RIP. Cell lysates were immunoprecipitated with anti-FLAG antibody, and the immunoprecipitates were subjected to in vitro kinase assays. The results indicated that TRAF7 increased the autophosphorylation of MEKK3 (Fig. 2C). In these experiments, TRAF7 did not have significant effects on autophosphorylation of MEKK2 and RIP (Fig. 2C). TRAF7 also did not affect kinase activity of MEKK1 and NIK (data not shown). These data suggest that TRAF7 specifically promotes the kinase activity of MEKK3.
JNK and p38 kinase pathways, which lead to activation of two transcription factors, AP1 and CHOP, respectively (15–20). We determined the effect of TRAF7 on MEKK3-induced AP1 and CHOP activation by reporter gene assays. The results indicated that TRAF7 itself had no effects on AP1 activation. However, TRAF7 could potentiate MEKK3-induced AP1 activation in a dose-dependent manner (Fig. 3A). Overexpression of MEKK3-mediated AP1 (C) and CHOP (D) activation. 293 cells (2 × 10⁵) were transfected with 100 ng of the indicated reporter plasmid, 200 ng of an expression plasmid for MEKK3 (empty bars) or control vector (filled bars), and 200 ng of the indicated TRAF7 mutant plasmids. Reporter gene assays were performed as described previously (14). E, inhibition of TRAF7 expression by a TRAF7 antisense construct. 293 cells (2 × 10⁵) were transfected with 100 ng of HA-TRAF7 plasmid, 100 ng of FLAG-MEKK3 plasmid, and 300 ng of TRAF7 antisense (TRAF7-AS) or control plasmid. Cell lysates were analyzed by Western blots with anti-HA (upper panel), anti-FLAG (middle panel), and anti-β-tubulin (lower panel) antibodies. F, inhibition of MEKK3-mediated AP1 activation by TRAF7-AS. 293 cells were transfected with AP1-luciferase reporter plasmid, TRAF7-AS or control plasmid, and MEKK3 or MEKK1 expression plasmid. Luciferase reporter assays were performed as described previously (14). G, inhibition of MEKK3-mediated CHOP activation by TRAF7-AS. The experiments were done similar to those in F.

We also analyzed which domain of TRAF7 is responsible for its potentiation of MEKK3-induced AP1 and CHOP activation. The results indicated that the WD40 repeats were essential for TRAF7 potentiation of both MEKK3-induced AP1 and CHOP activation (Fig. 3, C and D).

Depletion of TRAF7 by Antisense RNA Inhibits MEKK3-induced AP1 and CHOP Activation—To determine whether TRAF7 plays a physiological role in MEKK3 signaling, we determined the effects of depletion of TRAF7 on MEKK3-induced AP1 and CHOP activation. As shown in Fig. 3E, transfection of antisense TRAF7 construct inhibited expression of TRAF7. In reporter gene assays, this antisense construct significantly inhibited MEKK3-induced AP1 and CHOP activation (Fig. 3, F and G). TRAF7 antisense RNA did not inhibit MEKK1-induced AP1 activation (Fig. 3F) but inhibited MEKK1-induced CHOP activation (Fig. 3G).

TRAF7 induces apoptosis through caspase-dependent pathways—In our transient transfection experiments, we found that overexpression of TRAF7 caused cellular condensation, round-up, and detachment from the dish, characteristics of apoptotic cells (Fig. 4A). Deletion of the RING finger domain of TRAF7 significantly reduced its ability to induce cell death, suggesting that the RING finger domain of TRAF7 is important for its apoptotic activity (Fig. 4A). TRAF7-induced apoptosis is significantly inhibited by the caspase inhibitor crmA (Fig. 4A). Overexpression of TRAF7 also induced DNA fragmentation and this was inhibited by crmA, confirming that TRAF7 induces caspase-dependent apoptosis (Fig. 4B). In these experiments, overexpression of MEKK3 caused weaker apoptotic effects (Fig. 4, A and B).

DISCUSSION

In our experiments, a major fraction of overexpressed N-terminal tagged TRAF7 was identified in high molecular...
weight complexes or was processed into smaller fragments, with a major processed fragment of ~45 kDa (Figs. 2A and 3E). TRAF7 mutants were also accumulated in high molecular weight complexes or processed into fragments (Fig. 2B). Overexpression of independently cloned, C-terminal FLAG-tagged TRAF7 also led to similar patterns (data not shown). The processing of TRAF7 is not mediated by caspases because crmA or the pan-caspase inhibitor BD-fmk could not inhibit the processing. Moreover, mutation of the potential caspase cleavage sites, including Asp341, Asp350, Asp357, and Asp363, had no effects on the processing of TRAF7 (data not shown).

During the preparation of this manuscript for publication, an independent study also identified TRAF7 (21). In a large scale experiment to map an interaction network of signaling proteins of the TNF and NF-κB activation pathways, TRAF7 was identified as a protein interacting with MEKK3 and potentiating MEKK3-induced NF-κB and AP1 activation. This study also demonstrated that TRAF7 is an E3 ubiquitin ligase capable of self-ubiquitination (21). This study points to the possibility that the high molecular weight complexes observed in our study represent ubiquitinated TRAF7 and ubiquitination of TRAF7 leads to its limited processing.

Our studies suggest that TRAF7 contains two important domains. Its WD40 repeats are sufficient for potentiating MEKK3-mediated AP1 and CHOP activation, suggesting that the E3 ubiquitin ligase activity of TRAF7 is not required for its potentiation of MEKK3-mediated AP1 and CHOP activation. In contrast, the RING finger domain of TRAF7 is important for its apoptotic activity. The mechanisms responsible for TRAF7-mediated cellular effects and the upstream components to which TRAF7 links MEKK3 need to be further investigated.

REFERENCES

1. Wajant, H., Henkel, F., and Scheurich, P. (2001) Cell. Signal. 13, 389–400
2. Chung, J. Y., Park, Y. C., Ye, H., and Wu, H. (2002) J. Cell Sci. 115, 679–688
3. Bradley, J. R., and Pober, J. S. (2001) Oncogene 20, 6482–6491
4. Rothe, M., Wong, S. C., Henaiz, W. J., and Goeddel, D. V. (1994) Cell 78, 681–692
5. Hsu, H., Shu, H. B., Pan, M. G., and Goeddel, D. V. (1996) Cell 84, 299–308
6. Shu, H. B., Takeuchi, M., and Goeddel, D. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13973–13978
7. Yuasa, T., Ohno, S., Kehrl, J. H., and Kyriakis, J. M. (1998) J. Biol. Chem. 273, 22681–22692
8. Liu, Z. G., Hsu, H., Goeddel, D. V., and Kari, M. (1996) Cell 87, 565–576
9. Li, X., Yang, Y., and Ashwell, J. D. (2002) Nature 416, 345–347
10. Wang, C., Deng, L., Hong, M., Akkaraju, G. R., Inoue, J., and Chen, Z. J. (2001) Nature 412, 346–351
11. Trompesouki, E., Hatziavassiliou, E., Tsirchritis, T., Farmer, H., Ashworth, A., and Mostalos, G. (2003) Nature 424, 793–796
12. Malinin, N. L., Boldin, M. P., Kovalenko, A. V., and Wallach, D. (1997) Nature 385, 540–544
13. Nishimoto, H., Saitoh, M., Mochida, Y., Takeda, K., Nakano, H., Rothe, M., Miyazono, K., and Ichijo, H. (1998) Mol. Cell 2, 389–395
14. Han, K. J., Su, X., Xu, L. G., Bin, L. H., Zhang, J., and Shu, H. B. (January 22, 2004) J. Biol. Chem. 10.1074/jbc.M316292200
15. Ellinger-Ziegelbauer, H., Brown, K., Kelly, K., and Siebenlist, U. (1997) J. Biol. Chem. 272, 2668–2674
16. Deacon, K., and Blank, J. L. (1997) J. Biol. Chem. 272, 14489–14496
17. Deacon, K., and Blank, J. L. (1999) J. Biol. Chem. 274, 16604–16610
18. Nakamura, K., and Johnson, G. L. (2003) J. Biol. Chem. 278, 36989–36992
19. Johnson, G. L., and Lapadat, R. (2002) Science 298, 1911–1912
20. Garrington, T. P., and Johnson, G. L. (1999) Curr. Opin. Cell Biol. 11, 211–218
21. Bouwmeester, T., Bauch, A., Ruffner, H., Angrand, P. O., Bergamini, G., Crouchon, K., Craciut, C., Eberhard, D., Gagneur, J., Ghidelli, S., Hof, C., Huebs, B., Mangano, R., Michon, A. M., Schirle, M. Schlegl, J., Schwab, M., Stein, M. A., Bauer, A., Casari, G., Drewes, G., Gavlin, A. C., Jackson, D. B., Joly, G., Neubauer, G., Rick, J., Kuster, B., and Superti-Furga, G. (2004) Nat. Cell Biol. 6, 97–105
TRAF7 Potentiates MEKK3-induced AP1 and CHOP Activation and Induces Apoptosis
Liang-Guo Xu, Lian-Yun Li and Hong-Bing Shu

J. Biol. Chem. 2004, 279:17278-17282.
doi: 10.1074/jbc.C400063200 originally published online March 4, 2004

Access the most updated version of this article at doi: 10.1074/jbc.C400063200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 20 references, 8 of which can be accessed free at http://www.jbc.org/content/279/17/17278.full.html#ref-list-1