

MEKK1/MEKK4 are responsible for TRAIL-induced JNK/p38 phosphorylation

BO K. SUN^{1*}, JOO-HANG KIM^{1,2,3*}, HOAN N. NGUYEN¹, SEEUN OH^{1,3}, SO Y. KIM^{1,3},
SOOJIN CHOI¹, HYE J. CHOI^{1,2}, YONG J. LEE⁴ and JAE J. SONG¹

¹Institute for Cancer Research, Yonsei Cancer Center; ²Department of Internal Medicine;

³Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea;

⁴Department of Surgery and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Received August 3, 2010; Accepted October 11, 2010

DOI: 10.3892/or.2010.1079

Abstract. The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to activate mitogen-activated protein kinases (MAPKs) depending on caspase and mammalian sterile 20-like kinase 1 activations. However, the upstream molecule of MAPKs has not yet been identified. The mitogen-activated protein kinase kinase 1 (MEKK1) and the apoptosis signal-regulating kinase 1 (ASK1) are considered to be possible candidates for the action of MAPKKs induced by TRAIL and the possibility of reactive oxygen species involvement has also been investigated. We found that MEKK1/MEKK4 as opposed to ASK1, are responsible for TRAIL-induced c-Jun NH₂-terminal kinase (JNK) or p38 activation, and that their catalytic activity is repressed by the caspase-8 inhibitor, suggesting that the

caspase-8 activation induced by TRAIL is indispensable for MEKK activation. The 14-3-3 θ was also shown to interact with and to dissociate from MEKK1 by TRAIL treatment, thus implicating the 14-3-3 protein as a negative regulator of MEKK1 activation. Taken together, we show herein that the upstream molecule of the TRAIL-induced MAPK activation is MEKK, as opposed to ASK1, via the mediation of its signal through JNK/p38 in a caspase-8-dependent manner.

Introduction

The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), also known as the Apo2 ligand, is a type II transmembrane protein belonging to the TNF superfamily (1,2). The binding of TRAIL to its receptors (DR4/TRAIL-R1 and DR5/TRAIL-R2) results in receptor aggregation and in the recruitment of the Fas-associated death domain (FADD) adaptor proteins, which subsequently induce the formation of the death-inducing signaling complex (DISC) involved in the activation of the caspase-8 initiator (3,4). Activated caspase-8 cleaves Bid and/or caspase-3 and initiates the mitochondrial apoptotic pathway (intrinsic pathway) and/or the caspase cascade (extrinsic pathway), respectively, eventually leading to cell death (5).

Many studies have indicated that TRAIL destroys a variety of tumor cell lines, while leaving normal cells viable, thus suggesting that this protein could function as a specific cancer therapeutic agent (6). Although TRAIL is regarded as a potential anti-cancer agent, a considerable proportion of cancer cells, especially those in certain highly malignant tumors, are resistant to apoptosis induced by TRAIL, and other cancer cells that are initially sensitive to TRAIL-induced apoptosis can become resistant after repeated exposure (acquired resistance) (7). However, the exact mechanisms of acquired TRAIL resistance are still largely unknown.

Previously, we reported that Src, c-Cbl and PI3K are involved in the phosphorylation of Akt during TRAIL treatment, and that these phosphorylations are related to TRAIL-induced acquired resistance (8,9). In addition to the non-apoptotic TRAIL signaling of Akt phosphorylation, TRAIL also induces the activation of mitogen-activated protein kinase (MAPK) kinase pathways in a caspase-8-dependent

Correspondence to: Dr Jae J. Song, Institute for Cancer Research, Yonsei Cancer Center, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul, Republic of Korea
E-mail: jjs109@yuhs.ac

*Contributed equally

Abbreviations: TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PAGE, polyacrylamide gel electrophoresis; PARP, poly(ADP-ribose) polymerase; MAPK, mitogen-activated protein kinase; JNK, c-Jun NH₂-terminal kinase; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase; MEKK1, MEK kinase 1; ASK1, apoptosis signal-regulating kinase 1; SEK, stress-activated protein kinase/extracellular-signal regulated kinase; MLK, mixed lineage kinase; TAK, TGF- β -activated kinase; ROS, reactive oxygen species; GCK, germinal center kinase; TRAF2, tumor necrosis factor receptor-associated factor 2; Mst1, mammalian sterile 20-like kinase 1

Key words: mitogen-activated protein kinase kinase 1/4, tumor necrosis factor-related apoptosis-inducing ligand, c-Jun NH₂-terminal kinase, p38, 14-3-3

manner (10-12). However, the biological roles of c-Jun NH₂-terminal kinase (JNK) and p38 MAPK activations in TRAIL-induced signaling remain uncertain (5). Moreover, multiple mechanisms have been reported, by which JNK or p38 are activated by TRAIL. For example, Varfolomeev *et al* (11) suggested that FADD, caspase-8, RIP1 and TNF receptor-associated factor 2 (TRAF2) are recruited within the primary DISC, leading to the stimulations of JNK and p38, while Liu *et al* (13) concluded that mitogen-activated protein kinase kinase 1 (MEKK1) can be activated via TRAF2 and RIP to activate JNK in the absence of apoptotic conditions. In this study, we demonstrated the occurrence of mammalian sterile 20-like kinase 1 (Mst1)-mediated caspase-8-dependent activation of MAPKs during TRAIL incubation. However, the upstream entities of MAPKs remain unidentified.

The MAPKs are a family of kinases known to transduce external signals to the nucleus in order to determine the fate of the cell (14). Usually, conventional MAPKs consist of three family members, JNK, p38 and the extracellular signal-regulated kinase (ERK), which are involved in different cellular processes, including inflammation, cell proliferation and differentiation, and apoptosis (5). Notwithstanding the TRAIL-induced ERK activation, which is mainly associated with an anti-apoptotic function, the functions of JNK and p38 activation in TRAIL-induced signaling are varied and can be controversial depending on the cell types and cellular contexts involved (15-20). However, the upstream molecules of MAPKs have not yet been identified. It should be noted that, MAPKKs link a variety of extracellular stimuli to cytoplasmic and nuclear effectors by activating downstream MAPK pathways (21). MEKK1, apoptosis signal-regulating kinase 1 (ASK1), TGF- β -activated kinase (TAK)1, and mixed lineage kinase (MLK)2, are well-known MAP3 kinases (22). In this study, we clearly demonstrate that MEKK1 and 4 transmit TRAIL-induced signals to JNK or p38 MAPK in a caspase-8-dependent manner.

Materials and methods

Cell culture. The human prostate adenocarcinoma cell line, DU-145, was cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (HyClone, Logan, UT, USA) and 26 mM sodium bicarbonate. The cells were maintained in a humidified environment containing 5% CO₂ and air at 37°C.

Reagents and antibodies. Polyclonal anti-phospho-ERK, anti-ERK, anti-p38, monoclonal anti-phospho-p38 and anti-caspase-8, were purchased from Cell Signaling (Beverly, MA, USA), and anti-ACTIVE (phosphoT183 and Y185) JNK was purchased from Promega (Madison, WI, USA). Polyclonal anti-JNK1 and 14-3-3 β , γ , θ and ζ , were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Anti-MEKK1 was purchased from Assay Designs (Ann Arbor, MI, USA). Anti-ASK1 was purchased from Millipore (Billerica, MA, USA). Monoclonal anti-poly(ADP-ribose) (PARP) was purchased from Biomol International, L.P. (Plymouth Meeting, PA, USA). Anti-actin antibody was purchased from ICN (Costa Mesa, CA, USA). Caspase-8 inhibitor (Z-IETD-FMK) was purchased from Calbiochem

(San Diego, CA, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Protein extracts and polyacrylamide gel electrophoresis (PAGE). Cells were lysed with 1X Laemmli lysis buffer [2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.002% bromophenol blue and 62.5 mM Tris (pH 6.8)] and boiled for 10 min. Protein content was measured using BCA Protein Assay Reagent (Pierce, Rockford, IL, USA). The samples were diluted with 1X lysis buffer, and β -mercaptoethanol was added until a concentration of 350 mM was achieved. Then, equal amounts of protein were loaded onto 10% SDS polyacrylamide gels. SDS-PAGE analysis was performed according to the procedure of Laemmli using a Hoefer gel apparatus.

Immunoblot analysis. Proteins were separated by SDS-PAGE and were electrophoretically transferred onto nitrocellulose membranes. Each nitrocellulose membrane was blocked with 5% non-fat dry milk in PBS-Tween-20 (0.1%, v/v) at 4°C overnight. The membrane was then incubated with the primary antibody (diluted according to the manufacturer's instructions) for 2 h. Horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG was used as the secondary antibody. Immuno-reactive proteins were visualized by chemiluminescence (ECL, Amersham, Arlington Heights, IL, USA).

Immune complex kinase assay. For the immune complex kinase assay, DU-145 cells were lysed after TRAIL treatment in a buffer solution containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EGTA, 10 mM NaF, 1% Triton X-100, 0.5% deoxycholate, 2 mM DTT, 1 mM sodium orthovanadate, 1 mM PMSF and protein inhibitor cocktail solution (Sigma-Aldrich). Cell extracts were clarified by centrifugation, and the supernatants were immunoprecipitated with mouse anti-MEKK1 or anti-MEKK4 antibodies and protein G agarose (Santa Cruz Biotechnology, Inc.). The beads were washed twice with a solution containing 150 mM NaCl, 20 mM Tris-HCl (pH 7.5), 5 mM EGTA, 2 mM DTT, 1 mM sodium orthovanadate, 1 mM PMSF and protein inhibitor cocktail solution, washed once more with the kinase buffer solution, and then were subjected to kinase assays. In order to examine the catalytic activity of MEKK1 or 4, GST-tagged JNK1 and stress-activated protein kinase/extracellular signal-regulated kinase (SEK)1 proteins were used as substrates. For the purification of JNK1 or SEK1, the plasmid containing GST-human JNK1 for bacterial fusion protein was constructed into pGEX-4T-1 by inserting the *HindIII/XbaI* fragment, followed by Klenow treatment with pCDNA3-JNK1. The expression of GST-JNK1 protein was confirmed by Western blotting and purified using glutathione-Sepharose 4B (Amersham Biosciences). GST-SEK1 was purified from ten plates of 293 cells transfected with the pEBG/SEK1 (kindly provided by J.M. Kyriakis, Massachusetts General Hospital, Charlestown, MA), and the purification step was performed as described previously (23). For the measurement of the immune complex activity of MEKK1 or 4, 0.2 μ g of GST-SEK1 were first incubated with the immune complexes for 10 min at 30°C in a final volume of 25 μ l of a solution containing 20 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, and

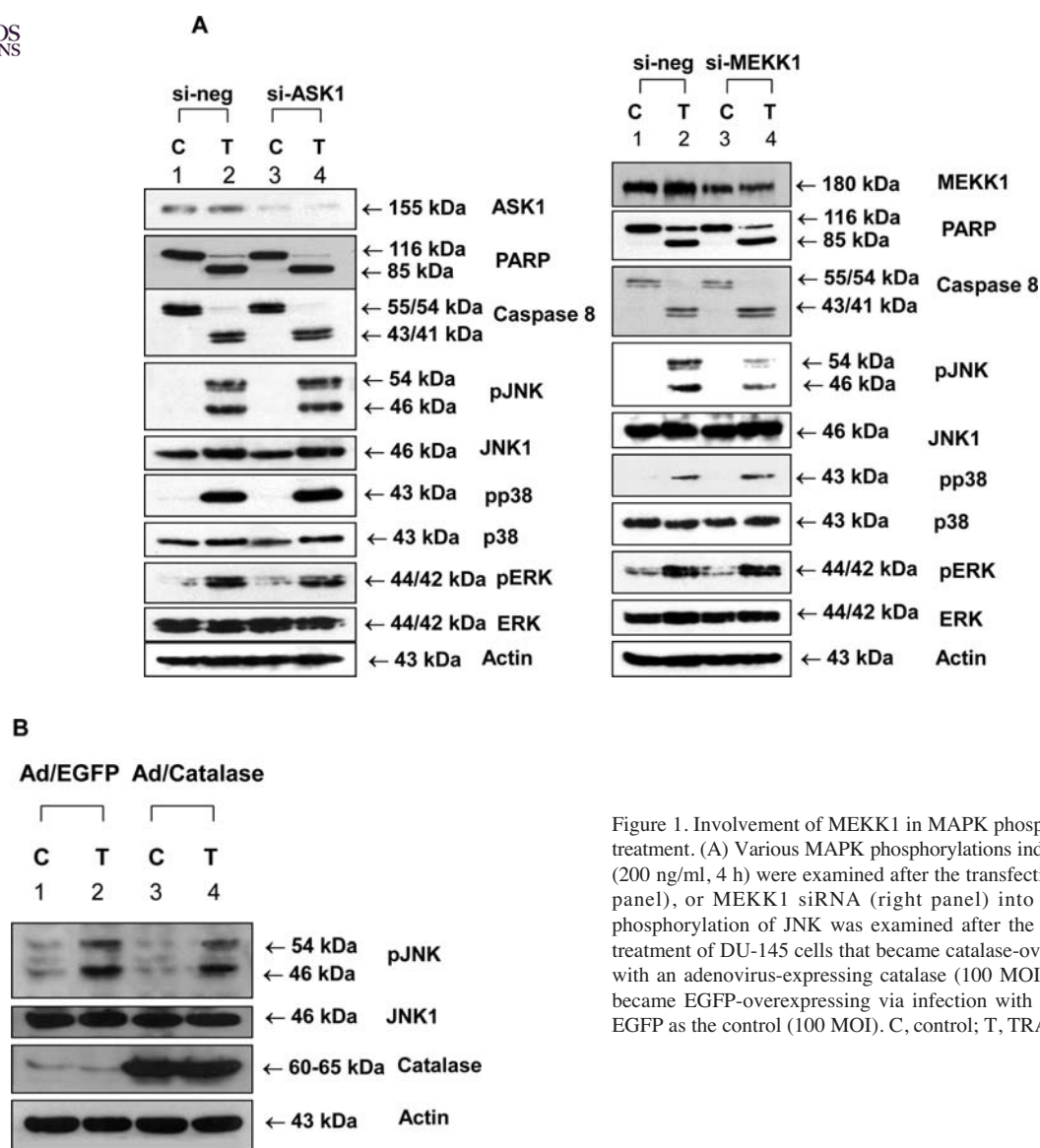


Figure 1. Involvement of MEKK1 in MAPK phosphorylation during TRAIL treatment. (A) Various MAPK phosphorylations induced by TRAIL treatment (200 ng/ml, 4 h) were examined after the transfection of control, ASK1 (left panel), or MEKK1 siRNA (right panel) into DU-145 cells. (B) The phosphorylation of JNK was examined after the TRAIL (200 ng/ml, 4 h) treatment of DU-145 cells that became catalase-overexpressing via infection with an adenovirus-expressing catalase (100 MOI) or of DU-145 cells that became EGFP-overexpressing via infection with an adenovirus-expressing EGFP as the control (100 MOI). C, control; T, TRAIL; si-neg, si-negative.

100 μ M ATP and then with 1 μ g GST-JNK1 for 10 min at 30°C. Thereafter, the activated complex was subjected to SDS-PAGE, and the phosphorylated JNK1 was analyzed using rabbit anti-ACTIVE JNK antibody.

RNA interference by siRNA of SEK1. In order to stably express siRNA for the long-term knockdown, *pSilencer* 2.1-U6 hygro vector (Ambion, Inc., Austin, TX) was used for clonal cell lines. The inserts for cloning hairpin siRNA into *pSilencer* were prepared by annealing two oligonucleotides. For human SEK1 siRNA, the top strand sequence was 5-GATCCACGCAAAGCACTGAAGTTGTTCAAGAGACAACCTTCAGTGCTTTGCGTTTTTTTTGGAAA-3, and the bottom strand sequence was 5-AGCTTTTCCAAAAAACGCAAAGCACTGAAGTTGTCTCTTGAACAACCTTCAGTGCTTTGCTGTG-3. The annealed insert was cloned into *pSilencer* 2.1-U6 hygro digested with *Bam*HI and *Hind*III. The correct structure of *pSilencer* 2.1-U6 hygro-SEK1 was confirmed by nucleotide sequencing. The resulting plasmid, *pSilencer*-SEK1, was transfected into DU-145 cells, and hygromycin B- (250 μ g/ml) resistant cell clones were isolated. The interference of SEK1

protein expression was confirmed by immunoblot analysis using anti-SEK1 (Cell Signaling) antibody. For the down-regulation of ASK1 and MEKK1 or 4, their respective siRNAs (Santa Cruz Biotechnology, Inc.) were used. Cells were transfected with each siRNA and incubated for 36 h. The interference of ASK1 and MEKK1 or 4 protein expression was confirmed by immunoblotting using the anti-ASK1, anti-MEKK1, or anti-MEKK4 antibodies, respectively.

Results

MEKK1 as opposed to ASK1 is the upstream molecule of TRAIL-induced JNK activation. We previously observed that TRAIL-induced MAPK activation is dependent on caspase activation, and that Mst1 mediates caspase activation and MAPK activation (12). However, the upstream molecule of MAPKs by TRAIL has not yet been identified. In this study, we show that TRAIL-induced JNK activation is dependent on MEKK1, as opposed to ASK1, and caspase-8 activations. First of all, we examined which MAPKKK is involved in the MAPK activation during TRAIL treatment. For this purpose,

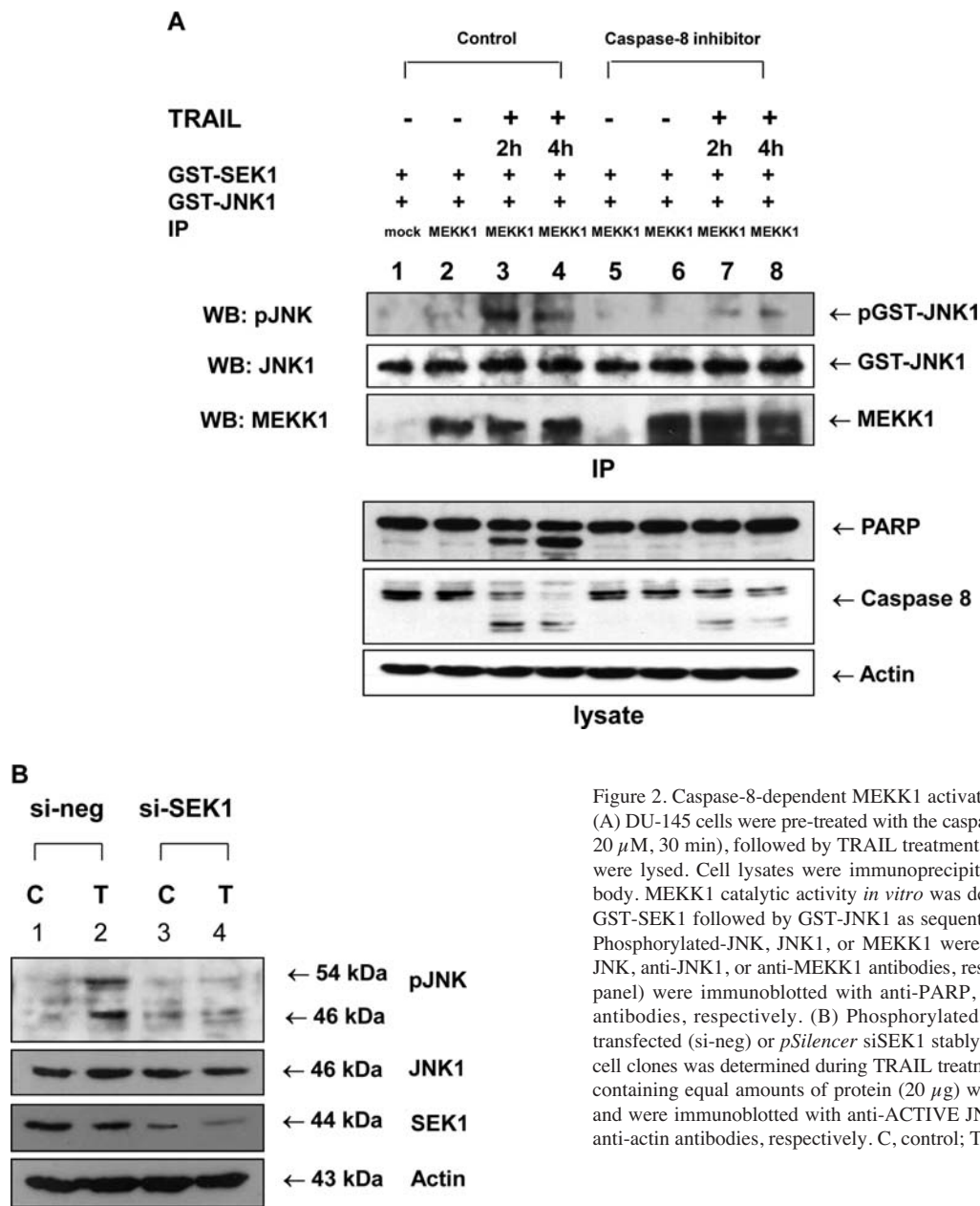


Figure 2. Caspase-8-dependent MEKK1 activation during TRAIL treatment. (A) DU-145 cells were pre-treated with the caspase-8 inhibitor (Z-IETD-FMK 20 μ M, 30 min), followed by TRAIL treatment (200 ng/ml) for 2 or 4 h, and were lysed. Cell lysates were immunoprecipitated with anti-MEKK1 antibody. MEKK1 catalytic activity *in vitro* was determined by incubation with GST-SEK1 followed by GST-JNK1 as sequential substrates (upper panels). Phosphorylated-JNK, JNK1, or MEKK1 were detected with anti-ACTIVE JNK, anti-JNK1, or anti-MEKK1 antibodies, respectively. Cell lysates (lower panel) were immunoblotted with anti-PARP, anti-caspase-8, or anti-actin antibodies, respectively. (B) Phosphorylated JNK in the control vector-transfected (si-neg) or *pSilencer* siSEK1 stably-transfected (si-SEK1) single cell clones was determined during TRAIL treatment of DU-145 cells. Lysates containing equal amounts of protein (20 μ g) were separated by SDS-PAGE and were immunoblotted with anti-ACTIVE JNK, anti-JNK1, anti-SEK1 or anti-actin antibodies, respectively. C, control; T, TRAIL; si-neg, si-negative.

MEKK1 and ASK1 were chosen and were down-regulated using either MEKK1 or ASK1 siRNA. Then, the phosphorylation of each of the MAPKs was examined. As shown in Fig. 1A and B, ASK1 and reactive oxygen species (ROS) production were not involved in the TRAIL-induced MAPK activation, whereas MEKK1 was closely related to the TRAIL-induced JNK activation.

MEKK1 catalytic activation induced by TRAIL is caspase-8-dependent. We then investigated whether the MEKK1 catalytic activation is really increased by TRAIL and whether this activation is related to caspase-8 activation, as suggested by many controversial studies. For the estimation of MEKK1 catalytic activity, its downstream substrates, SEK1 (MKK4) and JNK1, were used for the consecutive substrates, MEKK1 and SEK1, respectively. Then, immunoprecipitation of MEKK1 after TRAIL treatment was performed using the MEKK1 antibody for an *in vitro* immunocomplex kinase

assay. Based upon our previous experience (12), the results were as expected. The increased MEKK1 activity by TRAIL was repressed by caspase-8 activation (Fig. 2A), and SEK1 mediated the MEKK1 signaling cascade through JNK during TRAIL treatment (Fig. 2B).

14-3-3 θ interacts with MEKK1, and its dissociation from MEKK1 by TRAIL treatment is caspase-8-dependent. As reported by Widmann *et al* (24), full-size MEKK1 activated JNK independently of its truncated form (91 kDa) (Fig. 2A and B). MEKK1 (91 kDa) was only observed when TRAIL treatment was used on cells that overexpressed MEKK1 (data not shown). These results do not clearly elucidate the underlying mechanisms of MEKK1 activation. Widmann *et al* (24) suggested that the 14-3-3 proteins, which are a family of serine/threonine-binding proteins that are expressed ubiquitously (25), have anti-apoptotic functions exerted by directly sequestering pro-apoptotic proteins, such as Bad (26,27). We

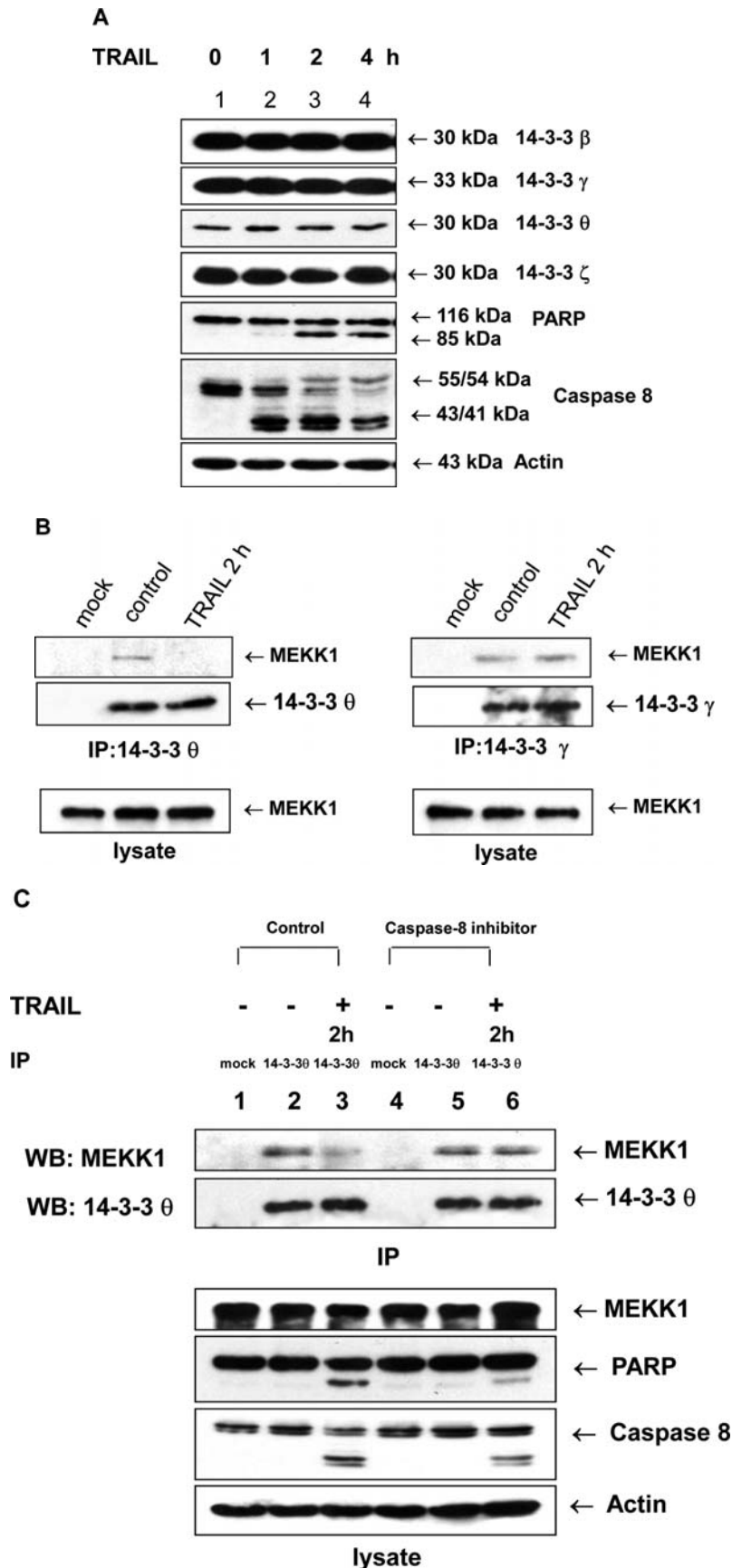


Figure 3. The 14-3-3 isotypes and MEKK1 during TRAIL treatment. (A) Various 14-3-3 isotypes were examined for cleavage by TRAIL (200 ng/ml) after various periods of time. (B) DU-145 cells were treated with TRAIL (200 ng/ml) for 2 h and lysed. Cell lysates were immunoprecipitated with anti-14-3-3 θ (left panel) or 14-3-3 γ (right panel) and immunoblotted with anti-14-3-3 θ/γ or anti-MEKK1 antibodies (upper panel). Cell lysates (lower panel) were immunoblotted with MEKK1 antibody. (C) DU-145 cells were pre-treated with caspase-8 inhibitor (Z-IETD-FMK 20 μM, 30 min), followed by TRAIL treatment (200 ng/ml) for 2 h, and were lysed. Cell lysates were immunoprecipitated with anti-14-3-3 θ antibody and immunoblotted with anti-MEKK1 antibody (upper panel). Cell lysates (lower panel) were immunoblotted with anti-MEKK1, anti-PARP, anti-caspase-8, or anti-actin antibodies, respectively.

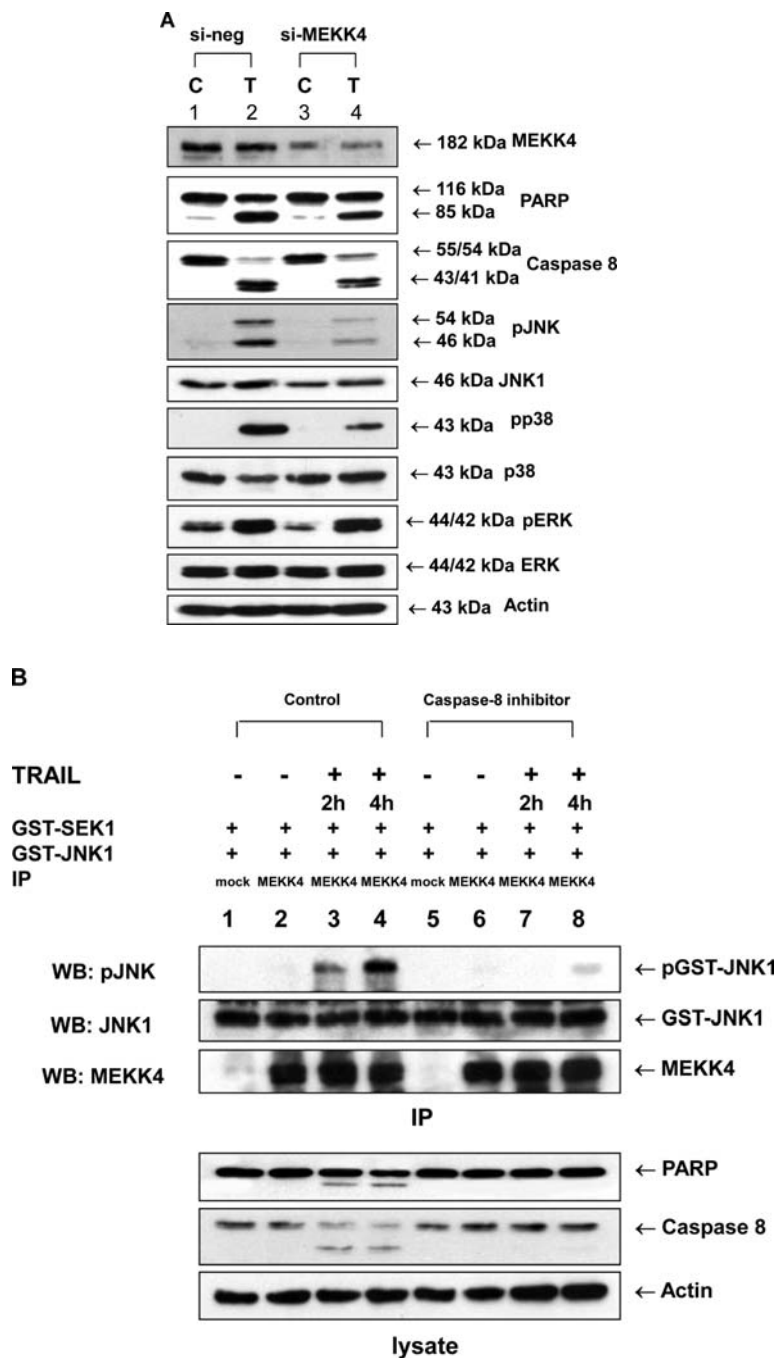


Figure 4. Involvement of MEKK4 in MAPK phosphorylation during TRAIL treatment. (A) MAPK phosphorylation induced by TRAIL treatment (200 ng/ml, 4 h) was examined after the transfection of control or MEKK1 (left) or MEKK4 siRNA (right) into DU-145 cells. (B) DU-145 cells were pre-treated with the caspase-8 inhibitor (Z-IETD-FMK 20 μ M, 30 min), followed by TRAIL treatment (200 ng/ml) for 2 or 4 h, and were lysed. Cell lysates were immuno-precipitated with anti-MEKK4 antibody. MEKK4 catalytic activity *in vitro* was determined by incubation with GST-SEK1 followed by GST-JNK1 as sequential substrates (upper panels). Phosphorylated-JNK1, JNK1, or MEKK4 were detected with anti-ACTIVE JNK1, anti-JNK1, or anti-MEKK4 antibodies, respectively. Cell lysates (lower panel) were immunoblotted with anti-PARP, anti-caspase-8, or anti-actin antibodies, respectively. C, control; T, TRAIL; si-neg, si-negative.

examined the same possibility of the sequestration of MEKK1 activity by 14-3-3 and the release of MEKK1 from 14-3-3. First, we examined whether various 14-3-3 isotypes are cleaved by TRAIL treatment and found that there was no cleavage of 14-3-3 (Fig. 3A). Then, we investigated the associations of MEKK1 and various 14-3-3 isotypes. As shown in Fig. 3B, only 14-3-3 θ showed a meaningful change with MEKK1 during TRAIL treatment. In DU-145 cells, the dissociation of MEKK1 from 14-3-3 was inhibited by the

caspase-8 inhibitor (Fig. 3C), implying that caspase-8 activity is necessary for the 14-3-3 release and subsequent MEKK1 phosphorylation and activation.

MEKK4 is responsible for p38 as well as JNK activation in TRAIL treatment. As a previous study indicated that MEKK4 can stimulate p38 as well as JNK activity (28), the MEKK4 involvement of p38 activation during TRAIL treatment was examined using MEKK4 siRNA. As shown in Fig. 4A, p38



phosphorylation were repressed during TRAIL treatment when MEKK4 expression was down-regulated.

Additionally, MEKK4 catalytic activity was also affected by caspase-8 activation (Fig. 4B).

Discussion

In this study, we found that the MEKK family is responsible for JNK and p38 phosphorylations during TRAIL treatment. In our previous study, we reported that the UV-induced JNK activation adopted the EGFR-Grb2-MEKK1-SEK1-JNK signaling cascade (29). However, we show here that the MEKK1/4-induced SEK1-JNK/p38 activation by TRAIL is caspase-8-dependent, suggesting that Mst1 could also play a role as an upstream molecule of MEKK1 and 4 phosphorylations (12,30). As has been shown in many studies, MEKK1 itself can be activated in various ways depending on the source of stimulation, e.g., UV light induces MEKK1 activation through EGFR-Grb2, whereas TRAIL induces MEKK1 activation through caspase-8-Mst1. In addition, MEKK1 can be also activated by the caspase-8-independent germinal center kinase (GCK)-MEKK1 signaling pathway mediated by TRAF2 (31). However, we do not and cannot know exactly why there are multiple cellular processes that can lead to the activation of MEKK1. It has been suggested that cells adopt specific signaling schemes to appropriately accommodate the stimulations they experience. In the case of the MAPK activation induced by TRAIL, Mst1 has been known to act as a putative MAPK kinase kinase (32), thus functioning in a positive feedback pathway that amplifies the apoptotic response through MAPK activation.

However, the biological significance of the MAPK activation has only been emphasized in regard to its pro-apoptotic effects, despite being known to have anti-apoptotic effects as well. Varfolomeev *et al* (11) suggested that kinase pathway activation by TRAIL was associated with increased production of the chemokines, IL-8 and MCP-1, and with enhanced macrophage migration for the promotion of chemokine-supported phagocytosis of apoptotic cells. It has also been reported that MCP-1 plays a role in prostate cancer invasion and metastasis (33,34), which suggests that the MAPK activation during TRAIL treatment could serve a number of functions in addition to its role in apoptosis. We have previously reported that cells from DU-145 developed acquired resistance to TRAIL-induced apoptosis after TRAIL treatment and that pAkt and Bcl-xL were involved in the process of acquired resistance (8). Many studies have shown that the ability to resist apoptosis can enhance metastasis (35,36). Therefore, in the future, we aim to investigate whether and/or to what extent the MAPK activation induced by TRAIL contributes to acquired TRAIL resistance, which allows for the progression to invasion or metastasis, by measuring MMP activity and MCP-1 and IL-6 levels *in vitro* and/or the incidence and intensity of metastasis *in vivo*.

Acknowledgements

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and

Technology (grant nos. 2009-0071809 and 2010-0007689, to J.J.S.), the Yonsei University Research Fund (grant no. 2009-0014, to J.J.S.) and by faculty research grants from Yonsei University College of Medicine (nos. 2009-0113 and 2010-0081, to J.J.S.). S.O. and S.Y.K. were supported by the Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea. This study was also supported by a NCI grant fund (no. CA140554, to Y.J.L.).

References

1. Wiley SR, Schooley K, Smolak PJ, *et al*: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3: 673-682, 1995.
2. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A and Ashkenazi A: Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem* 271: 12687-12690, 1996.
3. Peter ME, Krammer PH: The CD95(APO-1/Fas) DISC and beyond. *Cell Death Differ* 10: 26-35, 2003.
4. Kelley SK and Ashkenazi A: Targeting death receptors in cancer with Apo2L/TRAIL. *Curr Opin Pharmacol* 4: 333-339, 2004.
5. Falschlehner C, Emmerich CH, Gerlach B and Walczak H: TRAIL signalling: Decisions between life and death. *Int J Biochem Cell Biol* 39: 1462-1475, 2007.
6. Walczak H, Miller RE, Ariail K, *et al*: Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. *Nat Med* 5: 157-163, 1999.
7. Zhu H, Zhang L, Huang X, Davis JJ, Jacob DA, Teraishi F, Chiao P and Fang B: Overcoming acquired resistance to TRAIL by chemotherapeutic agents and calpain inhibitor I through distinct mechanisms. *Mol Ther* 9: 666-673, 2004.
8. Song JJ, An JY, Kwon YT and Lee YJ: Evidence for two modes of development of acquired TRAIL resistance: Involvement of Bcl-xL. *J Biol Chem* 282: 319-328, 2007.
9. Song JJ, Kim JH, Sun BK, Alcalá Jr MA, Bartlett DL and Lee YJ: c-Cbl acts as a mediator of Src-induced activation of the PI3K-Akt signal transduction pathway during TRAIL treatment. *Cell Signal* 22: 377-385, 2010.
10. MacFarlane M, Cohen GM and Dickens M: JNK (c-Jun N-terminal kinase) and p38 activation in receptor-mediated and chemically-induced apoptosis of T-cells: differential requirements for caspase activation. *Biochem J* 15: 93-101, 2000.
11. Varfolomeev E, Maecker H, Sharp D, Lawrence D, Renz M, Vucic D and Ashkenazi A: Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J Biol Chem* 280: 40599-40608, 2005.
12. Song JJ and Lee YJ: Differential cleavage of Mst1 by caspase-7/-3 is responsible for TRAIL-induced activation of the MAPK superfamily. *Cell Signal* 20: 892-906, 2008.
13. Liu ZG, Hsu H, Goeddel DV and Karin M: Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell* 87: 565-576, 1996.
14. Wada T and Penninger JM: Mitogen-activated protein kinases in apoptosis regulation. *Oncogene* 23: 2838-2849, 2004.
15. Jurewicz A, Matysiak M, Andrzejak S and Selmaj K: TRAIL-induced death of human adult oligodendrocytes is mediated by JNK pathway. *Glia* 53: 158-166, 2006.
16. Corazza N, Jakob S, Schaer C, Frese S, Keogh A, Stroka D, Kassahn D, Torgler R, Mueller C, Schneider P and Brunner T: TRAIL receptor-mediated JNK activation and Bim phosphorylation critically regulate Fas-mediated liver damage and lethality. *J Clin Invest* 116: 2493-2499, 2006.
17. Mühlenbeck F, Haas E, Schwenzler R, Schubert G, Grell M, Smith C, Scheurich P and Wajant H: TRAIL/Apo2L activates c-Jun NH2-terminal kinase (JNK) via caspase-dependent and caspase-independent pathways. *J Biol Chem* 273: 33091-33098, 1998.
18. Jin CY, Park C, Kim GY, Lee SJ, Kim WJ and Choi YH: Genistein enhances TRAIL-induced apoptosis through inhibition of p38 MAPK signaling in human hepatocellular carcinoma Hep3B cells. *Chem Biol Interact* 180: 143-150, 2009.

19. Zhang L, Zhu H, Davis JJ, Jacob D, Wu S, Teraishi F, Gutierrez A, Wang Y and Fang B: Lack of p38 MAP kinase activation in TRAIL-resistant cells is not related to the resistance to TRAIL-mediated cell death. *Cancer Biol Ther* 3: 296-301, 2004.
20. Lee MW, Park SC, Yang YG, Yim SO, Chae HS, Bach JH, Lee HJ, Kim KY, Lee WB and Kim SS: The involvement of reactive oxygen species (ROS) and p38 mitogen-activated protein (MAP) kinase in TRAIL/Apo2L-induced apoptosis. *FEBS Lett* 512: 313-318, 2002.
21. Enomoto A, Kido N, Ito M, Morita A, Matsumoto Y, Takamatsu N, Hosoi Y and Miyagawa K: Negative regulation of MEKK1/2 signaling by serine-threonine kinase 38 (STK38). *Oncogene* 27: 1930-1938, 2008.
22. Davis RJ: Signal transduction by the JNK group of MAP kinases. *Cell* 103: 239-252, 2000.
23. Yuasa T, Ohno S, Kehrl JH and Kyriakis JM: Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38. Germinal center kinase couples TRAF2 to mitogen-activated protein kinase/ERK kinase 1 and SAPK while receptor interacting protein associates with a mitogen-activated protein kinase kinase upstream of MKK6 and p38. *J Biol Chem* 273: 22681-22692, 1998.
24. Widmann C, Gerwins P, Johnson NL, Jarpe MB and Johnson GL: MEK kinase 1, a substrate for DEVD-directed caspases, is involved in genotoxin-induced apoptosis. *Mol Cell Biol* 18: 2416-2429, 1998.
25. Aitken A, Collinge DB, van Heusden BP, Isobe T, Roseboom PH, Rosenfeld G and Soll J: 14-3-3 proteins: a highly conserved, widespread family of eukaryotic proteins. *Trends Biochem Sci* 17: 498-501, 1992.
26. Masters SC, Subramanian RR, Truong A, Yang H, Fujii K, Zhang H and Fu H: Survival-promoting functions of 14-3-3 proteins. *Biochem Soc Trans* 30: 360-365, 2002.
27. Porter GW, Khuri FR and Fu H: Dynamic 14-3-3/client protein interactions integrate survival and apoptotic pathways. *Semin Cancer Biol* 16: 193-202, 2006.
28. Abell AN, Granger DA and Johnson GL: MEKK4 stimulation of p38 and JNK activity is negatively regulated by GSK3beta. *J Biol Chem* 282: 30476-30484, 2007.
29. Song JJ and Lee YJ: Differential activation of the JNK signal pathway by UV irradiation and glucose deprivation. *Cell Signal* 19: 563-572, 2007.
30. Graves JD, Draves KE, Gotoh Y, Krebs EG and Clark EA: Both phosphorylation and caspase-mediated cleavage contribute to regulation of the Ste20-like protein kinase Mst1 during CD95/Fas-induced apoptosis. *J Biol Chem* 276: 14909-14915, 2001.
31. Chadee DN, Yuasa T and Kyriakis JM: Direct activation of mitogen-activated protein kinase kinase kinase MEKK1 by the Ste20p homologue GCK and the adapter protein TRAF2. *Mol Cell Biol* 22: 737-749, 2002.
32. Souza PM and Lindsay MA: Mammalian sterile20-like kinase 1 and the regulation of apoptosis. *Biochem Soc Trans* 32: 485-488, 2004.
33. Lu Y, Cai Z, Galson DL, Xiao G, Liu Y, George DE, Melhem MF, Yao Z and Zhang J: Monocyte chemotactic protein-1 (MCP-1) acts as a paracrine and autocrine factor for prostate cancer growth and invasion. *Prostate* 66: 1311-1318, 2006.
34. Lu Y, Cai Z, Xiao G, Keller ET, Mizokami A, Yao Z, Roodman GD and Zhang J: Monocyte chemotactic protein-1 mediates prostate cancer-induced bone resorption. *Cancer Res* 67: 3646-3653, 2007.
35. Shtievelman E: A link between metastasis and resistance to apoptosis of variant small cell lung carcinoma. *Oncogene* 14: 2167-2173, 1997.
36. Takaoka A, Adachi M, Okuda H, Sato S, Yawata A, Hinoda Y, Takayama S, Reed JC and Imai K: Anti-cell death activity promotes pulmonary metastasis of melanoma cells. *Oncogene* 14: 2971-2977, 1997.