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Physiological Roles of SAPK/JNK Signaling Pathway

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Stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK) is activated by many types of cellular stresses and extracellular signals. Recent studies, including the analysis with knockout mice, have led to progress towards understanding the physiological roles of SAPK/JNK activation in embryonic development in addition to immune responses. SAPK/JNK activation plays essential roles in organogenesis during mouse development by regulating cell survival, apoptosis, and proliferation. Two SAPK/JNK activators, SEK1 and MKK7, are required for fetal liver formation and full activation of SAPK/JNK, which responds to various stimuli in an all-or-none manner. This article focuses on physiological roles of SAPK/JNK activation in fetal liver formation and in apoptosis regulation.

Key words: apoptosis, liver formation, MAP kinase, SAPK/JNK, stress.

Developmental programs and environmental agents trigger distinct and evolutionarily conserved kinases that relay signals mediating survival, death, proliferation, and cell cycle arrest. Mitogen-activated protein kinases (MAPKs) are evolutionarily conserved serine/threonine kinases involved in regulation of many cellular events. Several MAPK groups have been identified in mammalian cells, including extracellular signal-regulated kinase (ERK), p38, and SAPK/JNK. These MAPKs are activated by their specific MAPK kinases (MAPKKs): ERK by MEK1 and MEK2, p38 by MKK3 and MKK6, and SAPK/JNK by SEK1 (also known as MKK4) and MKK7 (SEK2). These MAPKKs are also activated by various MAPKK kinases (MAPKKKs) such as Raf, MLK, MEKK1, TAK1, and ASK1.

SAPK/JNK is ubiquitously expressed and is activated by many types of stress, including UV and γ-irradiation, protein synthesis inhibitors (anisomycin), hyperosmolarity, toxins, ischemia/reperfusion injury in heart attacks, heat shock, anticancer drugs (cisplatinum, adriamycin, or etoposide), ceramide, T-cell receptor stimulation, peroxide, and inflammatory cytokines such as TNFα. Recently, several *in vitro* and *in vivo* experiments have shown that SAPK/JNK is activated synergistically by SEK1 and MKK7. The SAPK/JNK stress pathway participitates in many different intracellular signaling pathways that control a spectrum of cellular processes, including cell proliferation, differentiation, transformation, apoptosis, migration, and cytoskeletal integrity. SAPK/JNK has been reported to phosphorylate transcription factors in addition to c-Jun, such as ATF-2, Elk-1, p53, and c-Myc, as well as nontranscription factors such as Bcl-2, Bcl-xL, paxillin, and MAP2 (*1*–*6*). This review summarizes recent progress in the SAPK/JNK signaling pathway in mouse development and the molecular mechanism of SAPK/JNK activation.

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Role of SAPK/JNK in mouse development

All three *Jnk* (*Jnk1*, *2*, and *3*), and *sek1* and *mkk7* loci have been knocked out. JNK1 and JNK2 are widely expressed in many tissues, but JNK3 is expressed predominantly in nervous system. Mice deficient in the single gene of *Jnk1*, *Jnk2*, or *Jnk3*, and *Jnk1*/*Jnk3*– or *Jnk2*/*Jnk3*–double mutant mice all survived normally. Mice lacking both JNK1 and JNK2 die around embryonic day 11 (E11) with severe dysregulation of apoptosis in the brain (*7*, *8*). Specifically, there was a reduction of cell death in the lateral edges of the hindbrain prior to neural tube closure. In contrast, increased apoptosis and caspase activation were found in the mutant forebrain. These results assign both pro-and anti-apoptotic functions to JNK1 and JNK2 in the development of the fetal brain.

Sek1$^{-/-}$ embryos die between E10.5 and E12.5 with impaired liver formation and massive apoptosis (*9*–*12*). We have recently shown that SEK1-mediated SAPK/JNK pathway downstream TNF-α receptor 1 (TNFR1) participates in embryonic hepatoblast proliferation and survival via a pathway different from NF-κB–induced anti-apoptosis (*13*). Furthermore, *mkk7*$^{-/-}$ embryos die between E11.5 and E12.5 with similar defects in liver formation (*14*). These results indicate that SEK1 and MKK7 cannot substitute for one another *in vivo* and that both are important for hepatoblast proliferation and survival during mouse embryogenesis (Fig. 1).

Role of SAPK/JNK in apoptosis regulation

It has been proposed that SAPK/JNK activation triggers the mitochondria-dependent apoptosis in response to many types of stress, including UV-irradiation. Both *Jnk1*$^{-/-}$ *Jnk2*$^{-/-}$ and *sek1*$^{-/-}$ *mkk7*$^{-/-}$ mouse embryonic fibroblasts (MEFs) exhibited profound defects in stress-induced apoptosis (*15*, *16*). These results strongly suggest that the SAPK/JNK activation directly regulates mitochondria-dependent apoptosis in the pro-apoptotic direction. In contrast, *sek1*$^{-/-}$ *mkk7*$^{-/-}$ ES cells show normal apoptotic responses, including DNA fragmentation and

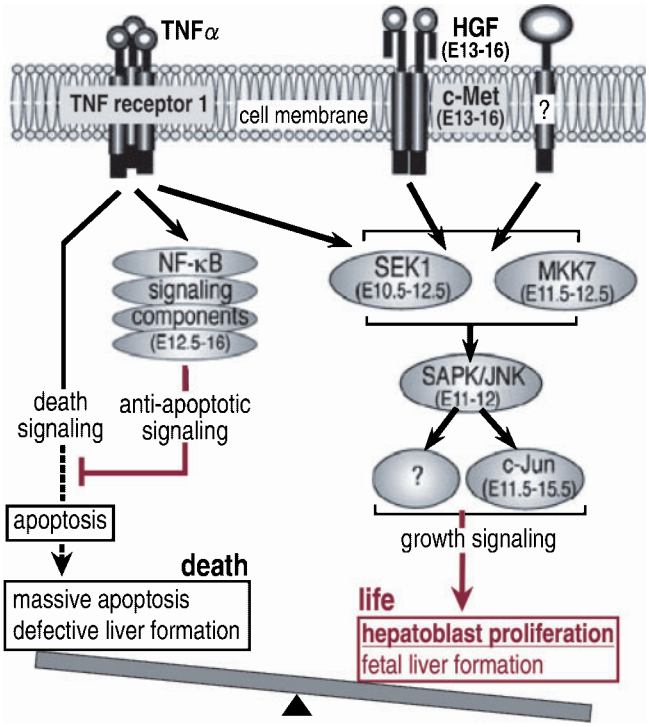

Fig. 1. **A proposed model for SAPK/JNK signaling pathway in hepatoblasts.** The numbers in parentheses are dates of embryonic lethality reported in previous papers. TNFα elicits a wide range of biological responses, such as inflammation, tumor necrosis, differentiation, cell proliferation, and apoptosis, through the stimulation of its receptor, TNFR1. The induction of apoptosis, NF-κB activation, and SAPK/JNK activation are simultaneously mediated through TNFR1. SAPK/JNK activation is involved in cell proliferation, while activation of NF-κB protects against the apoptosis in hepatoblasts (*13*).

caspase 3 activation, even though *apaf1*$^{-/-}$ ES cells exhibit profound defects in the mitochondria-dependent apoptosis (*17*). In those *sek1*$^{-/-}$ *mkk7*$^{-/-}$ ES cells, the SAPK/JNK activation by various stresses was completely abolished. Normal apoptotic responses without SAPK/JNK activation were also observed in fibroblasts derived from *sek1*$^{-/-}$ *mkk7*$^{-/-}$ ES cells. These results raised the question of whether SAPK/JNK activation is indeed required for the induction of cell death in response to apoptosis inducers. Thus, the physiological role of SAPK/JNK activation in cell survival and apoptosis is controversial, being suggested to have a pro-apoptotic, an anti-apoptotic, or no function (*18*).

From our recent results, it appears that the various roles of SAPK/JNK activation in apoptosis depend on the cell types and conditions observed. While late passage *mkk7*$^{-/-}$ MEFs are resistant to cell death in the same manner as *Jnk1*$^{-/-}$ *Jnk2*$^{-/-}$ and *sek1*$^{-/-}$ *mkk7*$^{-/-}$ MEFs (*15*, *16*), *mkk7*$^{-/-}$ MEFs at earlier cell passages (passages 1–4) undergo apoptosis in response to UV exposure with the same kinetics and to the same extent as wild-type MEFs (*14*). These results support the notion that SAPK/JNK activation is not always involved in apoptosis, but this activation rather regulates apoptosis in a signal-specific (and perhaps cell type-dependent) manner. Our results in MEFs indicate that the 'history' and passage number of

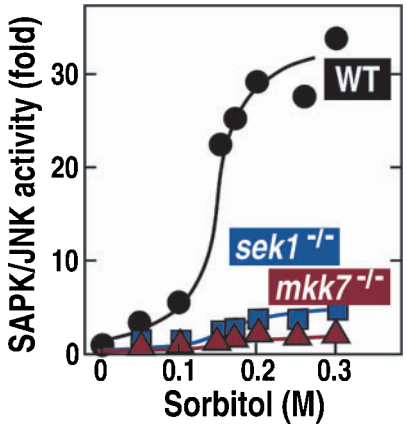

Fig. 2. **SAPK/JNK activation in response to hyper-osmolar stress (sorbitol) requires both SEK1 and MKK7 in ES cells.** Wild-type, *sek1*$^{-/-}$, and *mkk7*$^{-/-}$ ES cells were stimulated with the indicated concentrations of sorbitol for 30 min.

cells is a critical determinant of cell death susceptibility in the absence of MKK7 expression.

SAPK/JNK activation as a molecular switch in an all-or-none manner

Recently, Ferrell *et al.* have proposed the interesting concept that SAPK/JNK-signaling cascade could, in principle, function as a sensitivity amplifier, which converts graded inputs into more switch-like outputs, allowing the cascade to filter out noise and yet still respond decisively to supra-threshold stimuli (*19*, *20*). They have shown in *Xenopus* oocytes, HeLa cells, HEK293 cells, and Jurkat T cells that SAPK/JNK responds to physiological and pathological stimuli, such as progesterone and sorbitol, in an all-or-none manner. The activation of SAPK/JNK by the stimuli was graded at the level of a population of oocytes; however, at the level of an individual oocyte, the stimulatory response appeared to be switch-like. Indeed, we have also observed a very steep concentration-dependent response in the activation of SAPK/JNK by hyper-osmolar stress, sorbitol, in wild-type murine embryonic stem (ES) cells but not in *sek1*$^{-/-}$ and *mkk7*$^{-/-}$ cells (Fig. 2) (*21*). This suggests that the all-or-none type MAPK activation also occurs in mammalian cells at an individual cell level only when the two MAPKKs are simultaneously activated. Therefore, this MAPK signaling should strictly proceed without errors basically through two separate signals, one that activates SEK1 and one that activates MKK7. The full activation of SAPK/JNK by SEK1 and MKK7 may be required for hepatoblast proliferation (Fig. 1).

Molecular mechanism of SAPK/JNK activation in living cells

Activation of SAPK/JNK requires the dual phosphorylation of Tyr and Thr residues located in a Thr-Pro-Tyr motif in the activation loop between VII and VIII of the kinase domain. The phosphorylation is catalyzed by the dual specificity kinases, SEK1 and MKK7, which are capable of catalyzing the phosphorylation of both Thr and Tyr residues. Recent studies have shown that SEK1 preferentially phosphorylates the Tyr residue, and MKK7 the

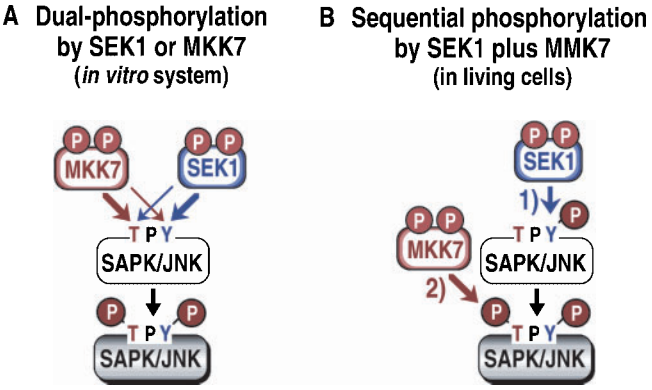

Fig. 3. **Schematic description of SAPK/JNK phosphorylation by SEK1 and MKK7 *in vitro* and *in vivo*.** A: Synergistic activation of SAPK/JNK by the dual-specificity kinase, SEK1 or MKK7, which has been reported in *in vitro* conditions (*22–24*). B: Synergistic activation of SAPK/JNK through sequential phosphorylation by SEK1 and MKK7 in murine living cells (*21*, *25*). TPY, Thr-Pro-Tyr motif.

Thr residue of SAPK/JNK *in vitro* (Fig. 3A) (*22–24*). Strong support for this activation mechanism has been obtained from studies of SEK1- and MKK7-gene disruption in ES cells (*21*, *25*). The severe impairment of SAPK/JNK activation observed in *mkk7*$^{-/-}$ ES cells was accompanied by a loss of the Thr-phosphorylation of SAPK/JNK, without marked reduction in its Tyr-phosphorylation level. On the other hand, Thr-phosphorylation of SAPK/JNK in *sek1*$^{-/-}$ ES cells was also attenuated, in addition to a decreased level of its Tyr-phosphorylation. These results indicate that the Tyr and Thr residues of SAPK/JNK are sequentially phosphorylated by SEK1 and MKK7, respectively, in stress-stimulated living cells (Fig. 3B).

Conclusion

SAPK/JNK activation by SEK1 and MKK7 is required for embryonic hepatoblast proliferation. The full activation of SAPK/JNK occurs only when the two MAPKKs are simultaneously activated. The Tyr and Thr residues of SAPK/JNK are sequentially phosphorylated by SEK1 and MKK7, respectively, in living cells.

SAPK/JNK may either protect or enhance sensitivity to apoptosis depending on the cell type, stimuli, and the latency of the activation of the MAPK. Our recent results in MEFs indicate that the "history" and passage number of cells are a critical determinant of cell death susceptibility in the absence of MKK7 expression. In this apoptotic pathway, SAPK/JNK seems to function through its effects on gene expression but not a direct effect on the effectors of apoptosis. These new findings could also solve the controversial data that have been obtained in different cell types and in different laboratories.

REFERENCES

1. Davis, R.J. (2000) Signal transduction by the JNK group of MAP kinases. *Cell* **103**, 239–252
2. Chang, L. and Karin, M. (2001) Mammalian MAP kinase signalling cascades. *Nature* **410**, 37–40
3. Weston, C.R. and Davis, R.J. (2002) The JNK signal transduction pathway. *Curr. Opini. Genes Dev.* **12**, 14–21
4. Manning, A.M. and Davis, R.J. (2003) Targeting JNK for therapeutic benefit: from junk to gold? *Nat. Rev. Mol. Drug Disc.* **2**, 554–565
5. Nishina, H., Nakagawa, K., Azuma, N., and Katada, T. (2003) Activation mechanism and physiological roles of stress-activated protein kinase/c-Jun NH_2-terminal kinase in mammalian cells. *J. Biol. Regul. Homeost. Agents*. **17**, 295–302
6. Wada, T. and Penninger, J.M. (2004) Mitogen-activated protein kinases in apoptosis regulation. *Oncogene* **23**, 2838–2849
7. Kuan, C.Y., Yang, D.D., Roy, D.R., Davis, R.J., Rakic, P., and Flavell, R.A. (1999) The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* **22**, 667–676
8. Sabapathy, K., Jochum, W., Hochedlinger, K., Chang, L., Karin, M., and Wagner, E.F. (1999) Defective neural tube morphogenesis and altered apoptosis in the absence of both JNK1 and JNK2. *Mech. Dev.* **89**, 115–124
9. Nishina, H., Fischer, K.D., Radvanyi, L., Shahinian, A., Hakem, R., Rubie, E.A., Bernstein, A., Mak, T.W., Woodgett, J.R., and Penninger, J.M. (1997) Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. *Nature* **385**, 350–353
10. Yang, D., Tournier, C., Wysk, M., Lu, H.T., Xu, J., Davis, R.J., and Flavell, R.A. (1997) Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH2-terminal kinase activation, and defects in AP-1 transcriptional activity. *Proc. Natl Acad. Sci. USA* **94**, 3004–3009
11. Ganiatsas, S., Kwee, L., Fujiwara, Y., Perkins, A., Ikeda, T., Labow, M.A., and Zon, L.I. (1998) SEK1 deficiency reveals mitogen-activated protein kinase cascade crossregulation and leads to abnormal hepatogenesis. *Proc. Natl Acad. Sci. USA* **95**, 6881–6886.
12. Nishina, H., Vaz, C., Billia, P., Nghiem, M., Sasaki, T., Pompa, J.L., Furlonger, K., Paige, C., Hui, C.-C., Fischer, K.D., Kishimoto, H., Iwatsubo, T., Katada, T., Woodgett, J.R., and Penninger, J.M. (1999) Defective liver formation and liver cell apoptosis in mice lacking the stress signaling kinase SEK1/MKK4. *Development* **126**, 505–516
13. Watanabe, T. Nakagawa, K, Ohata, S, Kitagawa, D., Nishitai, G., Seo, J., Tanemura, S., Shimizu, N., Kishimoto, H., Wada, T., Aoki, J., Arai, H., Iwatsubo, T., Mochita, M., Watanabe, T., Satake, M., Ito, Y., Matsuyama, T., Mak, T.W., Penninger, J.M., Nishina, H., and Katada, T. (2002) SEK1/MKK4-mediated SAPK/JNK signaling participates in embryonic hepatoblast proliferation via a pathway different from NF-κB-induced antiapoptosis. *Dev. Biol.* **250**, 332–347
14. Wada, T., Joza, N., Cheng, H.-Y.M., Sasaki, T., Kozieradzki, I., Bachmaier, K., Katada, T., Schreiber, M., Wagner, E.F., Nishina, H., and Penninger, J.M. (2004) MKK7 couples stress signaling to G2/M cell cycle progression and cellular senescence. *Nat. Cell Biol.* **6**, 215–226
15. Tournier, C., Hess, P., Yang, D.D., Xu, J., Turner, T.K., Nimnual, A., Bar-Sagi, D., Jones, S.N., Flavell, R.A., and Davis, R.J. (2000) Requirement of JNK for stress-induced activation of the cytochrome c–mediated death pathway. *Science* **288**, 870–874
16. Tournier, C., Dong, C., Turner, T.K., Jones, S.N., Flavell, R.A., and Davis, R.J. (2001) MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. *Genes Dev.* **15**, 1419–1426
17. Nishitai, G., Shimizu, N., Negishi, T., Kishimoto, H., Nakagawa, K., Kitagawa, D., Watanabe, T., Momose, H., Ohata, S., Tanemura, S., Asaka, S., Kubota, J., Saito, R., Yoshida, H., Mak, T.W., Wada, T., Penninger, J.M., Azuma, N., Nishina, H., and Katada, T. (2004) Stress induces mitochondria-mediated apoptosis independent of SAPK/JNK activation in ES cells. *J. Biol. Chem.* **279**, 1621–1626
18. Lin, A. (2002) Activation of the JNK signaling pathway: breaking the brake on apoptosis. *Bioessays* **25**, 17–24

19. Bagowski, C.P. and Ferrell, J.E. (2001) Bistability in the JNK cascade. *Curr. Biol.* **11**, 1176–1182
20. Bagowski, C.P., Besser, J., Frey, C.R., Ferrell, J.E. (2003) The JNK cascade as a biochemical switch in mammalian cells: Ultrasensitive and all-or-none responses. *Curr. Biol.* **13**, 315–320
21. Kishimoto, H., Nakagawa, K., Watanabe, T., Kitagawa, D., Momose, H., Seo, J., Nishitai, G., Shimizu, N., Ohata, S., Tanemura, S., Asaka, S., Goto, T., Fukushi, H., Yoshida, H., Suzuki, A., Sasaki, T., Wada, T., Penninger, J.M., Nishina, H., and Katada, T. (2003) Different Properties of SEK1 and MKK7 in Dual Phosphorylation of Stress-induced Activated Protein Kinase SAPK/JNK in Embryonic *Stem Cells*. *J. Biol. Chem.* **278**, 16595–16601
22. Lawler, S., Fleming, Y., Goedert, M., and Cohen, P. (1998) Synergistic activation of SAPK1/JNK1 by two MAP kinase kinases *in vitro*. *Curr. Biol.* **8**, 1387–1390
23. Lisnock, J., Griffin, P., Calaycay, J., Franz, B., Parsons, J., O'Keefe, S.J., and LoGrasso, P. (2000) Activation of JNK3α1 requires both MKK4 and MKK7: Kinetic characterization of *in vitro* phosphorylated JNK3α1. *Biochemistry* **39**, 3141–3148
24. Fleming, Y., Armstrong, C.G., Morrice, N., Paterson, A., Goedert, M., and Cohen, P. (2000) Synergistic activation of stress-activated protein kinase 1/c-Jun N-terminal kinase (SAPK1/JNK) isoforms by mitogen-activated protein kinase 4 (MKK4) and MKK7. *Biochem. J.* **352**, 145–154
25. Wada, T., Nakagawa, K., Watanabe, T., Nishitai, G., Seo, J., Kishimoto, H., Kitagawa, D., Sasaki, T., Penninger, J.M., Nishina, H., and Katada, T. (2001). Impaired synergistic activation of stress activated protein kinase SAPK/JNK in mouse embryonic stem cells lacking SEK1/MKK4. *J. Biol. Chem.* **276**, 30892–30897