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Nitric oxide inhibits an interaction between JNK1 and c-Jun through nitrosylation

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Abstract

Nitric oxide (NO) has been shown to negatively regulate c-Jun N-terminal kinase (JNK) through *S*-nitrosylation. Here, we show that disruption of an interaction between JNK and its substrate c-Jun is an important mechanism underlying the NO-mediated inhibition of JNK signaling. Endogenous NO, which was generated by interferon-γ treatment, suppressed anisomycin-stimulated JNK activity in microglial BV-2 cells. The interferon-γ-induced suppression of JNK1 activation in BV-2 cells was prevented completely by treatment with N^G-nitro-L-arginine, an inhibitor of NO synthase. A NO donor *S*-nitro-*N*-acetyl-DL-penicillamine (SNAP) inhibited JNK activity in vitro, and this inhibition was reversed by a thiol-reducing agent, dithiothreitol. Nitric oxide disrupts a physical interaction between JNK and its substrate c-Jun both in vitro and in intact cells without affecting an interaction between SEK1 and JNK. Collectively, our results suggest that the inhibition of the interaction between JNK and c-Jun may be an integral part of the mechanism underlying the negative regulation of the JNK signaling pathway by NO.

Keywords: JNK; NO; Nitrosylation; c-Jun

Nitric oxide (NO) is a highly diffusible intercellular signaling molecule, which has been implicated in a wide range of biological effects [1–4]. It is generated by the enzyme nitric oxide synthase (NOS), which catalyzes the oxidation of L-arginine into NO and citruline [4]. Three distinct NOS isoforms have been identified; in neurons, endothelial cells, and macrophages. Endothelial and neuronal NOS (eNOS and nNOS) are constitutively expressed enzymes, whereas macrophage NOS is in an inducible form (iNOS) [5–8]. Each NOS monomer has the N-terminal oxygenase domain with the binding sites for a P450-like cysteine thiolate-ligated heme, tetrahydrobiopterin cofactor (H4B), and L-arginine. The C-terminal reductase domain harbors the binding sites for FAD, FMN, and the NADPH cofactor. These two domains are linked by a functional peptide consisting of 20–25 amino acids, which binds to calmodulin (CaM) and performs a key function of the enzyme [5–8]. The constitutive isoforms (eNOS and nNOS) generate low amounts of NO. Their enzymatic activities are regulated by intracellular calcium flux, or by calmodulin. The expression and function of iNOS can be induced by a variety of cytokines, growth factors, and inflammatory stimuli on target cells. This induction of the enzyme results in the release of high amounts of NO and is involved in the immune response of the host [9–12].

The mitogen-activated protein (MAP) kinase-signaling pathways are primary mediators of extracellular signals. MAP kinases include the extracellular signal-regulated kinases (ERKs), p38 kinases, and c-Jun N-terminal kinases (JNKs) [13–16]. The MAP kinases are involved in a host of cellular functions, including proliferation, differentiation, migration, cell death, and inflammatory responses

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[17–27]. The activation of the MAP kinase pathway often occurs in response to the growth factor stimulation of receptor tyrosine kinases, which are coupled to the activation of Ras G-proteins via Src homology 2 domain-containing proteins, including Shc and Grb2, as well as guanine nucleotide exchange factors, including SOS [28]. The active GTP-bound form of Ras can activate protein kinases, such as Raf-1 and MEK kinase-1 (MEKK1), which phosphorylate and activate the MAP or ERK kinases (MEKs) that in turn phosphorylate and activate MAP kinase [29–34]. The MAP kinases regulate the diverse range of biological functions via the phosphorylation of transcription factors, various protein kinases, structural proteins, and other signaling proteins [13–16]. JNKs/SAPKs are activated in cellular response to a variety of environmental stresses, including UV irradiation, osmotic stress, heat shock, and metabolic inhibitors [35–40]. The JNK/SAPK signaling pathway also includes two MAP2Ks, SEK1/MKK4 and MKK7, and several MAP3Ks, including MEKK1 and ASK1 [41–48]. The JNK/SAPK signaling cascade appears to play a role in a variety of cellular processes, including gene expression, cell proliferation, cell survival, and apoptosis [49–57].

We previously showed that interferon-γ induces the NO-mediated suppression of JNK via *S*-nitrosylation in macrophages and microglial cells [58]. In this study, we have demonstrated that interferon-γ-induced endogenous NO inhibits anisomycin-stimulated JNK activity. IFN-γ suppresses the phosphorylation of c-Jun, but not that of JNK. Furthermore, NO disrupts a physical interaction between JNK and its substrate c-Jun without affecting an interaction between SEK1 and JNK. The inhibition of the binding between JNK and c-Jun may be important in the mechanism underlying the negative regulation of the JNK signaling pathway by NO.

Materials and methods

Cell culture and transfection. HEK293 and BV2 murine microglial cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in 5% CO_2 at 37 °C. For DNA transfections, the cells were plated on 100-mm dishes at 1×10^6 cells per dish, grown overnight, and then transfected with the indicated expression vectors, using either lipofectamine (Invitrogen) or calcium phosphate method.

Immune complex kinase assay. The cultured cells were harvested and lysed in lysis buffer. The cell lysates were subjected to centrifugation at 12,000*g* for 10 min at 4 °C. The soluble fraction was immunoprecipitated with the appropriate antibodies, and the immunopellets were assayed for the enzymatic activities of indicated protein kinases, as was previously described. The phosphorylated proteins were separated via SDS–PAGE and quantified with a Fuji BAS 2500 PhosphorImager. Purified GST-cJun(1–79) was used as substrate for JNK. Protein concentrations were determined via the Bradford method (Bio-Rad).

Immunoblot analysis. The cells were harvested and lysed in RIPA buffer (50 mM Tris–HCl (pH 7.5)), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, 1 mM DTT, and 2 μg/ml leupeptin and aprotinin) for 30 min. The cell lysates were subjected to 20 min of centrifugation at 12,000*g* at 4 °C. The resulting soluble fraction was then boiled in Laemmli buffer and subjected to SDS–PAGE. After gel electrophoresis, the separated proteins were transferred via electroblotting onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were then blocked with Tris-buffered saline solution (pH 7.4) containing 0.1% Tween 20 and 5% nonfat milk. The blotted proteins were probed with anti-phospho JNK1 or anti-phospho c-Jun antibody (Cell Signaling), and then incubated with anti-mouse horseradish peroxidase-conjugated secondary antibodies. The blots were developed using an enhanced chemiluminescence (ECL) system (Pierce).

Coimmunoprecipitation. The cells were lysed in 1 ml RIPA buffer for 30 min at 4 °C. After 20 min of centrifugation at 12,000*g*, the supernatants were subjected to immunoprecipitation with the appropriate antibodies, coupled to protein A–Agarose beads. The resulting immunoprecipitates were then washed three times in phosphate-buffered solution (pH 7.4). Laemmli sample buffer was then added to the immunoprecipitated pellets; the pellets were heated for 5 min at 95 °C and analyzed via SDS–PAGE. Western blotting was conducted with the indicated antibodies [59].

In vitro binding assay. The recombinant GST-SEK1 and GST-c-Jun proteins were expressed in the *Escherichia coli* BL21 strain, using the pGEX system as indicated. The GST fusion proteins were then purified with glutathione–Agarose beads (Sigma), in accordance with the manufacturer's instructions. Hexahistidine (6His)-tagged JNK1 was expressed and purified as previously described [59]. Equal amounts of GST-SEK1 or GST-c-Jun fusion proteins were incubated with immobilized 6His-JNK1. After incubation, the beads were washed three times with ice-cold PBS and boiled with 5× Laemmli protein sample buffer. The pull-down proteins were analyzed and visualized via Coomassie's brilliant blue staining.

Results

IFN-γ suppresses JNK activation in microglial cells through NO generation

We previously showed that IFN-γ, by inducing iNOS expression and increasing NO production, suppresses the activation of JNK1 in murine macrophage cells and that this suppression is achieved by means of *S*-nitrosylation [58]. In order to test the inhibitory effect of NO production on JNK1, JNK2, and JNK3, we treated BV-2 murine microglial cells with IFN-γ and then examined the actions of IFN-γ on the activity of each JNK isoform. IFN-γ treatment resulted in a marked decrease in the anisomycin-stimulated activities of all three JNK isoforms in BV-2 microglial cells (Fig. 1). Furthermore, N^G-nitro-L-arginine (L-NNA), a NOS inhibitor, abolished the inhibitory effect of IFN- on the anisomycin-induced activation of JNK1, JNK2, and JNK3 (Fig. 1). These data suggest that the IFN-γ, by inducing NO generation, suppresses the activation of all of JNK isoforms.

SNAP suppresses all isoforms of JNKs through a thiol-redox mechanism

We next examined whether NO directly inhibits the kinase activities of each isoform of JNKs. We isolated JNK1, JNK2, and JNK3 by immunoprecipitating the lysates of HEK293 cells with the corresponding antibodies after the cells were untreated or treated with anisomycin. We then examined whether *S*-nitro-*N*-acetyl-DL-penicillamine (SNAP), an NO donor, could directly inhibit the kinase activities of the isolated JNK1, JNK2, and JNK3 immunopellets. In vitro kinase assay data revealed that SNAP suppressed the activities of all three JNK isoforms and that this suppression by SNAP was reversed by a

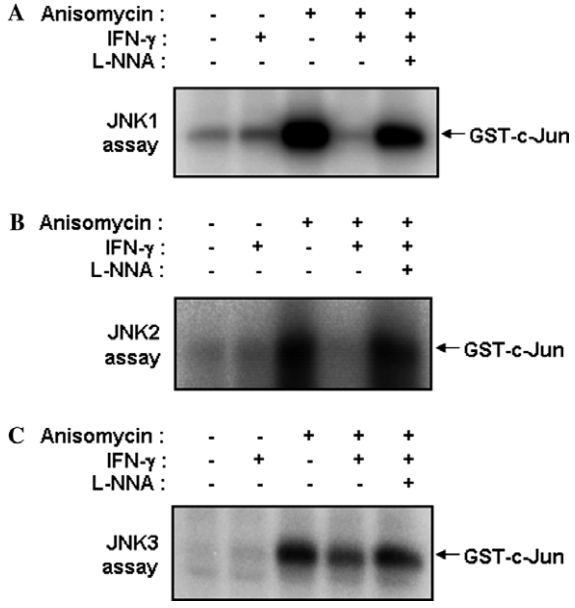

Fig. 1. Effect of IFN-γ on JNK activities in microglial BV2 cells. (A–C) BV2 murine microglial cells were pretreated with IFN-γ (100 U/ml) for 16 h and then incubated with 2 mM L-NNA for 30 min. The cells were then exposed to 10 μM anisomycin for 15 min at 37 °C. Cell lysates were subjected to immunoprecipitation with antibodies to JNK1 (A), JNK2 (B), or JNK3 (C). The resulting immunoprecipitates were examined by immunocomplex kinase assays using GST-c-Jun as substrate.

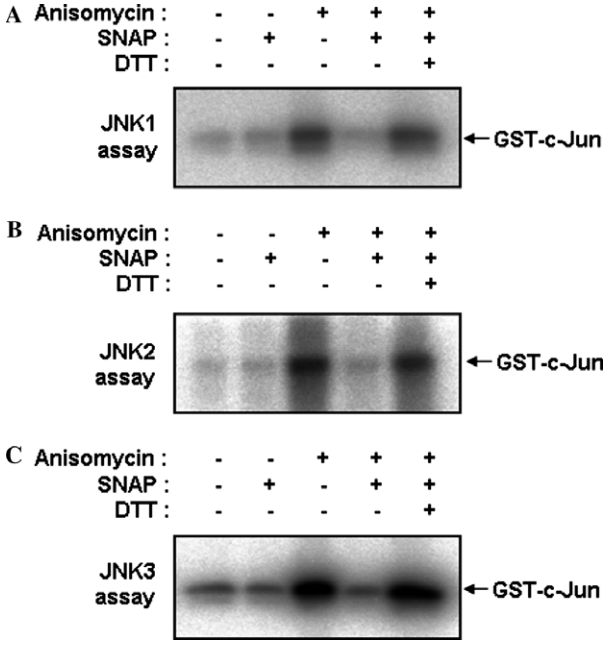

Fig. 2. In vitro effect of SNAP on JNK activity. HEK293 cells were exposed to 10 μM anisomycin for 15 min at 37 °C. The cell lysates were then subjected to immunoprecipitation using (A) anti-JNK1, (B) anti-JNK2, or (C) anti-JNK3 antibody, as indicated. The resultant immunopellets were treated with 100 μM SNAP on ice for 20 min, incubated for additional 20 min with 10 mM DTT on ice where indicated, and then examined for the kinase activities of JNK1 (A), JNK2 (B), or JNK3 (C) by immunocomplex kinase assays.

thiol-reducing agent dithiothreitol (Fig. 2). These data suggest that NO inhibits all three isoforms of JNKs (JNK1, JNK2, and JNK3) through a thiol redox mechanism. In order to better understand the mechanism of the NO-mediated inhibition of JNK activation, we next examined the effect of INF-γ on the phosphorylation status of either JNK or c-Jun in anisomycin-treated cells. Treatment of HEK293 cells with anisomycin enhanced the phosphorylation of JNK1, and this enhancement was not affected by IFN-γ (Fig. 3A). These findings suggest that IFN-γ-induced NO generation did not affect the activation process of JNK1 catalyzed by its upstream kinase such as SEK1. In contrast, IFN-γ suppressed the anisomycin-induced phosphorylation of c-Jun, suggesting that IFN-γ-induced NO generation inhibits the JNK-mediated phosphorylation of c-Jun (Fig. 3B).

NO inhibits a physical interaction between JNK and c-Jun in vitro

In order to test the effect of NO on the association between JNK1 and c-Jun, we performed in vitro binding assays. Purified 6His-JNK1 was pre-incubated with purified GST-SEK1 in the absence or presence of ATP and then 6His-JNK1 was bound to Nickel–NTA beads. The 6His-JNK1 immobilized on Ni–NTA beads was then incubated with purified GST-c-Jun. Our results revealed that 6His-JNK1 physically associated with GST-c-Jun and this interaction was higher in the presence of ATP. SNAP inhibited the interaction between JNK1 and c-Jun regardless of the presence of ATP (Fig. 4A). Next, we examined the effect of NO on the interaction SEK1 and JNK1. In vitro binding assays using 6His-JNK and GST-SEK1 showed that SNAP did not affect the physical association between SEK1 and JNK1 (Fig. 4B).

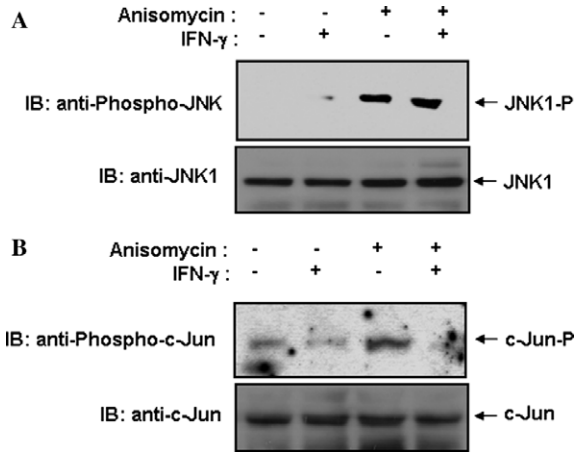

Fig. 3. IFN- γ treatment inhibits the anisomycin-induced phosphorylation of c-Jun, but not that of JNK, in BV-2 cells. BV2 murine microglial cells were pretreated with IFN-γ (100 U/ml) for 16 h, then exposed to 10 μM anisomycin for 15 min at 37 °C. Cell lysates were subjected to immunoblot analysis with antibodies to phospho JNK1 antibody (A) or anti-phospho c-Jun (B).

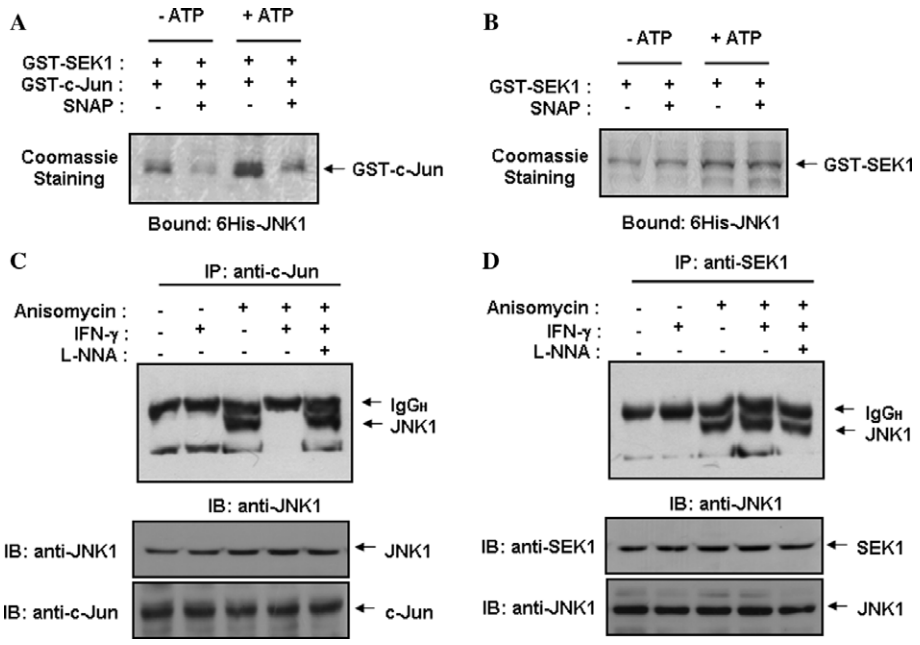

Fig. 4. NO inhibits the physical interaction between JNK and c-Jun both in vitro and in intact cells. (A) Recombinant 6His-JNK1 proteins were immobilized onto Nickel–NTA–Agarose beads. Purified GST-SEK1 was applied to 6His-JNK1 immobilized on the beads in the absence or presence of ATP. The beads were exposed to 100 μM SNAP for 20 min on ice. The beads were washed extensively and then incubated with GST-c-Jun in the absence or presence of ATP. Proteins bound to 6His-JNK1 were eluted and visualized by Coomassie brilliant blue staining. (B) Recombinant 6His-JNK1 proteins were immobilized onto Nickel–NTA–Agarose beads. Purified GST-SEK1 was applied to 6His-JNK1 immobilized on the beads in the absence or presence of ATP. The beads were exposed to 100 μM SNAP for 20 min on ice. Proteins bound to 6His-JNK1 were eluted and visualized by Coomassie's brilliant blue staining. (C,D) BV2 murine microglial cells were pretreated with IFN-γ (100 U/ml) for 16 h, incubated with 2 mM L-NNA for 30 min, and then exposed to 10 μM anisomycin for 15 min at 37 °C as indicated. The cell lysates were subjected to immunoprecipitation with antibodies to c-Jun (C) or SEK1 (D), and then the resulting precipitates were subjected to immunoblot analysis with anti-JNK1 antibody.

IFN-γ inhibits the physical interaction between JNK1 and c-Jun in intact cells

Next, we examined the effect of IFN-γ on the physical interaction between two endogenous JNK1 and c-Jun proteins in BV-2 microglial cells by co-immunoprecipitation. IFN-γ treatment resulted in a marked inhibition of the interaction between JNK1 and c-Jun (Fig. 4C). This inhibitory effect of IFN-γ on the interaction between JNK1 and c-Jun was reversed completely by L-NNA. These data suggest that NO mediates the inhibitory effect of IFN-γ. Next, we examined the effect of IFN-γ on the physical interaction between SEK1 and JNK1 in BV2 cells. Co-immunoprecipitation data showed that IFN-γ failed to affect the interaction between endogenous SEK1 and endogenous JNK1 in intact cells (Fig. 4D).

Discussion

Nitric oxide (NO) has been implicated as a key regulator of a variety of developmental and cellular signaling protocols, operating through the thiol modification of a host of intracellular signaling proteins [1–4]. We previously showed that NO negatively regulates JNK1 by a thiol redox mechanism [58]. Herein, we have attempted to understand two questions: (i) can all three JNK isoforms be regulated in a redox-sensitive manner? and (ii) what are the characteristics of the protein–protein interaction and phosphorylation status of JNK in the presence of NO? Our findings in this study revealed that all three JNK isoforms are inhibited by NO both in intact cells and in vitro, and that this inhibition occurs through a thiol redox mechanism in a reversible manner.

JNK acquires its activity via the well-known canonical signaling cascade from a variety of stimuli. The sequential phosphorylation of JNK1 occurs via the MEKK1–SEK1 pathway, and transfers this signal to c-Jun via both physical interaction and protein modification [41–48]. Prior to the transfer of the phosphate group to the downstream effector, a protein kinase should be bound to the substrate. Our data indicate that NO inhibits the physical interaction between JNK1 and c-Jun without affecting the association between SEK1 and JNK1. NO appears to modify JNK and to reduce its kinase activities, but is not capable of preventing the phosphorylation of JNK by SEK1. These findings thus suggest that the NO-mediated inhibition of JNK results from the suppression of physical interaction between JNK and its downstream substrate such as c-Jun.

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References

[1] O. Arancio, E.R. Kandel, R.D. Hawkins, Activity-dependent long-term enhancement of transmitter release by presynaptic 3′,5′-cyclic GMP in cultured hippocampal neurons, Nature 376 (1995) 74–80.
[2] S. Dimmeler, J. Haendeler, M. Nehls, A.M. Zeiher, Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases, J. Exp. Med. 185 (1997) 601–607.
[3] B. Kuzin, I. Roberts, N. Peunova, G. Enikolopov, Nitric oxide regulates cell proliferation during *Drosophila* development, Cell 87 (1996) 639–649.
[4] C. Nathan, Q.W. Xie, Nitric oxide synthases: roles, tolls, and controls, Cell 78 (1994) 915–918.
[5] Y. Wang, P.A. Marsden, Nitric oxide synthases: gene structure and regulation, Adv. Pharmacol. 34 (1995) 71–90.
[6] R. Hattori, K. Sase, H. Eizawa, K. Kosuga, T. Aoyama, R. Inoue, S. Sasayama, C. Kawai, Y. Yui, K. Miyahara, et al., Structure and function of nitric oxide synthases, Int. J. Cardiol. 47 (1994) S71–S75.
[7] W.C. Sessa, The nitric oxide synthase family of proteins, J. Vasc. Res. 31 (1994) 131–143.
[8] H.H. Schmidt, J.S. Pollock, M. Nakane, U. Forstermann, F. Murad, Ca^{2+}/calmodulin-regulated nitric oxide synthases, Cell Calcium 13 (1992) 427–434.
[9] S.C. Lee, C.F. Brosnan, Cytokine Regulation of iNOS Expression in Human Glial Cells, Methods 10 (1996) 31–37.
[10] D.L. Eizirik, F. Leijerstam, The inducible form of nitric oxide synthase (iNOS) in insulin-producing cells, Diabetes Metab. 20 (1994) 116–122.
[11] J. Gao, D.C. Morrison, T.J. Parmely, S.W. Russell, W.J. Murphy, An interferon-gamma-activated site (GAS) is necessary for full expression of the mouse iNOS gene in response to interferon-gamma and lipopolysaccharide, J. Biol. Chem. 272 (1997) 1226–1230.
[12] E.W. Alley, W.J. Murphy, S.W. Russell, A classical enhancer element responsive to both lipopolysaccharide and interferon-gamma augments induction of the iNOS gene in mouse macrophages, Gene 158 (1995) 247–251.
[13] Y. Shi, M. Gaestel, In the cellular garden of forking paths: how p38 MAPKs signal for downstream assistance, Biol. Chem. 383 (2002) 1519–1536.
[14] R.K. Barr, M.A. Bogoyevitch, The c-Jun N-terminal protein kinase family of mitogen-activated protein kinases (JNK MAPKs), Int. J. Biochem. Cell. Biol. 33 (2001) 1047–1063.
[15] E. Cano, L.C. Mahadevan, Parallel signal processing among mammalian MAPKs, Trends Biochem. Sci. 20 (1995) 117–122.
[16] R.J. Davis, MAPKs: new JNK expands the group, Trends Biochem. Sci. 19 (1994) 470–473.
[17] C. Bradham, D.R. McClay, p38 MAPK in development and cancer, Cell Cycle 5 (2006) 824–828.
[18] B. Kaminska, MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits, Biochim. Biophys. Acta 1754 (2005) 253–262.
[19] J.Y. Fang, B.C. Richardson, The MAPK signalling pathways and colorectal cancer, Lancet Oncol. 6 (2005) 322–327.
[20] W. Kolch, M. Calder, D. Gilbert, When kinases meet mathematics: the systems biology of MAPK signalling, FEBS Lett. 579 (2005) 1891–1895.
[21] M.A. Bogoyevitch, I. Boehm, A. Oakley, A.J. Ketterman, R.K. Barr, Targeting the JNK MAPK cascade for inhibition: basic science and therapeutic potential, Biochim. Biophys. Acta 1697 (2004) 89–101.
[22] W.T. Gerthoffer, C.A. Singer, MAPK regulation of gene expression in airway smooth muscle, Respir. Physiol. Neurobiol. 137 (2003) 237–250.
[23] W. Zhang, H.T. Liu, MAPK signal pathways in the regulation of cell proliferation in mammalian cells, Cell Res. 12 (2002) 9–18.
[24] C.A. Hazzalin, L.C. Mahadevan, MAPK-regulated transcription: a continuously variable gene switch? Nat. Rev. Mol. Cell Biol. 3 (2002) 30–40.
[25] J.M. English, M.H. Cobb, Pharmacological inhibitors of MAPK pathways, Trends Pharmacol. Sci. 23 (2002) 40–45.
[26] R. Seger, E.G. Krebs, The MAPK signaling cascade, FASEB J. 9 (1995) 726–735.
[27] J. Inglese, W.J. Koch, K. Touhara, R.J. Lefkowitz, G beta gamma interactions with PH domains and Ras-MAPK signaling pathways, Trends Biochem. Sci. 20 (1995) 151–156.
[28] K. Shen, R.F. Novak, DDT stimulates c-erbB2, c-met, and STATS tyrosine phosphorylation, Grb2-Sos association, MAPK phosphorylation, and proliferation of human breast epithelial cells, Biochem. Biophys. Res. Commun. 231 (1997) 17–21.
[29] N. Dumaz, R. Marais, Integrating signals between cAMP and the RAS/RAF/MEK/ERK signalling pathways. Based on the anniversary prize of the Gesellschaft fur Biochemie und Molekularbiologie Lecture delivered on 5 July 2003 at the Special FEBS Meeting in Brussels, FEBS J. 272 (2005) 3491–3504.
[30] F. Chang, L.S. Steelman, J.T. Lee, J.G. Shelton, P.M. Navolanic, W.L. Blalock, R.A. Franklin, J.A. McCubrey, Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention, Leukemia 17 (2003) 1263–1293.
[31] R.A. Hilger, M.E. Scheulen, D. Strumberg, The Ras-Raf-MEK-ERK pathway in the treatment of cancer, Onkologie 25 (2002) 511–518.
[32] N. Agell, O. Bachs, N. Rocamora, P. Villalonga, Modulation of the Ras/Raf/MEK/ERK pathway by Ca(2+), and calmodulin, Cell. Signal. 14 (2002) 649–654.
[33] W. Kolch, Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions, Biochem. J. 351 (Pt. 2) (2000) 289–305.
[34] R. Marais, C.J. Marshall, Control of the ERK MAP kinase cascade by Ras and Raf, Cancer Surv. 27 (1996) 101–125.
[35] V. Adler, A. Schaffer, J. Kim, L. Dolan, Z. Ronai, UV irradiation and heat shock mediate JNK activation via alternate pathways, J. Biol. Chem. 270 (1995) 26071–26077.
[36] Y.R. Chen, X. Wang, D. Templeton, R.J. Davis, T.H. Tan, The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation. Duration of JNK activation may determine cell death and proliferation, J. Biol. Chem. 271 (1996) 31929–31936.
[37] B. Derijard, M. Hibi, I.H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, R.J. Davis, JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain, Cell 76 (1994) 1025–1037.
[38] J. Han, J.D. Lee, L. Bibbs, R.J. Ulevitch, A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells, Science 265 (1994) 808–811.
[39] C. Rosette, M. Karin, Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors, Science 274 (1996) 1194–1197.
[40] H.Y. Song, C.H. Regnier, C.J. Kirschning, D.V. Goeddel, M. Rothe, Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2, Proc. Natl. Acad. Sci. USA 94 (1997) 9792–9796.
[41] B. Derijard, J. Raingeaud, T. Barrett, I.H. Wu, J. Han, R.J. Ulevitch, R.J. Davis, Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms, Science 267 (1995) 682–685.
[42] I.N. Foltz, R.E. Gerl, J.S. Wieler, M. Luckach, R.A. Salmon, J.W. Schrader, Human mitogen-activated protein kinase kinase 7 (MKK7) is a highly conserved c-Jun N-terminal kinase/stress-activated protein

kinase (JNK/SAPK) activated by environmental stresses and physiological stimuli, J. Biol. Chem. 273 (1998) 9344–9351.
[43] P.M. Holland, M. Suzanne, J.S. Campbell, S. Noselli, J.A. Cooper, MKK7 is a stress-activated mitogen-activated protein kinase kinase functionally related to hemipterous, J. Biol. Chem. 272 (1997) 24994–24998.
[44] T. Moriguchi, F. Toyoshima, N. Masuyama, H. Hanafusa, Y. Gotoh, E. Nishida, A novel SAPK/JNK kinase, MKK7, stimulated by TNFalpha and cellular stresses, EMBO J. 16 (1997) 7045–7053.
[45] H. Nishina, K.D. Fischer, L. Radvanyi, A. Shahinian, R. Hakem, E.A. Rubie, A. Bernstein, T.W. Mak, J.R. Woodgett, J.M. Penninger, Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3, Nature 385 (1997) 350–353.
[46] C. Tournier, A.J. Whitmarsh, J. Cavanagh, T. Barrett, R.J. Davis, Mitogen-activated protein kinase kinase 7 is an activator of the c-Jun NH2-terminal kinase, Proc. Natl. Acad. Sci. USA 94 (1997) 7337–7342.
[47] C. Tournier, A.J. Whitmarsh, J. Cavanagh, T. Barrett, R.J. Davis, The MKK7 gene encodes a group of c-Jun NH2-terminal kinase kinases, Mol. Cell. Biol. 19 (1999) 1569–1581.
[48] M. Yan, T. Dai, J.C. Deak, J.M. Kyriakis, L.I. Zon, J.R. Woodgett, D.J. Templeton, Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1, Nature 372 (1994) 798–800.
[49] C. Huang, K. Jacobson, M.D. Schaller, A role for JNK-paxillin signaling in cell migration, Cell Cycle 3 (2004) 4–6.
[50] Y.T. Ip, R.J. Davis, Signal transduction by the c-Jun N-terminal kinase (JNK)–from inflammation to development, Curr. Opin. Cell Biol. 10 (1998) 205–219.
[51] G.L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases, Science 298 (2002) 1911–1912.
[52] H. Kanda, M. Miura, Regulatory roles of JNK in programmed cell death, J. Biochem. (Tokyo) 136 (2004) 1–6.
[53] N.J. Kennedy, R.J. Davis, Role of JNK in tumor development, Cell Cycle 2 (2003) 199–201.
[54] A. Lin, B. Dibling, The true face of JNK activation in apoptosis, Aging Cell 1 (2002) 112–116.
[55] H. Nishina, T. Wada, T. Katada, Physiological roles of SAPK/JNK signaling pathway, J. Biochem. (Tokyo) 136 (2004) 123–126.
[56] S. Vlahopoulos, V.C. Zoumpourlis, JNK: a key modulator of intracellular signaling, Biochemistry (Mosc.) 69 (2004) 844–854.
[57] C.R. Weston, R.J. Davis, The JNK signal transduction pathway, Curr. Opin. Genet. Dev. 12 (2002) 14–21.
[58] H.S. Park, S.H. Huh, M.S. Kim, S.H. Lee, E.J. Choi, Nitric oxide negatively regulates c-Jun N-terminal kinase/stress-activated protein kinase by means of *S*-nitrosylation, Proc. Natl. Acad. Sci. USA 97 (2000) 14382–14387.
[59] H.S. Park, J.S. Lee, S.H. Huh, J.S. Seo, E.J. Choi, Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase, EMBO J. 20 (2001) 446–456.