Redox-sensitive Transactivation of Epidermal Growth Factor Receptor by Tumor Necrosis Factor Confers the NF-κB Activation* 

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Cross-communication between different signaling systems allows the integration of the great diversity of stimuli that a cell receives under varying physiological situations. In this paper we have explored the possibility that tumor necrosis factor (TNF) receptor signal cross-talks with epidermal growth factor (EGF) receptor signal on the nuclear factor-κB (NF-κB) activation pathway. We have demonstrated that overexpression of the EGF receptor (EGFR) in NIH3T3 cells significantly enhances TNF-induced NF-κB-dependent luciferase activity even without EGF, that EGF treatment has a synergistic effect on the induction of the reporter activity, and that this enhancement is suppressed by AG1478, EGFR-specific tyrosine kinase inhibitor. We also have shown that TNF induces tyrosine phosphorylation and internalization of the overexpressed EGFR in NIH3T3 cells and the endogenously expressed EGFR in A431 cells and that the transactivation by TNF is suppressed by N-acetyl-L-cysteine or overexpression of an endogenous reducing molecule, thioredoxin, but not by phosphatidylinositol 3-kinase inhibitors and protein kinase C inhibitor. Taken together, this evidence strongly suggests that EGFR transactivation by TNF, which is regulated in a redox-dependent manner, is playing a pivotal role in TNF-induced NF-κB activation.

A cardinal concept in signal transduction is that receptors are activated by highly specific interactions with cognate ligands. Hence each receptor recognizes one or a few structurally related ligands, and each ligand stereochemically "fits" one or perhaps two structurally related receptors. The specificity of biological responses generated to extracellular signals depends perhaps two structurally related ligands, and each ligand stereochemically "fits" one or perhaps two structurally related receptors. The specificity of biological responses generated to extracellular signals depends on each other in what is termed receptor transactivation or cross-talk. Although receptor cross-talk is no doubt real, its biological significance remains unclear. Understanding the mechanism(s) by which inter-receptor communication occurs may offer the experimental means to explore the underlying biology.

The initial steps in the major pathway utilized by tumor necrosis factor (TNF) to activate the NF-κB are fairly well understood (1, 2). The NF-κB activation is usually initiated by the interaction of TNF with TNFR1 (p55), one of two distinct surface receptors, the other is TNFR2 (p75). Ligand-induced clustering of TNFR1 leads to the recruitment of TRADD to the intracellular portion of the receptor (3). Receptor-bound TRADD recruits both the protein kinase designated receptor-interacting protein (RIP) and the ring/zinc finger protein TNF receptor-associated factor 2 (TRAF2). These two adapter proteins lead to the activation of the NF-κB as well as the activation of the stress-activated protein kinases such as c-Jun N-terminal kinase and p38 MAPK (3). IL-1 also activates NF-κB and stress-activated protein kinases using a similar pathway initiated by ligand binding to the type 1 IL-1 receptor and involving adapter proteins MyD88, IL-1 receptor-associated kinase, and TRAF6 (4). The precise connection between RIP-TRAF2 or IL-1 receptor-associated kinase-TRAF6 complexes and NF-κB are less well established but are thought to involve the MAPK kinase kinase family members MEKK-1 or NF-κB-inducing kinase (NIK) (5). Both of these protein kinases can directly phosphorylate and activate IκB kinase (IKK) which is a multiprotein enzyme complex consisting of IKK-1, IKK-2, and NEMO (also known as IKKγ) subunits (6–9). IKK-α and IKK-β phosphorylate members of the IκB family that share a common mechanism for regulation of NF-κB. In resting cells, IκB proteins bind to and mask the nuclear localization sequence of NF-κB resulting in the retention of the transcription factor in the cytosol. Phosphorylation of IκB by IKKs targets this protein for rapid ubiquitination and degradation by the proteasome (2, 10). This process releases NF-κB which then translocates to the nucleus and induces transcription. MEKK-1, but not NIK, catalyzes the phosphorylation and activation of yet other MAPK kinases and is probably responsible for the parallel activation of stress-activated protein kinases (11–13).

Recently, an alternative signaling pathway has been described in some cells in which Akt directly phosphorylates IKKs leading to the activation of NF-κB, independent of MEKK-1 and NIK (14). This pathway thus allows growth factors such as platelet-derived protein kinase to activate NF-κB (15). Moreover, TNF and IL-1 have been found to activate the phosphatidylinositol 3-kinase (PI3K/Akt pathway, potentially providing an alternative pathway for TNF and IL-1 to activate NF-κB that is independent of NIK or MEKK-1. The importance of the nuclear factor-κB, EGFR, EGF receptor; TRX, thioredoxin; ROI, reactive oxygen intermediate; MAPK, mitogen-activated protein kinase; NAC, N-acetyl-l-cysteine; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; TRAF, TNF receptor-associated factor; IL, interleukin; NIK, NF-κB-inducing kinase; IKK, IκB kinase; MEKK, MAPK/extracellular signal-regulating kinase kinase kinase; GFP, green fluorescent protein; EGFP, enhanced GFP; TRADD, TNF receptor-associated death domain.

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§ The abbreviations used are: TNF, tumor necrosis factor; EGF, epidermal growth factor; NF-κB, nuclear factor-κB; EGFR, EGF receptor; TRX, thioredoxin; ROI, reactive oxygen intermediate; MAPK, mitogen-activated protein kinase; NAC, N-acetyl-l-cysteine; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; TRAF, TNF receptor-associated factor; IL, interleukin; NIK, NF-κB-inducing kinase; IKK, IκB kinase; MEKK, MAPK/extracellular signal-regulating kinase kinase kinase; GFP, green fluorescent protein; EGFP, enhanced GFP; TRADD, TNF receptor-associated death domain.

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of this alternative NF-κB pathway compared with the previously described TRADD/RIP/TRAF2 or MyD88/IL-1 receptor-associated kinase/TRAF6 cytokine signaling pathways is unknown and may vary with the cell type. TNF and IL-1 also activate MAPK, but this pathway is even less well defined.

The epidermal growth factor (EGF) receptor is ubiquitously expressed in various tissues and is thus positioned to influence a wide range of responses, depending on the coordinate expression of the cognate ligand (16, 17). A number of reports have demonstrated that various extracellular stimuli, unrelated to EGF-like ligands, can also activate the EGF receptor (18). These diverse stimuli include numerous agonists such as carbachol, bombesin, thrombin, and lysophosphatidic acid for heparin-like G protein-coupled receptors, cytokine receptors such as prolactin, growth hormone adhesion receptors such as integrins, membrane-depolarizing agents such as KCl, and environmental stress factors such as ultraviolet irradiation, γ-irradiation, oxidants, heat shock, and hyperosmotic shock (19–21). Activation of EGF receptors by such seemingly unrelated and indirect means is potentially biologically significant (18–20).

Together, this evidence prompted us to investigate a possibility of cross-talk between TNFα-induced signals and EGF-mediated signals on the NF-κB activation. In this paper, we have demonstrated that TNFα transactivates EGFR in a redox-sensitive manner, and this activation enhances the NF-κB activation.

EXPERIMENTAL PROCEDURES

Cell Lines and Reagents—A murine embryonic fibroblast cell line NIH3T3 was obtained from Dr. H. Sabe (Osaka Bioscience Institute, Osaka, Japan). A human embryo kidney cell-derived cell line HEK293 and human carcinoma A431 cells were from the American Type Culture Collection (Manassas, VA). Cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and antibiotics (50 units/ml penicillin and 100 μg/ml streptomycin) at 37 °C under a humidified atmosphere of 5% CO2. Recombinant human TNFα and recombinant human EGF were purchased from Roche Molecular Biochemicals. Monoclonal antibodies raised against EGF receptor (E12120) and activated EGF receptor (E12120) were from BD Transduction Laboratories. Tyrosin kinase AG1478, wortmannin, and tyrphostin AG1478 (E12020) and activated EGF receptor (E12120) were from BD Transduction Laboratories.

Molecular Biochemicals. Whole cell lysates (50 μg) were separated on 7.5% SDS-polyacrylamide gels. Western blotting analysis was performed following the protocol described previously (24, 26). Briefly, after electrophoresis, the polyvinylidene difluoride membranes (Millipore, Bedford, MA) were treated with 5% (w/v) skim milk in TBS-T (20 mM Tris-HCl, pH 7.6, 157 mM NaCl, 0.5% Tween 20) and incubated with antigen-specific antibodies against EGF receptor (1:2500) or activated form EGF receptor (1:1000), followed by incubation with peroxidase-conjugated anti-mouse IgG (1:2000) (Amersham Pharmacia Biotech). The epitope was visualized with an ECL Western blot detection kit (Amersham Pharmacia Biotech).

Confocal Microscopy—NIH3T3 cells were plated onto chamber slides (LabTech) and transfected by pEGFP-N3 or pEGFR-EGFP. After 12 h, cells were serum-starved (0.1% serum) for 12 h and then stimulated for 15 min in the absence or presence of TNF or EGF. Cells were fixed in 4% paraformaldehyde and mounted in 90% glycerol with 1 mg/ml p-phenylenediamine and examined with a confocal microscope, MRC-1024 (Bio-Rad) (24).

Statistical Analysis—Statistical significance between two groups was tested using the unpaired Student’s t test. Differences were considered statistically significant at a value of p < 0.05.

RESULTS

Overexpression of EGF Receptor Enhances TNFα-induced NF-κB Transcriptional Activation in NIH3T3 Cells—Because NIH3T3 cells express EGFR only in low density, we can examined the effects of EGFR on the TNFα-induced NF-κB activation by overexpression of EGFR. In Fig. 1A, pEGFR-EGFP enhanced the NF-κB-dependent luciferase activity compared with pEGFP-N3 (lane 2). The concentration of serum did not cause any significant differences (lanes 2 and 5). EGF has been shown to activate NF-κB in rat aortic smooth muscle cells (28) and A431 carcinoma cells (29) but not in human microvascular endothelial cells (30). Therefore, we examined the effect of EGF on the NF-κB activation in NIH3T3 cell. Treatment with EGF by itself did not have any significant effects (lane 3) but made significant synergistic effect with TNF treatment (lane 4). This enhancing effect of EGF was in a dose-dependent manner for the pEGFR-EGFP plasmid (Fig. 1B).

Kinase Inhibitors Suppress TNFα-induced NF-κB Transcriptional Activation in NIH3T3 Cells and HEK293 Cells—The EGFR is a transmembrane protein, and the ligation of EGF to the receptor results in an increase in the tyrosine kinase activity of the cytoplasmic domain and the autophosphorylation of tyrosine residues (31). Various protein kinases such as PKC, MAPK, and EGFR itself are considered to be involved in the tyrosine phosphorylation of EGFR indirectly or directly (16). In order to explore the molecular mechanisms, we used a series of kinase inhibitors (Fig. 2A). The effect of expression of pEGFR-EGFP was suppressed dose-dependently by treatment with tyrphostin AG1478 (right columns of lanes 2–4), which is a rather selective EGFR tyrosine kinases inhibitor. AG1478 treatment did not have significant effects on TNFα-induced NF-κB activation in pEGFP-N3-transfected NIH3T3 cells (left columns of lanes 2–4). In contrast, both PI3K inhibitors, LY294002 and wortmannin, and a PKC inhibitor, GF109203X, suppressed the NF-κB activation in both pEGFP-N3- and pEGFR-EGFP-transfected NIH3T3 cells (lanes 5–8, 11, and 12). Because a line of reports have demonstrated that reactive oxygen intermediates (ROI) play a pivotal role in the TNF-induced NF-κB activation, we tried a potent antioxidant NAC in this assay. Interestingly, NAC almost completely abolished EGF-mediated enhancement of the NF-κB activation induced by TNF (lanes 9 and 13). In contrast, the MEK-1 inhibitor, PD98059, did not have any significant effects on the NF-κB activation (lanes 9 and 10). Treatment with AG1478 or NAC caused similar suppressive effects of the NF-κB activation in HEK293 cells (Fig. 2B) as well as in pEGFR-EGFP-transfected NIH3T3 cells.

We then examined the effect of overexpression of kinase-dead EGFR (K721A) on the TNF-induced NF-κB-dependent
gene expression in HEK293 cells. As shown in Fig. 2C, expression of the EGFR(K721A)-EGFP chimera blocked the NF-κB-dependent gene expression in a plasmid dose-dependent manner. Together with the results using A1478, the tyrosine phosphorylation of EGFR plays very critical roles in TNF-induced NF-κB activation process.

EGF Transactivation by TNF Is Not Suppressed by Inhibitors of PI3K and PKC but by NAC Treatment and TRX Expression—Next we examined whether EGFR could be activated by TNFα or not by using an antibody that specifically recognizes the “activated” EGFR from which tyrosine residues were phosphorylated (32). We detected significant signals of activated EGFR (upper row) in pEGFR-EGFP-transfected NIH3T3 cells treated by EGF (Fig. 3A, lane 4) or TNF (lane 5). Neither 100 μM LY294002 (lane 7) nor 10 μM GF109203X (lane 8) affected TNF-induced EGFR activation. In contrast, NAC dose-dependently suppressed the EGFR phosphorylation by TNF (lanes 9 and 10). EGFR endogenously expressed in A431 cells were also phosphorylated by TNF treatment (Fig. 3B, lane 3), and this phosphorylation was suppressed by AG1478 (lane 4) or NAC treatment (lane 5) as well as exogenously expressed EGFR in

**FIG. 1.** Enhancement of the TNFα-induced NF-κB transcriptional activation by overexpression of EGF receptor in NIH3T3 cells. NIH3T3 cells (5 × 10⁴ cells/well) were transfected by pEGFR-EGFP or pEGFP-N3 plasmids. 12 h after transfection, cells were treated with 100 ng/ml TNF (lanes 2, 4, and 5) and/or 500 ng/ml EGF (lanes 3 and 4). The cells then underwent the luciferase assay following the protocol given under “Experimental Procedures.” In lane 5, the culture media was replaced with the media with 0.5% serum. The results are the means ± S.D. and presented as fold increases in luciferase activity over the baseline seen in the mock transfectant without treatment. This is a representative of two independent experiments that were done in triplicate. #, p < 0.05.

**FIG. 2.** Effects of kinase inhibitors on the TNFα-induced NF-κB transcriptional activation. NIH3T3 cells were transfected by reporter plasmid and pEGFR-EGFP- or pEGFP-N3 (A). 12 h after transfection, cells were pretreated with AG1478, LY294002, wortmannin, PD98059, GF109203X, or NAC for 1 h and then treated with TNF. HEK293 cells (B and C) were transfected by reporter plasmid and pEGFR-EGFP, pEGFR-EGFP(K721A), or pEGFP-N3 plasmids as indicated (C) and pretreated with AG1478 or NAC for 1 h (A). 6 h after incubation, cells underwent the luciferase assay following the protocol under “Experimental Procedures.” The results are the means ± S.D. and are presented as fold increases in luciferase activity over the baseline seen in the column without TNF treatment. This is a representative of two independent experiments that were done in triplicate. A, %, p < 0.05 compared with pEGFP-N3 treated with TNF; #, p < 0.05 compared with pEGFR-EGFP treated with TNF; B and C, #, p < 0.05.
NIH3T3 cells. Moreover, expression of an endogenous protein with antioxidant property, TRX blocked phosphorylation of EGFR expressed in NIH3T3 cells (Fig. 3).

If the observed tyrosine phosphorylation reflects EGFR activation, it should lead to multimerization and internalization of EGFR (17). To examine whether stimulation of TNFR leads to internalization of transactivated EGFR, we examined the intracellular localization of overexpressed EGFR-EGFP in NIH3T3 cells with confocal immunofluorescence microscopy. Fig. 4 shows that in unstimulated cells transfected with pEGFR-EGFP, EGFR localizes primarily to the cell surface (Fig. 4B). TNFα (500 ng/ml) treatment of these cells, however, leads to an increase in EGFR localized inside the cells (Fig. 4C). EGF (500 ng/ml) treatment also leads to the internalization (Fig. 4D). GFP was homogeneously expressed in cells and did not have any particular localization pattern (Fig. 4A).

TRAF2-induced NF-κB Activation Is Sensitive but IKKa-induced NF-κB Activation Is Not Sensitive to AG1478 in HEK293 Cells—

In this paper, we have described the EGFR transactivation by TNF and its involvement in TNF-induced NF-κB activation. We have demonstrated that the transactivation is redox-sensitive but dependent on activity of neither P13K nor PKC. In contrast, the NF-κB activation process is redox-sensitive and dependent on the activity of PI3K and PKC but not MAPK. The EGFR is a transmembrane glycoprotein, and the ligation of EGF to the receptor results in the dimer formation, an increase in the tyrosine kinase activity of the cytoplasmic domain, and the autophosphorylation of several tyrosine residues (33, 34). Intracellular tyrosine kinases such as Src and focal adhesion kinase and EGFR itself are considered to be involved in the tyrosine phosphorylation (18). Under certain circumstances, intracellular tyrosine kinase activity is dependent on other kinases such as PKC, P13K, and MAPK. Our results of EGFR transactivation assay using anti-activated EGFR anti-

DISCUSSION

expression of TRAF2 induced significantly NF-κB-dependent gene expression, and this was suppressed by treatment with AG1478 (250 nm) (Fig. 5A). In contrast, AG1478 (250 nm) did not have any significant effect on the IKKα-induced NF-κB activation (Fig. 5B).

TRAF2 Induced EGFR Phosphorylation in a Redox-dependent Manner in NIH3T3 Cells—

TRAF2-induced NF-κB Activation Is Sensitive but IKKa-induced NF-κB Activation Is Not Sensitive to AG1478 in HEK293 Cells—To explore the AG1478-sensitive step in the NF-κB activation, we overexpressed signaling intermediate molecules, TRAF2 and IKKa, in HEK293 cells (Fig. 5). Over-

expression of TRAF2 induced significantly NF-κB-dependent gene expression, and this was suppressed by treatment with AG1478 (250 nm) (Fig. 5A). In contrast, AG1478 (250 nm) did not have any significant effect on the IKKα-induced NF-κB activation (Fig. 5B).

TRAF2 Induced EGFR Phosphorylation in a Redox-dependent Manner in NIH3T3 Cells—We next examined the effect of TRAF2 on the EGFR phosphorylation. HEK293 cells were transfected by TRAF2 expression vector with or without NAC treatment. As shown in Fig. 6, EGFR phosphorylation was significantly enhanced by TRAF2 overexpression (lane 3), and the enhancement was abolished by NAC treatment (lane 4). H2O2 by itself enhanced EGFR phosphorylation (lane 2).
catalogic sites containing reactive cysteine residues, which form a thiol-phosphatase intermediate during catalysis, oxidation of these residues leads to their inactivation. Thiol-mediated redox systems such as glutathione and TRX systems have been demonstrated to be involved in these processes (22, 24, 26, 43). Overexpression of TRX suppressed the TNF-induced EGFR phosphorylation (Fig. 3C) as did the treatment with NAC. We have shown that redox-sensitive steps in the TNF-induced NF-κB signaling pathway are down from TRAF2 and up to IKK in HEK293 cells (27). In addition, in this study, we have shown that TRAF2 overexpression induces EGFR phosphorylation in a redox-dependent manner and TRAF2-induced NF-κB activation is AG1478-dependent (Figs. 5 and 6). Consistent with this observation, Liu et al. (44) recently showed that not only TNF but also just overexpression of TRAF2 fosters the production of ROI in transfected cells and that overexpression of TRX or treatment with antioxidants suppressed part of the downstream signaling cascades. Rac1 is an essential subunit of the NADPH oxidase system even in non-phagocytic cells, and overexpression of the constitutively active mutant of Rac1 confers generation of ROI in certain cells, and overexpression of dominant negative mutant suppresses ROI generation by growth factor such as EGF and platelet-derived protein kinase as well as by TNF (45–49). Together with our results (Figs. 4 and 5), the ROI of downstream TNFR and TRAF2 may play a role in the EGFR transactivation process. In this study we have clearly demonstrated that treatment with AG1478 significantly suppresses TRAF2-induced NF-κB-dependent gene expression but not IKKα-induced gene expression (Fig. 5). Moreover, TRAF2 induces EGFR phosphorylation in a redox-dependent manner (Fig. 6). In addition to signaling by protein-to-protein interaction, the diffusible second messenger, ROI, mainly H2O2 downstream of the TNFR-TRAF2 complex may certainly contribute to this signaling pathway.

Considering the ubiquitous expression of EGFR in various types of cells, particularly in cancer cells, transactivation of EGFR by TNF may play very important roles in the activation of NF-κB in physiological and pathophysiological scenarios. Further studies on the molecular mechanism of this phenomenon may lead to a better understanding of the TNF signaling pathway. In particular, it may be very interesting to determine whether TNF-induced transactivation of EGFR involves proteolytic cleavage of membrane-bound EGFR ligands such as HB-EGF in the case of G protein-coupled receptor ligands including carbachol, bombesin, and thrombin (50). Moreover, it also seems interesting and significant to determine whether the transactivation potentiates other signaling pathways downstream of TNF such as MAPKs activation.

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REFERENCES

1. Vandenabeele, P., Declerck, W., Beyeart, R., and Fiers, W. (1995) Trends Cell Biol., 5, 392–399
2. Karin, M. (1999) J. Biol. Chem., 274, 27339–27342
3. Liu, Z.-G., Hsu, H., Goeddel, D. V., and Karin, M. (1996) Cell 87, 565–576
4. Cao, Z., Xiong, J., Takeuchi, M., Kurama, T., and Goeddel, D. V. (1996) Nature 383, 443–446
5. Malinin, N. L., Boldin, M. P., Kevalenko, A. V., and Wallach, D. (1997) Nature 385, 540–544
6. Zandi, E., Rothbard, D. M., Delhase, M., Hayakawa, M., and Karin, M. (1997) Cell 91, 243–252
7. Mercier, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. R., Barbosa, M., Mann, M., Manning, A., and Ren, A. (1997) Science 278, 860–866
8. Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Aoug, F., Kirk, H. E., Kay, R. J., and Israel, A. (1998) Cell 80, 1231–1240
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9. Werniecz, J. D., Gao, X., Cao, Z., Rothe, M., and Goeddel, D. V. (1997) Science 278, 866–869
10. Lee, S. E., Peters, R. T., Dang, L. C., and Maniatis, T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9319–9324
11. Minden, A., Lin, A., Clarett, F. X., Abo, A., and Karin, M. (1995) Cell 81, 1147–1157
12. Ichijo, H., Nishida, E., Irie, K., ten Dijke, P., Saitoh, M., Morimichi, T., Takagi, M., Matsumoto, K., Miyazono, K., and Gotoh, Y. (1997) Science 275, 90–94
13. Yao, Z., Diener, K., Wang, X. Z., Zukowski, M., Matsumoto, G., Zhou, G., Ma, R., Sasaki, T., Nishina, H., Hui, C. C., Tan, T. H., Woodgett, J. R., and Penninger, J. M. (1997) J. Biol. Chem. 272, 32378–32383
14. Ozes, O. N., Mayo, L. D., Gustin, J. A., Pfeffer, S. R., Pfeffer, L. M., and Donner, D. B. (1999) Nature 401, 82–85
15. Romashkova, J. A., and Makarov, S. S. (1999) Annu. Rev. Biochem. 68, 1–29
16. Carpenter, G. (1987) J. Biol. Chem. 262, 16433–16436
17. Carpenter, G. (1999) EMBO J. 18, 7632–7644
18. Daub, H., Weiss, F. U., Wallasch, C., and Ullrich, A. (1996) Nature 379, 557–560
19. Rosette, C., and Karin, M. (1996) Science 274, 1194–1197
20. Hirota, K., Matsui, M., Iwata, S., Nishiyama, A., Mori, K., and Yodoi, J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3633–3638
21. Shinkura, R., Kitada, K., Matsuda, F., Tashiro, K., Ikuta, K., Suzuki, M., Kogishi, K., Serikawa, T., and Honjo, T. (1999) Nat. Genet. 22, 74–77
22. Hirota, K., Murata, M., Sachi, Y., Nakamura, H., Takeuchi, J., Mori, K., and Yodoi, J. (1999) J. Biol. Chem. 274, 27881–27887
23. Hirota, K., Murata, M., Itoh, T., Yodoi, J., and Fukuda, K. (2001) FEBS Lett. 489, 134–138
24. Hirota, K., Matsui, M., Murata, M., Takashima, Y., Chen, F. S., Ichihashi, T., Fukuda, K., and Yodoi, J. (2002) Biochem. Biophys. Res. Commun. 274, 177–182
25. Takeuchi, H., Hirota, K., Ichihashi, T., Shinkura, K., Kitada, K., Yodoi, J., Nambara, T., and Fukuda, K. (2000) Antioxid. Redox Signal. 2, 83–92
26. Oka, T., Hirota, K., Yudoh, K., Katsuda, H., Hikasa, T., Kato, H., Miyata, M., and Tanaka, H. (1996) Biochem. Biophys. Res. Commun. 224, 27–32
27. Sun, L., and Carpenter, G. (1998) Oncogene 16, 2095–2102
28. Izumi, H., Ono, M., Ushiro, S., Kung, H.-F., and Kuwano, M. (1994) Exp. Cell Res. 214, 564–566
29. Van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Annu. Rev. Cell Biol. 10, 251–337
30. Barbier, A. J., Poppleton, H. M., Yigzaw, Y., Mullenix, J. B., Weitz, G. J., Bertics, P. J., and Patel, T. B. (1999) J. Biol. Chem. 274, 14067–14073
31. Carpenter, G. (1990) J. Biol. Chem. 265, 7709–7712
32. Carpenter, G. (2000) BioEssays 22, 697–707
33. Li, N., and Karin, M. (1999) FEBS Lett. 443, 1137–1143
34. Finkel, T. (1999) FEBS Lett. 461, 248–253
35. Finkel, T. (2000) FASEB J. 14, 51–54
36. Lee, S.-R., Kwon, K.-S., Kim, S.-R., and Rhee, S. G. (1997) J. Biol. Chem. 272, 15366–15372
37. Kamata, H., Shibukawa, Y., Oka, S.-L., and Hirata, H. (2000) Eur. J. Biochem. 267, 1933–1944
38. Wang, X., McCullough, K. D., Franke, T. F., and Holbrook, N. J. (2000) J. Biol. Chem. 275, 14624–14631
39. Devary, Y., Gottlieb, R. A., Smeal, T., and Karin, M. (1992) Cell 71, 1081–1091
40. Gamou, S., and Shimizu, N. (1995) FEBS Lett. 357, 161–164
41. Nakamura, H., Nakamura, K., and Yodoi, J. (1997) Annu. Rev. Immunol. 15, 351–369
42. Liu, H., Nishihara, H., Ichijoh, H., and Kyriakis, J. M. (2000) Mol. Cell. Biol. 20, 2198–2208
43. Sundaresan, M., Yu, Z.-X., Ferrans, V. J., Irani, K., and Finkel, T. (1995) Science 270, 296–299
44. Sulciner, D. J., Iian, Y., Yu, Z.-X., Ferrans, V. J., Goldschmidt-Clermont, P., and Finkel, T. (1996) Mol. Cell. Biol. 16, 7155–7121
45. Irani, K., Xia, Y., Zweier, J. L., Soliot, S. J., Der, C. J., Fearon, E. R., Sundaresan, M., Finkel, T., and Goldschmidt-Clermont, P. J. (1997) Science 275, 1649–1652
46. Bae, Y. S., Kang, S. W., Lee, M. S., Baines, I. C., Tekle, E., Chock, P. B., and Rhee, S. G. (1997) J. Biol. Chem. 272, 217–221
47. Kang, S. W., Chae, H. Z., Lee, M. S., Kim, K., Baines, I. C., and Rhee, S. G. (1998) J. Biol. Chem. 273, 6297–6302
48. Finkel, T. (1996) BioEssays 18, 633–636
49. Finkel, T. (1998) FEBS Lett. 434, 83–92
50. Preznel, N., Zwick, E., Daub, H., Leser, M., Abraham, R., Wallasch, C., and Ullrich, A. (1999) Nature 402, 884–888
