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Homocysteine and α-Lipoic Acid Regulate p44/42 MAP Kinase Phosphorylation in NIH/3T3 Cells

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ABSTRACT

Biological thiols can regulate cell signal transduction. The effects of two biothiols, homocysteine (Hcy), a risk factor for cardiovascular disease, and α-lipoic acid (αLA), a therapeutic antioxidant, on p44/42 mitogen-activated protein kinases (MAPK) phosphorylation were examined in NIH/3T3 fibroblasts. Cells grown in serum-containing media had constitutive levels of MAPK phosphorylation as determined by Western blot analysis using the phospho-specific MAPK antibody. Treatment of cells with 20 μM Hcy for 0–60 min resulted in a transient enhancement of MAPK phosphorylation. In contrast, 20 μM αLA inhibited serum-mediated phosphorylation of MAPK. The differential effects of these two thiols are not due to their redox states as oxidized Hcy (Hcy thiolactone) enhanced MAPK phosphorylation. The effect of αLA appears to be serum-dependent because Hcy or αLA treatment of serum-deprived cells activated MAPK phosphorylation. Thus, αLA and Hcy can either induce common signal transduction pathways or differentially modulate MAPK phosphorylation, depending on the state of the cell. This relationship may be important to understand how some biothiols are associated with pathogenic events while others offer potential as therapeutic agents. Antiox. Redox Signal. 1, 123–128.

INTRODUCTION

THIOL-CONTAINING BIOLOGICAL MOLECULES play various roles in physiology. Homocysteine (Hcy) can act as a pro-oxidant and is a putative pathogenic agent in cardiovascular disease. Glutathione and α-lipoic acid (αLA) serve as antioxidants and appear useful in therapeutic interventions. Mechanisms by which various biothiols elicit differential actions in cells are not understood.

Hcy, generated during the metabolism of methionine, appears to be involved in the pathogenesis of arteriosclerosis (McCully, 1996). Hereditary hyperhomocysteinemia (homocystinuria; >100 μM plasma Hcy) is associated with defects of cystathionine synthase, Hcy methyltransferase and methylenetetrahydrofolate reductase, and the rapid development of arteriosclerosis. Moderate hyperhomocysteinemia (≥14 μM plasma Hcy) is recognized as an independent risk factor for cardiovascular disease (Clarke *et al.*, 1991; Selhub *et al.*, 1995). It has been postulated that the production of reactive oxygen species (ROS) may be involved in the pathogenesis of Hcy-

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mediated disease (Olszewski and McCully, 1993). Hcy promotes the proliferation of vascular smooth muscle cells (Tsai *et al.*, 1994; Dalton *et al.*, 1997), an action that may be mediated via production of ROS (Sundaresan *et al.*, 1995; Irani *et al.*, 1997).

αLA is essential in mitochondrial dehydrogenase reactions catalyzing the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate, and branched chain α-keto acids. αLA and its reduced form dihydrolipoic acid (DHLA) appear also to serve as biological antioxidants. DHLA has been shown to prevent microsomal lipid peroxidation (Bast and Haenen, 1988; Scholich *et al.*, 1989) and αLA and DHLA scavenge hydroxyl radicals, although only DHLA reacts with superoxide (Suzuki *et al.*, 1991). Therefore, αLA, either directly or via reduction to DHLA, may act as an antioxidant. αLA is employed therapeutically in diabetes and other diseases in which the pathogenic role of oxidative stress has been documented (Packer *et al.*, 1995).

p44 and p42 mitogen-activated protein kinases (MAPK), also referred to as extracellular signal-regulated kinases (ERK) 1 and 2 play important roles in cell growth, apoptosis, and migration (Davis, 1993). Various growth factors, cytokines, and hormones elicit a cascade of events that involve phosphorylation and activation of MAPK. We have examined the influence of Hcy and αLA on MAPK phosphorylation in a fibroblast cell line.

MATERIALS AND METHODS

Cell culture

Stable transfectants of NIH/3T3 cells (gift from Dr. Marcio Chedid, National Cancer Institute) with control pCEV29 vector or constitutively active mutant of $p21^{ras}$ (Lys substituted for Gly at amino acid 12) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, gentamicin (0.5 mg/ml), and geneticin (0.25 mg/ml) in an atmosphere of 5% CO_2 at 37°C in humidified air. Cells grown at 80–90% confluency were used for experiments. D,L-Homocysteine, L-homocysteine thiolactone, *R,S*-α-lipoic acid, and L-ascorbic acid (vitamin C) (Sigma Chemical Company, St. Louis, MO) were dissolved in DMEM to make 0.2 m*M* stock solutions within 1 hr of being used for experiments.

Western blot analysis

Cells were solubilized with 50 m*M* HEPES solution pH 7.4 containing 95 m*M* sodium fluoride, 2.7 m*M* sodium orthovanadate, 10 m*M* tetrasodium pyrophosphate, 4 m*M* EDTA, 1% Triton X-100, 0.35 mg/ml phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, and 10 μg/ml aprotinin (Sigma Chemical). Following centrifugation in a microfuge, protein concentrations were determined in supernatants using the method described by Bradrod (1976). Cell lysates (10 μg protein) were electrophoresed through a reducing (5% β-mercaptoethanol) 10% SDS polyacrylamide gel and electroblotted onto a nitrocellulose membrane. The membrane was incubated with the rabbit polyclonal immunoglobulin G (IgG) for phosphorylated p44/42 MAPK (New England Biolabs, Beverly, MA) or p44 MAPK protein (Santa Cruz Biotechnology, Santa Cruz, CA). The detection was made with horseradish peroxidase (HRP)-linked secondary antibody using the ECL system (Amersham Life Science, Arlington Heights, IL). Experiments were repeated at least six times to confirm the results.

Electrophoretic mobility shift assays

To prepare nuclear extracts, cells were washed in phosphate-buffered saline (PBS) and incubated in 10 m*M* HEPES pH 7.8, 10 m*M* KCl, 2 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.1 m*M* PMSF, and 5 μg/ml leupeptin. IGEPAL CA-630 (Sigma Chemical) was then added at a final concentration of 0.6% (vol/vol), mixed vigorously, and centrifuged. Pelleted nuclei were resuspended in 50 m*M* HEPES pH 7.8, 50 m*M* KCl, 300 m*M* NaCl, 0.1 m*M* EDTA, 0.1 m*M* PMSF, and 10% (vol/vol) glycerol, then mixed and centrifuged. The supernatant was harvested and protein concentrations determined. Binding-reaction mixtures contained 2 μg of protein of nuclear extract, 1 μg poly(dI-dC)·poly(dI-dC) (Pharmacia Biotech, Piscataway, NJ), and ^{32}P-labeled double-stranded oligonucleotide containing the serum response element [5′-GGA TGT CCA TAT TAG GAC

ATC T-3'] in 50 m*M* NaCl, 0.2 m*M* EDTA, 0.5 m*M* DTT, 10% (vol/vol) glycerol, and 10 m*M* Tris-HCl pH 7.5. Electrophoresis of samples through a native 6% polyacrylamide gel was followed by autoradiography. Supershift experiments were performed with the rabbit polyclonal IgG for serum response factor (Santa Cruz Biotechnology).

High-pressure liquid chromatography analysis of ascorbate and dehydroascorbate

Cellular ascorbate was determined by the reverse-phase high-pressure liquid chromatography (HPLC) (Okamura, 1980). Cells were washed with cold Hank's balanced salt solution (HBSS) and scraped into 2% metaphosphoric acid (MPA) solution. The sample was sonicated, centrifuged, and 20 μl of supernatant was injected onto a C-18 column (Microsorb-MV, Rainin). Ascorbic acid was eluted isocratically with a mobile phase containing 30 m*M* sodium acetate, 0.26 m*M* EDTA, and 0.025% octylamine pH 5.0 and measured at 0.6 V with electrochemical detection. Dehydroascorbate was measured after mixing the supernatant with phosphate buffer pH 8.0 containing 1.5 mg/ml dithiothreitol (DTT). The difference in peak area between the reduced and nonreduced samples reflects dehydroascorbate content.

RESULTS

Cells grown in DMEM containing 10% FBS exhibited constitutive levels of tyrosine phosphorylation of both p44 (ERK-1) and p42 (ERK-2) MAPK at amino acid position 204 (Sturgill *et al.*, 1988) as shown in the representative Western blot in Fig. 1. Treatment of cells with Hcy (20 μ*M*) for 0–60 min caused transient enhancement of p44/42 MAPK phosphorylation with the peak level of activation observed at 10 min. The protein levels of p44/42 MAPK did not change in response to Hcy treatment as illustrated by experiments using the antibody for p44 MAPK protein which also cross-reacts with p42 MAPK.

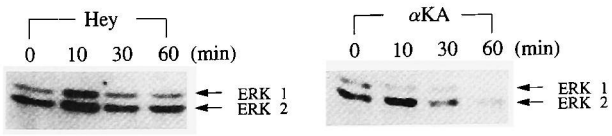

FIG. 1. Hcy enhances but αLA inhibits p44/42 MAPK phosphorylation in cells grown in serum containing media. NIH/3T3 cells grown in DMEM containing 10% FBS were treated for 0–60 min with Hcy (20 μ*M*) or αLA (20 μ*M*). Cell lysates were subjected to immunoblot analysis using the antibody for phospho-p44/42 MAPK (ERK1/2). The protein levels of p44/42 MAPK did not change in response to Hcy treatment as determined by using the antibody which reacts with p44/42 MAPK proteins (data not shown).

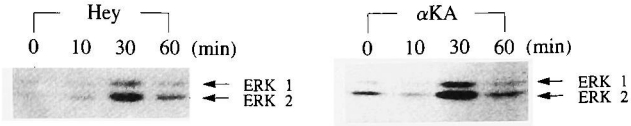

FIG. 2. Hcy and αLA induce phosphorylation of p44/42 MAPK in serum-deprived cells. NIH/3T3 cells were serum starved for 18 hr and treated for 0–60 min with Hcy (20 μ*M*) or αLA (20 μ*M*). Cell lysates were subjected to immunoblot analysis using the antibody for phospho-p44/42 MAPK (ERK 1/2). The protein levels of p44/42 MAPK did not change in response to Hcy or αLA treatment as determined by using the antibody which reacts with p44/42 MAPK (data not shown).

In contrast to stimulatory effects of Hcy on p44/42 MAPK phosphorylation, αLA inhibited serum-dependent p44/42 MAPK phosphorylation. Significant inhibition was detected by 30 min and phosphorylation levels were undetectable by 60 min (Fig. 1). The protein levels of p44/42 MAPK did not change in response to αLA treatment. The differential actions of Hcy and αLA on p44/42 MAPK phosphorylation are not due to their redox states because treatment of cells with the oxidized form of Hcy, Hcy thiolactone (20 μ*M*) caused a transient enhancement of serum-dependent p44/42 MAPK phosphorylation.

Serum-deprived cells are often employed in investigations of growth factor-mediated p44/42 MAPK activation to eliminate basal background signals and to synchronize cells into a quiescent state (York *et al.*, 1998). Incubation of serum-deprived NIH/3T3 cells with Hcy (20 μ*M*) resulted in phosphorylation of p44/42 MAPK (Fig. 2). Phosphorylation of p44/42 MAPK by Hcy was transient with the peak level of activation achieved at 30 min. Similar to the action of Hcy, αLA (20 μ*M*) induced activation of p44/42 MAPK phosphorylation in serum-deprived cells (Fig. 2). The kinetics of αLA-mediated p44/42 MAPK acti-

vation was also transient with maximal activation occurring at 30 min. The protein levels of p44/42 MAPK did not change in response to Hcy or αLA treatment.

Ras GTPase-activated cells (Macara *et al.*, 1996) was employed to study the nature of the serum-stimulated cells in which differential influences of Hcy and αLA were observed. Serum-deprived NIH/3T3 cells expressing constitutively active $p21^{ras}$ mutant (lysine substituted for glycine at amino acid 12) had constitutive levels of phosphorylated p44/42 MAPK (Fig. 3). Although both Hcy and αLA slowly increased MAPK phosphorylation, effects exerted by the two thiols in cells grown in serum, particularly inhibition by αLA, were not observed in active Ras mutant-expressing cells.

Activation of p44/42 MAPK has been shown to elicit stimulation of transcription factors including serum response factor (SRF) (Leaman *et al.*, 1996). A significant constitutive level of SRF DNA binding activity was observed without Hcy treatment in cells grown in serum. This serum-dependent SRF activity was further enhanced in a dose-response fashion by 20 and 100 μ*M* Hcy (Fig. 4A). αLA (20 or 100 μ*M*) did not enhance SRF activity (data not shown).

Cells expressing active $p21^{ras}$ mutant also have constitutive SRF activity, even in the absence of serum (Fig. 4B), indicating the Ras-dependent activation of SRF. In contrast to the action of Hcy in serum-stimulated cells, treatment of active Ras mutant expressing cells with Hcy (20 μ*M*) inhibited Ras-dependent SRF activity by 30 min, and effects sustained up to 60 min. αLA had no effect on SRF in Ras-activated cells.

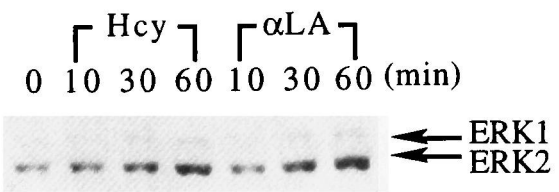

FIG. 3. Effects of Hcy and αLA on p44/42 MAPK phosphorylation in cells expressing active $p21^{ras}$ mutant. NIH/3T3 cells stably transfected with the gene for constitutively active mutant of $p21^{ras}$ (Lys-12) were serum starved for 18 hr and treated for 0–60 min with Hcy (20 μ*M*) or αLA (20 μ*M*). Cell lysates were subjected to immunoblot analysis using the antibody for phospho-p44/42 MAPK (ERK 1/2). The protein levels of p44/42 MAPK did not change in response to Hcy or αLA treatment as determined by using the antibody which reacts with p44/42 MAPK (data not shown).

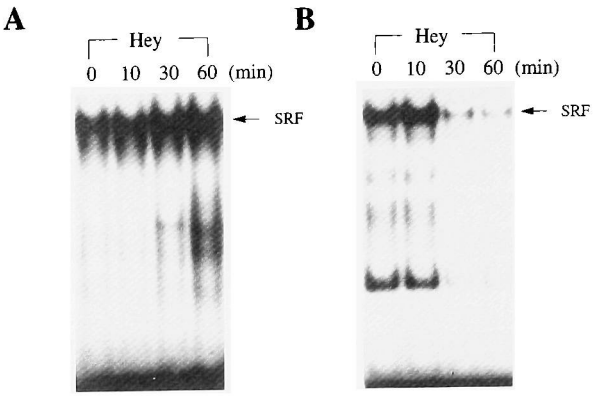

FIG. 4. Effects of Hcy on SRF activity. A. NIH/3T3 cells grown in DMEM containing 10% FBS were treated for 0–60 min with Hcy (100 μ*M*) **B.** NIH/3T3 cells stably transfected with the gene for constitutively active mutant of $p21^{ras}$ were serum starved for 18 hr and treated for 0–60 min with Hcy (20 μ*M*). Nuclear extracts were subjected to electrophoretic mobility shift assays. Supershift experiments using the SRF antibody confirmed the serum response element binding protein was SRF (data not shown).

Vitamin C (20 μ*M*) also inhibited SRF activity in cells expressing constitutively active $p21^{ras}$ (data not shown). However, the percent dehydroascorbate of total vitamin C detected by HPLC after vitamin C treatment was similar in control (20.0 ± 6.3%, $n = 4$) and constitutively active mutant $p21^{ras}$ expressing cells (19.7 ± 3.0%, $n = 4$).

DISCUSSION

In serum-deprived cells, Hcy and αLA both elicited the phosphorylation of p44/42 MAPK. However, in cells grown in serum-containing media, 20 μ*M* Hcy stimulated, but 20 μ*M* αLA inhibited p44/42 MAPK phosphorylation. These differential actions of Hcy and αLA are not due to differences in redox states of the thiols because the oxidized Hcy thiolactone enhanced the phosphorylation. Further, the differential effects do not appear to be cell cycle-dependent (Newberry and Pike, 1995) because both Hcy and αLA slowly increased MAPK phosphorylation in Ras-activated cells.

Hcy, at concentrations found in individuals with moderate hyperhomocysteinemia, acti-

vating p44/42 MAPK phosphorylation may have relevance to its role as a risk factor for cardiovascular disease. The inductive action of Hcy on signal transduction may be related to its capacity to promote vascular smooth muscle cell proliferation, a critical step in arteriosclerosis (Tsai *et al.*, 1994). Because SRF participates in the regulation of c-*fos* gene transcription (Leaman *et al.*, 1996), Hcy-mediated phosphorylation of p44/42 MAPK and subsequent activation of SRF may also be relevant to signaling for c-*fos* gene expression and cell proliferation (Dalton *et al.*, 1997).

αLA is employed as a therapeutic antioxidant in treating diabetes and other conditions associated with elevated oxidative stress status (Packer *et al.*, 1995). Inhibition of NF-κB-dependent gene expression by αLA (Suzuki *et al.*, 1992) may be related to its efficacy in preventing blood cell adhesion to vascular endothelium (Roy *et al.*, 1998). Serum-dependent inhibition of p44/42 MAPK by αLA also suggests this biothiol may be useful in attenuating pathogenic cell growth and apoptosis. Our results with active Ras transfectants suggest the target of αLA inhibition of serum-dependent MAPK phosphorylation may reside upstream of Ras. Alternatively, serum-αLA interactions may generate inhibitors of MAPK phosphorylation.

Hcy activated SRF in control NIH/3T3 cells, but inhibited SRF DNA binding activity induced by transfection of constitutively active mutant of $p21^{ras}$. NIH/3T3 cells expressing active mutant of $p21^{ras}$ exhibit elevated mitogenic activity that can be inhibited by antioxidants (Irani *et al.*, 1997). Similarly, vitamin C inhibited SRF activity in cells expressing constitutively active $p21^{ras}$ although, as dehydroascorbate levels were not different from control cells, ROS may not be involved in Ras-dependent SRF activation. The mechanism of Hcy and vitamin C actions may be analogous to antioxidant-inhibition of signal transduction for NF-κB activation that is independent of ROS scavenging properties (Anderson *et al.*, 1994; Suzuki *et al.*, 1994).

In contrast to Irani *et al.* (1997), we found serum-deprived fibroblasts expressing a constitutively active $p21^{ras}$ mutant have higher basal levels of p44/42 MAPK than controls. This observation is more consistent with the well-accepted mechanism where $p21^{ras}$ activates p44/42 MAPK (Leaman *et al.*, 1996). Furthermore, Ras-dependent signaling events elicited by the constitutively active point mutant appear distinct from those induced by serum.

Antioxidants appear to reduce the risk of many chronic diseases by preventing ROS-induced damage to DNA, proteins, and lipids (Halliwell and Gutteridge, 1985). Although ROS can inhibit many elements of cell function, the capacity of ROS to stimulate signal transduction processes (Suzuki *et al.*, 1997) may also be relevant to their pathogenic mechanisms of action. Irani *et al.* (1997) found that ROS induce cell growth by serving as signaling molecules and suggested that antioxidants prevent abnormal cell growth by eliminating oxidant signals. However, this hypothesis does not account for the actions of antioxidants that elicit signal transduction events such as the Ras-ERK-SRF pathway described by Müller *et al.* (1997). We report here that pro- and antioxidant biothiols can induce common signal transduction pathways or act to modulate cell signal transduction differentially depending upon the state of the cell.

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REFERENCES

ANDERSON, M.T., STAAL, F.J.T., GITLER, C., and HERZENBERG, L.A. (1994). Separation of oxidant-initiated and redox-regulated steps in the NF-κB signal transduction pathway. Proc. Natl. Acad. Sci. USA **91,** 11527–11531.

BAST, A., and HAENEN, G.R.M.M. (1988). Interplay between lipoic acid and glutathione in the protection

against microsomal lipid peroxidation. Biochim. Biophys. Acta **963,** 558–561.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248–254.

CLARKE, R., DALY, L., ROBINSON, K., NAUGHTEN, E., CAHALANE, S., FOWLER B., and GRAHAM, I. (1991). Hyperhomocysteinemia: An independent risk factor for vascular disease. New Engl. J. Med. **324,** 1149–1155.

DALTON, M.L., GADSON JR., P.F., WRENN, R.W., and ROSENQUIST, T.H. (1997). Homocysteine signal cascade: Production of phospholipids, activation of protein kinase C, and the induction of c-fos and c-myb in smooth muscle cells. FASEB J. **11,** 703–711.

DAVIS, R.J. (1993). The mitogen-activated protein kinase signal transduction pathway. J. Biol. Chem. **268,** 14553–14556.

HALLIWELL, B., and GUTTERIDGE, J.M.C. (1985). *Free Radicals in Biology and Medicine.* (Clarendon Press, Oxford).

IRANI, K., XIA, Y., ZWEIER, J.L., SOLLOTT, S.J., DER, C.J., FEARON, E.R., SUNDARESAN, M., FINKEL, T., and GOLDSCHMIDT-CLERMONT, P.J. (1997). Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science **275,** 1649–1652.

LEAMAN, D.W., LEUNG, S., LI, X., and STARK, G.R. (1996). Regulation of STAT-dependent pathways by growth factors and cytokines. FASEB J. **10,** 1578–1588.

MACARA, I.G., LOUNSBURY, K.M., RICHARDS, S.A., MCKIERNAN, C., and BAR-SAGI, D. (1996). The Ras superfamily of GTPases. FASEB J. **10,** 625–630.

MCCULLY, K.S. (1996). Homocysteine and vascular disease. Nature Med. **2,** 386–389.

MÜLLER, J.M., CAHILL, M.A., RUPEC, R.A., BAEUERLE, P.A., and NORDHEIM, A. (1997). Antioxidants as well as oxidants activate c-*fos* via Ras-dependent activation of extracellular-signal-regulated kinase 2 and Elk-1. Eur. J. Biochem. **244,** 45–52.

NEWBERRY, E.P., and PIKE, L.J. (1995). Cell-cycle-dependent modulation of EGF-receptor-mediated signaling. Biochem. Biophys. Res. Commun. **208,** 253–259.

OKAMURA, M. (1980). An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. Clin. Chim. Acta **103,** 259–268.

OLSZEWSKI, A.J., and MCCULLY, K.S. (1993). Homocysteine metabolism and the oxidative modification of proteins and lipids. Free Rad. Biol. Med. **14,** 683–693.

PACKER, L., WITT, E.H., and TRITSCHLER, H.J. (1995). Alpha-lipoic acid as a biological antioxidant. Free Rad. Biol. Med. **19,** 227–250.

ROY, S., SEN, C.K., KOBUCHI, H., and PACKER, L. (1998). Antioxidant regulation of phorbol ester-induced adhesion of human Jurkat T-cells to endothelial cells. Free Rad. Biol. Med. **25,** 229–241.

SCHOLICH, H., MURPHY, M.E., and SIES, H. (1989). Antioxidant activity of dihydrolipoate against microsomal lipid peroxidation and its dependence on α-tocopherol. Biochim. Biophys. Acta **1001,** 256–261.

SELHUB, J., JACQUES, P.F., BOSTOM, A.G., D'ANGOSTINO, R.B., WILSON, P.W.F., BELANGER, A.J., O'LEARY, D.H., WOLF, P.A., SCHAEFER, E.J., and ROSENBERG, I.H. (1995). Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. New Engl. J. Med. **332,** 286–291.

STURGILL, T.W., RAY, L.B., ERIKSON, E., and MALLER, J.L. (1988). Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. Nature **334,** 715–718.

SUNDARESAN, M., YU, Z.X., FERRANS, V.J., IRANI, K., and FINKEL, T. (1995). Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. Science **270,** 296–299.

SUZUKI, Y.J., TSUCHIYA, M., and PACKER, L. (1991). Thioctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species. Free Rad. Res. Comms. **15,** 255–263.

SUZUKI, Y.J., AGGARWAL, B.B., and PACKER, L. (1992). αLipoic acid is a potent inhibitor of NF-κB activation in human T cells. Biochem. Riophys. Res. Commun. **189,** 1709–1715.

SUZUKI, Y.J., MIZUNO, M., and PACKER, L. (1994). Signal transduction for nuclear factor-κB activation. Proposed location of antioxidant-inhibitable step. J. Immunol. **153,** 5008–5015.

SUZUKI, Y.J., FORMAN, H.J., and SEVANIAN, A. (1997). Oxidants as stimulators of signal transduction. Free Rad. Biol. Med. **22,** 269–285.

TSAI, J.-C., PERRELLA, M.A., YOSHIZUMI, M., HSIEH, C.-M., HABER, E., SCHLEGEL, R., and LEE, M.-E. (1994). Promotion of vascular smooth muscle cell growth by homocysteine: A link to atherosclerosis. Proc. Natl. Acad. Sci. USA **91,** 6369–6373.

YORK, R.D., YAO, H., DILLON, T., ELLIG, C.L., ECKERT, S.P., MCCLESKEY, E.W., and STORK, P.J.S. (1998). Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. Nature **392,** 622–626.

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2. Kate Petersen Shay, Régis F. Moreau, Eric J. Smith, Anthony R. Smith, Tory M. Hagen. 2009. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1790**:10, 1149-1160. [CrossRef]
3. Yuichiro J. Suzuki, Vivek Jain, Ah-Mee Park, Regina M. Day. 2006. Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. *Free Radical Biology and Medicine* **40**:10, 1683-1692. [CrossRef]
4. K. Robert, C. Pagès, A. Ledru, J. Delabar, J. Caboche, N. Janel. 2005. Regulation of extracellular signal-regulated kinase by homocysteine in hippocampus. *Neuroscience* **133**:4, 925-935. [CrossRef]
5. Kazumi Kitta , Regina M. Day , Jane Remeika , Jeffrey B. Blumberg , Yuichiro J. Suzuki . 2001. Effects of Thiol Antioxidants on Hepatocyte Growth Factor Signaling in Cardiac Myocytes. *Antioxidants & Redox Signaling* **3**:5, 911-918. [Abstract] [Full Text PDF] [Full Text PDF with Links]