Signal transduction of reactive oxygen species and mitogen-activated protein kinases in cardiovascular disease

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Abstract : Reactive oxygen species (ROS), generated by reduction-oxidation (redox) reactions, have been recognized as important chemical mediators that regulate signal transduction. It has been reported that increase in ROS generation may relate to a risk for cardiovascular diseases such as atherosclerosis, angina pectoris, and myocardial infarction. Therefore, understanding the ROS-generating biological processes and ROS-induced intracellular signaling will be informative to gain insights into the pathogenesis of these diseases. In this review, we focus on the sources and reactions of ROS in the cardiovascular system and the role of mitogen-activated protein (MAP) kinase pathway in redox-mediated signal transduction. Clinical implications of ROS and MAP kinase are then described to provide insight into the pathogenesis of various redox-sensitive cardiovascular diseases. The pathways responsible for ROS generation in the cardiovascular system may provide novel therapeutic targets. J. Med. Invest. 48 : 11-24, 2001

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INTRODUCTION

Signal transduction is an event of conversion of signals from extracellular stimuli to intracellular responses that lead to change in gene expressions and cellular phenotypic modulations. Reactive oxygen species (ROS), generated by a variety of extracellular and intracellular mechanisms, have gained attention as novel signal mediators that regulate signal transduction events. Accumulating evidence suggests that ROS may be relevant to various cardiovascular diseases (1-3). In this review, we focused on the generation of ROS in cardiovascular systems and discussed ROS-mediated cell signaling, especially the role of mitogen-activated protein (MAP) kinases, in cardiovascular diseases.

ROS has been implicated as a major cause for the pathogenesis of myocardial ischemia and reperfusion injury (4, 5). ROS including hydrogen peroxide (H_2O_2), superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and peroxynitrite ($ONOO^-$) have been shown to increase upon reperfusion of the heart following ischemia (5-7). In ischemia and reperfused hearts, many alterations such as contractile dysfunction, arrhythmias, and changes in gene expression have been observed (8, 9). These alterations in the myocardium during ischemia-reperfusion were suggested to be, in part, due to oxidative stress. Clinically, antioxidants are believed to counteract with ROS and reduce the incidence of coronary artery disease (10). Intake of vitamin E decreased the incidence of cardiovascular events in the population of ischemic heart disease in the Cambridge Heart Antioxidant Study (CHAOS) (11). In addition, a multivitamins and probucol (MVP) trial revealed that an antioxidant, probucol was effective for prevention of restenosis after percutaneous transluminar coronary angioplasty (PTCA) procedure (12).

Based on the above studies, it is suggested that ROS may also be pathogenic for vascular diseases

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such as atherosclerosis and restenosis. *In vivo* experiments revealed that the generation of $O_2^{\bullet -}$ increased in aortas from hyperlipidemic rabbits and coronary arteries from balloon-injured pigs compared with control vessels (13). It was also reported that administration of vitamins C and E decreased $O_2^{\bullet -}$ production from injured vessels (14). It is now apparent that change in the redox (reduction-oxidation) state is involved in the pathogenesis of various cardiovascular diseases.

SOURCES AND REACTIONS OF ROS

One of the major sources for ROS is mitochondria in cells. Mitochondrial respiration converts carbohydrates into high-energy metabolites such as adenosine triphosphate (ATP). This requires the sequential oxidation and reduction of the substrates using respiratory complexes. After oxidation by mitochondrial and plasma membrane oxidases, oxygen is reduced and the superoxide radical $O_2^{\bullet -}$ is formed (Fig. 1). Endoplasmic (sarcoplasmic) reticulum and nuclear membranes have also been shown to produce $O_2^{\bullet -}$ by a reaction of oxidative phosphorylation. The cell's endogenous defense system is dismutation of $O_2^{\bullet -}$ by superoxide dismutase (SOD) to produce hydrogen peroxide, H_2O_2. In biological systems, H_2O_2 is less reactive than $O_2^{\bullet -}$ and is metabolized by catalase or by peroxidase to form O_2 and H_2O (Fig. 1). Other important sources of ROS within cells are the metabolic by-products from biogenic cascade of arachidonic acid by cyclooxygenase, lipoxygenase, and cytochrome p-450 monooxygenase. Xanthine oxidase and NADH/NADPH oxidase are also $O_2^{\bullet -}$ generating enzymes in cells. Xanthine oxidase is converted from xanthine dehydrogenase and mediates nucleotides metabolism, such as xanthine, to form $O_2^{\bullet -}$ and uric acid. NADH/NADPH oxidase is a multi-subunit enzyme that may be a major source of $O_2^{\bullet -}$ generation in the vasculature. The role of NADH/NADPH oxidase in vascular diseases will be discussed below. Two other ROS that would be important in vascular injury are hydroxyl radical ($^{\bullet}OH$) and peroxynitrite ($ONOO^-$) (Fig. 1). In the presence of nitric oxide (NO), $O_2^{\bullet -}$ reacts more rapidly with NO than with SOD, to form $ONOO^-$. $ONOO^-$ readly reacts with tyrosine to form nitrotyrosine which may impair the vascular responses to stimuli. $^{\bullet}OH$ is a very reactive ROS that does not exist in large amounts under physiological conditions. However, under the presence of iron which may be released from red blood cells, $^{\bullet}OH$ would be generated via the Fenton reaction (or Haber-Weiss reaction) from H_2O_2.

ROS-MEDIATED MAP KINASE SIGNALING

ROS mediate activation of MAP kinases in a variety of cells leads to changes in gene expressions. MAP kinases are serine and threonine protein kinases that are activated by various extracellular stimuli and are encoded by a multigene family (Fig. 2) (15). MAP kinases are activated by phosphorylation on

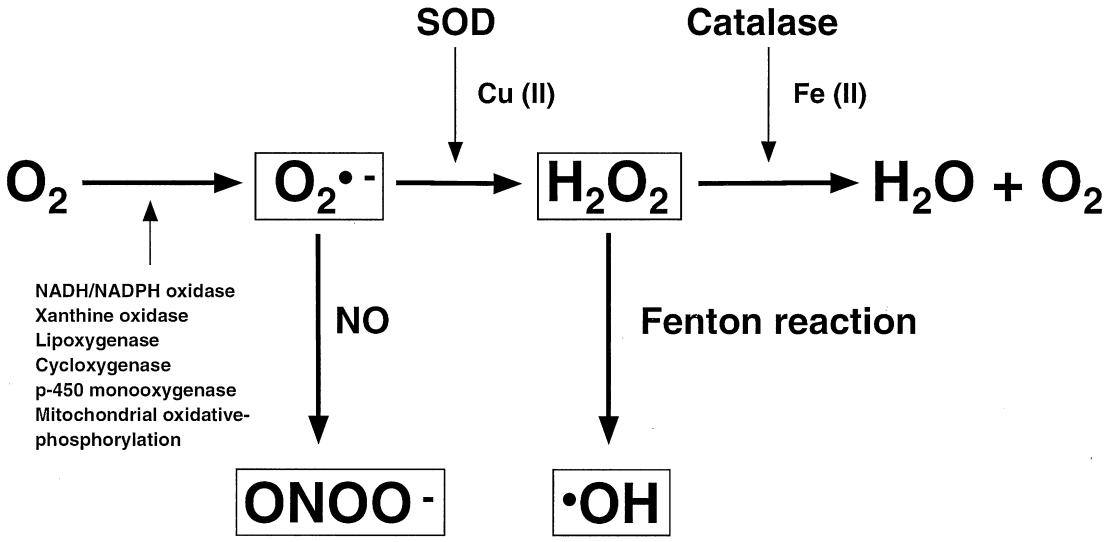

Fig.1. Sources of reactive oxygen species (ROS) generated endogenously by cardiovascular cells and key metabolic pathways for these species. Multiple enzymes may induce ROS generation in cardiovascular cells, these include NADH/NADPH oxidase, xanthine oxidase, lipoxygenase, cyclooxygenase, p-450 monooxygenase, and the enzymes of mitochondrial oxidative phosphorylation. ($O_2^{\bullet -}$, superoxide anion radical; H_2O_2, hydrogen peroxide; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; SOD, superoxide dismutase.) (Modified from Ref. 1 and 3 with permission.)

Threonine (T) and Tyrosine (Y) residues within a T-X-Y phosphorylation motif, where "X" can be Glu (E), Pro (P), or Gly (G). Three major classes of dual-specificity MAP kinases can be defined, based on their activation motifs (TEY, TPY, and TGY), which is termed ERK1/2 and BMK1 (also called ERK5), c-Jun N-terminal protein kinases (JNK, also called SAPK), and p38, respectively. Activation of the three classes of MAP kinases is charactreristic for particular stimuli. For example, growth factors and phorbol myristate acetate (PMA) activate ERK1/2 strongly, but JNK and p38 kinases, weakly (16). Hyperosmolar stress and TNF-α are strong stimuli for p38 (17). Berk *et al*. have shown that growth factors and angiotensin II are powerful activators of ERK1/2 in vascular smooth muscle cells (VSMC) (18). They also found that ROS activated ERK1/2 when the ROS was $O_2^{\bullet -}$ (19). In other systems, ROS activate JNK strongly (20). The specificity for MAP kinase activation is elucidated, in part, by members of the MAP kinase and ERK kinase (MEK) family that exhibit unique pairing with downstream MAP kinases. MEK1 and MEK2 activate ERK1/2, MKK3 and MKK6 activate p38, and SEK1 (MKK4) and MKK7 activate JNK. The specificity of activation of MAP kinases by individual stimuli is reiterated by specific substrates for each class (Fig. 2). Common substrates for the MAP kinases are transcription factors that, upon phosphorylation, induce changes in gene expression. ERK1/2 phosphorylates ternary complex factor (TCF)/Elk-1 on sites essential for transactivation (21). JNK phosphorylates c-Jun and increases its transcriptional activating potential (22). ATF2 is phosphorylated and activated by both JNK

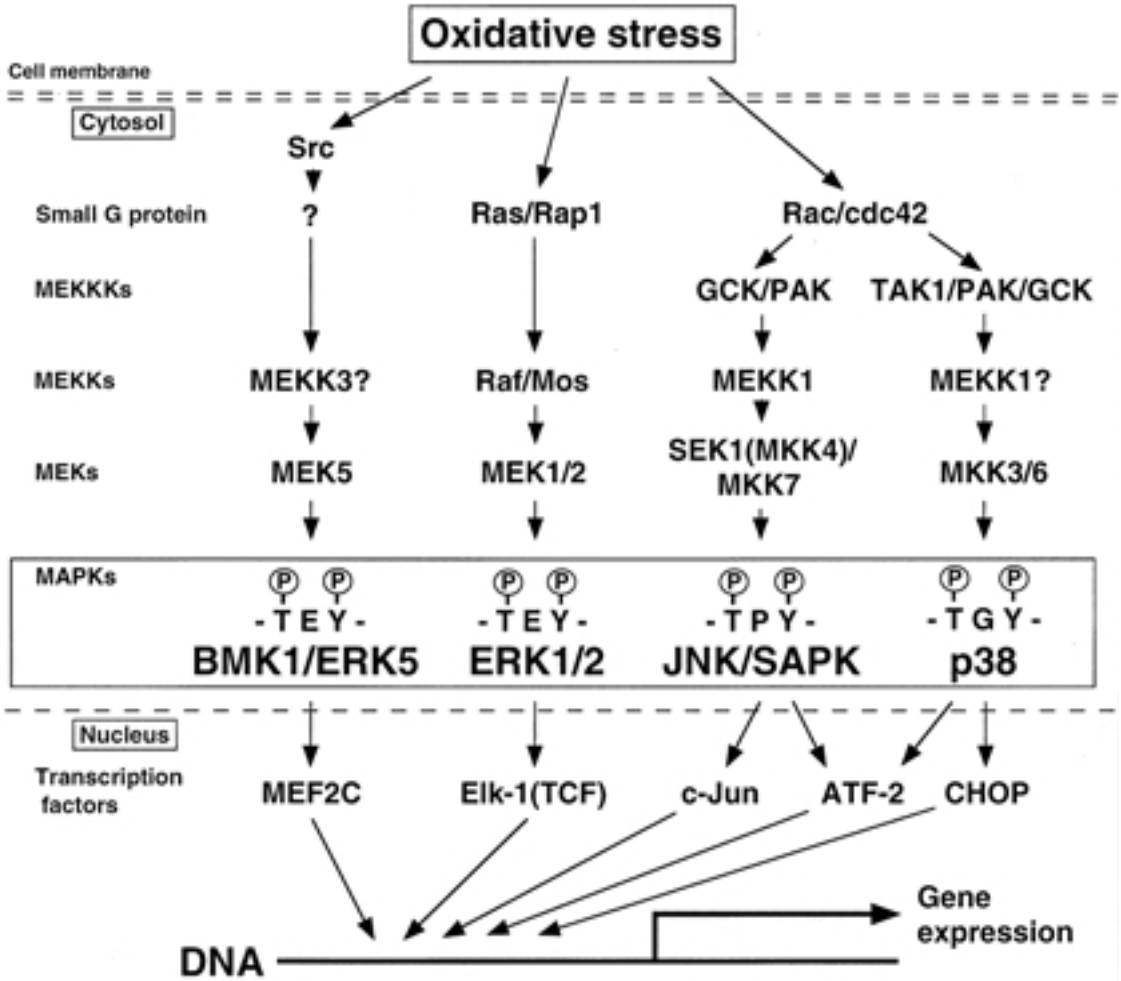

Fig.2. Signal transduction pathways for mitogen-activated protein kinases (MAPKs) by oxidative stress in cardiovascular cells. Shown in a highly schematic linear pattern are modules of kinases that regulate each other in a kinase cascade. For example, oxidative stress is proposed to activate the small G protein Ras and thereby stimulate MAPK and ERK kinase (MEK) kinase (MEKK) and subsequent activation of MEK1, which is a regulator of the MAP kinase, ERK1/2. Additionally, it has been shown that other MAPKs including BMK1, JNK, and p38 may be regulated by oxidative stress. Among the targets for these MAP kinases are various transcription factors including Elk-1 (ternary complex factor, TCF), c-Jun, ATF-2, and CHOP. (Modified from Ref. 1 and 3 with permission.)

and p38 (23, 24). BMK1 activates MEF2C transcription factors (25).

ROLE OF MAP KINASES IN ISCHEMIA AND REPERFUSION INJURY IN THE HEART

There is growing evidence that multiple MAP kinases are activated during ischemia and/or reperfusion and may contribute to the structural and functional alterations after myocardial ischemia (2). Myocyte loss during the acute stage of myocardial infarction involves both apoptotic and necrotic cell death (26-28). Therefore, it is reasonable to conceive that the balance of cell survival and death is critical during the pathological evolution of postischemic cardiac dysfunction. Recent findings elucidated the possible role of MAP kinases in the balance of cell survival and death in ischemic myocardium. As shown in Fig. 3, Yu *et al*. revealed that three MAP kinase members (ERK1/2, JNK and p38) were activated in cardiomyocytes subjected to ischemia and reperfusion (29). They also showed that ERK1/2 is part of a survival pathway whereas JNK and p38 mediate the death pathway in the myocardium, based on experiments with their specific inhibitors. Among the substrates of ERK, p90 ribosomal S6 kinase (p90RSK) may mediate ERK-induced antiapoptotic signaling pathway. p90RSK is known to regulate gene expression via phosphorylation of transcription factors including c-Fos, cAMP-responsive element-binding protein (CREB) and to regulate protein synthesis by phosphorylation of polyribosomal proteins and glycogen synthese kinase-3. Recently, Bonni *et al*. (30) and Tan *et al*. (31) reported that p90RSK phosphorylated the proapoptotic protein BAD at serine 112, which specifically suppressed BAD-mediated apoptosis. These findings may imply that p90RSK and ERK promote cell survival by both inhibiting components of the cell death machinery (e.g. BAD) and increasing transcription of prosurvival genes (e.g. CREB). BMK1 has also been reported to be an antiapoptotic molecule from the findings of MEK5 inhibition, immediately upstream from BMK1 (25, 32). It was reported that BMK1 was activated in ischemia and reperfusion of the guinea pig heart (33). MEK5 and BMK1 are highly expressed in cardiomyocytes (33). MEK5-dependent BMK1 activation results in the phosphorylation of MEF2A and MEF2C, transcription factors that belong to the myocyte enhancer factor-2 (MEF-2) family (25), which are important regulators of cardiac gene expression.

It has been suggested that the pathways regulated by JNK and p38 contribute to apoptosis. The mechanisms by which JNK and p38-induced apoptosis are commonly cell and stimulus specific. For example, apoptosis induced by growth factor deprivation principally mediated through the activation of p38 rather than JNK (34). In some cells, JNK-mediated apoptosis has been shown to be involved in ubiquitination and degradation of p53, which may be regulated by JNK-mediated phosphorylation of p53 (35). However, p53 does not appear to be important for hypoxia-induced apoptosis in cardiomyocytes from the experiments using p53 knockout mice (36). Therefore, the mechanism of JNK-induced apoptosis remains unclear. The role of p38 in cardiomyocyte apoptosis is controversial because p38 can also induce cardiac hypertrophy. One reported way to explain the role of p38 in apoptosis is through its effect on cyclin D1 expression during cell cycle. It has been reported that the coexpression

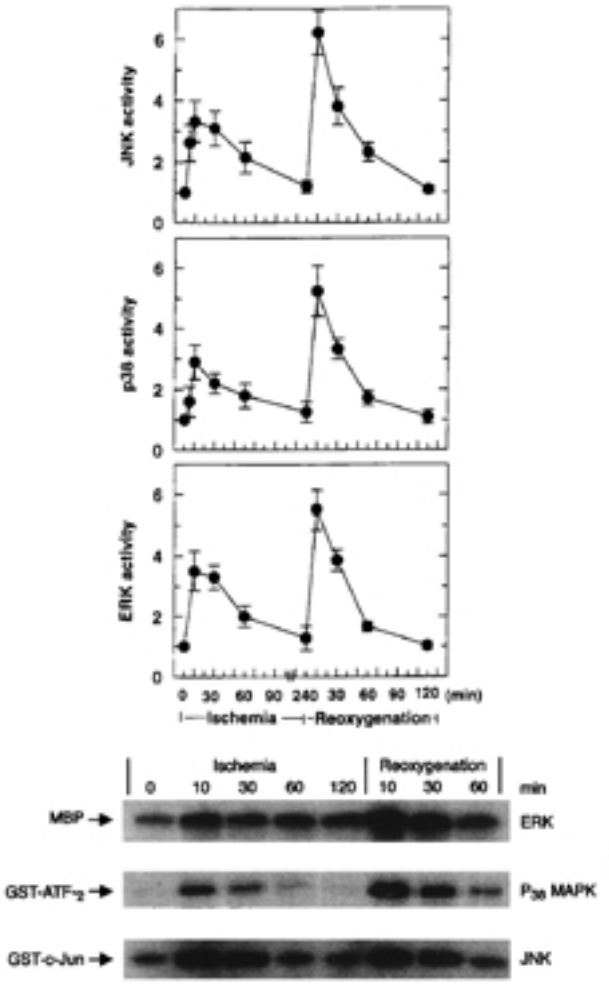

Fig.3. Activation of ERK, JNK, and p38 in cardiomyocytes subjected to ischemia and reperfusion. Myocytes were exposed to ischemia or ischemia followed by reoxygenation for the indicated time period. Cells were harvested and extracted. Endogeneous MAPKs were immunoprecipitated, and the activities of ERK, JNK, and p38 were measured. Top, densitometric analysis of MAPKs activation. Bottom, representative autoradiogram. (Cited from Ref. 29 with permission.)

of MKK3 along with p38 inhibits mitogen-induced cyclin D1 expression (37). However, contrasting findings have been reported that p38 phosphorylates MAPK-activated protein kinase 2 (MAPKAPK2), which in turn phosphorylates heat shock protein 27 (HSP27) and protects against apoptosis during ischemia (38). Activation of this pathway is cytoprotective and overexpression of HSP27 confers protection against ischemia in cardiomyocytes (39, 40). It was also reported that activation of p38 and JNK by anisomycin is cardioprotective (41). The controversies among studies may be explained by the existence of different p38 isoforms such as p38α which is proapoptotic whereas p38β may be antiapoptotic in neonate rat cardiomyocytes (42). The current evidence for JNK and p38 suggest multiple roles in ischemia and reperfusion, preconditioning, and hypertrophy of the heart. Further studies are needed to define the precise role of these MAP kinases in ischemia and reperfusion injury in the heart.

MAP KINASE-MEDIATED VASCULAR REMODELING REGULATED BY ROS

Vascular remodeling is a well described response of blood vessels to both physiologic and pathologic stimuli. It was originally defined by the findings that atherosclerotic vessels undergo a compensatory enlargement during atherosclerosis by the analysis of pathologic specimens (43). Recently, vascular remodeling was recognized to occur in animal models of ballon injury (13) and in patients after the PTCA procedure (44), and has been suggested to relate to restenosis. Several lines of evidence indicated that ROS and MAP kinases are involved in vascular remodeling under various pathological conditions. For example, we previously showed that exposure of vascular smooth muscle cells (VSMC) to H_2O_2 altered MAP kinase activity (Fig. 4) (45). In VSMC, ROS alters MAP kinase activity (19), protooncogene expression, and cell growth (46). Recently, it became clear that intracellular ROS generated by growth factors and cytokines may act as second messengers. Platelet-derived growth factor (PDGF) has been reported to increase intracellular ROS in VSMC (47). Increasing the intracellular levels of the scavenging enzyme catalase or the antioxidant glutathione prevented the PDGF-mediated increase in ROS. These ROS scavengers also blocked PDGF-induced tyrosine phosphorylation, ERK1/2 activation, DNA synthesis,

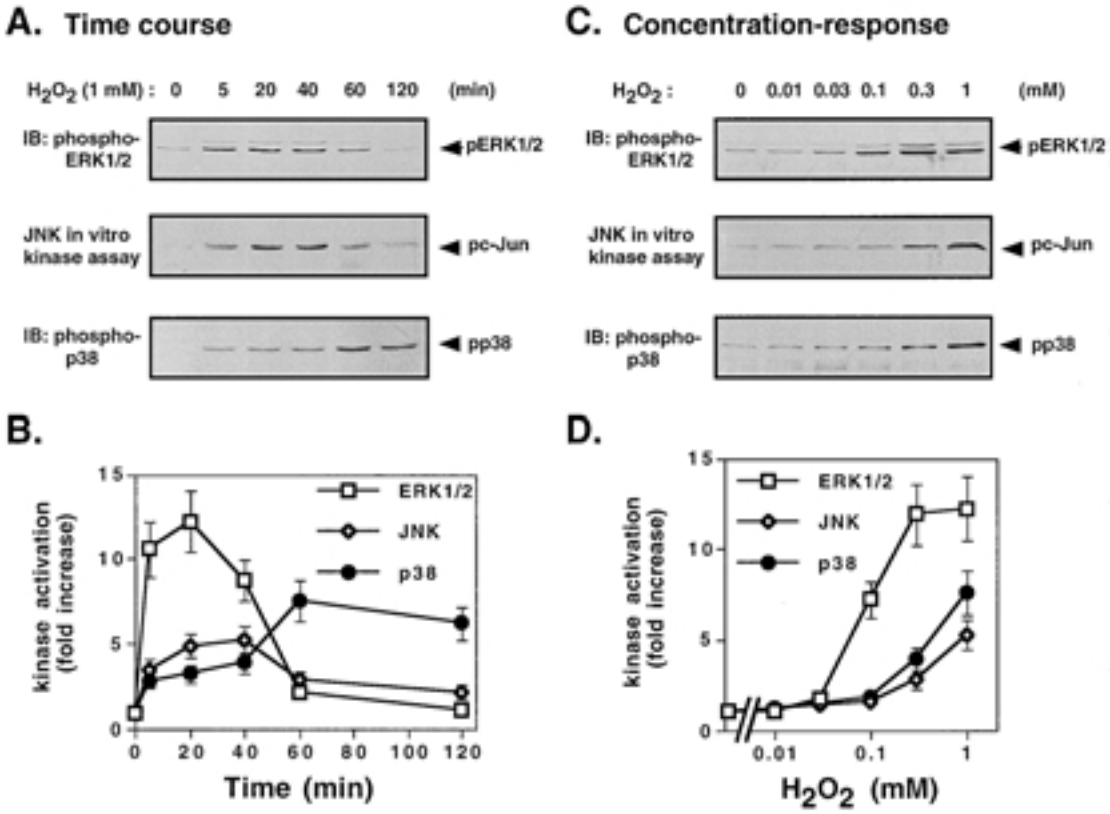

Fig.4. ERK, JNK, and p38 activation by hydrogen peroxide (H_2O_2) in vascular smooth muscle cells (VSMC). Time course (A, B) with 1 mM H_2O_2 and the concentration-response curve (C, D) (20 min for ERK1/2, 40 min for JNK, and 60 min for p38) are shown. Panels A and C are representative autoradiograms. Panels B and D are densitometric analysis of MAPKs activation. (Modified from Ref. 45.)

and chemotaxis, suggesting the role of ROS as second messengers. Activation of ERK1/2 has also been implicated in vascular endothelial growth factor (VEGF)-induced vascular endothelial cell (EC) survival (48). More recently, a general role for ROS in cell growth was suggested by the findings that v-Ras transformation was associated with increased ROS and that augmenting antioxidant defenses with catalase and SOD inhibited transformation and growth (49).

JNK and p38 are also sensitive to redox modulation (Fig. 4) (45). Members of the Rho family of small GTPases including Rac1 regulate these kinases (50). In contrast to ERK1/2, JNK and their downstream target c-Jun have been implicated in H_2O_2 and other stress-induced apoptosis of ECs (51). Moreover, p38 has been implicated in EC upregulation of intracellular adhesion molecule-1, and therefore, endothelial dysfunction (52). In VSMC, ROS mediated activation of p38 has also been implicated in cell migration (53).

Angiotensin II and endothelin are other activators of MAP kinases in VSMC and are believed to cause vascular remodeling (54, 55). Very recently, Griendling *et al*. showed that angiotensin II rapidly increased production of ROS via NADH/NADPH oxidase that reside in cell membranes (Fig. 5) (56). In blood vessels, a plasma membrane NADH/NADPH oxidase accounts for more than 90% of $O_2^{\bullet -}$ formation (57). Interestingly, angiotensin II potently activates ERK1/2 and p38 ; however, only p38 is sensitive to both inhibition of NADH/NADPH oxidase activity and catalase overexpression in VSMC (58, 59). We obtained similar findings that antioxidants preferentially inhibits JNK and p38 activation by angiotensin II but not ERK1/2 activation (unpublished observation). p22phox is a critical component of NADH/NADPH oxidase activity and transfection of antisense p22phox cDNA to VSMC inhibited angiotensin II-induced VSMC hypertrophy via a p38-dependent manner (60). It has also been reported that thrombin, TNF-α, and lactosylceramide also stimulate NADH/NADPH oxidase-dependent $O_2^{\bullet -}$ generation in VSMC (61-63). In ECs, mechanical forces stimulate NADH/NADPH oxidase (64), but agonist responsiveness remains to be elucidated. Of note, NADH/NADPH oxidase is regulated at the protein level and may be increased by hormonal stimuli. For example, TNF-α increases NADH/NADPH oxidase activity in VSMC over 24 hours, an event that is dependent on increased transcription of p22phox (62). p22phox mRNA and $O_2^{\bullet -}$ production are upregulated in the aortas of rats made hypertensive by angiotensin II infusion (65). It has become obvious that various vasoactive substances activate NADH/NADPH oxidase and produce ROS as second messengers in vascular cells. However, involvement of other sources of ROS, such as xanthine oxidase, lipoxygenese, and cyclooxygenase cannot be ruled out. It was recently reported that lipoxygenase metabolites of arachidonic acid mediate angiotensin II stimulation of the NADH/NADPH oxidase in VSMC (66). Further information concerning the activation mechanisms of the oxidase and protein targets of the oxidase-derived ROS should be clarified.

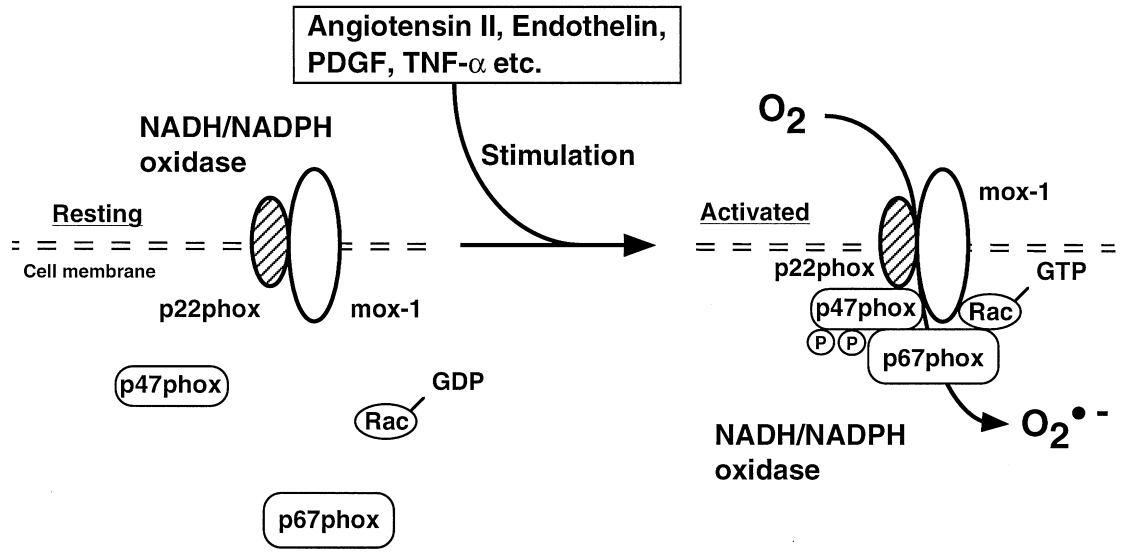

Fig.5. Structure and components of NADH/NADPH oxidase. Left side panel shows the scheme of the resting state of the components. Mitogenic oxidase-1 (mox-1) and p22phox form the electron transfer components of the oxidase, and p47phox and p67phox are cytosolic components that interact with these 2 proteins to modulate its activity. The small G protein Rac also serves a regulatory function. Right side panel shows the activated state of NADH/NADPH oxidase induced by agonists such as angiotensin II, endothelin, and PDGF etc.

OTHER SIGNALING MOLECULES ACTIVATED BY ROS

Although MAP kinases play a central role in ROS-mediated signal transduction in cardiovascular diseases, other signaling molecules also involved in ROS-mediated phenomena in cells. ROS participate in signal transduction by generating classic second-messengers (calcium and lipid mediators) that transmit the signal to intracellular mediators in both the cytoplasm and nucleus (Fig. 6).

Calcium-dependent signal transduction by ROS

ROS stimulate increases in calcium via at least three pathways. First, intracellular calcium release from endoplasmic (sarcoplasmic) reticulum occurs by generation of IP_3, which binds to an IP_3-sensitive calcium channel in endoplasmic (sarcoplasmic) reticulum. In addition, ROS may directly increase the open probability of calcium release channels. Previously, we reported that angiotensin II and endothelin, potent activators of NADH/NADPH oxidase as mentioned above, increased intracellular calcium in cardiomyocytes (67) and VSMC (68, 69). Moreover, a calcium-ATPase is required to maintain calcium within the IP_3-sensitive pool, and inhibiting this ATPase would increase intracellular calcium (70). Secondly, in several cell types, ROS have been shown to stimulate extracellular calcium entry by effects on channels (71) and the sodium-calcium exchanger (72). Thirdly, mitochondria also store intracellular calcium, and significant oxidative stress inhibits this ability resulting in a release of calcium, especially in response to oxidized pyridine nucleotides (73, 74).

Phospholipid-dependent signal transduction by ROS

The finding that ROS increased intracellular calcium indicated that phospholipases were activated by ROS, because generation of IP_3 from PIP_2 is a well established mechanism in cardiovascular cells for the release of calcium from intracellular stores (75). At least three important phospholipases have been shown to be activated by ROS : phospholipase A_2 (PLA_2), phospholipase C (PLC), and phospholipase D (PLD). PLA_2 is likely activated by increases in intracellular calcium. It is important to note that many lipids generated by PLA_2 may themselves generate ROS through the action of monooxygenases. PLC is a calcium-dependent phospholipase that hydrolyses PIP_2 to generate IP_3 and diacylglycerol. Although there are no published

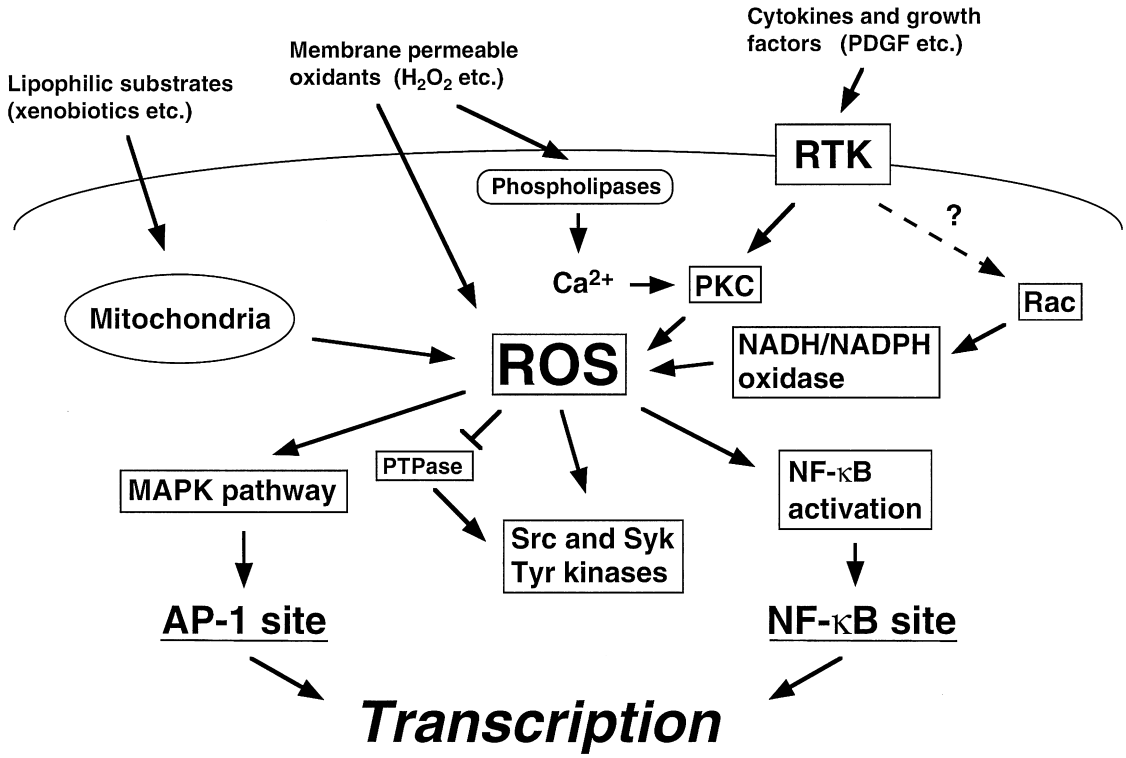

Fig.6. Redox regulation of signal transduction components. In addition to generation of intracellular $O_2^{\bullet -}$ by the enzymes, discussed in Fig. 1, extracellular stimuli including lipophilic substrates, membrane permeable oxidants (H_2O_2), cytokines and growth factors may modulate cellular redox state. The increased levels of reactive oxygen species (ROS) may act as second messengers to activate MAPKs, tyrosine kinases, and NF-κB, as well as inhibit protein tyrosine phosphatase (PTPase). As a consequence, there are alterations in the phosphorylation and activity of transcription factors, resulting in promotion of gene expression. (RTK, receptor tyrosine kinase ; PKC, protein kinase C) (Modified from Ref. 1 and 3 with permission.)

findings to suggest that ROS directly activate PLC, generation of ROS may be an early event in growth factor dependent signaling and activation of PLC. PLD has been shown to be stimulated by H_2O_2, fatty acid hydroperoxides (75, 76), and 4-hydroxynonenal in endothelial cells. Further studies are required to elucidate the mechanisms by which phospholipases are activated.

Tyrosine kinases as mediators of ROS-sensitive pathways

Tyrosine kinases are important as intracellular mediators because they are among the kinases most rapidly activated by many stimuli including ROS. Many growth factor receptors contain tyrosine kinase domains in their cytoplasmic portions (Fig. 6). Thus, a proximate signal event in growth factor signal transduction is receptor dimerization and activation of intrinsic tyrosine kinase. To date, there is no direct evidence that ROS stimulate receptor tyrosine kinases; however, three mechanisms have been proposed by which ROS activate tyrosine kinases. Firstly, tyrosine kinases may be activated by ROS directly by altering protein-protein interactions depending on sulfhydryl groups. Secondly, inhibition of phosphotyrosine phosphatases (PTPases) may be another important mechanism by which ROS activate tyrosine kinases. All PTPases contain a redox-sensitive cysteine at their active site (77), and oxidation of the sulfhydryl group inactivates the PTPase. Since many tyrosine kinases are inactivated by PTPases, oxidation of PTPases would stimulate tyrosine kinases. Thirdly, oxidation has been shown to stimulate proteolysis of regulatory proteins that may inhibit tyrosine kinase activation. Several tyrosine kinases are activated by ROS, among which Src family kinases have been described most frequently. p60Src, p56Lck, and p59Fyn of the Src family members have been found to be activated by H_2O_2 and other ROS. Two other ROS-sensitive tyrosine kinases identified in white blood cells are Syk and ZAP-70 (78). Of note, specific Src family kinases stimulate specific pathways leading to selective activation of MAP kinases. We previously found that c-Src mediates H_2O_2-induced JNK activation in VSMC (45). Abe *et al*. reported that BMK1 is activated via c-Src (58) whereas ERK1/2 is activated by Fyn (79). These findings indicate that c-Src and Fyn have separate role in ROS-mediated signal transduction. In addition, another tyrosine kinase Pyk2, a FAK related kinase, has been shown to be activated by ROS (80) and may activate other MAP kinases.

Small G proteins in ROS-sensitive signal transduction

Other important mediators of the ROS-sensitive signaling pathway are small G proteins. From the experiments with recombinant p21Ras *in vitro*, it was found that ROS directly promoted guanine nucleotide exchange on p21Ras (81). Furthermore, H_2O_2 activation of ERK2 was abolished by expression of dominant negative RasN-17 (49) or by treating with a farnesyltransferase inhibitor. Another small G protein, Rac may also regulate ROS production (Figs. 2, 6) (82). Expression of constitutively active V12-Rac1 in HeLa cells or stimulation with cytokine resulted in a significant increase in intracellular ROS. Treatment of cells with antioxidants inhibited the increase in ROS that occurred following V12-Rac1 expression. Potential mechanisms for small G protein activation include alterations of the lipid environment and inhibition of post-transcriptional modifications, such as palmitoylation, which may regulate small G protein activity.

ROS-induced activation of transcription factors: NF-κB and AP-1

Transcription factors are proteins that move to the nucleus upon activation and trigger gene expressions (Fig. 6). Nuclear factor-κB (NF-κB) is an inducible transcription activator that is a likely target for ROS signal transduction. NF-κB is a heterodimer containing a 50- and a 65-kD subunit (termed p50 and p65). There are two forms of NF-κB in the cell, an inactive form in the cytosol and an active form in the nucleus. Cytosolic NF-κB activation can be brought about by a variety of stimuli including cytokines, physical stress such as UV and ionizing radiation, and oxidants such as H_2O_2 (82-84). Activation of NF-κB by ROS may be pathogenic for atherosclerosis, as NF-κB can be activated in response to low-density lipoproteins (LDLs), an atherogenic diet, and advanced glycation end products (AGEs). In addition, recognition sequences for NF-κB are present in genes whose expression causes monocyte adhesion to endothelial cells and activation, such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1).

In addition to NF-κB, activator protein-1 (AP-1) appears to be activated by ROS (85). Regulation of AP-1 by ROS has been shown to involve ERK1/2 (86) and JNK (87) in a cell-and stimulus-specific manner. For example, metabolic oxidative stress in the liver increased AP-1 activity and stimulated binding of c-Fos, c-Jun, ATF-2, JunB, and JunD (87). ERK1/2 was shown to be necessary for phosphorylation of

the transcription factor Elk-1 and induction of the c-fos serum response element (86). In vascular smooth muscle, expression of mRNAs for c-fos and c-jun are rapidly induced by H_2O_2 and by arachidonic acid metabolites including 15-HETE and linoleic acid metabolites such as hydroperoxyoctadecadienoic acids (HPODEs) (88-90). Since these same stimuli also activate ERK1/2 in smooth muscle (19), it is likely that ERK1/2 play an important role in AP-1 activation. We also observed that endothelin-1 stimulates AP-1 activation probably through the activation of ERK1/2 in VSMC (55). Other transcription factors such as hypoxia-inducible factor-1 (HIF-1) and early growth response-1 (Egr-1) may be involved in ROS-mediated events and were described elsewhere (91, 92).

CLINICAL IMPLICATIONS OF ROS AND MAP KINASES IN CARDIOVASCULAR DISEASE

Although the role of MAP kinases in cardiac hypertrophy and apoptosis has been described in *in vivo* and in animal models, the role in human disease is not well documented. Recently, it was demonstrated that the expression of three MAP kinase members, ERK1/2, JNK, and p38 were confirmed in human heart (93). A potential clinical relevance for their action was reported by an increased activity of JNK and p38 in heart failure secondary to ischemic heart disease which may relate to ROS generation. It was also reported that activation of ERK1/2, JNK, and p38 was observed in human heart during coronary artery bypass grafting surgery (94). However, the clinical significance of MAP kinase activation in ischemia and heart failure of humans still remains to be elucidated. Gene transfer techniques provide insight into the role of MAP kinases in cardiac disease. In the gene transferred rat of dominant-negative SEK1, immediately upstream from JNK, cardiac hypertrophy was inhibited as evaluated by echocardiography and biochemical markers (95). In addition, an upstream regulator of p38, TAK1 was activated in cardiac hypertrophy of transgenic mice and TAK1 activation resulted in heart failure (96). These findings suggest the role of MAP kinases in cardiac disease, and therefore, MAP kinases are potential drug targets in the treatment of the disease.

Three vascular process in which ROS are likely to play a pathogenic role are hypertension, atherosclerosis, and vascular remodeling. Recently, it was shown that angiotensin II-induced hypertension was associated with increased vascular $O_2^{\bullet -}$ production and that treatment with SOD reduced blood pressure by 50 mmHg in angiotensin II-infused rat (97). In addition, it was reported that the increase in MAP kinase activity was sustained in the angiotensin II-infused rat (98). These findings suggest that hypertension caused by chronically elevated angiotensin II and MAP kinase activity is mediated in part by $O_2^{\bullet -}$. Increases in ROS may affect the pathogenesis of atherosclerosis. Hypercholesterolemic animals and patients exhibit impaired endothelial dependent relaxation that can be restored with antioxidants (99). Clinically, it was also observed that vascular superoxide production by NADH/NADPH oxidase is associated with endothelial dysfunction in patients with hypercholesterolemia (100). ROS also cause oxidative modification of LDL, promoting uptake by the scavenger receptor and foam cell formation (101). These findings strongly suggest that the vascular redox state plays a pathogenic role in atherosclerosis. Vascular remodeling is also a ROS-sensitive phenomenon occuring clinically. It was reported that p22phox, a component of NADH/NADPH oxidase, expressed highly in the atherosclerotic remodeled specimens of the coronary artery (102). NADH/NADPH oxidase is a potent mediator of MAP kinase activation as mentioned above. Recent findings revealed that remodeling occurs in patients after the PTCA procedure (44) and remodeling is a key process for restenosis (103). The findings that antioxidants inhibit restenosis both *in vivo* (13) and clinically (12) may imply the role of ROS in vascular remodeling.

FUTURE DIRECTIONS

As discussed above, significant progress has been made in identifying and characterizing the sources of ROS and the role of MAP kinases in cardiovascular diseases. However, significant work should be performed to resolve the remaining issues. Firstly, additional information is required to clarify what molecule is really responsible for ROS generation in different cardiovascular cells including EC, VSMC, and cardiomyocytes. In addition, identification of the enzymatic and protein targets of ROS should be important. Of course, the ultimate goal is the development of new therapeutic strategies for the treatment of cardiovascular diseases of which ROS may be involved, in part, or in total. Understanding the molecular mechanisms of ROS generation and ROS-mediated alterations of signaling molecules including MAP kinases may provide insights into the pathogenesis of various cardiovascular diseases. Searching for ROS generating and ROS-mediated signaling molecules

is now ongoing in our labs and will inform help identify targets for the emerging therapeutic paradigm of gene therapy.

During the revision of this review, it has been reported that $G_{\alpha i}$ and $G_{\alpha o}$, subunits of heterotrimeric G protein, are potential targets of oxidative stress for activation of ERK (104).

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