Targeting NAD(P)H Oxidase
Ets-1 Regulates p47\textsuperscript{phox}

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Reactive Oxygen Species (ROS) have been shown to modulate vascular signaling in endothelium, smooth muscle, and adventitia, regulate vascular hypertrophy, inflammation, remodeling, intracellular calcium, and disturb nitric oxide bioactivity.\textsuperscript{1,2} Since Griendling et al discovered the activation of NAD(P)H oxidase by angiotensin II (Ang II),\textsuperscript{3} researches have focused on the regulation of this enzyme in Ang II signaling/Ang II-induced hypertension both in vitro and in vivo. Vascular NAD(P)H oxidase consists of multiple subunits including p22\textsuperscript{phox}, p40\textsuperscript{phox}, p47\textsuperscript{phox}, p67\textsuperscript{phox}, Rac1, and unique catalytic subunits, Nox isoforms (gp91\textsuperscript{phox} homologue).\textsuperscript{4} The signaling mechanisms for the rapid activation of NAD(P)H oxidase by Ang II have been identified using cultured aortic vascular smooth muscle cells (VSMCs). Ang II rapidly activates PLC to increase intracellular calcium and diacylglycerol levels, which causes the activation of protein kinase C (PKC). PKC phosphorylates p47\textsuperscript{phox} and releases ROS from Nox subunits. Subsequently, ROS activates cSrc, EGF-receptor, PI3-kinase, and Rac1, leading to the secondary activation of NAD(P)H oxidase to augment the intracellular ROS levels.\textsuperscript{4} These events occur within 30 minutes in cultured VSMCs. Considering the ROS generation associated with hypertension in vivo, the latter transcriptional upregulation of NAD(P)H oxidase subunits by Ang II might be more important. Ang II upregulates the expressions of NAD(P)H oxidase subunits after more than 4 hours including p22\textsuperscript{phox}, Nox2 (gp91\textsuperscript{phox}), p47\textsuperscript{phox}, and p67\textsuperscript{phox},\textsuperscript{5} however the mechanisms of transcriptional regulations for NAD(P)H oxidase subunits have not fully been elucidated yet.

In this issue of Circulation Research, Ni and colleagues identified that Ets-1 was a critical transcriptional regulator of p47\textsuperscript{phox} induced by Ang II in vitro and in vivo.\textsuperscript{6} Ets-1 has been known as a proto-oncogene transcription factor to induce matrix-degradation proteins such as collagenase, plasminogen activation inhibitor-1 (PAI-1), and matrix-metallopainases. Ets-1 can be induced by TNF-\alpha, endothelin-1, prostanoid(s), and platelet-derived growth factor,\textsuperscript{7-8} suggesting an implication in vascular inflammation. Indeed, this group previously reported that Ets-1 was a critical factor needed to induce cyclin-dependent kinase, PAI-1, vascular cells adhesion molecule-1, and monocyte chemotactant protein-1 in response to Ang II. In Ets-1\textsuperscript{-/-} mice, the vascular inflammation by Ang II infusion, which was represented by the recruitment of T cell and macrophage to vessel wall, was blunted, although hypertensive response was preserved.\textsuperscript{9} In this article, the authors carefully seek the molecular mechanisms to regulate the expression of NAD(P)H oxidase subunits by Ets-1. The augmentation of superoxide and hydrogen peroxide (H$_2$O$_2$) generations by Ang II were markedly attenuated in aorta from Ets-1\textsuperscript{-/-} mice or VSMCs with siRNA for Ets-1. siRNA for Ets-1 also blunted the upregulation of p47\textsuperscript{phox} without affecting the expression of Nox1, Nox4, Rac1, p22\textsuperscript{phox}, and p67\textsuperscript{phox} by Ang II. They used gel-shift assay, luciferase reporter assay, and chromatin immunoprecipitation assay with deletion mutant of p47\textsuperscript{phox} promoter, and identified the $-45$ Ets-1-binding promoter region as essential for the induction of p47\textsuperscript{phox}. They developed peptides to inhibit ETS-1 bindings (DN-Ets-1 peptides) and delivered them to the Ang II–infused mice in vivo. DN-Ets-1 peptides attenuated medial hypertrophy and aortic ROS generation without affecting hypertensive response to Ang II.

This article impacts the field of hypertension research in 3 major ways. First, the authors demonstrate the importance of p47\textsuperscript{phox} induction for aortic ROS generation in Ang II–induced hypertension. p47\textsuperscript{phox} is phosphorylated at S359/S370/S379 by PKC, which causes association with p22\textsuperscript{phox}. S303/S304 of p47\textsuperscript{phox} were also phosphorylated to augment the catalytic activity of NAD(P)H oxidase.\textsuperscript{4} We expressed S303A/S304A mutant p47\textsuperscript{phox} in VSMCs to suppress the redox-sensitive signal by Ang II.\textsuperscript{10} The posttranslational modifications of p47\textsuperscript{phox} by Ang II are critical for the rapid activation of NAD(P)H oxidase.

The importance of p47\textsuperscript{phox} expression in Ang II–induced hypertension was also shown by Landmesser and colleagues.\textsuperscript{11} An increase in superoxide generation of aorta by Ang II was blunted in p47\textsuperscript{phox}\textsuperscript{-/-} mice but rose 3-fold in control. Hypertensive response to Ang II was modestly decreased in p47\textsuperscript{phox}\textsuperscript{-/-} mice. Consistent with these observations and the results by Ni and colleagues,\textsuperscript{6} p47\textsuperscript{phox} induction is essential for aortic ROS generation and vascular inflammation in Ang II–infusion, whereas it is not required for the hypertensive response. Because Ets-1 is a critical transcriptional regulator for p47\textsuperscript{phox}, it is a potential therapeutic target for vascular inflammation.

Second, this study showed the importance of transcriptional regulation of the NAD(P)H oxidase. The authors clearly show the specific induction of p47\textsuperscript{phox} by Ets-1 without affecting the expressions of other subunits by Ang II. AP-1 was shown to regulate the expression of p67\textsuperscript{phox} in
Redox-Sensitive Thiol Targets for NAD(P)H Oxidase

Although the molecular mechanisms for the redox-sensitive signaling are not fully known, growing evidence indicates the importance of thiols modification by ROS. ROS can modify the redox-sensitive thiols to sulfenylation (RSOH), sulfinylation (RSO2H), and S-glutathiolation (RSSG). We modify the redox-sensitive thiols to sulfenylation (RSOH), sulfinylation (RSO2H), and S-glutathiolation (RSSG). We previously found that Ang II increased ROS generation from NAD(P)H oxidase, which caused the activation of Ras via S-glutathiolation at Cys118 in VSMCs. Because many of the oncogene/transcription factors can be upregulated by the activation of Ras, the mechanism may be implicated in the ROS-mediated transcriptional regulations. There are several other candidates for thiol-containing proteins targeted by ROS. Phosphatases have critical redox-sensitive thiols in the center of catalytic sites and the oxidation of them by ROS decreases their activity. Rac1 contains a redox-sensitive thiol to modulate its function. Caspase can be inhibited by S-glutathiolation, and Thioredoxin (Trx) and glutaredoxin (Grx) can reverse thiol-modifications of Ras, phosphatases, and caspases. Interestingly, another Trx superfamily, protein disulfide isomerase, is associated with NAD(P)H oxidase and supports ROS generation. Transcriptional factors can be also regulated by ROS via redox-sensitive thiols. Cys179 on IKKβ is S-glutathiolated by ROS from Nox1, which causes NF-κβ activation. ROS oxidizes the reactive thiol on Keap1 to dissociate Nrf-2, leading to the Nrf-2-transcription. In contrast, many transcription factors such as AP-1 and NF-κβ have redox-sensitive thiols in the DNA-binding sites and their modifications disturb DNA binding. The effects of ROS on transcription may differ according to the localization of ROS.

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Third, the authors used unique peptides to block Ets-1 transcription for in vivo models. DN-Ets-1 peptides contained HIV-TAT protein membrane-transduction domains to promote intracellular delivery, especially to the nucleus. In the past, Pagano and colleagues generated gp91-ds-tat peptides, which inhibited the interaction between Nox2 and p47phox and efficiently decreased vascular ROS from NAD(P)H oxidase. Similar peptide drugs may be useful for targeting proteins related with NAD(P)H oxidase. With recent advances for understanding the regulation of NAD(P)H oxidase, we can create multiple strategies to modulate NAD(P)H oxidase activity (Figure). The AT1 receptor blocker attenuates Ang II signaling. Statin, a HMG-CoA reductase inhibitor, decreases farnesyl/geranylgeranyl pyrophosphate, leading to the suppression of Ras/Rac1. Inhibition of glucose 6-phosphate dehydrogenase decreases intracellular NADPH and ROS from NAD(P)H oxidase, although it may weaken the antioxidant defenses. Many of the redox-sensitive thiols and thiol-reducing enzymes (Trx/Grx/PDI) can be the downstream targets. Moreover, redox-sensitive transcriptional factors can be new targets for the suppression of NAD(P)H oxidase.

Sources of Funding
The work was supported by Grants-in-Aid for Scientific Research (B:19390090) (to T.A.) and for Creative Scientific Research 17GS0419 (to T.A. and M.S.). T.A. and M.S. are core members of Global Center-of-Excellence (GCOE) for Human Metabolomics Systems Biology from MEXT. M.Y. is a research fellow supported by New Energy and Industrial Technology Development Organization.
Disclosures
None.

References

Key Words: Ests-1  ■  NAD(P)H oxidase  ■  Angiotensin II  ■  Hypertension
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Circ Res. 2007;101:962-964
doi: 10.1161/CIRCRESAHA.107.164434

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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