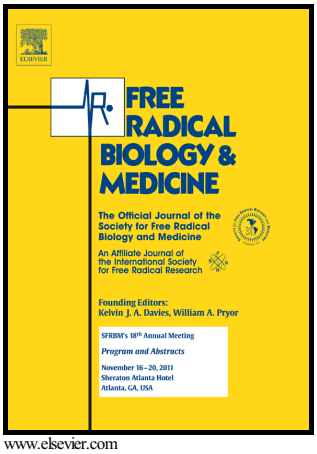

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Down-regulation of NOX2 activity in phagocytes mediated by ATM-kinase dependent phosphorylation

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Running head: NOX2 down-regulation by ATM kinase

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Keywords: NADPH oxidase; neutrophil; NOX; phosphorylation; ataxia telangiectasia mutated (ATM); NOX-specific Insertion Sequence (NIS)

Abbreviations: NOX, NADPH oxidase; ROS, reactive oxygen species; CGD, chronic granulomatous disease; PMA, Phorbol 12-myristate 13-acetate; fMLP, formyl-methionylleucyl-phenylalanine; DMF, dimethylformamide; DFP, diisopropylfluorophosphate; DPI, diphenylene iodonium; INT, iodonitrotetrazolium, SOD, superoxide dismutase; NOX, NADPH oxidase; $O_2^{\cdot-}$, superoxide, ATM, ataxia-telangectasia mutated

Abstract

NADPH oxidases (NOX) have many biological roles, but their regulation to control production of potentially toxic ROS molecules remains unclear. A previously identified insertion sequence of 21 residues (called NIS) influences NOX activity, and its predicted flexibility makes it a good candidate for providing a dynamic switch controlling the NOX active site. We constructed NOX2 chimeras in which NIS had been deleted or exchanged with those from other NOXs (NIS1, 3 and 4). All contained functional heme and were expressed normally at the plasma membrane of differentiated PLB-985 cells. However, NOX2-ΔNIS and NOX2-NIS1 had neither NADPH-oxidase nor reductase activity and exhibited abnormal translocation of $p47^{phox}$ and $p67^{phox}$ to the phagosomal membrane. This suggested a functional role of NIS. Interestingly after activation, NOX2-NIS3 cells exhibited superoxide overproduction compared with wild-type cells. Paradoxically, the V_{max} of purified unstimulated NOX2-NIS3 was only one-third of that of WT-NOX2. We therefore hypothesized that post-translational events regulate NOX2 activity and differ between NOX2-NIS3 and WT-NOX2. We demonstrated that Ser486, a phosphorylation target of ataxia telangiectasia mutated kinase (ATM kinase) located in the NIS of NOX2 (NOX2-NIS), was phosphorylated in purified cytochrome b_{558} after stimulation with phorbol 12-myristate-13-acetate (PMA). Moreover, ATM kinase inhibition and a NOX2 Ser486Ala mutation enhanced NOX activity whereas a Ser486Glu mutation inhibited it. Thus, the absence of Ser486 in NIS3 could explain the superoxide overproduction in the NOX2-NIS3 mutant. These results suggest that PMA-stimulated NOX2-NIS phosphorylation by ATM kinase causes a dynamic switch that deactivates NOX2 activity. We hypothesize that this downregulation is defective in NOX2-NIS3 mutant because of the absence of Ser486.