

Direct ATM Activation by Sulfhydryl-Reactive Inflammatory Cyclopentenone Prostaglandins

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ATM (ataxia-telangiectasia mutated) is essential for cellular response to double strand breaks in vertebrate cells. However, ATM is activated by a variety of noxious agents, including oxidative stress, and ATM deficiency results in anomalous cellular response to oxidative stress. While this defective response to oxidative stress may underlie the pathogenesis of cerebellar ataxia, premature aging and cancer predisposition in ataxia-telangiectasia, mechanisms for ATM activation by oxidative stress remain to be established. Furthermore, it is not clear whether or not ATM responds to oxidative DNA damage or rather responds to a change in intracellular redox state independent of DNA damage. To address these questions, we studied ATM activation by protein sulfhydryl (SH)-group-modifying agents, N-methyl-N'-nitro-nitrosoguanidine (MNNG) and 15-deoxy- Δ 12,14-prostaglandin J₂ (15d-PGJ₂), in chicken DT40 cells deficient for *NBS1* or *MSH6* generated by targeted disruption. We found that ATM is effectively activated with MNNG and 15d-PGJ₂ in *NBS1*^{-/-} and *MSH6*^{-/-} cells. We further found that ATM is directly activated by treatment of chromatin-free ATM immunoprecipitates with MNNG or 15d-PGJ₂ through modification of SH groups and that 15d-PGJ₂ covalently binds to ATM. Interestingly, 15d-PGJ₂-induced ATM activation leads to p53 phosphorylation and apoptosis but not Chk2 phosphorylation in human tumor cells. These results indicate that ATM is activated through direct modification by free SH-group-modifying reagents independently of DNA damage, resulting in apoptotic downstream response. However, it remains to be established how ATM regulates intracellular oxidative stress and how ATM abnormality leads to various defective manifestations in oxidative stress response. More extensive proteomics analysis is, thus, required for establishing ATM functions in oxidative stress response. For such analysis, 15d-PGJ₂ might be a useful agent, since ATM activation by 15d-PGJ₂ does not result in the activation of DNA damage-linked Chk1 and Chk2 phosphorylation.