Direct ATM Activation by Sulfhydryl-Reactive Inflammatory Cyclopentenone Prostaglandins

Masahiko Kobayashi, Hiroto Ono, Keiko Mihara, Hiroshi Tauchi, Kenshi Komatsu, Hiroko Shimizu, Koji Uchida, and Ken-ichi Yamamoto

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 sulfhydril (SH)-group-modifying agents, N-methyl-N'-nitro-nitrosoguanidine (MNNG) and 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2), in chicken DT40 cells deficient for *NBS1* or *MSH6* generated by targeted disruption. We found that ATM is effectively activated with MNNG and 15d-PGJ_2 in *NBS1*$^{-/-}$ and *MSH6*$^{-/-}$ cells. We further found that ATM is directly activated by treatment of chromatin-free ATM immunoprecipitates with MNNG or 15d-PGJ_2 through modification of SH groups and that 15d-PGJ_2 covalently binds to ATM. Interestingly, 15d-PGJ_2-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-PGJ_2 might be a useful agent, since ATM activation by 15d-PGJ_2 does not result in the activation of DNA damage-linked Chk1 and Chk2 phosphorylation.