Direct ATM activation by toxic metabolites

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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 agent, including oxidative stress, and ATM deficiency results in an 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 ATM responds to oxidative DNA damage or to a change in the 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). 15d-PGJ_2 is a reactive cyclopentenone-type prostaglandin D_2 metabolite generated during inflammatory processes, and is a potent inducer of intracellular oxidative stress and directly modulates the activity of several biologically important molecules through the specific alkylation of free sulfhydryl (SH) groups on cysteine residues of the target proteins. An alkylating agent, MNNG, is another potential oxidative stress inducer that reacts with free SH groups, although MNNG is well known to methylate DNA, and effectively induces checkpoint activation through the activation of both ATM and ATR with the subsequent activation of Chk1 and Chk2. We found that MNNG and 15d-PGJ_2 effectively activate ATM in *NBS1*- or *MSH6*- deficient chicken DT40 cells. We further found that ATM is also activated by treating chromatin-free immunoprecipitated ATM with MNNG or 15d-PGJ_2, and that 15d-PGJ_2 binds covalently to ATM. Interestingly, 15d-PGJ_2-induced ATM activation leads to p53 activation and apoptosis, but not to Chk2 or H2AX phosphorylation. 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. 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.