Roles for ATM in cellular response to oxidative stress

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ATM (ataxia telangiectasia-mutated) is activated in response to DNA double strand breaks (DSBs) after genotoxic treatments such as X-ray irradiation and regulates homologous recombination repair and cell cycle checkpoints. However, the molecular mechanisms underlying DNA damage recognition and subsequent activation of ATM are still unclear. The apoptosis of post-mitotic cerebellar Purkinje cells in AT patients indicates that ATM has other role than in the response to DSBs. Recent study has shown that ATM is a component of a complex of BRCA1-associated proteins including mismatch repair (MMR) enzymes. BRCA1 and MMR enzymes were revealed to be related to the pathway of transcription-coupled repair (TCR) by which a part of oxidative DNA damages is repaired. In addition, MMR enzymes recognize 8-oxo-guanine in DNA, which is generated by endogenous reactive oxygen species and has highly mutagenicity. Furthermore the elevated oxidative damages and the lower expression level of catalase were observed in AT cells compared with wild type. These evidences suggest that AT cells are unable to response to oxidative stress and repair oxidative damages adequately. Therefore we addressed to the study on the signal transduction pathway from oxidative stress to the activation of ATM.

In this study we used an isogenic set of stable chicken B cell line, DT40, for the sake of knockout availability and high compatibility to human cell. By colony assay the differences of sensitivities to hydrogen peroxide and paraquat, which induce different oxidative stress respectively, were examined among wild type, ATM-/-, BRCA1 and newly constructed MSH6-/- and Rad17-/- strains. ATM-/- and BRCA1-/- cells were highly sensitive to hydrogen peroxide and only Rad17-/- cells were sensitive to paraquat, suggesting that the protective process to hydrogen peroxide is different from that to superoxide radical generated by paraquat. MSH6-/- cells showed similar sensitivities to wild type. Moreover we prepared anti-ATM serum to analyze the responses of ATM to the stimuli directly. Anti-ATM serum was collected from the rabbit immunized with purified His-tagged recombinant partial chicken ATM. The ATM-recognition of the anti-ATM serum was confirmed by Western blotting and the irradiation of X-ray activated ATM kinase in wild type cells but not ATM-/-. At present we are analyzing the activation of ATM by the oxidative stress and other genotoxic reagents in the strains described above by kinase assay.