Msh6 and Nbs1 are required for ATR-mediated Chk1 activation induced by DNA replication stalling in higher vertebrate cells

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MutSα, which consists of Msh2 and Msh6, is known to recognize O^6-MeG generated by MNNG and to bind to this DNA lesion. This is consistent with the recent findings that Msh2 and Msh6 interact with ATR and is involved in ATR-mediated Chk1 phosphorylation induced by MNNG. We confirmed the requirement of Msh6 for MNNG-induced and ATR-mediated Chk1 phosphorylation. However, MNNG-induced Chk2 phosphorylation did not require Msh6, and was rather enhanced in *MSH6*$^{-/-}$ DT40 cells. Nbs1, in addition to its role in DSB-induced ATM activation, is involved in ATR-mediated Chk1 phosphorylation induced by HU and UV. Thus, consistent with these findings, we found that MNNG-induced Chk1 phosphorylation on Ser-345 was severely diminished in *NBS1*$^{-/-/-}$ cells. We also observed MNNG-induced Chk2 phosphorylation, which was previously shown to be independent of human ATM and Mlh1 mismatch repair proteins, particularly with higher MNNG concentrations such as those used in the present study. However, we observed greatly reduced MNNG-induced Chk2 phosphorylation in *NBS1*$^{-/-/-}$ cells, indicating that ATM and mismatch repair proteins are dispensable but Nbs1 is required for Chk2 phosphorylation induced by high concentrations of MNNG. Many recent studies have provided evidence for a role for Nbs1 as a damage sensor or activator acting upstream of ATM in cellular response to DSB. There is also evidence for a direct physical interaction between ATM and Nbs1. In addition, both of Nbs1 and ATM are involved in DSB repair by the homologous recombinational DNA repair system. These findings explain shared clinical features of A-T and NBS (Nijmegen breakage syndrome caused by a hypomorphism in *NBS1*), such as immunodeficiency, radiosensitivity, chromosome instability and cancer predisposition. However, the results presented in the present study as well as those reported recently by other investigators indicate that Nbs1 is also involved in ATR-mediated Chk1 phosphorylation, which is induced by MNNG, HU, UV or cisplatin, which cause replication stalling. It is also likely that a kinase involved in Msh6/ATM- independent but Nbs1-dependent Chk2 phosphorylation induced by high concentrations of MNNG is ATR. This functional relationship between Nbs1 and ATR may explain the embryonic lethality of *NBS1* knockout in mice, which is distinct from the non-essential feature of ATM in mice and human.