Involvement of the ATR- and ATM-dependent checkpoint responses in cell cycle arrest evoked by pierisin-1

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Piersin-1 identified from the cabbage butterfly, *Pieris rapae*, is a novel mono ADP-ribosylating toxin that transfers the ADP-ribose moiety of NAD at N^2 of dG in DNA. Resulting mono ADP-ribosylated DNA adducts cause mutations and the induction of apoptosis. However, little is known about checkpoint responses elicited in mammalian cells by the formation of such bulky DNA adducts. In the present study, it was demonstrated that DNA polymerases were blocked at the specific site of mono ADP-ribosylated dG which might lead to the replication stress. Pierisin-1-treatment of Bcl-2-overexpressing HeLa cells was found to induce the S-phase arrest and the G2/M-phase delay. In the colony survival assays, Rad17$^{-/-}$ DT40 cells showed greater sensitivity to pierisin-1-induced cytotoxicity than wild type and ATM$^{-/-}$ DT40 cells, possibly due to defects of checkpoint responses, such as the Chk1 activation. Further investigation of HeLa cells confirmed that Chk1 was activated ATR- and Rad17-dependently and that mitotic delay was inhibited in ATR- and Rad17-knockdown HeLa cells, but not in ATM-knockdown cells. The results thus suggest ATR-Rad17-Chk1 pathway mainly contributes to the S-phase arrest and G2/M-phase delay induced by pierisin-1. Simultaneously, pierisin-1-treatment activated Chk2 pathway ATM-dependently and ATM-independently. Furthermore characteristic 50 kbp DNA fragmentation, known to be blocked by Bcl-2 overexpression was observed and activation of ATM and Chk2 was only partially inhibited. Thus, the roles of ATM-Chk2 or Chk2 pathways appear minor in cell cycle arrest, but may be involved in the induction of apoptosis. From these findings, it is suggested that mono ADP-ribosylation of DNA causes a specific type of fork blockage which induces checkpoint activation and signaling.