133 Molecular analysis of p53 protein expression through signal transduction pathway in normal human cells following X-irradiation.

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p53 is a nuclear phosphoprotein whose function is required for transactivation of a set of genes involved in the regulation of cell cycle checkpoints, controlling DNA replication and activating apoptosis of cells exposure to ionizing radiation. Although it is hypothesized that ATM is involved in a specific IR-indicible signal transduction pathway upstream of the p53, the mechanism through which transduced signal accumulates p53 protein is not yet clear. Therefore, we investigated signal transduction pathway which led to p53 accumulation in normal human embryonic cells following X-irradiation. Cells were treated with specific PKC and several inhibitors during X-irradiation and incubated for 2 hours because maximum accumulation of p53 was observed at 2 hours after X-ray with 6 Gy. We found that quercetin could suppress the accumulation of p53 but calphostin-C had no effect. Wortmannin also suppressed the accumulation of p53 and 1 min. or less than 1 min. treatment was enough for the suppression. Our results suggest that protein kinase(s) rather than PKC is responsible for transduction of the X-ray induced signal to p53 protein.

134 Mechanism of Interaction of c-Abl with ATM Protein and DNA-PK.

The gene mutated in ataxia telangiectasia, ATM, encodes a putative protein kinase that plays a major role in DNA-damage response by ionizing radiation. The DNA-dependent protein kinase (DNA-PK) requires DNA double strand breaks for its activation and is also essentially involved in response to DNA damage by ionizing radiation. Recently, it has been reported that c-Abl protein tyrosine kinase is activated in response to ionizing radiation by interaction with ATM protein and DNA-PK. In the present study, we investigated mechanism of protein-protein interaction of c-Abl with ATM protein and DNA-PK. We constructed two glutathione-S-transferase (GST)-Abl fusion proteins. One GST-Abl fusion protein contained intact SH2 and SH3 domains and in the other fusion protein 70% of the SH3 domain was deleted. After cell lysate from RKO and AM5BIVA cells were incubated with the GST-Abl fusion proteins, ATM protein and DNA-PK that bound a GST-Abl fusion protein were detected by Western blotting. We show that both ATM protein and DNA-PK interact with not only the GST-Abl fusion protein containing intact SH3 domain but also the fusion protein containing the deleted SH3 domain. This finding suggests that intact SH3 domain of c-Abl is not always required for its interaction with ATM protein and DNA-PK.

135 p53 function and PLDR


p53 is the most frequently mutated gene in human tumors. The p53 gene is involved not only in suppression of tumor phenotype of transformed cells, but also in the response of cells to DNA damage and radiation sensitivity. They are transcriptional regulation caused by sequence specific DNA binding, cell cycle control and induction of apoptosis. In this study, therefore, we examined the effects of p53 protein on cell death and mutation induction by radiation. We found that all cells which were deficient in p53-function (SAOS-2, H1299 and anti-p53 KMST cells) did not have PLDR repair capacity. However, these cells showed the almost same survival as that of irradiated normal HE49 cells. Anti-p53 KMST cells (#3 and #8) showed extremely elevated sensitivity in mutation induction. From these results, we may expect that (1) p53 is involved in the response of cells to repair process (PLDR) for X-ray induced damage and (2) for genetical stability in human cells.