121 Sequence alterations of the *ATM* gene and related cell-cycle checkpoint genes in human tumor cell lines

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Mutation on both alleles of the *ATM* gene is responsible for the human radiosensitive disease ataxia-telangiectasia (A-T). *ATM* gene is also involved in the development of sporadic human cancer like T-PLL or B-CLL. In this study, sequence alterations of the *ATM* gene and related cell-cycle checkpoint genes (*hRad1*, *hRad9*, *hRad17*, *hHus1*, *Chk1*, *CHES1*) were examined in a panel of 25 human solid tumor cell lines. Sequence alterations were analyzed by PCR-SSCP method by using genomic DNA for *ATM*, or using reverse-transcribed cDNA for the other genes. Homozygous inactivation of *ATM* like that seen in A-T patients or lymphoid tumors were not detected in any of the cell lines. The most prominent change was the generation of abnormal transcript (497del22, 1236del372) due to the deletion within the intronic mononucleotide repeats. For the other six *ATM*-related genes, sequence alterations were rare (one missense change in *hRad1* and two in *CHES1*, respectively), indicating relative stability of these genes in human cancer. In *hRad17*, a polymorphism was noted at a highly conserved codon. High incidence of rare polymorphic alleles was also noted for *ATM*, suggesting its possible relevance to cancer susceptibility.

122 *Atm*-disrupted (*Atm*-/-) mice showed no effect of fractionation in acute intestinal death and crypt cell survival after X-irradiation.

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Atm-disrupted mice were subjected to total body irradiation (TBI) with 200 kV X-rays at 72 cGy/min and observed for 30 days. $LD_{50/8}$ was approximately 4.5 Gy, 11 Gy and 11 Gy for *Atm*-disrupted (*Atm*-/-), heterozygous (*Atm*+/-) and wild type (*Atm*+/+) mice, respectively. Wild type mice were irradiated with 12 Gy divided into 2 equal fractions (6 Gy + 6 Gy) at 8 hour interval. They died between 6th and 13th day after irradiation, significantly longer than the mice irradiated single with 12 Gy. *Atm*-/- mice were irradiated with 6 Gy divided into 2 equal fractions (3 Gy + 3 Gy) at 8 hour interval. They died between 4th and 7th day after irradiation, as the mice with single 6 Gy irradiation. Mice were subjected to TBI and sacrificed at 3 and 3/4 days after irradiation to obtain crypt cell survival curves for single doses and fractionated doses. The results collectively indicate that there is no recovery from radiation damage of *Atm*-/- TBI mice in terms of both acute intestinal death and crypt cell survival.

123 Repression of DNA synthesis in UV-irradiated xeroderma pigmentosum variant cells is more severe than normal cells

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Xeroderma pigmentosum (XP) variant cells are slightly more sensitive than normal to killing, but much more sensitive than normal cells to the induction of mutations by ultraviolet light (UV). However, it is not clear what is a cause of this high mutability of variant cells. Previously, it has been reported that DNA replication at UV-damaged DNA site is abnormal in variant cells. Cell including abnormality in DNA replication should be easy to produce mutation. It is likely that variant cells show any deficiency in signal transudaction pathway related cell cycle control. Therefore, we examined dynamics of the proteins that control progress of cell cycle such as p53, p21 and Rb, in UV irradiated cells by Western blotting method.p53 and p21 increased by UV irradiation in normal and variant cells. Amount of phosphorylated Rb decreased by UV in both cells. However, there was no difference in dynamycs of accumulation and resolution of cell cycle related proteins after UV irradiation. In normal cell, DNA synthesis was suppressed at 6hr after UV irradiation and it recovered to normal level by 48hr after UV irradiation. In XP variant cells, however, DNA synthesis was suppressed more severe than normal cells. This result is similar to recent Maher's in that suppression of DNA synthesis is variant cells is severe and bypass replication ability is lower than normal cells.