185 Analysis of the cell death after irradiation in the cells with defective DNA repair

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DNA double strand break (dsb) by ionizing radiation causes cell death if it is not repaired. There are at least two repair pathways for dsb: homologous recombination (HR) and non-homologous end joining (NHEJ). To investigate the relationship between repair pathway and death signaling, we analyzed cell death after treatment with DNA damaging agents using the chicken DT40 cell lines in which genes involved in dsb repair (Ku70 for NHEJ, or Nbs1 for HR/NHEJ) was disrupted. In sharp contrast with their similar radiation sensitivity, Nbs1–/–/– cells were more sensitive to alkylating agent than Ku70–/–. No apparent effect on induction of apoptosis within 72 h post irradiation were observed in Ku70–/– cells although the induction of apoptosis was suppressed in Nbs1–/–/– cells. Because the DT40 cells do not express p53, these observations suggest that aberrant NHEJ may not directly affect on induction of apoptosis, and that the Nbs1 might be involved in a p53-independent apoptotic signaling.

186 Delayed apoptosis in mouse embryos fertilized by X-irradiated spermatozoa

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We have previously shown p53 dependent checkpoint, which suppresses the rate of pronuclear DNA synthesis in zygotes fertilized with irradiated sperm. The present study is undertaken to determine the stage where p53 induced apoptosis in embryos fertilized with irradiated sperm. Sexually mature male mice were X-irradiated at 6 Gy and mated with female. Embryos were recovered from the pregnant mice on day 3.5. Cleavage speed, apoptosis, micronuclei frequency and p53 expression were studied. The early cleavage in the embryos was not affected by the X-irradiated sperm. However, 35% embryos showed delayed development on day 3.5, which is higher than control. Remaining embryos developed further but the quality of blastocysts and average cell number in the blastocyst were less than those of the control. We observed high incidence of apoptosis in embryos fertilized with irradiated sperm and the apoptosis was higher in blastocysts than in arrested or slow cleaving embryos. Our study indicates that function of apoptosis begin only at blastocyst stage

187 The protective effect of a fermented milk Kefir to radiation-induced apoptosis in rat colon

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The aim of this study was to evaluate the effect of a fermented milk Kefir on radiation-induced apoptosis in the rat colon. Seven-week-old male Wistar rats were used. The Kefir-treated group drank a fermented milk Kefir ad libitum for 12 days, whereas the control group drank water. Rats from each group received whole-body X-ray irradiation and a single dose of 0.25, 0.5, 1, 2 and 4 Gy was given. 1, 2, 4 and 6 h after irradiation, the colon was immediately resected and the incidence of cell death was quantified by counting the number of apoptotic cells in each crypt by microscopic examination of H&E stained section. Apoptotic fragments were confirmed by TUNEL staining. Active caspase-3 expession was observed by immunostaining. The number of apoptotic cells in the colon was significantly decreased at 2 h after 1 Gy irradiation ($p < 0.01$) in Kefir -treated group. TUNEL positive cells and the expression of active caspase-3 in the kefir-treated rats were lower than that in control rats. This study indicated that Kefir suppresses radiation-induced apoptosis in the colon of rats at a dose of 1 Gy. This suppression effect of apoptosis by kefir may be due to caspase-3 inhibition.

188 Caspase-3-independent programmed cell death and apoptosis induced by X-rays

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Human leukemic MOLT-4 cells undergo apoptosis after X-irradiation through p53-dependent pathway. X-ray-induced apoptosis in MOLT-4 is inhibited by the inhibitor of caspase-3-like proteases, Ac-DEVD-CHO. To characterize the mode of cell death induced by the inhibition of caspase-3, we have studied the effects of Ac-DEVD-CHO, on the structural changes in X-irradiated MOLT-4 cells. The hallmarks of apoptosis, i.e., nuclear condensation and DNA ladder formation, were depressed. However, a new type of nuclear morphology appeared. The morphology was also observed by the treatment with Ac-IETD-CHO. The sum of the frequencies of apoptosis and new type of nuclear structure corresponded to the frequency of X-ray-induced apoptosis for cells incubated in the absence of Ac-DEVD-CHO. Removal of Ac-DEVD-CHO during the course of post-irradiation incubation increased apoptotic nuclear condensation accompanied by only a slight decrease in the frequency of the new type of nuclear structure. These results suggest that the morphology observed in the presence of Ac-DEVD-CHO is an apoptosis-related structure, and that the cell death observed is a programmed cell death independent of caspase-3-like proteases.