

RADIATION-INDUCED TRANSGENERATIONAL GENOMIC INSTABILITY IN MICE

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Among untargeted effects of ionizing radiation there is the so-called phenomenon of transgenerational genomic instability. This phenomenon encompasses diverse observations in the mouse, including an increased somatic mutation rate and tumor susceptibility in the offspring of irradiated fathers, which cannot be explained by mendelian inheritance. However, some unrepeated experiments and the lack of a mechanistic understanding make the phenomenon still highly controversial.

Here we exploited a tumor susceptible mouse model, heterozygous for a null mutation of the *Ptch1* gene, highly prone to develop spontaneous and radiation-induced medulloblastoma, to test the hypothesis of transgenerational genomic instability induced by irradiation of differentiating spermatogonia.

Ptch1 wild type mice were irradiated with 1 Gy X rays and mated 42 days later with unirradiated *Ptch1*^{+/-} females. Their *Ptch1*^{+/-} progeny was tested for the spontaneous incidence of medulloblastoma and for the incidence of tumors induced by irradiation (1 Gy) at postnatal day 2 (P2), a well-characterized window of cerebellum radiation sensitivity. In parallel, the level of spontaneous and radiation-induced (1 and 2 Gy) DNA damage and repair in P2 cerebellum cells was analyzed by comet assay in the progeny of irradiated and unirradiated fathers. In addition, the possible transmission of chromosomal instability was investigated in bone marrow and spleen cells of adult progeny by comet and micronucleus assays.

Data collected so far showed a borderline statistically significant increase of the incidence of radiation-induced medulloblastoma in the progeny of irradiated fathers with respect to the incidence measured in a matched group of mice born from unirradiated parents, whereas no difference was observed in the spontaneous tumour incidence between the progeny of irradiated and unirradiated fathers.

The results on the spontaneous and radiation-induced level of DNA damage in P2 cerebellar cells, and its repair, did not show an effect of paternal irradiation, suggesting that neither increased DNA fragility nor compromised repair are involved in transgenerational carcinogenicity.

Similarly, the comet assay analysis of baseline DNA damage in bone marrow and spleen cells did not reveal an effect of paternal irradiation. Consistently with these data, the micronucleus test in bone marrow erythrocytes of the F1 progeny did not show an effect of paternal irradiation on either the spontaneous or the radiation-induced (0.1 Gy) level of chromosome instability.

To elucidate the molecular pathways of possible transgenerational carcinogenesis, studies are in progress on the genetic/epigenetic mechanisms of *Ptch1* wild type allele inactivation in tumor samples, and on the characterization of epigenetic changes at the miRNA and DNA methylation level in sperm descendant of irradiated spermatogonia.