

Transgenerational Effects of Chronic Low-Dose Irradiation in a Medaka Fish Model System

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There are major gaps in our knowledge about the genetically-based mechanisms underlying the radiobiological response of living systems to low doses of ionizing radiation in the dose range that is relevant to workplace exposure limits or to increased background radiation levels from various man-made or natural sources. The overall goal of this project is to gain an understanding of how gene activity and mutations in microsatellite DNA in a promising model organism [Japanese Medaka fish (*Oryzias latipes*)] change in response to low doses of gamma rays (low-LET radiation) delivered either: a) to parent fish and not offspring; or b) to parent fish and continuously throughout a number of progeny generations, and how this genetic response relates to certain processes and biological effects. Medaka specimens are being irradiated at selected dose-rates and total doses in the Low-Dose Irradiation Facility (LoDIF) at the Savannah River Ecology Laboratory (SREL). Studies on markers for mutations in microsatellite DNA from irradiated Medaka are being conducted at SREL and selected tissues from these specimens are shipped to Colorado State University (CSU) for genetic analysis. The project was started in October, 2005.

In preparation for transgenerational studies at CSU, we have conducted mechanistic studies to define the relationship between specific DNA damage products and the gene products that are known to act on these damages. The studies have been carried out *in vivo* using young adult Medaka fish (~24 weeks old). DNA damage induced by acute irradiation at doses of 0 to 30 Gy and a dose-rate of 2.5 Gy/min was assessed by measurements of 8-hydroxyguanine (8-OHG) levels and double strand breaks in the genomic DNA. Tissue was collected 5 minutes after the start of irradiation for all doses except 30 Gy where the current dose rate necessitated a 12 min irradiation. Dose-dependent expression patterns of genes involved in the excision of oxidized DNA bases and double strand break repair processes were also determined under the same conditions.

We found that the dose-yield response of the unrepaired 8-OHG in the DNA of muscle tissues is linear up to 0.5 Gy and non-linear from 0.5 to

30 Gy. The radiation yield of unrepaired 8-OHG is 0.66 ± 0.19 8-OHG per 10^5 dG/Gy. The dose-response of unrepaired DNA double strand breaks in muscle tissue was linear to 2.5 Gy. In the case of the liver tissue the dose dependency of unrepaired DNA double strand breaks was linear from 0.5 - 30 Gy. The frequency of double-strand breaks for 0.1 Gy was not significantly different from that in the control.

Steady-state mRNA levels of DNA double-strand break repair-related genes p53, Ku70, and a putative Medaka glycosylase that may be involved in excision of 8-OHG were measured by Quantitative Real-Time PCR. Levels of 8-OHG and the transcript level for the putative glycosylase had a significantly high degree of correlation whereas both p53 and Ku70 did not show any significant changes at this short time after irradiation.

At SREL, experiments using acute irradiation have been done to determine the dose response of the frequency of germ line mutations in medaka. We were interested in detecting the lowest dose at which effects can be observed and in individual genetic responses of the studied medaka families to radiation exposure. Nine highly variable microsatellite loci (mutation rates of 10^{-2}) identified in previous studies were used. Prior to the exposure, medaka pairs were bred, and 96 hatchlings per pair were collected to serve as controls for microsatellite analyses. The same medaka breeding pairs (two per treatment) were exposed later to four doses of acute ionizing radiation (0.1, 0.5, 2.5, and 5 Gy) and then allowed to breed again. The DNA was extracted, the microsatellite loci were amplified and the genotypes were identified for the parents and all hatchlings produced before and after the exposure. The mutation frequencies were calculated for each locus and compared among all treatments and control. We expected increases in the germline mutation rates with increasing dose to the parents. We also expected variation in the response (i.e., differing genetic sensitivity) among families, but tested against the null model of no differences among families. Our preliminary results suggest that mutations are significantly elevated in hatchlings of medaka after their parents were exposed to any of the four radiation doses applied when compared to the mutation frequencies of the offspring of the same parents before exposure (Fisher's exact test $P < 0.05$). Germline mutation rates increased significantly even at the lowest dose of 0.1 Gy raising concerns for the induction of germline mutation rates by environmentally-relevant radiation doses.