Low-Dose Ionizing Radiation Alters the Epigenome of the A^{vy} Mouse

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Background:

Humans have evolved and thrived amidst constant low-dose (0-10 cGy) background radiation exposure from natural sources. Currently, however, the frequency of exposures to low doses of radiation is increasing due to man-made sources such as diagnostic imaging and nuclear power. This increased exposure has led to concerns amongst the general public and the government about the impact that low-dose radiation has on human health. To date, epigenetic analyses of high doses of radiation have shown epigenetic disruption, but analyses of low-dose ionizing radiation *in vivo* is minimal. Epigenetic aberrations can disrupt normal development and are involved in the occurrence and progression of numerous diseases, including cancer. Epigenetic changes have also been determined to be essential for the persistence of the bystander effect in response to high doses of ionizing radiation. The goal of this project is to further define the health effects of low-dose ionizing radiation exposure by examining its ability to alter the epigenome *in vivo* at metastable epialleles and at imprinted genes in a dose-response manner.

Methods:

The viable yellow agouti (A^{vy}) mouse was exposed *in utero* to 0, 0.6, 1.2, 2.5, 5.0, or 10.0 cGy of acute X-ray irradiation on day 4.5 of gestation. Upon weaning, mice were examined for coat color (an indication of methylation) and tail, liver, kidney, and brain tissues were extracted methylation analyses. The Sequenom platform was used to analyze the CpG sites upstream of the cryptic promoters in the A^{vy} allele and in the *Cdk activator binding protein* ($Cabp^{IAP}$) allele, which are regions shown to be biomarkers for exposures that alter the epigenome. Methylation was also examined in the long terminal repeat of intracisternal a particles (IAP), genomic elements in which epigenetic disruption can lead to genomic instability. Tail tissue samples were also used for the analyses of imprinted genes. The Sequenom platform was used to identify and analyze differentially methylated regions (DMR) in *Igf2/H19* and *Nnat* genes. Multifactorial ANOVA statistical analyses were performed to look for main effects of dose and interactions between dose, sex, and CpG site on methylation changes. Chi-square analyses were performed to look at shifts in population coat color.

Results:

Analyses of all doses at the A^{vy} allele show that the sex of the animal, radiation dose, and CpG site interact to affect methylation, with males showing greater changes in methylation (ANOVA, $p<.0001$). In males, 0.6 cGy of radiation has no effect on coat color or methylation; 1.2 cGy shifts the coat color towards pseudoagouti (X^2, $p<0.05$), but does not significantly increase methylation; 2.5 and 5.0 cGy shift the coat color to pseudoagouti (X^2, $p<0.05$) and significantly increase methylation (ANOVA, $p<0.05$); 10.0 cGy shifts the coat color to heavily mottled (X^2, $p=0.045$), but only increases methylation in 3/10 CpG sites (ANOVA, $p<0.05$).

Imprinted genes also demonstrate increased methylation. The *Igf2/H19* imprint control region shows increased methylation at three CpG sites in response to 0.6 cGy of radiation and at two CpG sites in response to 2.5 cGy of radiation (ANOVA, $p<0.05$). *Nnat* also shows increases in methylation in response to radiation exposure at 2.5 cGy at all CpG sites within the DMR (ANOVA, $p<0.05$).

Other loci demonstrate disrupted methylation patterns. At the $Cabp^{IAP}$ allele and at global IAP elements, methylation is either increased or decreased depending on the applied dose and CpG site (ANOVA, $p<0.05$).

Interpretation:

Low-dose radiation increases the methylation at several loci, including imprinted genes of A^{vy} mice. Males are more affected at the A^{vy} allele, while sex does not play a factor for the other loci examined. Increased methylation at these low doses of radiation is in contrast to other published data that show hypomethylation in response to high doses of radiation. These results may support a biphasic dose response curve. Such a curve also might explain why the A^{vy} mice exposed to 10.0 cGy are more similar to the control population. This dose could lie in the transition zone- a zone in which the radiation exposure is switching between opposing effects (hypermethylation to hypomethylation). The methylation changes in imprinted genes are particularly important since these genes are also imprinted in humans and intimately involved in growth and development. Further investigation of additional imprinted genes will help to translate the risk of low-dose radiation exposure from mice to the human population.