

γ H2AX Foci after Low Dose-Rate Irradiation Reveal a Defect in DNA DSB Processing in Cells from Unaffected Parents of Retinoblastoma Patients, *ATM* Heterozygotes, and Other Apparently Normal Humans

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We have investigated the use of the γ -H2AX assay, reflecting the presence of DNA double strand breaks, as a possible means for identifying individuals who are mildly hypersensitive to ionizing radiation such as some *ATM* heterozygotes. Previously we reported that cells from unaffected parents of Retinoblastoma (RB) patients in all 5 families tested are mildly hypersensitivity to radiation induced G₁ arrest and cell killing, and display a distinctive gene expression profile. The study of cells from parents of RB patients suggested the possibility of an enhanced germline mutation rate, perhaps resulting from some mild defect in genome maintenance.

We have now compared levels of γ -H2AX foci after irradiation in cells from 18 apparently normal individuals as well as from parents of 5 Retinoblastoma patients and individuals from two separate A-T families including the proband, mother, father, and three unaffected siblings in each family. After 24 hours of continuous irradiation at a dose rate of 10 cGy/hour, appreciable increases in foci per cell were observed for cells from all the RB parents, from 6 of 18 apparently normal individuals and from all the individuals with *ATM* +/- and *ATM* -/- genotypes, as compared with controls. The pooled results for the A-T families are shown in figure 1. For the heterozygotes, only the parents (obligate heterozygotes) were included in this figure but, 2 of the 6 unaffected siblings had mean foci per cell similar to that observed for the parents, while the other 4 had mean foci per cell similar to controls. We determined independently that those 2 siblings with mean foci per cell in the range of *ATM* heterozygotes bore the mutant allele, while the four siblings with a normal number of foci per cell after irradiation had normal alleles. A more limited set of experiments using lymphoblastoid cell strains in the low dose-rate assay also revealed distinct differences for normal vs. *ATM* heterozygotes of the same families, and opens the possibility of utilizing peripheral blood lymphocytes as a more suitable material for an assay to detect mild hypersensitivities to radiation among individuals.

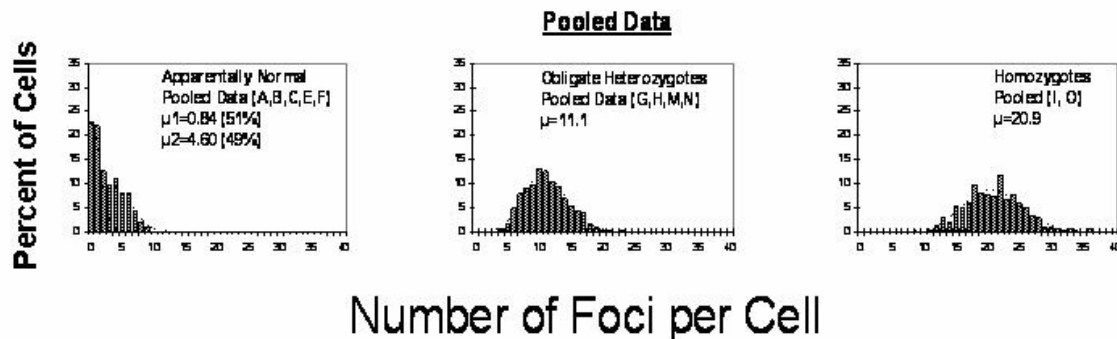


FIGURE 1--Pooled results for normal, *ATM*+/- and *ATM*-/- individuals from two A-T families. The histograms show the proportion of cells with various number of γ H2AX foci per cell immediately after irradiation at 10 cGy/h for 24 hours.

The summary of the RB and A-T studies showing the spectrum of radiosensitivities is illustrated in figure 2. The data from the 24 hour low dose rate γ H2AX assay clearly reveal a distinct spectrum of sensitivities in this assay, showing that perhaps as high as one-third of the samples from "apparently normal" individuals, and all the parents of RB patients were as sensitive as AT heterozygotes.

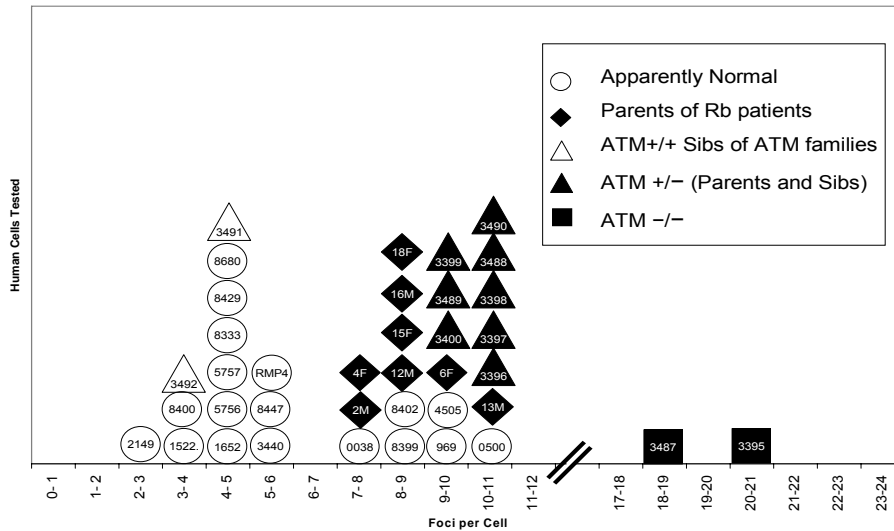


FIGURE 2

It has been pointed out on several occasions (eg., *Pharoah, et al., Nature Genetics 31, 33-36, 2002*) that while a small proportion of individuals may have a high risk of cancer, an allele that may confer a weaker predisposition but which is present in, say 20 or 30 % of the population can have for a much greater impact on the overall cancer incidence in the whole population. Similar arguments would apply for susceptibility to radiation induced cancer. To the extent that such susceptibility can be detected, at least in part by DNA repair defects, the γ H2AX assay could be useful for screening not only mildly and more severely radiosensitive individuals, but also individuals who may comprise the normal or more resistant fraction of the population. In fact, it is not known whether the presence of a relatively high proportion of mildly hypersensitive individuals may be a major factor driving risk estimates, especially for low dose and low dose-rate exposures.