Individual Genetic Susceptibility

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Loss of function of DNA repair genes has been implicated in the development of many types of cancer, but for the vast majority of cases there is no link to specific germline mutations. In the last several years heterozygosity leading to haploinsufficiency for proteins involved in DNA repair pathways was shown to play a role in genomic instability and carcinogenesis after DNA damage is induced. Since the effect of heterozygosity for one protein is relatively small, we hypothesize that predisposition to cancer could be a result of the additive effect of heterozygosity for two or more genes critical to pathways that control DNA damage signaling, repair or apoptosis. We investigated the role of heterozygosity for *Atm*, *Rad9* and *Brca1* on cell transformation, apoptosis a and cataractogenesis. Our results show that cells heterozygous for both *Atm* and *Rad9* or *Atm* and *Brca1* are more resistant to apoptosis and *Atm* and *Rad9* cells are more sensitive to transformation by radiation when compared with wild-type controls or those cells haploinsufficient for only one of these proteins. Radiation induced cataractogenesis also depends on the genotype and at least for ATM and Rad9 genes is enhanced by combined haploinsufficiency for both genes.

METHODS

Mice: *Atm*, *Mrad9* and *Brca1* heterozygous mice, as well as corresponding wild-type animals, were derived and maintained as described previously (Elson, 1996, Hopkins, 2004, Ludwig, 1998). Double heterozygotes for both *Atm-Mrad9 and Atm-BRCA1* were created by mating singly heterozygozygous mice.

Cell transformation: MEFs were irradiated in suspension and plated in 100-mm plates at a density of 6,000 cells/plate over a feeder layer of 70,000 cells prepared from the same embryo but irradiated previously with a supralethal dose. After 2 weeks of growth the cells were fixed, stained, and yields of transformed clones scored. The scoring criteria were developed and examined by preliminary experiments, where embryo cells were irradiated and plated with the same density. The clones which seemed dense and had stellate-shaped piled cells were isolated with cloning cylinders. These clones were expanded and injected into nude mice. Those that caused the development of tumors were designated as transformed. Clones that matched their shape and dimensions were scored as transformed in later experiments.

Apoptosis assay
For in vitro assays, thymocytes were isolated, irradiated and apoptosis was measured after 6 hrs by Annexin V staining. For in vivo measurements of apoptosis, mice from indicated genotypes were irradiated with 5Gy of γ-rays. 24 hours later, thymuses from irradiated and unirradiated control mice were isolated and labeled with CD4 and CD8

specific antibodies followed by flow cytometry. Cell survival for CD4/CD8 positive cells was compared with nonirradiated controls and the apoptosis was calculated from these numbers.

Catararactogenesis:
The right eye of 28 day old mice of each genotype was exposed to 0.5 Gy of 250 kVp X-rays. Unirradiated animals of each genotype were also utilized as additional controls. Mice were examined every 2-4 weeks for 55 weeks post irradiation. Lens opacification was monitored by slit-lamp biomicroscopy and analyzed using a modified version of Merriam-Focht scoring.

CONCLUSIONS

1. Under normal conditions we do not see differences in cell transformation, apoptosis or cataractogenesis between wild type and heterozygous states, but cataracts appear at an earlier time in double heterozygous animals.
2. After irradiation, we observed differences in all endpoints including reduced apoptosis, elevated transformation and increased cataractogenesis in the double heterozygous genotypes. A general conclusion is that stress response depends on the genotype and that combined heterozygosity may have additive negative effect.
3. The apoptosis results give us direct link to the efficiency of DNA dsb detection and repair. Similar damage results in different levels of apoptosis, which indicates that the overall cell reaction to damage depends on the genotype. The reduced apoptotic levels for some genotypes indicates that cells with unprepared DNA survived which may lead to increased transformation.
4. The data from this model shows that there are at least 3 low frequency high penetrance genes, mutation in which confers radiosensitivity to various endpoints including one in a highly organized normal tissue, namely ocular cataracts. Individuals with mutations in one or more of these genes constitute a radiosensitive subpopulation.