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"Mutagenic Effects of Ionizing Radiation on Immature Rat Oocytes"

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Study Findings

This study, using rats, examined deletion mutations*, which serve as a marker for determining genetic effects of radiation. We screened more than one million DNA fragments from the offspring (F_1) of female rats whose immature oocytes had received 2.5 Gy of radiation and a similar number from the offspring of non-irradiated controls. We did not find any deletion mutations that were derived from the irradiated female rats. These results suggest that immature oocytes are less sensitive than mature oocytes to mutation induction.

***This type of mutation occurs when a portion of chromosome is lost. It is considered the major type of mutation induced by radiation.**

Explanation

Risk assessment of genetic effects of radiation in humans is mainly based on the results of mouse experiments. In male mice, much data are available after radiation exposure of their spermatogonial cells. In female mice, the immature oocytes, which are the analogous female target of study, are highly sensitive to radiation and die in a process called apoptosis, leaving the F_1 offspring derived from the surviving immature oocytes essentially mutation-free. In the case of human females, however, such high sensitivity to radiation-induced apoptosis is not observed in immature oocytes. Given that apoptotic death eliminates damaged oocytes in mice and prevents viable offspring with mutations, the possibility exists that mutations may occur at a high frequency in situations where such a clearance mechanism does not function (e.g., in humans). To explain the results of RERF's genetic studies of A-bomb survivors and their offspring (F_1), experimental data from female animals are indispensable, and hence we have searched over many years for appropriate experimental animals that can be used as a model for radiation exposure in human females. Since our investigation into this issue revealed that rat immature oocytes are not as sensitive to apoptosis as mouse immature oocytes and can therefore serve as an animal model, we conducted a genetic effects

study on F_1 populations derived from irradiated female rats and nonirradiated female rats utilizing two-dimensional electrophoresis (2DE method).*

***This method cuts genes (DNA) into segments with a given restriction enzyme and performs an electrophoresis to separate the DNA segments by size (restriction enzymes cut DNA at given sequences of 4-8 base pairs). The second separation, again by size, occurs with a different restriction enzyme. Thus, if there are deletions in autosomal DNA (the numbered chromosome pairs [22] as opposed to the sex chromosome pair [1]) caused by radiation, the copy number of corresponding DNA is reduced by one-half, as only one chromosomal DNA of the autosomal DNA pair is affected).**

1. Objectives

To determine the mutation rates induced by radiation in immature oocytes in rats irradiated with 2.5 Gy and to characterize the mutations observed.

2. Methods

(1) Materials: Female Sprague-Dawley (SD) rats* were irradiated with 2.5 Gy of gamma radiation. To exclude offspring derived from mature or maturing oocytes at the time of irradiation, irradiated females were mated with nonirradiated Brown Norway (BN)* males starting at 80 days following irradiation. These F_1 rats comprised the irradiated group, while the F_1 rats born to non-irradiated pairs were considered controls. At three weeks of age, the spleen, kidney, and liver were collected from the F_1 rats, quickly frozen with liquid nitrogen, and stored at -80℃.

***SD is a laboratory rat strain established long ago and has been used in many experiments. Due to the rich store of data regarding these rats, they represent an easy-to-use strain. On the other hand, BN is a pure laboratory rat strain used in recent genome analyses. Since large numbers of genomic differences exist between SD and BN rats, these differences are useful in determining the parental origin of any mutations observed in F_1 rats.**

(2) Two-dimensional electrophoresis: DNA isolated from the spleen of F_1 rats was digested with *Not*I and *Eco*RV restriction enzymes, and the breakpoints were isotope labeled. Two types of gels containing *Not*I-*Eco*RV fragments of 1–4 kb and 4–10 kb were prepared. We conducted two-dimensional electrophoresis of these gels, dried them, and visualized the DNA fragments on X-ray film by autoradiography. Computer-based analysis of the obtained electrophoresis images was conducted to detect aberrant fragments that would indicate a deletion mutation.

3. Results

(1) Selection of DNA fragments to be examined: Detection of a total of about 3,000 DNA fragments (spots) is possible with use of the aforementioned two types of autoradiograms. From among the spots, 162 from female SD rats, 179 from male BN rats, and 1,387 shared by both SD and BN strains were found to be suitable for mutation screening (equivalent to testing of 1,549 and 1,566 gene loci, respectively, for SD and BN rats). With regard to a total of about 3,000 images obtained from 750 rats each in the F_1 group (which was derived from 2.5 Gy-irradiated female rats) and the control group, we examined DNA fragments by means of computer image analysis. Radiation emitted from the isotope contained in DNA fragments hitting silver particles turned them black on the film, and hence we determined as possible mutation cases those that had lost spot intensity by one-half as well as cases that had lost DNA fragments unique to SD or BN.

(2) Detected mutations: About 1.13 million spots were screened in each of the irradiated group and the control group. As a result, a total of 50 germ cell mutations, 18 and 32 mutations in the irradiated group and control group, respectively, were detected. A majority of the 50 mutations were caused by changes in number of repeats of 2–8 core sequences that constitute genetically unstable loci called microsatellites, which our previous research suggest were not caused by radiation. A total of three mutations, two in the control group and one in the irradiated group, were base-change mutations* at restriction enzyme recognition sites. This type of mutation is also less sensitive to radiation, and hence radiation exposure is unlikely to have been involved.

***This type of mutation occurs when one DNA base pair is replaced by another.**

With regard to deletion mutations, which serve as a marker for determining genetic effects of radiation, we detected one and three such mutations, respectively, in the control group and the 2.5 Gy-irradiated group, none of which could be established as being derived from the irradiated females (two of the three mutations in the irradiated group were derived from males, and the parental origin of the remaining mutation could not be determined). The aforementioned results suggest that the paucity of mutations in “mouse” immature oocytes is not attributable to apoptotic elimination of radiation-damaged cells, because rat immature oocytes that are more resistant did not indicate a higher radiation sensitivity for mutation induction. Therefore, immature

oocytes may be refractory (in other words, resistant) by nature to mutation induction. Due to the limited number of mutations observed, however, we plan to conduct research in the future using a different method that would enable screening of a larger number of genomic sites.

The Radiation Effects Research Foundation has studied A-bomb survivors and their offspring in Hiroshima and Nagasaki for more than 60 years. RERF's research achievements are considered the principal scientific basis for radiation risk assessment by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) and for recommendations regarding radiation protection standards by the International Commission on Radiological Protection (ICRP). RERF expresses its profound gratitude to the A-bomb survivors and survivors' offspring for their cooperation in our studies.

[§]***Radiation Research,*** which is an official monthly journal of the Radiation Research Society, publishes original, peer-reviewed papers and review articles on radiation effects and related issues in the fields of physics, chemistry, biology, and medicine. (Impact factor in 2013: 2.445)