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"Radiation-induced deletions in mouse spermatogonia are usually large (over 200kb) and contain little sequence similarity at the junctions"

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Study Findings

It has long been understood that radiation exposure induces germ cell mutations, but few studies have focused on the properties of radiation-induced mutations at the DNA level.

This study analyzed 33 de novo mutations (27 deletions and six duplications) among offspring sired by irradiated male mice and their non-irradiated controls to determine the characteristics of their base sequences.

The results of the study determined that deletions could be classified into three groups according to the size of deletion and the length of homologous sequences at the rejoined junctions:

Group 1: This group consisted of nine deletions, varying in size from 1,000 bp (1 kb) to 2,000 kb (2 Mb), with rejoined junctions containing blocks of homologous sequences longer than 200 bp. (This length indicates the homologous recombination.)

Group 2: This group consisted of five deletions shorter than 200 kb, with short blocks of homologous sequences (0–7 bp) around the junctions. (This length may indicate recombination based on short-sequence homologies.)

Group 3: This group consisted of 12 deletions characterized by their large size (>200 kb) and few homologous sequences (0–2 bp) around the junctions. (One deletion was excluded from this classification.)

Group 1 deletions were found in both irradiated and unirradiated genomes; a majority of Group 2 deletions occurred in unexposed female genomes; and Group 3 deletions occurred mainly in irradiated male genomes.

The present results indicate that Group 3 deletions could have been induced by radiation. On the other hand, no associations were indicated between irradiation and duplications.

Explanation

It is known that radiation induces various types of DNA damage, and that the most hazardous damage to living organisms is DNA double-strand breaks (DSBs). Two major types of cellular mechanism exist to repair such DSBs: One mechanism, "homologous recombination" (HR), utilizes similar or identical sequences as a template base for repair, and it is commonly considered to be error-free. The other mechanism, "non-homologous end joining" (NHEJ), ligates the ends of the break by force, and it is considered to be error-prone.

Repair via the HR pathway is less likely to bring about the many mutations that occur through the NHEJ pathway, and thus a majority of radiation-induced deletions are considered attributable to DSB repair via the NHEJ pathway.

Previously, we examined about 1,000 genes per mouse by applying two-dimensional gel electrophoresis to DNA to detect deletions among offspring sired by irradiated mice. Recently, we also prepared microarrays based on mouse genome data and screened at least one million sites in the genome by comparative genomic hybridization (CGH).

To assess the mutations detected by these methods (27 deletions and six duplications), we determined the sequences around the rejoined junctions by referring to mouse genome data. We could also infer which changes occurred during the rejoining process of two DSBs that initiated deletions.

Study Results

Among the 27 deletions we examined, 12 were characterized by their large size (>200 kb) and by short stretches of homologous sequences (0–2 bp) around the rejoined junctions; these deletions were considered to be radiation-induced mutations that occurred through repair via the NHEJ pathway. The characteristics of these deletions were different from those of deletions observed in the genome of control mice, as well as those observed at the junctions for polymorphic deletions in the human genome.

The results of this study suggest that large deletion size (>200 kb) and scarcity of homologous sequences around the rejoined junctions are the hallmarks of radiation-induced deletions in mouse spermatogonia.

Study Significance

This study revealed that there are qualitative differences between spontaneous deletions and radiation-induced deletions. In other words, radiation-induced deletions are characterized by their large size (>200 kb) and short stretches of homologous sequences around the rejoined junctions (0–2 bases). On the other hand, mechanistic differences in production of spontaneous deletions bring about two different types—small deletions measuring <200 kb (with several shared base sequences at the recombination site) and deletions of various sizes (ranging from several kb to over 1,000 kb with long homologous sequences of at least 200 bases at the recombination site)—suggesting that size is not the only determinant. Thus, to identify whether the newly found deletion-type mutations are radiation induced, deletion size alone is insufficient, with nucleotide sequence information at the DNA recombination site also necessary.

The Radiation Effects Research Foundation has studied A-bomb survivors and their offspring in Hiroshima and Nagasaki for around 70 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.

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