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“Creation of mice bearing a partial duplication of HPRT gene marked with a GFP gene and detection of revertant cells in situ as GFP-positive somatic cells”

Asao Noda, Hirofumi Suemori, Yuko Hirai, Kanya Hamasaki, Yoshiaki Kodama, Hiroshi Mitani, Reid D. Landes, Nori Nakamura

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Study Findings

We created transgenic mice that allow *in situ* (i.e., in tissue) visualization of mutations in any cell throughout the body of the animal. The mice are technically called HPRT-dup-GFP knock-in mice. We used this knock-in mouse model to detect mutant cells arising in organs, including the liver, pancreas, small intestine, large intestine, lungs, thyroid, and testes. Large differences among individuals in terms of spontaneous mutation frequencies were observed in somatic cells. Such high spontaneous frequencies impeded detection of mutations induced by radiation. Lowering the background mutation frequency is the next challenge for better use of this system in radiation research.

1. Objectives

One objective was to create mice to assist in the detection of radiation-induced mutations in somatic and germ-line cells. Cells developing a mutation in this mouse model survive and fluoresce green (because they contain green fluorescent protein, or GFP). Another objective was to use this mouse model to observe where radiation-induced mutant cells occur and grow in the body. The final objective was to establish a system for monitoring the genetic effects of radiation in individual organisms.

2. Methods

Embryological engineering techniques were used for manipulating the mouse HPRT gene (a structural gene involved in nucleic acid synthesis) to be partially duplicated in conjunction with the GFP gene on the X chromosome, thereby creating transgenic mice. Recombination-mediated reversion from the partial duplications of the *HPRT* (i.e., reactivation of gene activity) in the cells of these mice led to expression of the HPRT-GFP fusion protein and caused the affected cells to fluoresce green.

Radiation exposure effects were evaluated using fluorescence to determine mutation frequency in the mouse cells after the mice were exposed to radiation.

3. Results

- (1) We successfully created transgenic mice (HPRT-dup-GFP mice) by double gene knock-in (the insertion of a gene in a specific location in a chromosome) in mouse embryonic stem cells (cells from the early embryo that can differentiate into any cell type). On the HPRT-dup-GFP locus of the mice, *in vivo* arising, somatic mutant cells, which are revertants from the partial duplicated allele of the *HPRT* gene, were confirmed as originating from gene deletion via normal homologous recombination repair. Mutant cells in any of the tissues except the brain fluoresced green, but the frequencies of spontaneous mutations differed among tissues.
- (2) Large individual differences were seen in the frequency of somatic cell mutations, even in the mice not exposed to radiation. Around 1% of the mice developed mutations too numerous to be countable. We used the term “extreme jackpot mutations” to describe such cases. The large differences in mutation frequency among individual mice may depend on when during development and growth cycles mutant cells arise. The investigation thus can be conceived as a fluctuation test of mutations at the level of the individual.
- (3) Somatic cell mutation frequencies were measured in different tissues of mice 3 months after exposure to a dose of 3 Gy as a model of radiation exposure. Mutation frequency was observed to be little changed in certain tissues (pancreas and lymphocytes) but two-fold higher in others (e.g., liver, small intestine).

Study Significance

We successfully created mice whose living cells fluoresce when developing a mutation. Although the model is useful, background noise from spontaneous mutations will have to be reduced for the model to be a reliable indicator of the effects of radiation exposure. Our findings strongly suggest that many of the somatic cells making up our bodies already contain mutations that give us a genetically mosaic appearance.

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.

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