*Delayed Adverse Effects of Drugs and Toxicological Risk Assessment (T. Fujii and T. Yanagita, eds.)
Session I. Symposium : Evidence Accumulated on the Delayed Adverse Effects of Drugs.*

DELAYED EFFECTS AND INDUCTION OF GENETIC INSTABILITY BY RADIATION

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1. Introduction

Carcinogenesis is the most widely recognized delayed effect of radiation in irradiated individuals. It has been considered that mutations induced by DNA damage are directly responsible for radiation carcinogenesis. However, as discussed below, some features of radiation carcinogenesis suggest that radiation induces cancer not through mutation due to DNA damage, but through indirect actions such as induction of genetic instability in irradiated cells.

2. Latency of radiation-induced cancer

Radiation induced malignancies can be classified into two groups according to their dose incidence relationships and latencies. Dose responses of radiation induction of leukemia among humans are often linear-quadratic, while those of solid tumors are linear (Preston *et al.*, 1994; Thompson *et al.*, 1994). In addition, the latencies for solid tumors among atomic bomb survivors are usually much longer than those for leukemia, with the tumors developing when the exposed individuals reach the so-called "cancer age" (Shimizu *et al.*, 1990).

It is widely accepted that carcinogenesis requires multiple hits to oncogenes and tumor suppressor genes (Fearon and Vogelstein, 1990).

This paper was presented at the 4th Satellite Symposium of the Japanese Society of Toxicological Sciences on July 17, 1995 in Tokyo.

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In agreement with this hypothesis, the incidence of cancers increases with power of age (Armitage and Doll, 1954). If radiation induces cancer by providing one of the hits, then one may expect that irradiated individuals would require less additional hits for the development of cancer. This would lend to the prediction that cancer should develop with shorter latency in irradiated individuals. However, as discussed above, the latencies of solid tumors are similar for non-irradiated and irradiated human populations. This is also true for experimental animals such as mice. This suggests that carcinogenic mutation occurs not at the time of irradiation, but long after the exposure to radiation.

3. Types of radiation-induced cancer

Radiation-induced cancers are not of one specific type. Most solid tumors seen in the unexposed population occur at higher levels in the exposed population of atomic bomb survivors, and the excess relative risk of these tumors distributes between 0 and 1.5 at 1 Sv (Thompson *et al.*, 1994). Also, radiation-induced solid tumors in mice are the same as seen in unirradiated groups, except they occur at higher frequencies in irradiated mice. Thus, radiation seems to enhance spontaneously occurring tumors.

4. Frequencies of radiation carcinogenesis

It is well known that radiation-induced somatic mutation at single-copy genes occurs at frequencies of 10^{-5} to 10^{-4} . In contrast, initiation

of cancer by radiation in rats and in tissue culture cells occurs at much higher frequencies, such as 10^{-1} (Kennedy *et al.*, 1980 ; Kamiya *et al.*, 1995). Incidence of lung cancer increases in mice born to irradiated parents (Nomura, 1982), suggesting involvement of germline mutation. The increase of frequency of this transgenerational carcinogenesis is in the order of 10^{-1} . In contrast, the frequency of radiation-induced germline mutation at single-copy genes is in the order of 10^{-4} (Russell and Kelly, 1982). These facts again suggest that carcinogenic events in germ cells occur at much higher frequency.

5. Radiation-induced germline mutation at a hypervariable minisatellite locus in mice

We have analyzed radiation-induced germline mutation at the mouse hypervariable minisatellite Pc-1 locus, among F1 mice born to irradiated parents. This locus consists of tandem repeat of GGGCA and is mapped on mouse chromosome 4 (Mitani *et al.*, 1990 ; Kelly *et al.*, 1989). The Ms6hm locus is highly unstable among laboratory mice. Male mice of the C3H/HeN strain were irradiated with gamma-rays and mated with female C57BL/6N mice. The matings were done immediately, 2–3 weeks and 10–11 weeks after irradiation, to see the effect of radiation on the spermatozoa, spermatids, spermatogonia stages of germ cells. DNA was extracted from the tail tissue of F1 mice, digested with HaeIII, and analyzed by Southern blotting. Mutants were identified as those carrying the Pc-1 allele with difference in length from the parental mice (Sadamoto *et al.*, 1994).

The dose relationship of mutation induction at the Pc-1 locus was analyzed for spermatozoa, spermatids, and spermatogonia irradiation (Fan *et al.*, 1995). The spontaneous mutation frequencies in 131 unirradiated F1 mice were 9.1% for the C3H/HeN allele and 12% for the C57BL/6N allele. Analysis of F1 mice born under the condition of spermatozoa irradiation revealed that the male and female allele mutation frequencies were elevated to 14% and 15%, respectively. However, these values were statistically not significant. Spermatid stage germ cells were sensitive to radiation, and the male allele mutation frequency rose to 22%, 28% and 28% for 1 Gy, 2 Gy, and 3 Gy irradiation, respectively ; these

values were statistically significant. The female allele were unaffected by spermatid irradiation. The male allele mutation frequency for spermatogonia irradiation was 16%, but this value was not statistically significant. The increase of over 10% in mutation frequency for 1 Gy of radiation was at least three orders higher than those of single copy genes (Russell and Kelly, 1982). Distribution of DNA damage per dose of radiation can be calculated to yield 1 single-strand break per 3,000 kb DNA for 1 Gy of radiation. In contrast, the size of the Pc-1 locus is around 3 kb. Thus, a mutation frequency of 10^{-3} can be expected for this locus at 1 Gy, while a mutation frequency of 10% was actually observed in this study.

These results suggest that the length change mutation at the Pc-1 locus was not due to the direct effect of radiation. Rather, it is likely that radiation induced genetic instability in the cells which in turn induced mutation at the Pc-1 locus. The minisatellite sequences are known to mutate by gene conversion or homologous recombination (Monkton *et al.*, 1994). Thus, radiation might affect the frequency of recombination through induction of genetic instability in the cell.

6. Induction of minisatellite instability in cancer cells

The Pc-1 locus is quite stable in somatic cells. However, it becomes highly unstable in certain cancer cells. We have analyzed methylcholanthrene-induced sarcomas in F1 mice between C3H/HeN and C57BL/6N. The Pc-1 locus mutated frequently, especially in sarcomas with amplification of the c-myc gene (Niwa *et al.*, 1995). These sarcomas with the amplified c-myc gene also carry point mutation of the K-ras gene. Thus, cancer cells have built-in instability of the genome, which may be an important factor in the progression and acquisition of more malignant phenotypes.

7. Mechanism of radiation carcinogenesis

As discussed, the frequencies of initiation in radiation carcinogenesis and transgenerational carcinogenesis are much higher than those of single-copy gene mutations. Therefore, it is possible that radiation induces genetic instability in the cells and thus the mutation frequency of

the irradiated cells is increased for long time after irradiation. Cells would undergo malignant conversion automatically when they acquire the mutations conferring growth advantage.

It has been accepted that DNA-damaging agents induce cancer by directly inducing carcinogenic mutations. However, one more pathway of carcinogenesis by these agents is the indirect one, i.e. induction of genetic instability. Thus, elucidation of the molecular mechanism of genetic instability induced by radiation would facilitate our understanding of initiation of carcinogenesis.

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