Skin and Bone Tumors Induced by Repeated Beta-Irradiation of Mice:
Threshold Effect and p53 Mutations

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INTRODUCTION

The question as to whether a threshold dose exists in radiation carcinogenesis is important for the estimation of radiation risk at low doses. Although the relationship between the radiation dose and tumor incidence fits a multihit or linear-quadratic model, a threshold model can not be excluded. In fact, the radiation dose at which no increase in tumor incidence is recognized over the background level can be seen in the induction of solid tumors and leukemia with single whole body exposure to X-rays or gamma rays, as well as in pulmonary tumors caused by partial irradiation with single doses of up to 2.5 Gy of X rays in mice. When irradiation is protracted or the total dose fractionated, no apparent increases in tumor incidence in skin cancer in mice and rats occurs, or in ovarian tumor and leukemia in mice. Error-free repair of DNA damaged by a low dose, low dose rate, or fractionated exposure is considered to be efficient.

We established the conditions of irradiation that yield 100% cumulative tumor incidence by repeated beta-ray radiation to the backs of mice 3 times a week. I here summarize our results which show the threshold effect in radiation carcinogenesis under our conditions and the associated p53 gene mutations.

EXPERIMENTAL

Various organs of rodents have been the targets in studies of radiation carcinogenesis. The lung, liver, ovary, mammary gland, bone, lymphoid tissue, skin, and pituitary and harderian glands have all been examined. Because inbred mice have spontaneous tumors, careful selection of the target organ is important.

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in examinations of tumors induced by a low dose or low dose rate of radiation. In our experiment, we used the skin of female ICR strain mice as the target organ, because spontaneous skin tumors are very rare in mice. Moreover, the setting of the exposure area and observation of tumor emergence are easy (Figure 1a). We used a $^{90}$Sr-$^{90}$Y applicator (40 mCi loaded on a 2-cm diameter disk, maximum beta-ray energy 2.24 MeV). As the penetration of beta-particles into tissue is weak, the influence of radiation scattered to other body parts is negligible.

![Beta-ray source](image)

Figure 1. Irradiation method and scheme. a. ICR female mice (Charles River Japan) 7 weeks of age were irradiated. The backs of the mice were freed of hair and irradiated with beta-rays from $^{90}$Sr-$^{90}$Y (Max. energy 2.24 MeV). b. Irradiation was repeated 3 times a week until a tumor appeared or until the mice died without developing tumors.

**THRESHOLD RESPONSE FOR TUMOR INDUCTION**

Cumulative tumor incidences for each dose group are shown in Figure 2. In groups irradiated at doses of 2.5 to 11.8 Gy per exposure, tumors began to emerge about the 200th day after the start of irradiation. At about the 500th day the cumulative tumor incidence became 100%. Emergence of tumors in the 1.5 Gy group began later, eventually reaching 100%. No tumors were generated in any of the mice in the 0.5 Gy group up to the end of their life spans. The threshold response is more clearly seen when the median time for tumor emergence is plotted against the beta-ray dose per exposure (Figure 3). In addition, immunological responses (mitogen in proliferation of lymphocytes, mixed lymphocyte reaction, killer T-cell activity, and NK-cell activity) did not decrease after repeated irradiation (Sado, Ootsuyama and Tanooka, unpublished observation).

Hecker et al. showed that the dose response for a tumor promoter is expressed in a threshold manner in their study of the chemical carcinogen DMBA (dimethylbenzanthracene) with 3TI (3-o-tetradecanoyligenol) as the promoter. Furthermore, Fujiki et al. showed that the activity of ODC (ornithinedecarboxylase), which is an index of the promotion effect, was elevated in skin exposed to beta-rays. We observed a significant delay in skin tumor emergence in a study in which tumors were induced by repeated irradiation combined with the continuous administration of DFMO ($\alpha$-difluoromethylornithine), an inhibitor of tumor...
promotion, in the drinking water\textsuperscript{18}). The results indicate that repeated irradiation with beta-rays has a tumor promotion effect and that the promoting action gives a threshold-like dose response.

Blum et al. observed a threshold-like response with repeated UV irradiation\textsuperscript{19}. Shortening the UV irradiation interval made the threshold-like response even more clear. DeGruilj et al.\textsuperscript{20} and Forbes et al.\textsuperscript{21}, however, did not find a threshold response in their studies with UV. This difference may be due to the choice of UV wave length.

Figure 2. Cumulative tumor incidences in mice exposed to repeated beta radiation plotted against time. Numbers of mice: a, 50; b, 31; c, 30; d, 22; e, 31; f, 21; g, 15; h, 31; i, 18; and the controls, 31. The cumulative tumor incidences were calculated by the Kaplan-Meier method\textsuperscript{22}. (Data redrawn from Ref.15)

Figure 3. Relationship between the dose per exposure and the time required for 50% tumor incidence. The time required for 50% tumor incidence was nearly constant in the range of 2.5 to 11.8 Gy and was rapidly prolonged at lower doses. (Data redrawn from Ref.22)
HISTOLOGICAL TYPES OF BETA-RAY INDUCED TUMORS

The radiation-induced tumors found in our examinations were classified histologically as squamous cell carcinoma, basal cell carcinoma of epidermal origin, fibrosarcoma of dermal origin, and osteosarcoma of bone origin (Figure 4a-d). The number of each type of tumor is shown in Table 1. The osteosarcomas emerged from the lumbar vertebrae of the mice because the beta-rays reached not only skin but also lumbar vertebrae (80% of the surface dose at the top of the lumbar vertebrae)\(^\text{[23]}\). Adnexal tumors of the skin, seen in rats\(^\text{[22,24]}\), were not found in our examination. Albert et al. reported that mouse hair-follicle cells are more sensitive to radiation than those of the rat, therefore tumors of this origin are rare in mice\(^\text{[25]}\). None of these tumors were found in the non-irradiated control mice. No predominance of a specific type of tumor was found in each group, except for the slight predominance of bone tumors in the 2.5 and 3.5 Gy groups\(^\text{[14]}\).

Figure 4. Histological types of tumors induced by repeated beta-irradiation. a. squamous cell carcinoma; b. basal cell carcinoma; c. fibrosarcoma; d. osteosarcoma. Tumors were dehydrated using the common methodology after fixation with 10% formalin, then paraffin embedded, and stained with hematoxylin-eosin.

p53 MUTATIONS IN RADIATION-INDUCED TUMORS

In our examination\(^\text{[23]}\), mutations in the p53 gene of tumors induced by repeated irradiation were found in exons 4 to 8 (Figure 5, Table 2), the same distribution as in human cancers\(^\text{[27]}\). A frame shift mutation was
found in 3 deletions and 2 insertions. In tumors No. 4, 5 and 12, the mutated genes seemed to produce only 55% to 70% of the complete p53 protein, which protein is thought to have lost the nuclear localization signal (NLS) region. It is speculated that truncated p53 protein cannot migrate into the nucleus, resulting in the reduction of its ability to inhibit cellular transformation. Immunohistochemical staining showed that the p53 protein accumulated in all the tumors, except No. 12 (Figure 6).

All the base substitutions except one were in the conserved region. There were 4 substitutions in the CpG site, and 3 of these occurred in codon 122. This is the site at which spontaneous mutation occurs most frequently in human tumors. Codon 122 is thought to be the hot spot of mutation in the p53 gene in the laboratory mouse. We found 3 types of the p53 pseudogene in 5 species of mice, and all had the same base substitution in codon 122.

As for the spectrum of p53 mutations, no previous reports have described the high frequency of deletions, insertions and base substitutions in the p53 gene found in ionizing radiation-induced tumors. Most p53 mutations in experimental tumors induced by UV or chemicals are base substitutions, and most of the p53 mutations in human skin cancers also are base substitutions. Deletions have been reported in the p53 gene of lung cancers in uranium miners, which may be the result of long term exposure to ore dust. The mutations in the mouse p53 gene found in our study may be unique to tumors induced by the repeated irradiation used in our experimental regimen.

Our experimental system is unique in that irradiation is repeated over the entire life span of the animals. These conditions can be extrapolated to daily exposure with very low dose radiation, and they simulate environmental exposure to ionizing radiation.

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Table 1. Number of tumors of different histological types induced by repeated radiation

| Histological type          | Total number of tumors |
|----------------------------|------------------------|
| Squamous cell carcinoma    | 42                     |
| Basal cell carcinoma       | 12                     |
| Fibrosarcoma               | 46                     |
| Osteosarcoma               | 57                     |

(Data from Ref. 26)

Figure 5. Site of mutations in the p53 cDNA of radiation-induced mouse tumors. Candidate DNA fragments for p53 mutation were selected by the SSCP method and directly sequenced. Deletions (1-24 base), base substitutions (4-8 base). Mutation sequences are shown in Table 2.

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Table 2. Mutation sequences

| Codon | Exon | Mutation type |
|-------|------|---------------|
| 0     | 1    | 8             |
| 1     | 2    | 123           |
| 2     | 3    | 456           |
| 3     | 4    | 789           |
| 4     | 5    | 012           |
| 5     | 6    | 345           |
| 6     | 7    | 678           |
| 7     | 8    | 890           |
| 8     | 9    | 901           |
| 9     | 10   | 0123          |
| 10    | 11   | 1234          |
| 11    | 12   | 2345          |
| 12    | 13   | 3456          |
| 13    | 14   | 4567          |

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Threshold in tumor induced by radiation
Table 2. p53 mutations of mouse tumors induced by repeated radiation.

| Type of p53 mutation | Sample number | Dose per exposure (Gy) | Tumor histology | Base(s) altered | IHC |
|----------------------|---------------|------------------------|-----------------|-----------------|-----|
| Deletion             | 1             | 1.0                    | SCC             | GCC CC          | +   |
|                      | 2             | 1.0                    | SCC             | T TC            | +   |
|                      | 3             | 1.5                    | FS              | C GCC ACA CCT CCA GCT GGG AGC CG | - |
|                      | 4             | 1.5                    | FS              | G              | +   |
|                      | 5             | 2.5                    | FS              | CAC AG          | +   |
|                      | 6             | 3.0                    | OS              | GT GCC GGC GCC A | + |
|                      | 7*            | 3.0                    | SCC             | T GGG AAC CTT CTG GGA CGG GAC AG | + |
|                      | 8             | 3.5                    | FS              | C TTA TCC GG    | +   |
|                      | 9             | 4.5                    | FS              | C AGC TTT GAG GTT | + |
|                      | 10*           | 8.0                    | FS              | TAC TCT CCT CCC CTC AAT AAG | + |
|                      | 11            | 8.0                    | OS              | T GGA AG        | +   |
| Insertion (repeated sequence) | 12          | 1.0                    | SCC             | C CTT           | -   |
|                      | 13            | 3.0                    | FS              | TC TG C C       | +   |
|                      | 14            | 3.0                    | SCC             | A CTG GAA G     | +   |
| Base substitution    | 15            | 1.0                    | SCC             | ACG → ATG       | +   |
|                      | 16            | 1.5                    | SCC             | ACG → ATG       | +   |
|                      | 17            | 1.5                    | FS              | ACG → ATG       | +   |
|                      | 18            | 2.5                    | OS              | CGC → TTC       | +   |
|                      | 19            | 3.0                    | FS              | CTT → CCT       | ND  |
|                      | 20            | 3.0                    | SCC             | GCC → GAT       | +   |

SCC: Squamous cell carcinoma, FS: Fibrosarcoma, OS: Osteosarcoma. IHC: Immunohistochemical analysis. CM-1 staining for p53. *: Splicing mutation. (Data from Ref. 26)

Figure 6. Immunostaining of tumor tissue with p53-antibody CM-1 (Ref. 44). a, Tumor No.13; b, Tumor No.20.
This review supplements previous reviews of our radiation carcinogenesis works\textsuperscript{45,46} with new p53 mutation data.

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