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REVIEW

MUTATION AND CANCER IN RELATION TO THE ATOMIC-BOMB RADIATION EFFECTS

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INTRODUCTION

Epidemiologic studies of the atomic bomb survivors[1-4] have provided valuable information on radiation-induced cancer and mutation in man. The results of these studies contain many clues useful for elucidating mechanisms of induction of cancer and mutation in man after exposure to radiation. In the present paper, these clues are considered together with recent developments in our knowledge of carcinogenesis and mutagenesis in *Drosophila*, mice, rats and cultured human cells, and a working hypothesis is presented for radiation carcinogenesis in man. The basis of this hypothesis is as follows.

The incidence of most cancers observed among A-bomb survivors increases approximately linearly with increasing radiation dose.[2, 4] If the occurrence of somatic mutations is the first step in the formation of these cancers, this linear relation is easily explained, as the frequency of radiation-induced mutations increases linearly with increasing dose.[5-9] However, the presumed radiation-induced mutation must be recessive in producing cancer, because radiation predominantly induces deletions,[10, 11] rarely base substitutions,[12-14] and most deletions produce recessive traits.[15] Hence, most radiation-induced oncogenic mutations should express their oncogenic characters only after they are converted from heterozygosity to homozygosity at some time in the host's life, an indication that two mutational events are required to initiate radiation carcinogenesis. Based on data of somatic mutations in A-bomb survivors and on the two-mutation model, I construct an equation for the frequency of production of a preleukemic cell in the human body as a function of dose.

The obtained theoretical equation can explain fairly well the actual dose-response relation for leukemia in A-bomb survivors except in the low dose range, where an apparent threshold effect exists. Whether the threshold effect really exists in A-bomb survivors cannot be concluded from epidemiologic data alone. To supplement the epidemiologic studies, relevant reports on experimental carcinogenesis are reviewed. From detailed comparative analyses of human and experimental cancers after irradiation, a promoter model emerges in which radiation at doses above a threshold value promotes the development of cancer.

I. ATOMIC-BOMB RADIATION EFFECTS

In this section, the genetic and oncogenic effects of the A-bomb radiations are reviewed and summarized to provide a basis for development of the present theory.

I.1. Genetic Effects on Children of Atomic Bomb Survivors

Painstaking and long-term studies from 1948 to 1987 have been unable to detect any significant increase in genetic abnormalities in the children of A-bomb survivors.

I.1.1. Untoward pregnancy outcomes[1)]

The incidence of untoward outcomes (stillbirth, major congenital defect, death during first postnatal week) was 4.78% (408/8537) in children with an average parental dose of ca. 50 rem per person and 4.75% (2924/61545) in control children.

I.1.2. Death of live-born children[1)]

The frequency of deaths through the age of 17 years was 6.30% (737/11736) in children with an average parental dose of ca. 50 rem per person and 6.40% (2494/38953) in control children.

I.1.3. Chromosome abnormalities[16)]

The frequency of aneuploidy plus structural rearrangements was 0.52% (30/5762) among children with an average parental dose of 45 rem per person and 0.49% (20/5058) among control children.

I.1.4. Base-substitution mutations altering electrophoretic mobility of blood proteins[17, 18)]

The mutation frequency was 4.5×10^{-6} (3/670,000) (/locus/generation) among children with an average parental dose of 24 rem per person and 6.4×10^{-6} (3/450,000) (/locus/generation) among control children.

I.2. Genotoxic Effects on Atomic Bomb Survivors

The following three somatic effects showed approximately linear dose-response relationships, though a threshold dose was found in some cases.

I.2.1. Mental retardation[19, 20)]

The incidence of mental retardation among Hiroshima children, exposed *in utero* and tested at the age of 17 years, significantly increased at a rate of ca. 10^{-3}/rad after a threshold dose of 10–30 rad.

I.2.2. Chromosome aberrations of stable types in peripheral lymphocytes[21, 22)] — markers for mutant clones originating in hemopoietic stem cells

Chromosome aberrations have been examined most extensively to investigate the genotoxic effects in A-bomb survivors. In individual donors within subgroups exposed to equal doses, the frequencies of these aberrations were scattered widely. However, clear dose-response relations were found when the mean frequencies for subgroups in different dose intervals were plotted against dose. The mean frequency per cell of aberrations increased approximately linearly with increasing dose in the case of Hiroshima survivors, whereas it increased with increasing dose only above an apparent threshold dose in the case of Nagasaki survivors. The average frequency of reciprocal translocations, the major type among aberrations present in 1968–71 samples, increased approximately linearly with increasing dose of the Hiroshima A-bomb radiation as follows[21, 22)]:

$$y=e+fx, \tag{1}$$

$$e=6\times10^{-3}\text{(/cell)}$$
$$=6\times10^{-6}\text{(/chromosome pair)}, \tag{2a}$$

$$f=6\times10^{-4}\text{(/cell/rem)}$$
$$=6\times10^{-7}\text{(/chromosome pair/rem)}, \tag{2b}$$

where y is the frequency of translocations per cell, e the spontaneous rate, f the induced rate per unit dose, and x the radiation dose. In Equations (2a) and (2b), the translocation per chromosome pair is obtained from the translocation per cell by dividing by 10^3 ($=45\times45/2$), an estimate of all possible combinations of non-homologous chromosome pairs among 46/45 chromosomes in a somatic cell. The e and f values are not significantly different for cells sampled in 1968–1971 and those sampled in 1978–1981.[23)] This supports the assumption that the observed translocations are replicas of prototype translocations produced by A-bomb radiation several decades ago in long-lived stem cells of the donors.

I.2.3. Somatic mutations at the glycophorin A locus in A-bomb survivors[3)]

Recent advances in flow cytometry have made it possible to detect variant cells with specific fluorescent-dye-labeled monoclonal antibodies. Blood samples from Hiroshima A-bomb survivors heterozygous for codominant alleles M and N of glycophorin A (a cell-surface glycoprotein on erythrocytes) were used to detect erythrocytes of variant (named mutant hereafter) types, M/O (having the M form but lacking the N form), N/O (N without M), M/M (expressing twice the M form) and N/N. The results reported by Jensen and coworkers[3)] can be approximated by the following equations:

$$y=a+bx, \tag{3}$$

$a = 12 \times 10^{-6}$(/locus);
for mutation M/O or N/O, (4a)
$a = 9 \times 10^{-6}$(/chromosome pair);
for mutation M/M, (4b)
$b = 5 \times 10^{-7}$(/locus/rad);
for mutation M/O or N/O, (5a)
$b = 3 \times 10^{-7}$(/chromosome pair/rad);
for mutation M/M, (5b)

where y is the frequency of mutant cells in samples from persons exposed to dose x, a is the frequency in the control, and b is an average rate per rad of increase in the frequency. The b values in Eqs. (5a) and (5b), which are about twice as large as the corresponding values originally reported,[3] are based on a reassessment of samples from the same donors using the new dosimetry system, DS86, and a modified technique with a single beam flow sorter.[24]

I.3. Cancers in Atomic Bomb Survivors

Attention will be focused on three points: (1) the classification of dose-response relations, (2) the cancer incidence at low doses and (3) the latent period of cancer.

I.3.1. Dose-incidence relations for cancers

Using the new dosimetry system (see below), estimates of the cancer mortality rate for the period 1950–1985 have recently been appraised in a preliminary report.[4] Dose-response curves are quoted from this report and reproduced with minor modification in Fig. 1. For testing the two-step (initiation and promotion) model of carcinogenesis, these curves were classified visually into linear, sigmoidal or quadratic types. They are listed in Table I.

I.3.2. Apparent threshold effects in induction of cancers after low doses of A-bomb radiation

Analyses of the dose-response relationships of cancers and other indicators in atomic bomb survivors were carried out for the first time by Kato *et al.*[25] in the range from 1–5 to 20–49 rad. Now, in the same low dose range but in terms of the new dosimetry system DS86, dose-response curves for the incidence of death from various types of cancer are available[4] as partly shown in Fig. 1. As can be seen from Fig. 1, the dose-response curves of most cancers have troughs at 1–5, 6–19 or 20–49 rad. In this paper, the existence of such troughs is referred to as an apparent threshold effect. Whether threshold effects really occurred in A-bomb survivors cannot be concluded from these epidemiologic data alone because of the large statistical uncertainty at each trough. With this reservation in mind, these apparent threshold effects are classified into four categories (−, non-existent; +, small; ⧺, intermediate; and ⧻, large) as given in Table I. Apparent threshold effects (⧺ or ⧻) in Nagasaki exist in 3 out of 5 types of cancers, and in Hiroshima in 1 of 5. Thus, available data suggest that apparent threshold effects might be more frequently found in persons exposed to the Nagasaki A-bomb radiation than in those exposed to the Hiroshima A-bomb radiation.

At the high dose of 300 rad, the induced rates of death from four of five types of cancer were higher in Hiroshima A-bomb survivors than in Nagasaki A-bomb survivors (see Table I). The Nagasaki A-bomb radiation produced seemingly sigmoidal dose-response relations, except for a possible linear relation for stomach cancer, whereas the Hiroshima A-bomb radiation produced seemingly linear dose-response relations, except for a possible quadratic relation for colon cancer (Table I).

All in all, there seem to exist qualitative as well as quantitative differences in the dose-response relations for the production of cancers and genetic effects after the Nagasaki A-bomb and the Hiroshima A-bomb. If the differences are real, some of them could be attributed to differences between the Nagasaki and Hiroshima populations, and some to differences in radiation quality. Here, I consider only the factors related to radiation quality.

In terms of DS86 (the dosimetry system revised and accepted in 1986 by the US-Japan Atomic Bomb Radiation Dosimetry Reassessment Committee for estimating radiation doses from the Hiroshima and Nagasaki atomic bombs),[26] the ratio of fission neutrons to gamma rays is greatly decreased for Hiroshima, approaching that of Nagasaki. Preston and Pierce[27] concluded, in consequence, that any apparent city difference in the dose-response should be much less attributable to RBE (relative biological effectiveness; see Section II. 4) of fission neutrons. However, the neutron component is still considerably larger for Hiroshima than for

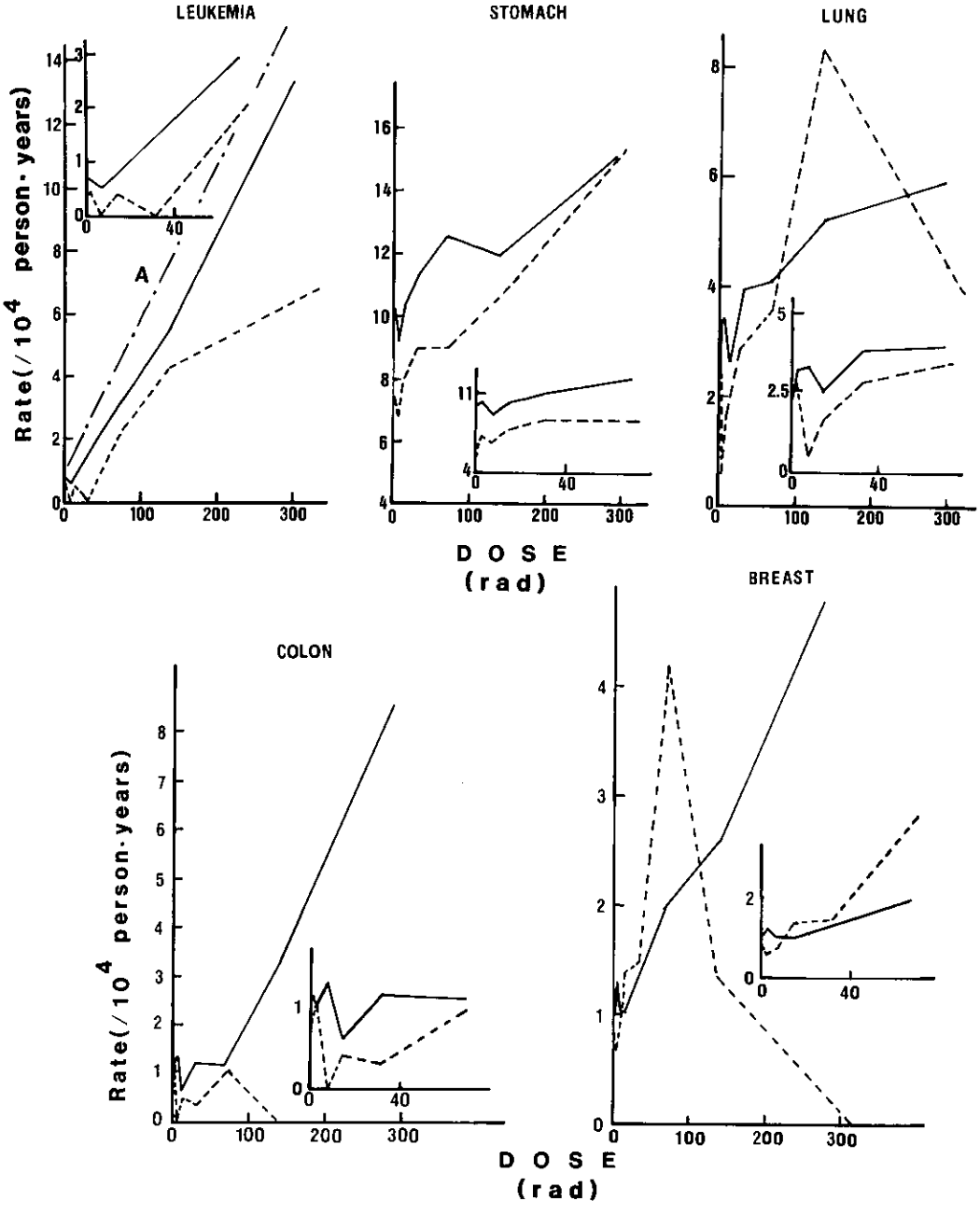

Fig. 1. Dose-response curves for the rates of deaths from five types of cancer in Hiroshima (——) and Nagasaki (----) A-bomb survivors (reproduced with minor modification by permission from Shimizu *et al.*[4]). The theoretical curve A in the panel 'LEUKEMIA' is obtained from Eq. (15) in the text.

Table I. Characteristics of Dose-Incidence Relations for Cancer Mortality[a]

City Cancer	Type of response	Spontaneous rate (/10^4 person·years)	Induced rate at 300 rad (/10^4 person·years)	Threshold effect[c]
Nagasaki				
Leukemia	Sigmoidal	0.4	6	##
Colon	Sigmoidal	0.8	−0.7	###
Stomach	Linear	6.3	8.5	−
Breast	Sigmoidal	1	(2)[b]	+
Lung	Sigmoidal	2.6	(1)[b]	##
Hiroshima				
Leukemia	Linear	0.8	12.5	+
Colon	Quadratic	1.2	7	##
Stomach	Linear	10	5	+
Breast	Linear	1.0	3.5	−
Lung	Linear	2.4	2.5	−

a) Extracted from Fig. 1.
b) Average of a value at 300 rad, which happened to be close to zero, and a maximal value at about 100 rad (see Fig. 1).
c) −, Non-existent; +, small; ##, intermediate; ###, large.

Nagasaki. Furthermore, DS86 disclosed that the energy spectrum of the fission neutrons from the Hiroshima A-bomb was considerably shifted toward lower energies.[26] This raises the possibility that the RBE of fission neutrons in Hiroshima could have been considerably larger than that in Nagasaki. This possibility is compatible with the empirical rule that fast neutrons with lower energy produce more damaging effects (or higher RBE values) on experimental animals and plants, and on cultured human cells.[28, 29]

I.3.3. Latent periods of cancers

A-bomb survivors were exposed to radiation for a very short time and their exposure dates are exactly known. Followup studies over more than 40 years have provided reliable information on the latent period for radiation-induced cancer.[2]

Leukemia appeared 2–3 years after exposure and reached a peak in 1950–51 in Hiroshima and slowly decreased thereafter; it occurred significantly above the control level even in 1979–1982. In Nagasaki, the incidence reached a peak in 1959–62 and decreased thereafter rather rapidly, coming down to the control level in 1971–74.

The mean interval from the A-bomb irradiation to death from leukemia was shorter in the group exposed at over 100 rad than in the 1–99 rad group or the control group,[30, 31] whereas the latent periods of solid tumors were not significantly affected by radiation.[31] In other words, solid tumors developed after exposed persons reached the ages when the same tumors developed in control persons; the latent periods of solid tumors were shorter for persons exposed at old ages than for persons exposed at young ages.[31] In contrast, chronic and acute myelogenous leukemia had a shorter latent period for persons exposed at young ages than those exposed at old ages.[30]

II. Experimental Mutagenesis and Carcinogenesis Relevant to the A-bomb Radiation Effects

II.1. Radiation Mutagenesis in Spermatogonia of Mice

Russell and Kelly[5–7] carried out extensive studies on the induction of recessive mutations in the germ cells of irradiated mice. They focused on mutation at seven specific loci. Homozygous mutants show specific coat color or short ears. In spermatogonia, the frequency y of mutations per locus (mean number of mutations per locus) increased linearly with increasing radiation dose x:

$$y = c + dx, \tag{6}$$

where c (/locus/generation) denotes the

spontaneous frequency of germline mutations and d (/locus/rad) the rate per unit dose of induced mutations.

II.1.1. Dose-rate dependence and independence of radiation mutagenesis

From extensive experiments, the following empirical rules and parameter values were established for radiation mutagenesis in spermatogonia:

$$c=8.1\times10^{-6}\text{(/locus/generation)}, \quad (7)$$

$$d=2.2\times10^{-7}\text{(/locus/rad)};$$

after acute exposure, (8a)

$$d=0.73\times10^{-7}\text{(/locus/rad)};$$

after chronic exposure. (8b)

The rate per rad of induced mutations was high at a dose rate of 90 rad/min (Eq. (8a)) and decreased with decreasing dose rate. The induced rate at 0.8 rad/min was only a third of the high value (compare (8b) with Eq. (8a)) but decreased no further when the dose rate was lowered, using Cs-137 gamma rays, from 0.009 to 0.001 and to 0.0007 rad/min, as summarized in Eq. (8b). The latter data are the sole experimental evidence for the popular belief that radiation is hazardous however small the dose, i.e., that there is no threshold effect in radiation-induced mutagenesis.

II.1.2. Comparison with human data

All four genetic indicators studied in children born to A-bomb survivors failed to show any significant effect of radiation (Section I.1). The observation that biochemical mutations of the base-substitution type did not increase after the A-bomb radiation (Section I.1.4) is compatible with various lines of evidence obtained from laboratory experiments that radiation induces mostly deletions[10,11] and only rarely base substitutions.[12-14]

The development of DNA engineering techniques has revolutionized the precision of DNA sequence analysis. A new research project to apply these technologies to detect mutations in the F_1 of A-bomb survivors has been set up.[32] In the near future, we will have decisive evidence for or against the suggestion that humans are more resistant than mice to the genetic effects of radiation.

II.2. Gene Mutations in Somatic Cells of the Mouse

The fetuses of mice are sensitive to mutation by radiation or chemicals only during the period of organogenesis. The frequency of somatic mutations at specific coat color loci increased linearly from 10 rad to 100 rad of X rays with a doubling dose (the dose needed to double the spontaneous frequency) of 25 rad.[8]

II.2.1. Comparison with human data

In man, the somatic mutation rate at the glycophorin A locus is 5×10^{-7} (/locus/rad) (see Eq. (5a)), whereas in the mouse the averaged value of induced germline mutation rates at seven loci is 2×10^{-7} (/locus/rad) (see Eq. (8a)). At face value, somatic cells *in vivo* in man are about twice as sensitive as spermatogonia in the mouse to mutation by irradiation.

II.3. Mitotic Recombination in Drosophila melanogaster

Muller emphasized in his Nobel-prize lecture that the frequency of mitotic recombination in the larvae of *D. melanogaster* is very high. Mitotic recombination is induced in *Drosophila* larvae at a high frequency after irradiation.[33] Here I review our own work on somatic mutations produced by crossing over of chromosomes.

II.3.1. Chromosomal mutations caused by mitotic recombination after irradiation

Stocks heterozygous for the recessive marker *mwh* (multiple wing hairs) on the third chromosome were used.[9,33] Heterozygous *mwh*/+ larvae were irradiated and allowed to emerge. Clones of mutant cells that expressed the multiple-hair phenotype on the wing blade of adult flies (designated "Mwh" hereafter) were scored as chromosomal mutations resulting from crossing over between pairs of homologous chromatids. Sophisticated procedures[34] indicated that the Mwh mutants are practically all caused by mitotic recombination. The results obtained after X irradiation[9] can be summarized by the following equation:

$$y=g+hx, \quad (9)$$

$$g=3\times10^{-2}\text{(/wing)}$$

$$=1\times10^{-5}\text{(/chromosome pair)}, \quad (10a)$$

$$h=2\times10^{-3}\text{(/wing/rad)}$$

$$=7\times10^{-7}\text{(/chromosome pair/rad)}, \quad (10b)$$

where y stands for the total frequency of Mwh mutations, g the spontaneous rate of mitotic recombination, h the rate of induced mitotic

recombination per unit dose, and x the radiation dose. In Eqs. (10a) and (10b), the third terms are derived from the second ones after multiplication by 10 (average number of cells per Mwh mutant clone)/3×10^4 (the total number of cells per wing).

II.3.2. Comparison with mitotic recombination and reciprocal translocation in man

Mitotic recombinations that produce homologous M/M erythrocytes in human peripheral blood (Section I.2.3) depend on random collisions between single homologous pairs of human chromosome 4. Reciprocal translocations that are detected in human lymphocytes (Section I.2.2) depend on random collisions between ca. 1000 ($45\times45/2$) non-homologous pairs of 46 or 45 human chromosomes. Hence, to compare these two types of symmetrical interchanges on an approximately equal basis, the translocation frequencies are divided by 1000 to obtain translocation rates per chromosome pair as given in the third terms of Eqs. (2a) and (2b). Comparing these rates with spontaneous and induced rates of M/M mutant erythrocytes given in Eqs. (4b) and (5b), we find that in regard to either the spontaneous rate (/chromosome pair) or the induced rate (/chromosome pair/rad), reciprocal translocations and mitotic recombinations occur at an equal rate within a factor of 2. Furthermore, these spontaneous and induced rates in man agree, within a factor of 2, with the corresponding values of mitotic recombination in *Drosophila* given in Eqs. (10a) and (10b). Whether the agreement among these three types of somatic recombination is fortuitous is open for future study.

II.4. Relative Biological Effectiveness of Fission Neutrons

The possible difference in the dose-incidence relation for the occurrence of some types of cancer in Hiroshima and Nagasaki survivors may be, at least partly, explained by the differential abundance of fission neutrons in the radiation from the two A-bombs as argued in Section I.3.2. A convenient quantity for measuring the effectiveness of neutrons relative to that of X or gamma rays is RBE defined as follows:

$$\text{RBE (of neutrons)} = D_x/D_n, \qquad (11)$$

where D_x and D_n denote, respectively, X-ray (or gamma-ray) and neutron doses required to produce the same degree of biologic effect.

II.4.1. RBE values of fission neutrons

The RBE values of fission neutrons are about 8 for specific locus mutations in spermatogonia of mice,[5] about 5–6 for chromosome aberrations in peripheral lymphocytes after irradiation *in vivo* in an accident,[29] about 5–10 for the production of dominant mutation "Minutes," which are thought to be caused by small chromosomal rearrangements,[30] about 20–50 for the induction of mutations in plants after irradiation of seeds or pollens,[28, 29] and, most interestingly, no less than 5 for mitotic recombination in *Drosophila*.[34]

It remains a challenge to radiation biologists to test how the RBE values of fission neutrons increase as their energy decreases by using metal with a thickness equivalent to the iron wall of the Hiroshima A-bomb and to apply the obtained RBE values to reassess the apparent differences in the radiation effects of the Hiroshima and Nagasaki A-bombs.

II.4.2. DNA repair in relation to high RBE of fission neutrons

In mouse spermatogonia, the mutation rate per unit dose after X- or gamma-irradiation decreases with decreasing dose rate (see Eqs. (8a) and (8b)) whereas the mutation rate by fission neutrons does not.[5] Hence, it is claimed that fission neutrons have an RBE value of 20[9] or 30[35] for irradiation at low dose rates or doses. Such large RBE values may explain the seemingly higher incidence of cancers in Hiroshima than in Nagasaki (Fig. 1 and Table I).

Most of the lesions produced in DNA by fission neutrons are not repaired, whereas most X- or gamma-ray induced DNA lesions are repaired. An estimate of the reparable fraction of X-ray-induced DNA lesions that cause mitotic recombination in *Drosophila* was made by using a strain with double repair-deficient alleles, *mei-9* and *mei-41*. The frequency of induced mitotic recombinations after larval X-irradiation in this strain was 12.5 times higher than that in DNA-repair proficient strains,[9] indicating that 92% ($=1-1/12.5$) or more of the X-ray induced DNA damage was repaired.

II.5. Carcinogenesis in Drosophila melanogaster

In *D. melanogaster,* at least 24 loci were mapped as the sites of recessive oncogenic mutations.[36, 37] Any one of these is sufficient to produce lethality in specific tissue(s) of homozygous mutant larvae. Cells taken from the anomalous tissue and implanted into the abdominal cavity of adult flies grow unrestrictedly and eventually kill the hosts. The homozygous mutant cells manifest various other types of anomalies that are very similar to the characteristics of mammalian tumor cells.[36, 37] Here I briefly review the characteristics of the two recessive mutations causing brain tumors.

II.5.1. Oncogenes *l(2)gl* and *l(3)mbt^{ts}-1* nullify the function of division-arrest

A temperature-sensitive mutant named *l(3)mbt^{ts}-1* was isolated; its temperature-dependent tumorigenesis is very interesting.[37] The *l(2)gl^{+}* gene was recently cloned.[38, 39] Various lines of evidence[36–41] suggest that the *l(2)gl^{+}* protein[41–43] and probably the *l(3)mbt^{ts}-1^{+}* protein also are involved in the arrest of the division of neuroblasts that is programmed to occur shortly before the larvae enter pupation.

Normal alleles of other recessive oncogenes in *Drosophila* may also be involved in the programmed division arrest of other primordial cells. Normal alleles of recessive oncogenes are referred to as antioncogenes. Most division-arrest genes may turn out to be antioncogenes.

II.5.2. Epigenetic changes induced in homozygous *l(2)gl* cells after transplantation *in vivo*

The recessive mutation *l(2)gl* causes the development of malignant tumors in the whole brain of homozygous larvae. A fragment of the mutant brain transplanted into an adult fly can kill the host in about 10 days.[36] While homozygous *l(2)gl/l(2)gl* cells *in situ* in the brain of mutant larvae showed no chromosome aberrations, about 10% of these cells showed chromosome aberrations after one generation of subculture *in vivo* in the adult abdomen.[42] Upon long-term culture of brain-tumor cells *in vivo,* the frequency of their chromosome aberrations increased with time of subculture and RNA virus-like particles with reverse transcriptase activity[43] were abundantly produced.[42] The RNA molecule is identical in sequence to the DNA of the movable element *copia* in the host chromosome. During the culture of *l(2)gl/l(2)gl* cells *in vivo* in the new microenvironment, *copia* genes seem to be brought to the active state of expression, resulting in mass production of *copia*-RNAs to produce virus-like particles. Further, mass production of cDNAs by reverse transcriptase from RNAs in virus-like particles led to amplification of *copia* genes.[42] These are typical epigenetic changes as gross changes in both the gene expression and the chromosome structure occur simultaneously. What is the main cause of such epigenetic changes? Release of the cells from the intrinsic territorial restraints may activate many genes including movable elements, whose activation can cause chromosome rearrangements and deletions as in the case of P elements.[44] The assumption that *l(2)gl/l(2)gl* cells are defective in cell-cell adhesion protein[41] is consistent with the idea that some oncogenes result from deletion of genes for territorial restraints.

II.6. Conversion of Heterozygotes for Recessive, Oncogenic Mutations to Homozygotes as a Model of Tumor Initiation

It is a general rule that recessive mutations more directly affect essential functions of living organisms than do dominant mutations.[45] Recently, various types of recessive human oncogenes have been identified. Here I briefly review their characteristics and then construct a model of radiation carcinogenesis assuming that tumor initiation results from the appearance of a cell homozygous for a recessive oncogene. The model will be applied to explain the incidence of leukemia in A-bomb survivors.

II.6.1. Recessive oncogenic mutations in man

Based on the fact that specific chromosomal deletions are associated with heritable human childhood tumors, such as retinoblastoma (chromosome 13q14) and Wilm's tumor (chromosome 11p13), Knudson[46, 47] proposed the hypothesis that recessive oncogenic mutations are involved in tumorigenesis. The advent of DNA engineering techniques has

provided decisive evidence that recessive deletion mutations are involved in retinoblastoma,[46-48] Wilm's tumor, embryonal rhabdomyosarcoma (11 p 11.5),[49] hepatoblastoma (11p), meningioma (22q), acoustic neuroma (22q), renal carcinoma (3p), small-cell carcinoma of the lung (3p),[50] and carcinoma of the colon (5q).[51]

Recently, from studies of cultured human cells *in vitro*, Smith and coworkers[52, 53] discovered other types of recessive oncogenes. They found that when human cancer cells were fused with normal cells, all the resultant hybrids produced from 22 cancer-cell lines ceased dividing, and suggested that the phenotype of cancerous immortality results from homozygosity of a recessive immortalization gene. By fusing different cancer-cell lines with each other, the 22 immortal lines were separated into 3 complementation groups. Therefore, the normal alleles of the presumed immortalization genes may be referred to as antioncogenes. They further showed that these presumed antioncogenes are involved in the constitutive production of DNA-synthesis-inhibiting proteins in senescent cells.[53] Thus, recessive immortalization genes are very similar to recessive oncogenes in *Drosophila* in the sense that both result from loss or mutation of the genes involved in the suppression of cell division.

II.6.2. Two-mutation model for radiation carcinogenesis

Knudson[46, 47] proposed that non-heritable retinoblastoma is produced in children as a consequence of two somatic mutations. We shall apply Knudson's model to radiation carcinogenesis. Since homozygous tumor cells result from mitotic recombination in most cases of heritable rhabdomyosarcoma,[49] we assume that one of the two oncogenic mutations is either a mitotic recombination or a recessive oncogenic mutation. Then, the frequency F of the production of a homozygous precancerous cell in the body some time after irradiation at dose D (rad) may be expressed as follows:

$$F=s(a'a''+a'b''D+b'Da'')+s(a'a'+a'b'D)/2, \quad (12)$$

where a' and b' stand for spontaneous and radiation-induced rates of recessive oncogenic deletion, respectively, a'' and b'' stand for spontaneous and induced rates of mitotic recombination, respectively, and s is the number of target cells per body. The two-mutation term $a'b''D$ stands for the induction of recombination by radiation in a cell in which an oncogenic deletion has already occurred. Spontaneous two-mutation events and the induction of deletion by radiation followed by the occurrence of spontaneous recombination are given by $a'a''$ and $b'Da''$, respectively. The terms $a'a'$ and $a'b'D$ stand for spontaneous two-deletion events and the occurrence of spontaneous deletion before or after induction of a deletion by radiation, respectively. The factor 1/2 is introduced in Eq. (12) because the first deletion can occur at either one of two antioncogene loci in a cell, whereas the second deletion must occur, in the same cell, at the antioncogene that is not deleted by the first deletion.

As previously proposed[54-56] on the basis of various lines of evidence, we assume that the target cells for tumorigenesis are stem cells. Stem cells are immortal. Hence, they can accumulate many mutations during a host's long life and have a good chance to acquire tumor characteristics during a long latent period after the initial precancerous mutation.[54-56]

As an example, we consider leukemogenesis. Then, s in Eq. (12) is the number of hemopoietic stem cells per person. Estimates of this number vary from 10^9 based on the measurement of colony forming cells on the spleen[57] to 10^6–10^7 based on the decrease in the observed frequencies of mutant erythrocytes in persons heavily exposed to A-bomb radiation.[3] The latter is adopted here as it is an estimate of multipotent hemopoietic cells whereas the former is an estimate of stem cells committed toward the myeloid lineage. Hence, we assume the following numerical values:

$$s=5\times10^6 \text{ (stem cells/person)}, \quad (13a)$$

$$a'=2\times12\times10^{-6},\ a''=9\times10^{-6},\ b'=2\times5\times10^{-7},\ b''=3\times10^{-7} \quad (13b)$$

where the values in Eq. (13b) are taken from the observed parameter values (4a, 4b, 5a and 5b) after multiplying by 2 when necessary. The factor 2 is introduced in the a' and b' values to account for the existence of two homologous, antileukemia genes per somatic cell. From Eqs. (12) and (13), we have the following equation:

$$F=2.52\times10^{-3}+1.44\times10^{-4}D$$
(preleukemic cells/person). (14)

We further assume that the production of one preleukemic cell in the bone marrow of a person eventually leads to the development of malignant leukemia. Then, Eq. (14) can be rewritten in terms of the annual rate of leukemia mortality f as follows:

$$f=0.87+0.050D$$ (leukemia mortality rate/10^4 person-years). (15)

This formula is obtained from Eq. (14) by dividing by 29, the mean follow-up years per person during the 1950–85 period of the epidemiologic surveys reported by Shimizu *et al.*[4] (for details, see p. 36 of reference 27).

As seen from the panel LEUKEMIA of Fig. 1, the slope of the theoretical dose-response curve (A) based on Eq. (15) is fairly close to those of the actual dose-responses of leukemia mortality in Hiroshima and Nagasaki except in the low dose range where apparent threshold effects appear. This agreement may, however, be fortuitous for the following reasons. In leukemia induced in mice after X-irradiation with 300 rad, only 30% of the animals with preleukemic cells developed overt leukemia.[58] In A-bomb survivors, the assumed spontaneous, preleukemic mutations must have occurred before the A-bomb irradiation. Hence, the recently observed a' value in Eq. (13b) is an overestimate. The number of antileukemia genes per human genome may be more than one, the number assumed to derive Eqs. (14) and (15). Furthermore, the dose-response curve for leukemia mortality in Nagasaki deviates very greatly from the theoretical curve (Fig. 1). However, this deviation might be greatly reduced by the use of a theoretical equation based solely on the data of somatic mutations in the Nagasaki population if the two-mutation model can really explain the first step in radiation leukemogenesis.

II.7. Experimental Carcinogenesis Relevant to Cancers in A-bomb Survivors

Here I review relevant reports on myeloid leukemia and cancers produced in the skin and digestive tract of rodents by irradiation, deduce some empirical rules in radiation carcinogenesis, and then apply the rules to explain carcinogenesis in A-bomb survivors.

II.7.1. Radiation leukemogenesis in mice and leukemia in A-bomb survivors

The RFM strain, which is highly susceptible to myeloid leukemia, was used by Upton and coworkers.[59] Among their many findings on experimental cancers in this strain after irradiation,[59] the following two are relevant to the mechanisms of radiation leukemogenesis. First, myeloid leukemia increased rapidly with increasing dose after acute X-irradiation whereas at the lowest dose rates of gamma-irradiation tested, 0.5–5 rad/day, no leukemogenic effects were evident even after 200 to 300 rad. Second, the latent period, as judged by mean age at death of mice with the disease, varied inversely in relation to dose. This finding is compatible with the dose-dependent shortening of the latent period of leukemia in the A-bomb survivors (see Section I.3.3).

The first finding mentioned above could be taken as evidence that threshold effects exist in the development of leukemia in mice after irradiation. However, if radiation acts as a tumor initiator by inducing only oncogenic mutations, there should be no threshold effect, as shown by Eq. (15). This contradiction may be resolved by assuming that radiation is not only an initiator but also a promoter for producing leukemia in mice. This assumption is supported by the following results.

To obtain a new insight in the processes that occur during the latent period of myeloid leukemia, Bessho and Hirashima[58] used the transplantation assay technique. Male RFM mice were exposed to 300 rad of X rays, killed at various times and examined hematologically. Spleen cells were extracted from mice with no overt leukemia, and transplanted at a dose of 10^7 cells into individual female RFM mice whose bone-marrow stem cells had been inactivated by whole-body irradiation. These irradiated recipients served for assaying preleukemic cells, which were defined as existing in the donors if the recipients developed overt leukemia within 3 months after the transplantation. Mice with preleukemic cells first appeared 18 days after the donors were irradiated (about 3 months earlier than the first appearance of overt leukemia in the donors); their incidence peaked sharply between 3 and 4 months, and then decreased rapidly. The last appearance of preleukemic cells

occurred 7 months after the donors were irradiated (about 3 months earlier than the last appearance of overt leukemia in the donors). The pattern of time-dependent incidence of overt leukemia in the irradiated donors, though greatly shortened in absolute time scale, was reminiscent of that of human leukemia after the Nagasaki A-bomb irradiation (Section I.3.3).

In a very close coincidence with the period of the appearance of preleukemic cells, granulocytic precursor cells (CFU-C: colony forming unit in culture) showed a depression in all examined mice, irrespective of the presence of preleukemic cells.[58]

From these findings, it is tempting to speculate that a high dose of radiation induces a disturbance in the cellular society of the bone marrow such that territorial restraints, which otherwise tightly regulate the behavior of each member of the CFU-C cells and of other related precursor cells in the bone marrow, are partly released, resulting in an increased freedom for the subsequent proliferation of various types of precursor cells. This might allow a small population of preleukemic stem cells to multiply and undergo gross, epigenetic changes. In fact, immortal mutant cells of *Drosophila* showed gross, epigenetic changes when they were simply transplanted into the adult abdominal cavity (see Section II.5.2). The long-term pressure of natural selection would tend to produce a specific clone of tumor cells best able to grow freely within the bone-marrow cell society governed by the absolute rule of altruism.[56] The processes of tumorigenesis share many common characteristics with those of the evolution of new species of living organisms.[54, 60]

The CFU-C assaying technique was applied to persons exposed to the fallout from a hydrogen bomb tested near Bikini Island 24 years after the exposure. The CFU-C cells in three of nine heavily irradiated persons were significantly depressed.[61] This suggests that the depression period of CFU-C cells in the bone marrow could have overlapped with the period of appearance of leukemia in A-bomb survivors. If so, radiation in the high dose range could have acted as a promoter to induce leukemia in A-bomb survivors also, implying that there could have been a threshold effect. In fact, apparent threshold effects in the incidence of leukemia and some other cancer types in A-bomb survivors (Section I.3.2) might be attributed to the promoting action of radiation.

II.7.2. Experimental carcinogenesis in skin and digestive tract

Human skin is resistant to carcinogenesis by a single radiation dose[31] but is rather sensitive to the production of cancers after repeated radiation doses.[62] Recently, by applying a beta-irradiation device to the skin of ICR/CRJ mice, Ootsuyama and Tanooka[63] obtained convincing evidence that repeated doses, but not a single high dose, induce skin cancers and that the number of repetitions is more important than the sum of repeated doses to induce thses cancers.

The stomach, small intestine, colon, and rectum in mice and rats are resistant to the induction of tumors after a single irradiation unless a very high dose is given, localized on the target organ; these animals are rather sensitive to the induction of carcinoma after repeated irradiation.[64] These modes of action of radiation are compatible with the assumption that a single high dose or repeated doses of radiation act as a promoter for radiation carcinogenesis in epithelial tissues by partly freeing their component cells from the restraints on their proliferation and movement. The rigidity of the cell-cell structure of the epithelial tissue is so well-organized that the territorial restraint on the behavior of individual members of the cellular society can be disrupted only after repeated doses given at appropriate intervals.

II.7.3. Threshold for degrading the ordered cell-cell structure of the tissue

I assume that disruption by irradiation of cell-cell interaction supporting the structure of the tissue is initiated only after more than a threshold number of cells at critical sites has been inactivated (see Ootsuyama and Tanooka[63] for supporting evidence) and that fission neutrons are far more effective than X or gamma rays for degrading the cellular orderliness in the tissue.

Summary and Perspective

The characteristics of recessive oncogenes in *Drosophila* and humans are similar. Radiation induces predominantly recessive mutation and frequently mitotic recombination. Thus, the following two-mutation model is proposed. Radiation induces a recessive oncogenic mutation in a stem cell, which will be later converted, by spontaneous mitotic recombination, to a precancerous homozygote. Radiation-induced mitotic recombination can convert a heterozygote with a spontaneous recessive oncogene into a homozygote. A similar homozygote can also be produced by combination of a spontaneous recessive oncogenic mutation and a radiation-induced one. Using data on somatic deletion and recombination in hemopoietic stem cells of A-bomb survivors, the two-mutation model is converted into a numerical equation for the frequency of the production of a preleukemic cell as a function of radiation dose. The slope of the theoretical dose-response curve obtained from this equation agrees fairly well with those of the actual dose-responses of leukemia in A-bomb survivors in Hiroshima and Nagasaki except at low doses (see Fig. 1).

In the low dose ranges, apparent threshold effects exist. Did threshold effects really occur in A-bomb survivors? The answer cannot be obtained from epidemiologic data alone. To supplement the epidemiological studies on A-bomb survivors, relevant reports on experimental carcinogenesis have been reviewed. Experimental evidence supports the model that radiation acts, at high doses above a threshold value, as a promoter to produce cancer. Obviously, the promoter model predicts a sigmoidal dose-response. In fact, 4 out of 5 types of cancer in Nagasaki show seemingly sigmoidal curves (see Table I and Fig. 1).

At face value, the mortality rate given in Fig. 1 for Nagasaki is lower among the group exposed in a limited low dose range than among the unexposed group for 4 out of 5 types of cancer. Such seemingly antioncogenic effects are loosely expressed, in this paper, as 'apparent threshold effects' but, in a strict sense, should be expressed differently, possibly as apparent beneficial effects. Whether low-level radiation really exerts beneficial effects is open for future study. Interestingly, there is increasing evidence that whole-body irradiation at low doses often stimulates defense mechanisms against various threats, including tumors.[65-68] The problem as to whether there is a beneficial or threshold effect of low-level radiation has never been seriously and systematically investigated; it is necessary to initiate a detailed study because not only is the answer of vital public importance but also it is essential for understanding the basic relation of mutation and cancer.

Experimental evidence supports the following model of radiation-induced tumor promotion. Radiation at a high dose degrades the ordered cell-cell structure of tissues and frees their component cells from territorial restraints on their proliferation. Under conditions favorable for free proliferation, cells rapidly accumulate epigenetic changes, the major cause of tumor progression.

Carcinogenesis is not a single-cell problem but a cell-society problem. The complexity of carcinogenesis is partly reflected in the proposed working hypothesis that radiation acts not only as an initiator, i.e., homozygous-mutant inducer, but also as a promoter, i.e., indirect inducer of epigenetic changes, to produce cancer in man.

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