S54 *Congenital Anomalies* 2000; **40**, S54–S67

SYMPOSIUM

Transplacental and transgenerational late effects of radiation and chemicals.

Taisei Nomura
Department of Radiation Biology and Medical Genetics, Graduate School of Medicine, Osaka University, Osaka, Japan

ABSTRACT ***In utero*** **exposure to radiation and chemicals has been shown to induce cancer and malformation in the offspring in man and animals. Cancer developed in various organs of mice and other experimental animals, when chemical carcinogens, urethane, dimethylbenz(a)anthracene, ethylnitrosourea diethylstilbestrol *etc.* were given after the organogenesis of each organ, while malformations were induced by the treatment of the organ at organogenesis stage. These animal experiments were supported by the fact that vaginal cancer was found in young women who had been treated with diethylstilbestrol for the threatened abortion. *In utero* exposure gave serious damage to the development of gonads and also to the organ function.**

Parental exposure to radiation and chemicals increased the incidence of cancer and malformation in the offspring. Radiation and chemical induced germ-line alterations causing tumors were transmitted to further generations. These findings have not been proven in the children of atomic bomb survivors in Hiroshima and Nagasaki, while higher risk of leukemia is reported in the children of fathers who were exposed to radionuclides at the nuclear reprocessing plants and to diagnostic doses of radiation. Contradiction between mice and men and between human populations are reconciled in part by the animal experiments. Offspring of mice exposed to radiation precon-ceptionally or at embryonic stages showed persistent hypersensitivity to cancer induction by the postnatal treatment with tumor promoting agents, urethane, 12-*O*-tetradecanoylphorbol-13-acetate, phenobarbital *etc.* Radiation or chemicals may imprint transmissible or genetic alterations in the embryo and germ cells for the future development of cancer by the postnatal environment.

Correspondence: Taisei Nomura, MD, PhD, Department of Radiation Biology and Medical Genetics, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan. E-mail:<nomura@radbio.med.osaka-u.ac.jp>

Key words: transplacental carcinogenesis, transgenerational effects, radiation, chemicals, man and animals, genetic instability, imprinting

INTRODUCTION

Why does cancer develop in a baby? Among the long term effects of exposures to various toxic agents during pregnancy, cancer is one of the most important subject to study in human diseases, because cancer is the 2nd highest cause of neonatal deaths in the well-developed countries. Parental exposure to various toxic agents may also cause cancer in the offspring derived from exposed germ cells.

After the middle of the twenty century, various studies have been accumulated on cancer induction by radiation and chemical exposures during pregnancy in man and experimental animals. Because placental transfer of chemical carcinogen to the fetus resulted in cancer induction in the offspring, these findings were referred to as transplacental carcinogenesis. Later, it was reported that these defects can be induced in the offspring by the parental *i.e.*, preconceptional exposure to radiation and chemicals. In this paper, the author reviews the original findings of transplacental and transgenerational late effects of radiation and chemicals in man and animals, and discusses the recent studies in this area at the molecular level.

IN UTERO EXPOSURE TO RADIATION AND CHEMICALS

When experimental animals are exposed to radiation and chemicals during pregnancy, various defects are observed in the embryo, fetus and live offspring, depending on the stage at exposure. **Fig. 1** summarizes such defects in scheme. Fertilized eggs die or are lost, when preimplantation stages are treated. Embryonic deaths and congenital malformations are induced, when the major organogenesis stage or early organogenesis stage of each organ is treated. In general, there is an apparent threshold for these defects except when the one cell stage is treated. When the later fetal stages after organo-

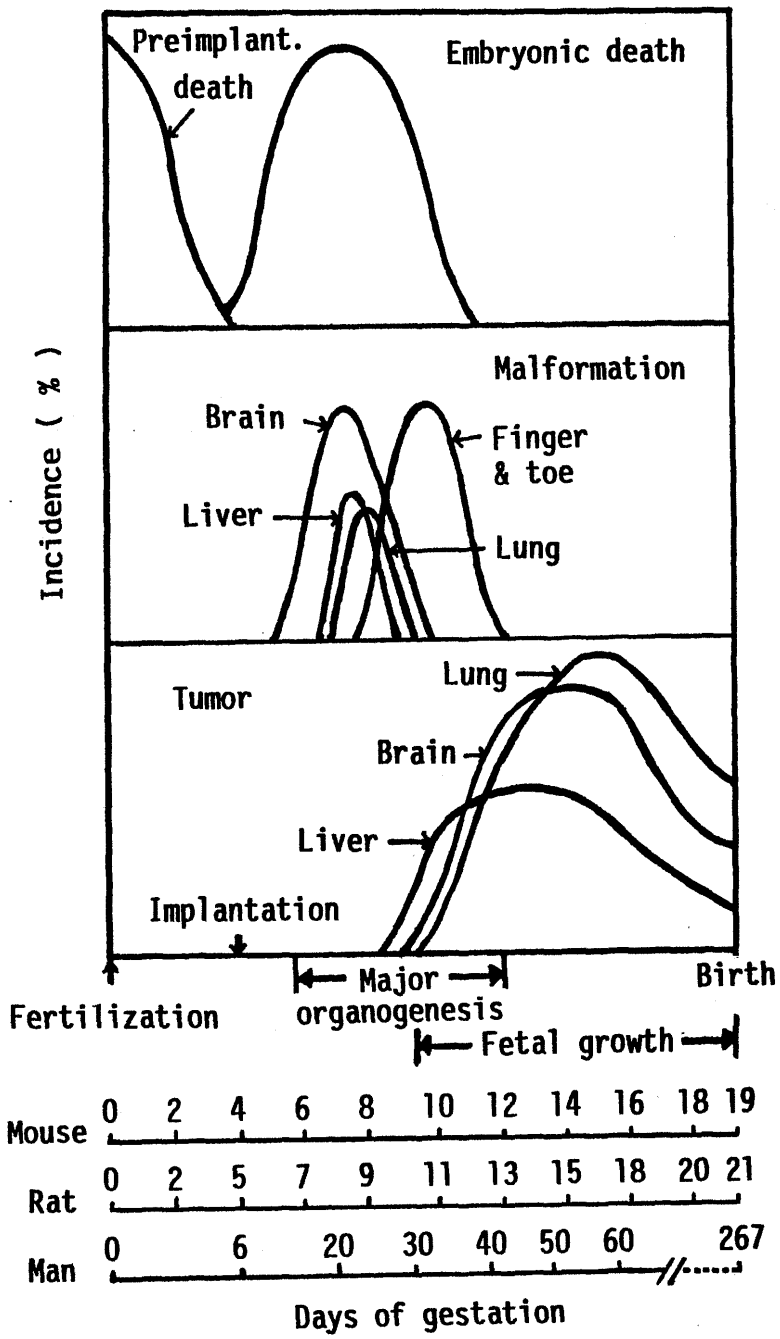

Fig. 1 Differential sensitivity of the developing embryo to mortality, malformation and neoplasm after *in utero* exposure to radiation and chemicals. (Nomura and Okamoto, 1972; Druckrey, 1973; Nomura, 1974a, b)

genesis are treated, healthy animals are delivered without any morphological abnormality. However, cancer develops in a year in the *in utero* exposed offspring. Differential sensitivity of fetal organs to tumor induction is shown with urethane in mice (Nomura and Okamoto, 1972; Nomura, 1974a; b) and with ethylnitrosourea (ENU) in rats (Druckrey, 1973). Tumors develop in the offspring, only when chemical carcinogens are given after the organogenesis of each organ but not before its organogenesis. Carcinogen treatment at the late gestational stage caused cancer induction via placenta and mother's milk (Nomura, 1973; Nomura *et al.*, 1973). Transplacental carcinogenesis was most extensively studied from late 1960's to middle 1970's. In experimental animals, 41 chemicals are proven transplacental carcinogens, and in human, diethylstilbestnal (DES) disaster was reported by Herbst *et al.* in 1971 (Nomura and Kanzaki, 1977; Nomura and Masuda, 1980). Radiation and chemical exposures during pregnancy may decrease enzymatic and metabolic activities in the offspring (Nomura, 1984a; 1987), resulting in the delayed and decreased decomposition of postnatally given toxic agents (*e.g.* carcinogens) followed by the increase of cancer incidence in the offspring (Nomura, 1973; 1984a; Nomura *et al.*, 1973).

Chemical exposure during pregnancy not only gives raise in cancer incidence in the offspring, but also it gives serious

damage to the gonads of the offspring, because most chemical carcinogens are estrogenic and act as endocrine disrupters. Rudimental ovary and testis were observed in the offspring after exposure to urethane, dimethylbenz(a)anthracene (DMBA), DES and other chemical carcinogens during the middle stage of pregnancy (Nomura, 1976; Nomura and Kanzaki, 1977). Histologically, there were no germ cells in the ovary and testis.

However, *in utero* exposure to radiation had not shown any increase of leukemia and cancer in experimental animals (Upton *et al.*, 1960; Rugh *et al.*, 1966) and also in atomic bomb survivors exposed *in utero* (Kato, 1978), although an increase of childhood cancer was suggested by some epidemiological studies (MacMahon, 1962; Bithell and Stewart, 1975). In 1978, Sasaki *et al.* found a small increase of adult type cancers but no leukemias by X-ray exposure during the late stages of pregnancy. Although no increase in cancer and leukemia had been reported in *in utero* exposed A-bomb survivors below the age of 40 (Kato, 1978), increased incidence of adult types cancer was confirmed, when A-bomb survivors became older than 40 years of age (Kato *et al.*, 1989; Yoshimoto *et al.*, 1991).

Embryonic mutagenesis predisposing to cancer

Although radiation exposure during pregnancy rarely induced tumors by itself in experimental animals, *in utero* exposure induced persistent hypersensitivity in fetal organs for developing tumors by postnatal treatment with tumor promoting agents.

In utero exposure to a low dose (0.36 Gy) of X-rays did not increase tumors in the offspring. However, large numbers of lung tumors were induced in the offspring which had been exposed to X-rays at early embryonic stages, but not at late fetal and neonatal stages, and postnatally treated with 0.5 mg/g body weight of urethane 3 weeks after birth (Nomura, 1984a). *In utero* exposure to radiation may imprint a transmissible memory in the mouse embryo and induce persistent hypersensitivity to postnatally given tumor promoting agents, resulting in the increase of tumor incidence (**Fig. 2**).

This finding was confirmed more precisely with a specific animal system to detect embryonic mutation and cancer. Recessive coat color mutant mice PT and HT were used. PT female (*a/a, b/b, p c^{ch}/p c^{ch}, d/d*) and HT male mice (*a/a, ln/ln, pa/pa, pe/pe*) were mated, and pregnant mice were exposed to radiation on the 10.5th day of gestation. $PTHTF_1$ embryos were heterozygous at 7 recessive coat color genes (*a/a, ln/+, pa/+, b/+, p c^{ch}/+ +, d/+, and pe/+*). If mutation occurs at one of the several thousands of pigment cells in the embryo, a small spot of mutant coat color appears on the black coat color back ground (**Fig. 3**). From the color, size, shape, and distribution of the pigment, mutated gene loci were determined. By the death of a pigment cell, a white spot appears on the midventral abdominal portion. The frequency of mutant spots increased linearly with doses of X-rays upto 1.2 Gy (Nomura, 1984b). Congenital malformations were also detected. Six weeks after birth, *i.e.*, after detecting somatic mutations and congenital malformations, some of the offspring were treated with tumor promoting agents such as sodium phenobarbital and 12-*O*-tetradecanoylphorbol-13-acetate (TPA).

In **Figs. 3 and 4**, $PTHTF_1$ fetuses were exposed to 0.3 and 1.03 Gy of X-rays at a dose rate of 0.54 Gy/min on the 10.5 day of gestation, and 10 μg of TPA was applied twice a week for 18 weeks on the back of the offspring where the hair was removed. When mice were euthanized one year after birth, an increase in the incidence of tumors was not observed after *in utero* X-ray exposure alone. By the postnatal-treatment with TPA, however, incidences of both hepatomas and skin tumors increased in proportion with *in utero* doses of X-rays (**Fig. 5**). Initial events by *in utero* irradiation must be kept in memory and cause tumors by the postnatal treatment with TPA. When *in utero* ^{60}Coγ-ray or ^{252}Cf neutron exposure was coupled with postnatal-treatment with TPA and/or 0.05 % Na phenobarbital in drinking water, incidences of hepatomas and skin tumors also increased in proportion with *in utero* doses of radiation (Nomura *et al.*, 1993; Nomura, 1998).

Contribution of *p53* and *ras* genes was examined in hepatomas induced by *in utero* radiation exposure and postnatal TPA or phenobarbital treatment. Only one K-*ras* mutation was detected at codon 13 in the hepatoma induced by *in utero* ^{60}Co irradiation coupled with postnatal phenobarbital treatment, but no mutations were detected in other 21 radiation induced hepatomas.

Even though radiation and chemical exposure during pregnancy was not or was very weakly carcinogenic by itself, it induced persistent transmissible hypersensitivity in fetal organs to develop cancer at older ages by postnatal exposure to cancer-promoting agents in the diet and/or environment (Nomura, 1973; 1984a; 1989; Nomura *et al.*, 1990) (**Fig. 2**). These results anticipated later increase of adult types cancer in *in utero* exposed atomic bomb survivors, because human beings are continuously exposed through their lives to various carcinogenic and/or promoting agents in the diet and environment, in contrast to experimental animals reared under specified condition without exposure to such carcinogenic and promoting agents (Nomura, 1984a). This is also a precaution to the researchers who set up animal experiments for the estimation of human risk.

TRANSGENERATIONAL EFFECTS OF RADIATION AND CHEMICALS

Germ cell exposure to radiation and chemicals causes cancer and congenital malformation in the offspring. We have done the first and largest experiments to know whether or not parental exposure to radiation and chemicals induces tumor and

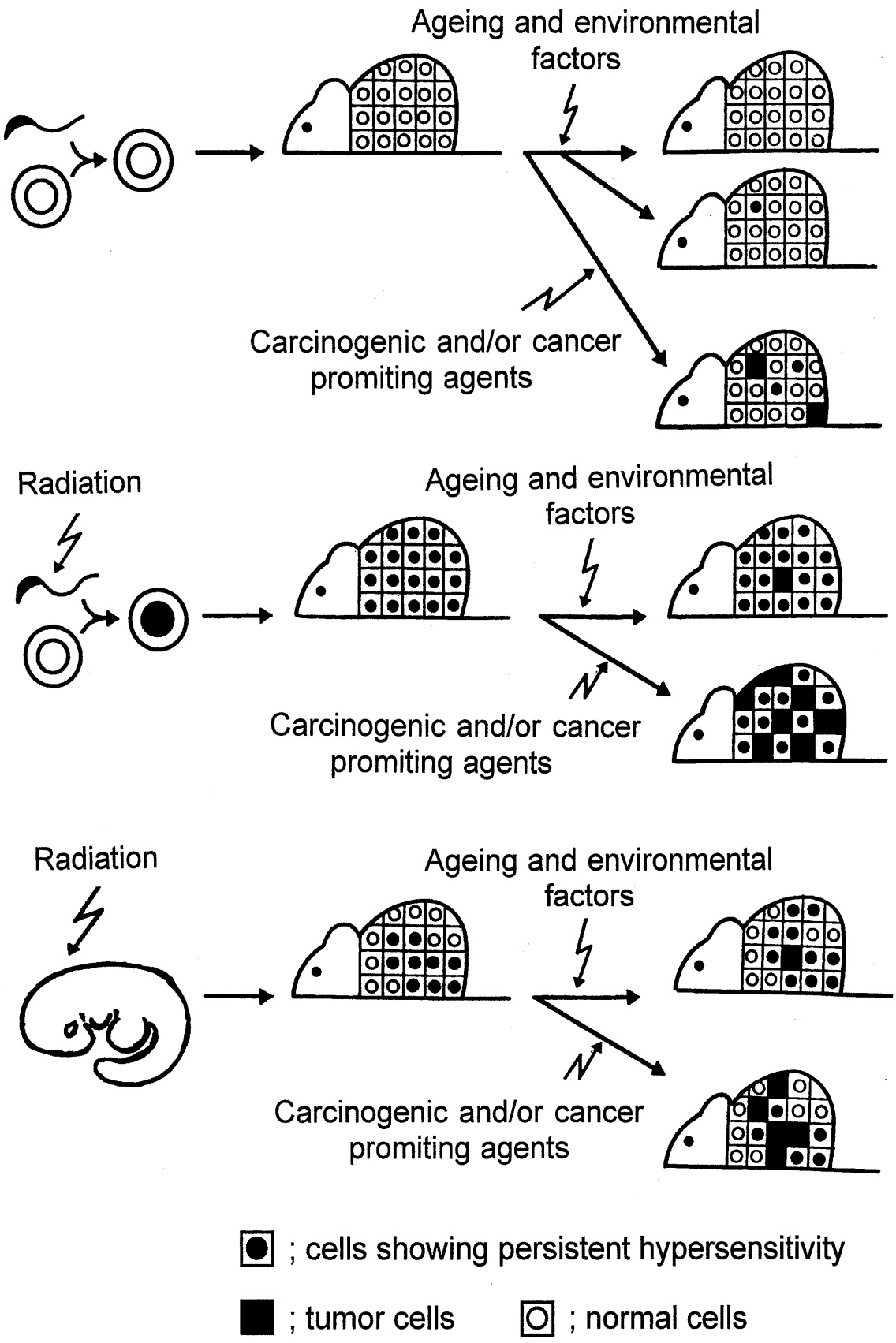

Fig. 2 Scheme of radiation induced persistent hypersensitivity in germ cells and embryos to tumor induction by postnatal tumor promoting agents in the diet and environment. Closed squares indicate tumor cells and closed circles indicate dormant cells which show hypersensitivity to postnatally given tumor promoting agents.

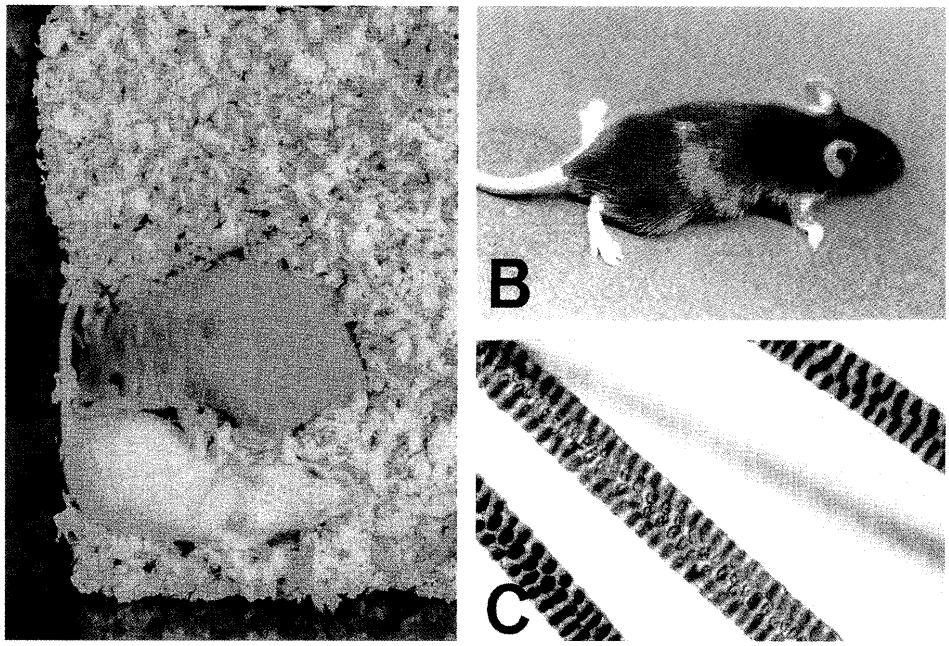

Fig. 3 Specific mouse system for the detection of *in vivo* mutation. **A**: HT (upper) and PT (lower) mice, **B**: colored spot on the black coat color background, **C**: hair (middle) showing mutation at *p* locus. Others are wild type hair.

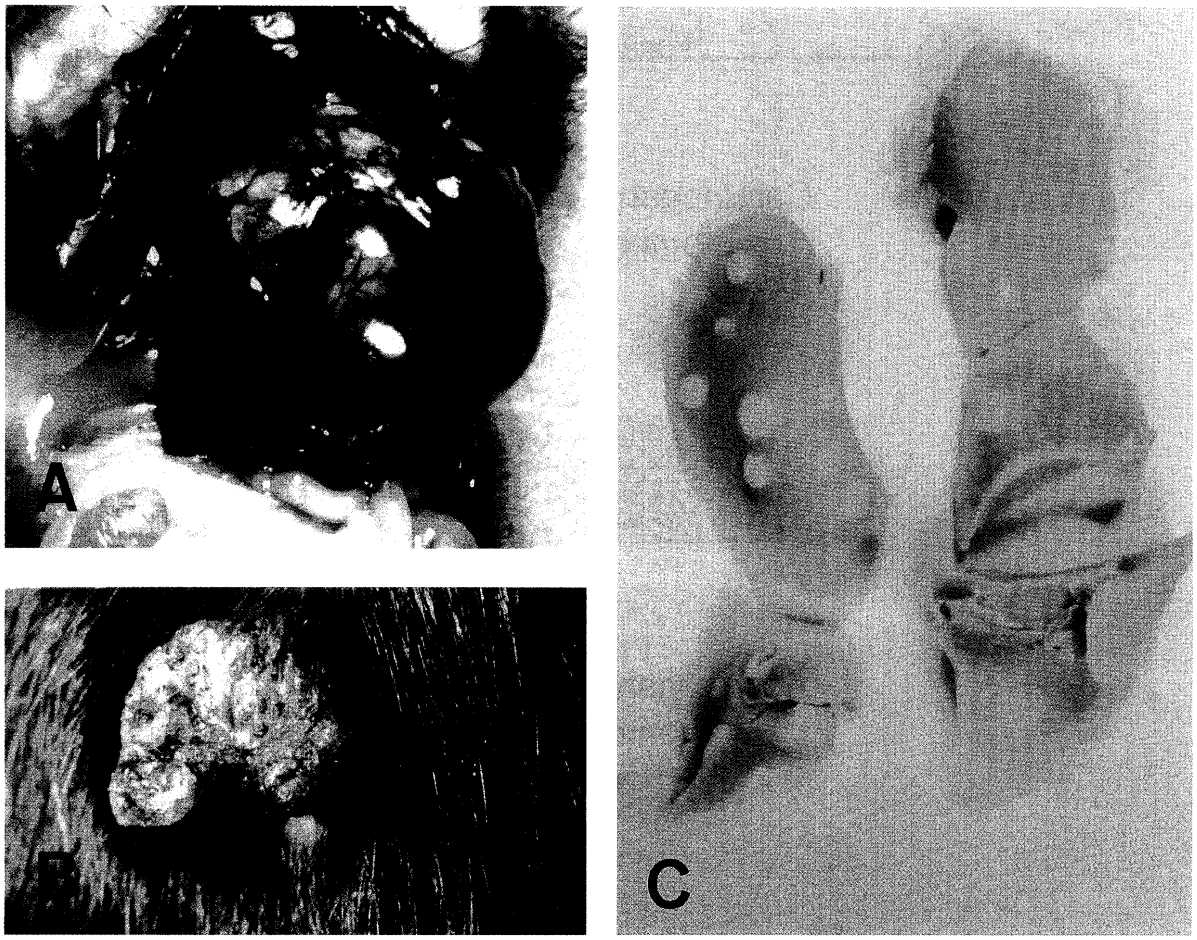

Fig. 4 Hepatoma (**A**) and skin tumor (**B**) induced in PTHTF$_1$ mice after *in utero* exposure to X-rays and a cluster of lung tumors (**C**) induced in ICR mouse after preconceptional exposure to X-rays. Mice were postnatally treated with TPA or urethane. No. of lung tumors in the offspring is one if at all by preconceptional and *in utero* exposure alone, and about 4 by postnatal urethane injection (0.5 mg/g body weight) alone (Nomura, 1983, 1984; Nomura *et al.*, 1990).

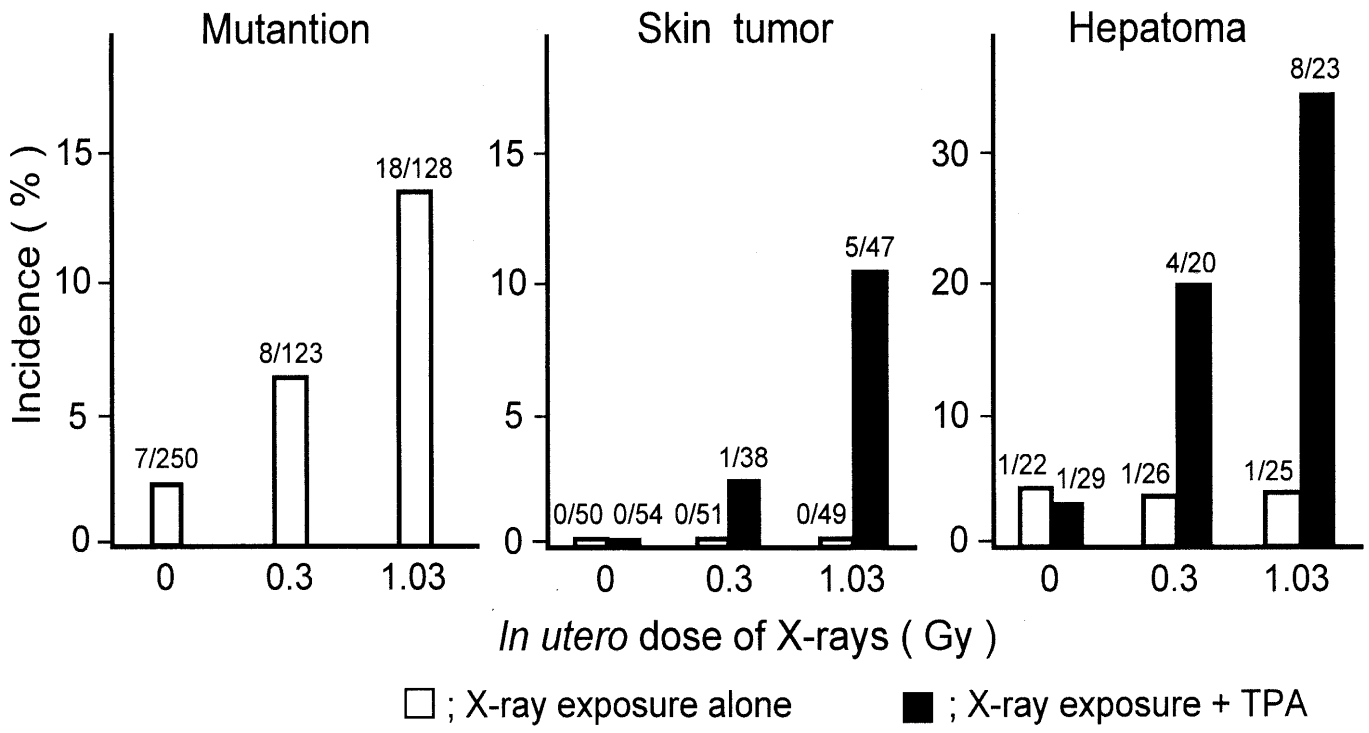

Fig. 5 *In vivo* mutation and tumors induced in the PTHTF$_1$ offspring by *in utero* exposure to X-rays on the 10.5th day of gestation coupled with postnatal treatment with 10 μg of TPA on the skin. TPA was given twice a week for 18 weeks starting at 6 weeks of age. Closed histograms indicate in utero exposure and postnatal TPA treatment. Open histograms indicate *in utero* exposure alone. Hepatomas were induced in male offspring. Details are given in Nomura *et al.*, 1990.

malformation in the offspring derived from exposed germ cells, and found that radiation and chemicals induced germ cell alterations causing tumors and malformations in the offspring (Nomura, 1975; 1978; 1982; UNSCEAR-Report, 1986).

Dominant lethals (abortion) and congenital malformations were induced in the offspring predominantly by the treatment of germ cells at the postmeiotic stages except for agents like ethyl-nitrosourea which induces more mutations and malformations by the treatment at spermatogonial stage (Nomura, 1975; 1982; 1984b; 1988; 1994b; Nomura *et al.*, 1990; Nagao and Fujikawa, 1990). Induced rate of congenital malformation by acute radiation was 12, 6.6 and 9.1 x 10^{-3}/gamete/Gy for spermatozoa, spermatogonia and mature oocyte, respectively (Nomura, 1984b; 1988). The increase in the incidence of malformations has been confirmed with radiation (Kirk and Lyon, 1984) and also with various chemicals (Nomura, 1975; 1982; 1988; 1994b; Nagao and Fujikawa, 1990). These studies were referred to as "Paternal Toxicology or Male-mediated Developmental Toxicology", although preconceptional exposure of females also induced such defects in the offspring (Nomura, 1982; 1988).

Several animal experiments have also suggested the increase of various types of tumors in the offspring after parental exposure to radiation and chemicals (Nomura, 1975; 1978; 1982). Dose dependent increases of tumors were observed in the F$_1$ offspring of male and female ICR mice exposed to acute doses of X-rays (0.36 - 5.04 Gy). Postmeiotic stages were twice as sensitive for tumor induction than spermatogonial stage, and oocytes were resistant to low doses (~ 1 Gy) of X-rays but very sensitive to higher doses. Even then, the incidence of both induced and spontaneous tumors in the offspring are 100 fold higher than those of ordinary mouse mutations (Russell *et al.*, 1958; Lyon *et al.*, 1972; Bartsch-Sandhoff, 1974; Russell and Kelly, 1982a, b; Ehling, 1984). The doubling dose of tumor-causing alterations in spermatogonia was about 1.5 Gy for adult type tumors in the lung and ovary (Nomura, 1986). Protracted irradiation (0.36 Gy at 2 hr intervals) reduced significantly the tumorigenic effects of irradiation in the offspring after exposure at the spermatogonia and mature oocyte stages (Nomura, 1978; 1982). However, such reduction was not observed, when spermatozoa and spermatrid stages were treated. The pattern of the radiation sensitivity of germ cell stages for tumor induction was similar to that for ordinary mouse mutations (Russell *et al.*, 1958; Lyon *et al.*, 1972; Russell and Kelly, 1982a, b; Ehling, 1984). Some of the above studies were confirmed with different strains of mice N5 and LT with X-rays (Nomura, 1986) and with urethane and 4-nitroguinoline l-oxide (4NQO) (Nomura, 1975; 1978; 1982). There were apparent strain differences in types of induced tumors in the offspring.

To examine the heritability of induced lung tumors, F$_1$ progeny of the treated parents were mated randomly and their F$_2$

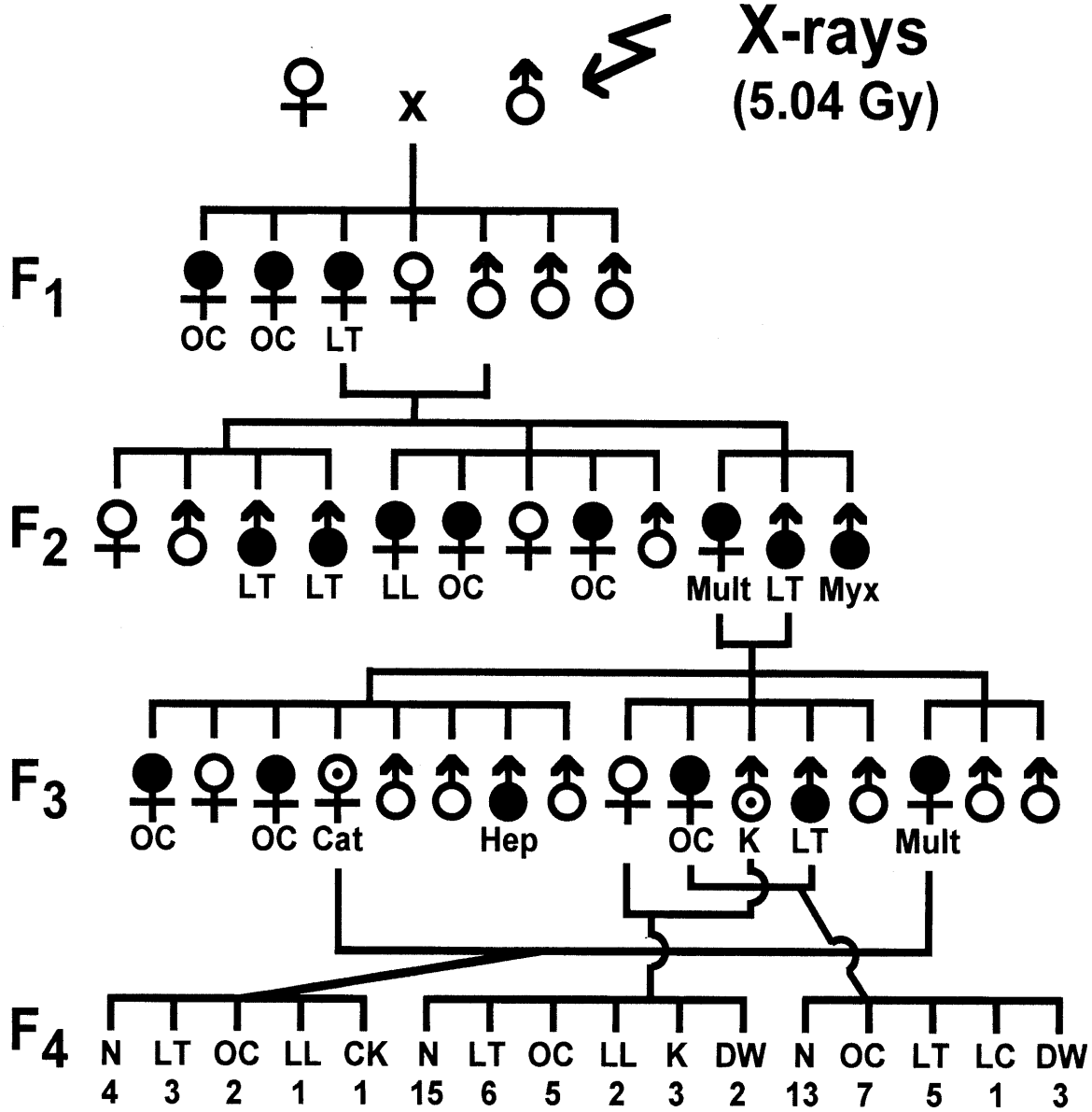

Fig. 6 Pedigree of tumor susceptible line originated from preconceptionally X-irradiated male N5 mouse. Tumors and malformations found in these descendants of male N5 mouse exposed to 5.04 Gy (0.54 Gy/min) of X-rays at spermatozoa stage were indicated by closed and dotted symbols, respectively. Offspring were randomly mated to obtain further generations, and their parental mice were euthanized at 12 months of age to detect tumors and malformations. Abbreviations used were; LT, lung tumor; OC, ovarian cystadenoma; Myx, pericardial myxoma; Mult, multiple embryonic tumors, LL, lymphocytic leukemia; Hep, hepatoma; K, kinky tail; Cat, cataract; DW, dwarf; LC, liver cyst; CK, cystic kidney; N, normal.

progeny were examined. The F_2 progeny were then classified to the retrospectively determined type of their parent, *i.e.*, F_1 parents with tumors and without tumors. The incidence of tumors in F_2 was significantly higher than controls only when either of parents had tumors. There were no differences in the tumor incidence when both parents had no tumors. The results were confirmed by continuing the experiment into the F_3 generation (Nomura, 1978; 1982).

During the course of the above experiments, several mutant lines which developed high incidence of tumors were established as shown in **Fig. 6**. Preconceptionally irradiated F_1 offspring with lung tumor was mated with her litter mate. In the F_2, there were variety of tumors, such as lung tumors, ovarian tumors, multiple embryonic tumors, myxoma and thymic lymphomas (lymphocytic leukemias). When the F_2 offspring with multiple embryonic tumor was mated with her litter mate with lung tumor, similar tumors and cataract were induced in F_3. From the F_3 offspring with cataract, a variety of tumors and malformations developed in F_4. The results suggest the inheritance of tumor susceptibility. Such germ-line changes must be concerned with mutations of tumor suppresser genes or changes in an array of genes modifying immunological, biochemical and physiological conditions which can lead to the elevation of tumor incidence.

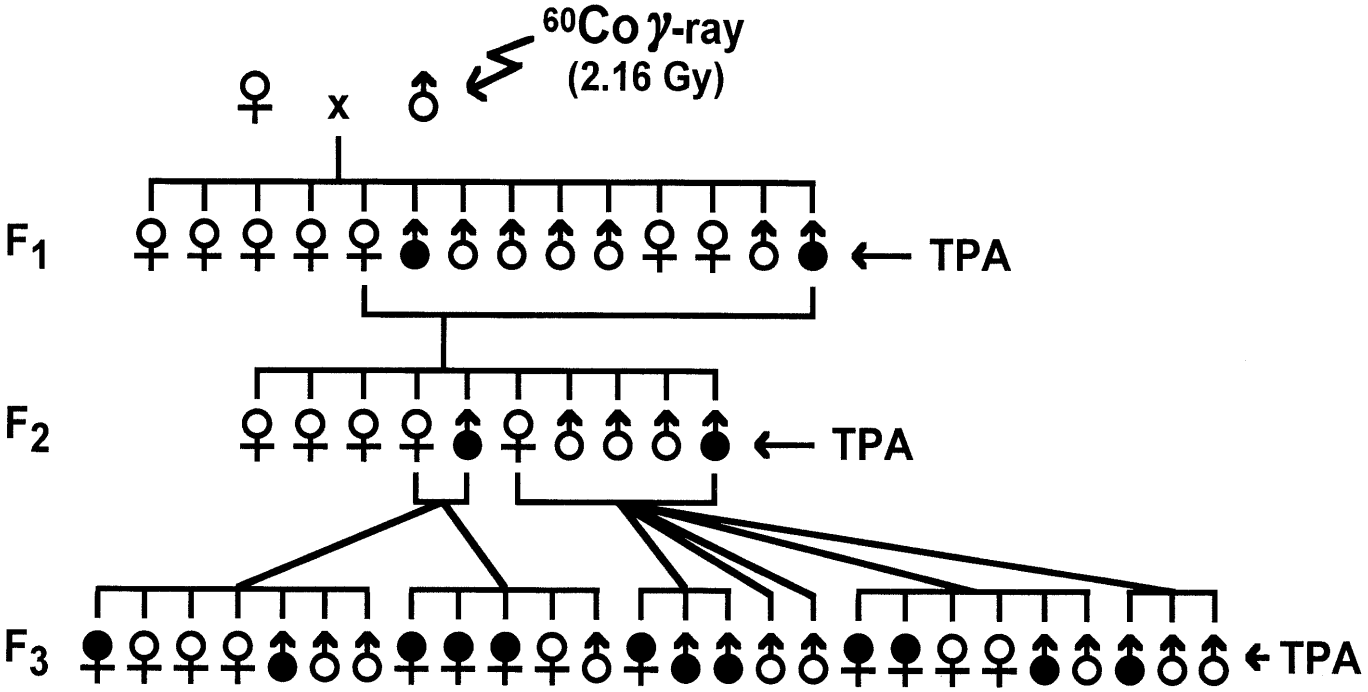

Fig. 7 Skin tumor susceptible line originated from male N5 mouse exposed to ^{60}Coγ-rays. Male N5 mouse was irradiated with 2.16 Gy of ^{60}Coγ-rays (0.57 Gy/min) at spermatogonia stage, and 20 μg of TPA was applied twice a week to the back of the offspring where the hair was removed. TPA was applied to all live offspring for 18 weeks starting at 6 weeks of age. Closed symbols indicate skin tumor-bearing offspring.

Germ-line alteration predisposing to cancer

If germ-line mutation can lead to cancer, all cells composing that organ must be mutated and have an equal likelihood to form tumors. However, only one tumor nodule was induced in the lung. Presumably, mutational change induced in the offspring by parental exposure to X-rays must be weakly carcinogenic by itself, and their expression will be influenced by aging, naturally existing carcinogenic and promoting agents in the diet and environment (**Fig. 2**). This hypothesis was proven by the fact that unusually large clusters of tumor nodules (**Fig. 3C**) developed in the lung after postnatal treatment with small amounts of urethane (Nomura, 1983). This study has been confirmed by Russian scientists with urethane (Vorobstova and Kitaev, 1988). Skin tumors were also induced in the offspring of parentally X-irradiated outbred SHR mice by postnatal treatment with TPA (Vorobstova *et al.*, 1993). This model was confirmed by preconceptional ^{239}Pu irradiation and postnatal methylnitrosourea treatment (Lord *et al.*, 1998). However, such enhancing effects were not observed when CBA/J male mice were irradiated and their offspring were postnatally treated with urethane (Mohr *et al.*, 1999).

To confirm these findings, N5 male mice were treated with 2.16 Gy of ^{60}Coγ-rays, and mated with untreated N5 females. Offspring (6 weeks old) were treated twice a week with TPA for 18 weeks. When skin tumor developed in the F_1 offspring, tumor-bearing offspring were mated with their litter-mates to examine the exact heritability of tumor-causing alterations in male germ cells. Significant increase of skin cancer was observed in the F_1 offspring. Higher incidence of skin tumors developed in F_3 generation, when skin tumor-bearing F_2 offspring were mated with their litter mates (**Fig. 7**). Thus, radiation appears to induce transmissible germ cell hypersensitivity to tumor induction by postnatal tumor promoting environment.

Relevant human data at nuclear plant

In 1990, Gardner *et al.* reported that there was about 6–8 fold higher risk of leukemia in the children of fathers who were employed at Sellafield nuclear reprocessing plant and had been exposed to 10–100 mSv of radiation before conception (**Table 1**). As a possible cause of leukemia induction, the sperm damage by fathers' exposure to radiation was considered from Nomura's mouse experiments (Nomura, 1982; 1983; 1986). However, it has not been supported in the children of atomic bomb survivors at Hiroshima and Nagasaki who were exposed to an average dose of 435 mSv (Yoshimoto and Mabuchi, 1991; Yoshimoto *et al.*, 1991), although some epidemiological studies reported (but have not proven) the increase of leukemia in the children of fathers who had been exposed to very low doses of diagnostic radiation (Graham *et al.*, 1966; Shiono *et al.*, 1980; Shu *et al.*, 1988).

The discrepancies between human populations in Hiroshima/Nagasaki and Sellafield and also between human and animals are reconciled partly by the following evidence: 1) Much

Table 1 Risk of leukemia and cancer in the F_1 offspring after paternal exposure to radiation in man and mice.

	Dose (mSv)	Relative risk	Doubling dose (mSv)	Induced rate/mSv (x 10^6)
Human				
Sellafield (Gardner *et al.*, 1990)				
All stages	≧ 100	6.24 (1.5 – 25.8)	11.9 (2.5 – 126)	22.2 (2.1 – 105)
Post-gonia*	5 – 9	3.54 (0.3 – 38.9)	1.5 (0.1 –)	179 (0 – 2680)
	≧ 10	7.17 (1.7 – 30.5)	1.0 (0.2 – 8.8)	260 (30 – 1250)
Hiroshima & Nagasaki (Yoshimoto and Mabuchi, 1991)				
Spermatogonia	435	1.24	900	0.23
Mouse				
ICR (Nomura, 1978, 1982)				
Spermatogonia	360 – 5040	1 (1.5 – 3.5)	–(1590)	0 (31.1)
Post-gonia	360 – 5040	1.9 – 3.2 (– 3.5)	950 (1920) **	1.9 (25.7) **
LT (Nomura, 1986, 1991, 1994)				
Spermatogonia	3600	1(2.7)	–(2090)	0 (38.2)
Spermatozoa	3600 – 5040	4.5 – 7.4 (– 2.3)	450 (4040)	9.0 (19.8)
N5 (Nomura, 1986, 1991, 1994a)***				
Spermatogonia	2160 – 5040	2.3 – 4.5 (1.7 – 2.7)	1520 (3250) **	4.6 (53.3) **
Spermatozoa	2160 – 5040	3.9 – 14.1 (1.5 – 2.1)	500 (2650) **	14.0 (65.2) **

Doubling doses in mice and men were calculated as uniparental exposure in contrast to the previous paper (Nomura, 1990), because fathers and mouse males were exposed to radiation in the Sellafield and mouse studies and conjoint parental gonad doses were used for the F_1 study of the atomic bomb survivors. Induced rate of leukemia per mSv in Sellafield study was calculated by the formula; (background incidence of leukemia relevant to Sellafield study, 53×10^{-5}) (Gardner, M. J., personal communication) x (excess risk value)/(paternal dose, mSv). External doses to employees were in the range of 100–150 mSv for all stages, 5–9 and 10–15 mSv for post-gonia (Gardner, M. J., personal communication). Average doses used for the calculation in this Table were 125, 7.5 and 12.5 mSv, respectively (modified from Nomura, 1990; 1991; 1994a). Figures in parentheses in mouse studies are for solid tumors, *i.e.*, tumors other than leukemia.

*Fathers' exposure during 6 months before conception indicates post-spermatogonial exposure, but includes spermatogonial exposure in part.

**Average value at 2160 and 5040 mSv.

***Unpublished data are included

higher doses in animal experiments (0.36~5 Gy) than those in epidemiological studies, 2) Human populations were exposed to radiation at the spermatogonial stage (in most parts) which is known to be less sensitive to radiation and chemicals than spermatozoa and spermatid stages, 3) Differences in the genetic predisposition in both human populations and mouse strains, 4) Postnatal exposure to radiation and/or chemically contaminated environment may enhance tumor incidence in the irradiated offspring as seen in mice, 5) Epidemiology focuses on childhood cancer and leukemia which develop at younger ages (below 20 years). Though the direct link to the sperm exposure has not been confirmed (COMARE, 1996), adult types cancer and adult diseases may increase at older ages in the human population, as it was seen in *in utero* exposed atomic bomb survivors (Kato, 1989; Yoshimoto *et al.*, 1991) and as anticipated by the animal experiments (Nomura, 1982; 1983; 1990; 1991; 1993; 1994a). In fact, induced rate of solid tumors in the offspring of mice exposed to radiation was much higher than that of leukemia (**Table 1**).

Very recently, a significant increase of stillbirths and specific congenital malformations was reported in the offspring of fathers who were exposed to radiation at Sellafield nuclear reprocessing plant (Parker *et al.*, 1999). Odds ratio were 1.21 to 1.69 at 100 mSv (**Table 2**). There are large differences in the exposed doses between Sellafield and mouse studies, as it was in leukemia study. Relative risk values for stillbirths and congenital malformations in mouse experiments were significantly high by post-spermatogonial exposure, but very low after spermatogonial exposure (**Table 2**). It was the case in the induced rate of congenital malformations in mouse experiments (Nomura, 1982; 1988; 2000).

Further animal experiments

After the Gardener's report in 1990, several studies have been carried out (see IARC Monograph 1999). High incidence of liver tumors was observed in the F_1 offspring of C3H male mice exposed to 0.5 Gy of ^{252}Cf (66 % neutron) and mated with C57BL/6 females (Takahashi *et al.*, 1992, Watanabe *et al.*, 1993). Slight increase of tumors was also observed in

Table 2 Estimated relative risk of congenital malformations and stillbirths in the offspring of male radiation workers at Sellafield nuclear reprocessing plant and of preconceptionally irradiated male mice

	Post-spermatogonia	Spermatogonia
1. Human (100 mSv)* (Parker *et al.*, 1999)		
All Stillbirths	—	1.26
Congenital Anomaly	—	1.43
Neural tube defects	—	1.69
Other specified cause	—	1.21
2. Human (10 mSv)** (Parker *et al.*, 1999)		
All Stillbirths	1.86	—
3. ICR mice (100 mSv/2.16 Gy) (Nomura, 1982)		
All Congenital Anomaly***	1.29	1.19
Lethal anomaly A	1.36	1.09
Lethal anomaly B	1.26	1.14
4. N5 mice (100 mSv/5.04 Gy) (Nomura, unpublished)		
All Stillbirths ****	1.31	1.09
Congenital anomaly	1.15	1.03

Values in ICR and N5 mice indicate the relative risk at 100 mSv, which is estimated from the induced rate of stillbirths and congenital malformations in the F_1 fetuses of ICR and N5 male mice exposed to 2.16 Gy (0.72 Gy/min) and 5.04 Gy (0.54 Gy/min) of X-rays, respectively. Morphological and functional defects were examined on the 18th day of gestation after caesarian operation.

* Total dose up to the time of conception (1961–1989; 7080 live and 73 stillbirths).

** Dose in the 90 days before conception (1950–1989; 9078 live and 130 stillbirths).

*** Total of lethal and viable anomalies. For the comparison with human stillbirth data, ICRmouse data were reanalysed and lethal anomalies were classified to 2 categories. A indicates all lethal anomaly in mice and B indicates types of anomalies which are lethal in mice but can be cured by the surgical operation in the case of human (*e.g.*, cleft palate, gastroschisis and some dwarfs).

**** Unresuscitated fetuses by caesarian operation on the 18th day of gestation. Late fetal deaths (> 16th days) were excluded.

$B6C3F_1$ offspring by ^{60}Co γ-rays (Kamiya, K., personal communication). A doubt was raised by Cattanach *et al.* (1995, 1998) for the lack of concurrent controls in my previous studies, because the results were published separately in a different calendar year, although the series of my large scale experiments were blind experiments and carried out in parallel with concurrent controls (Nomura, 1975; 1978; 1982; 1983). Cattanach *et al.* (1995, 1998) ascribed no significant increase but a seasonal change in the incidence of tumors in the offspring of BALB/cJ or C3H/HeH mice exposed to X-rays following the experimental protocol of Nomura (Nomura, 1982). Such a seasonal change is observed, when experiments are carried out in insufficient animal facilities, *e.g.*, animals are exposed to outdoor light. In fact, change of light-dark intervals significantly influenced tumor frequencies in mice (Nakajima *et al.*, 1994). The quality of animal facility is very important for carcinogenesis study, because, in addition to genetic predisposition, tumor incidence is influenced predominantly by the postnatal environment as in human cases.

Recently, another experiment was carried out in Canada with N5 mice provided by Nomura. Male N5 mice were irradiated in conditions close to those used by Nomura (1982, 1986) with 5 Gy of X-rays. The probability of dying from leukemia and overall survival were statistically different ($p<0.05$) between the offspring of X-ray-treated males and unirradiated controls. Earlier occurrence of leukemia was also observed in the F_1 offspring after the treatment of male N5 mice with tritiated water (Daher *et al.*, 1998).

A lifetime experiment showed a trend towards a higher incidence of tumors of the hematopoietic system and bronchoalveolar adenocarcinomas in the offspring of male CBA/JNCrj mice exposed to X-rays 1 week before mating (spermatozoa stage). However, no increase in tumor incidence was observed in the offspring of males irradiated 3 and 9 weeks before conception (Mohr *et al.*, 1999).

In general, there are apparent strain differences in the induced tumor types and tumor incidences in the preconceptionally irradiated offspring, indicating that pre-existing genetic predisposition *i.e.*, strain difference is essentially important even in transgenerational carcinogenesis.

Possible mechanism

What is the germ cell alteration causing tumors in the next generation? As described above, tumor incidence in the offspring was 100 fold higher than those of ordinary mouse mutations. For the possible mechanism, many gene loci concerning normal immunological, biochemical and physiological function might be involved, and the change in such genes may slightly elevate tumor incidence. Mutations of tumor suppresser genes like *p53* have also been detected in cancer prone descendants of mice exposed to radiation (**Fig. 6**). Alternatively, radiation induced genetic instability in germ cells and also in embryonic cells may elevate or enhance tumor occurrence in the offspring, as preconceptionally and *in utero* irradiated offspring showed persistent hypersensitivity to postnatal treatment with tumor promoting agents (**Fig. 2**). Paternal exposure to ^{60}Coγ-rays and ^{252}Cf fission neutron increased instability of repeat DNA sequences in their descendants. Significantly high frequency of simple-tandem repeat mutation was observed in F_1 offspring of γ(X)-ray or neutron irradiated male mice at both spermatozoa and spermatogonia stages and also in F_2 offspring of these unexposed F_1 offspring, suggesting indirect effects on genome instability in the next generation (Dubrova *et al.*, 1993; 1998a, b; 2000a, b), although Sadamoto *et al* (1994) and Niwa *et al* (1996) reported a slight increase of mutation at the same locus by postomeiotic but not spermatogonial γ-ray and neutron exposures. Frequency of germ line tandem repeat mutation was as high as that of offspring tumors. In human, it was also high in the offspring of inhabitants exposed to radionuclides after Chernobyl catastrophe (Dubrova *et al.*, 1996). However, it was higher by spermatogonial exposure than spermatozoa exposure and there were no dose rate effects (Dubrova, 1998a; 2000a), in contrast to the ordinary mouse mutations (Russell *et al.*, 1958, Lyon *et al.*, 1972; Bartsch-Sandhoff, 1974; Russell and Kelly, 1982a, b; Ehling, 1984) and transgenerational teratogenesis and carcinogenesis in mice (Nomura, 1978; 1982; 1983; 1988) Consequently, the direct link of genome instability to tumor incidence in the offspring has not been proven.

Germ-line alteration causing tumors may be ocurring in an array of normal genes concerning biochemical, immunological and physiological functions, and changes in such genes may slightly elevate tumor incidence. Consequently, changes of gene expression are now being examined by the GeneChip technology. Although data are not shown, 5500 functional genes were examined in the F_3 descendants (**Fig. 7**) of ^{60}Coγ-ray irradiated male N5 mice. About 0.5 % of genes showed 20 fold difference in the expression level (both suppression and over-expression) between the normal skin of preconceptionally irradiated and unirradiated F_3 descendants. Furthermore, there was a 20 fold difference in 2 % of genes between the normal skin and skin cancer lesion of the same F_3 mouse. Similar results were observed in the liver.

Dioxins

Dioxins, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a typical man-made environmental toxic agent. It is classified into the group 1 carcinogen to human (IARC, 1997), and acts as an endocrine disrupter as is the case with most chemical carcinogens. However, genotoxicity of TCDD has not been confirmed in bacteria, culture cells, *etc.* (IARC, 1997) and in human sperm (Kamiguchi and Ishii, 1999), although it decreases the numbers of male germ cells in mice by its damage to Sertoli cells (Inoue *et al.*, 1999). Nonetheless, possible transgenerational disorders are suspected in man and mice.

Significantly high incidence of congenital malformations was reported in the children of fathers who had been exposed to herbicides containing TCDD at the Vietnam War (Cân, 1984; Nomura, 1994b; Nomura *et al.*, 1999). To examine the transgenerational effects of TCDD, 50 or 100 ng/g bw of TCDD was given intraperitoneally to C3H/HeJ male mice and then mated with untreated C57BL/6J females. The spermatogonial stage was treated. With a high dose of TCDD, a significant reduction of implants and live births was observed. Although incidence of malformation increased in F_1 fetuses, the difference was marginally significant ($p = 0.057$). However, induced malformations were of the severe types (2 omophaloceles, testis defect, agnathy and eye defect) which were extremely rare in unexposed control mice. Similar results have been also observed with another strain of mice. Germ line mutation of the repetitive DNA sequence locus, *pc-3* was examined in B6C3 F_1 fetuses of TCDD treated and untreated male parents. About 25 % of individual F_1 fetus showed length differences. Although there were no significant differences between F_1 fetuses of TCDD treated and untreated parents, all malformed fetuses showed length alterations. Genomic instability might be involved in parent-mediated or transgenerational defects induced by TCDD (Nomura *et al*, 1999).

ACKNOWLEDGMENT

The author thanks Drs. Hiroo Nakajima, Haruko Ryo and Tadashi Hongyo for their help and Masumi Maeda, Khalida Wani, Yumi Kitagawa and Rie Tsuboi for their editorial assistance.

REFERENCES

Bartsch-Sandhoff M (1974) Skeletal abnormalities in mouse embryos after irradiation of the sire. *Humangenetik*, **25**: 93-100.

Bithell JF, Stewart AM (1975) Pre-natal irradiation and childhood malignancy: a review of British data from the Oxford Survey. *Br J Cancer*, **35**: 271-287.

Cân N (1984) Effect on offspring of parental exposure to herbicides. In: Westing AH (eds) *Herbicides in War - the Long Term Ecological and Human Consequences*. Pailor and Francis, Philadelphia, pp137-140.

Cattanach BM, Papworth D, Patrick G, Goodhead DT, Hacker T, Cobb L, Whitehill E (1998) Investigation of lung tumor induction of C3H/HeH mice, with and without tumor promotion with urethane, following paternal X-irradiation. *Mutat Res*, **403**: 1-12.

Cattanach BM, Patrick G, Papworth D, Goodhead DT, Hacker T, Cobb L, Whitehill E (1995) Investigation of lung tumor induction in BALB/cJ mice following paternal X-irradiation. *Int J Radiat Biol*, **67**: 607-615.

COMARE (1996) *Committee on Medical Aspects of Radiation in the Envrioment*. Fourth Report. Dept Health, London.

Daher A, Varin M, Lamontagne Y, Oth D (1998) Effect of pre-conceptional external or internal irradiation of N5 male mice and the risk of leukemia in their offspring. *Carcinogenesis*, **19**: 1553-1558.

Druckrey H (1973) Chemical structure and action in transplacental carcinogenesis and teratogenesis. *IARC Sci Publ*, **4**: 45-58.

Dubrova YE, Jeffreys AJ, Malashenko AM (1993) Mouse minisatellite mutations induced by ionizing radiation. *Nat Genet*, **5**: 92-94.

Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ (1996) Human minisatellilte mutation rate after the Chernobyl accident. *Nature*, **380**: 683-696.

Dubrova YE, Plumb M, Brown J, Boulton E, Goodhead D, Jeffreys AJ (2000a) Induction of minisatellite mutations in the mouse germline by low-dose chronic exposure to γ-radiation and fission neutrons. *Mutat Res*, **453**: 17-24.

Dubrova YE, Plumb M, Brown J, Fennelly J, Bois P, Goodhead D, Jeffreys AJ (1998a) Stage specificity, dose-response and doubling dose for mouse minisatellite germline mutation induced by acute radiation. *Proc Natl Acad Sci USA*, **95**: 6251-6255.

Dubrova YE, Plumb M, Brown J, Jeffreys AJ (1998b) Radiation-induced germline instability at minisatellite loci. *Int J Radiat Biol*, **74**: 689-696.

Dubrova YE, Plumb M, Gutierrez B, Boulton E, Jeffreys AJ (2000b) Transgenerational mutation by radiation. *Nature*, **405**: 37.

Ehling UH (1984) Methods to estimate the genetic risk. In: Obe G (eds) *Mutations in man*. Springer-Verlag, Berlin, pp292-318.

Gardner MJ, Snee MP, Hall AJ, Powell CA, Downes S, Terrell JD (1990) Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *Brit Med J*, **300**: 423-429.

Graham S, Levin ML, Lilienfield AM, Schuman LM, Gibson R, Dowd JE, Hemplemann L (1966) Preconception, intrauterine, and postnatal irradiation as related to leukemia. *Natl Cancer Inst Monogr*, **19**: 347-371.

Herbst AL, Ulfelder H, Poskanzer DC (1971) Adenocarcinoma of the vagina: Association of the maternal stilbestrol therapy with tumor appearance in young women. *New En l J H:ed*, **284**: 878-881.

IARC (1997) IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. Vol. 69: Polychlorinated Dibenzo-para-Dioxins and Polychlorinated Divenzofurans. IARC, Lyon.

IARC (1999) IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. Vol. 75: Physical Agents: Ionizing Radiation, Part I , X-rays, γ-rays and neutrons. IARC, Lyon.

Inoue M, Kurihara T, Ueda T, Nomura T (1999) Effects of dioxin on mouse germ cells. *Environ Mutagen Res*, **21**: 197-200. (Japanese)

Kamiguchi Y, Ishii Y (1999) Genetic effects of dioxin: from a viewpoint of the effects on human sperm chromosomes. *Environ Mutagen Res*, **21**: 201-205. (Japanese)

Kato H (1978) *Mortality of in utero children exposed to the A-bomb and of offspring of A-bomb survivors*. IARC-SM-224/603, IAEA, Vienna, pp49-60.

Kato H, Yoshimoto Y, Schull WJ (1989) Risk of cancer among children exposed to A-bomb radiation *in utero*: a review. In: Napalkov NP, Rice JM, Tomatis L, Yamasaki H (eds) *Perinatal and Multigeneration Carcinogenesis*, IARC Sci Publ No. 96, IARC, Lyon, pp365-374.

Kirk KM, Lyon MF (1984) Induction of congenital malformations in the offspring of male mice treated with X-rays at pre-meiotic and post-meiotic stages. *Mutat Res*, **125**: 75-85.

Lord BI, Woodford LB, Wang L, Stones VA, McDonald D, Lorimore SA, Papworth DP, Wright EG, Scott D (1998) Tumour induction by methyl-nitrosourea following preconceptional paternal contamination with plutonium-239. *Br J Cancer*, **78**: 301-311.

Lyon MF, Papworth DG, Phillips RJS (1972) Dose-rate and mutation frequency after irradiation of mouse spermatogonia. *Nature (London), New Biol*, **238**: 101-104.

MacMahon B (1962) Prenatal X-ray exposure and childhood cancer. *J Natl Cancer Inst*, **28**: 1173-1191.

Mohr U, Dasenbrock C, Tillmann T, Kohler M, Kamino K, Hagemann G, Morawietz G, Campo E, Cazorla M, Fernandez P, Hernandez L, Cardesa A, Tomatis L (1999) Possible carcinogenic effects of X-rays in a transgenerational study with CBA mice. *Carcinogenesis*, **20**: 325-332.

Nagao T, Fujikawa K (1990) Genotoxic potency in mouse spermatogonial stem cells of triethylenemelamine, mitomycin C, ethylnitrosourea, procarbazine, and propyl methanesulfonate as measured by F1 congenital defects.

Mutat Res, **229**: 123-128.

Nakajima H, Narama I, Matsuura T, Nomura T (1994) Enhancement of tumor growth under short light/dark cycle in the mouse lung. *Cancer Letters*, **78**: 127-131.

Niwa O, Fan YJ, Numoto M, Kamiiya K, Kominami R (1996) Induction of a germline mutation at a hypervariable mouse minisatellite locus by ^{252}Cf radiation. *J Radiat Res*, **37**: 217-224.

Nomura T (1973) Carcinogenesis by urethan via mother's milk and its enhancement of transplacental carcinogenesis in mice. *Cancer Res*, **33**: 1677-1683.

Nomura T (1974a) An analysis of the changing urethan response of the developing mouse embryo in relation to mortality, malformation, and neoplasm. *Cancer Res*, **34**: 2217-2231.

Nomura T (1974b) Differential sensitivity of the developing mouse embryo to mortality, malformation and neoplasm induced by urethane. *Biochem Soc Transactions*, **2**: 710-713.

Nomura T (1975) Transmission of tumors and malformations to the next generation of mice subsequent to urethan treatment. *Cancer Res*, **35**: 264-266.

Nomura T (1976) Comparison of tumour susceptibility among various organs of foetal,young, and adult ICR/Jcl mice. *Brit J Cancer*, **33**: 521-534.

Nomura T (1978) Changed urethan and radiation response of the mouse germ cell to tumor induction. In: Severi L (ed) *Tumors of Early Life in Man and Animals*. Perugia Univ Press, Perugia, pp873-891.

Nomura T (1982) Parental exposure to X rays and chemicals induces heritable tumors and anomalies in mice. *Nature*, **296**: 575-577.

Nomura T (1983) X-ray induced germ-line mutation leading to tumors: its manifestation in mice given urethane postnatally. *Mutation Res*, **121**: 59-65.

Nomura T (1984a) Induction of persistent hypersensitivity to lung tumorigenesis by *in utero*X irradiation in mice. *Environ Mutagen*, **6**: 33-40.

Nomura T (1984b) Quantitative studies on mutagenesis, teratogenesis, and carcinogenesis in mice. In: Tazima Y, Kondo S, Kuroda Y (eds) *Problem of threshold in chemical mutagenesis*. Environmental Mutagen Society of Japan, Shizuoka, pp27-34.

Nomura T (1986) Further studies on X-ray and chemically induced germ-line alterations causing tumors and malformations in mice. In: Ramel C (eds) *Genetic Toxicology of Environmental Chemicals, Part B: Genetic Effects and Applied Mutagenesis*, Alan R Liss, New York, pp13-20.

Nomura T (1987) Tumors and malformations in the offspring. In: Fujii T, Adams PM (eds) *Functional Teratogenesis*, Teikyo Univ Press, Tokyo, pp175-185.

Nomura T (1988) X-ray and chemically induced germ-line mutation causing phenotypical anomalies in mice. *Mutation Res*, **198**: 309-320.

Nomura T (1989) Role of radiation-induced mutations in multigeneration carcinogenesis. In: Napalkov NP, Rice JM, Tomatis L, Yamasaki H (eds) *Perinatal and Multigeneration Carcinogenesis*, IARC Sci Publ No. 96, IARC, Lyon, pp375-387.

Nomura T (1990) Of mice and men? *Nature*, **345**: 671.

Nomura T (1991) Paternal exposure to radiation and offspring cancer in Mice: Reanalysis and new evidences. *J Radiat Res*, **32 (Suppl 2)**: 64-72.

Nomura T (1993) Leukemia in children whose parents have been exposed to radiation. *Br Med J*, **306**: 1412.

Nomura T (1994a) Genetic effects of radiation and offspring cancer. In: Huilgol NG, Gopinath DV, Singh BB (eds) *Low Level Radiation and Living State*. Springer-Verlag, Berlin, pp40-56.

Nomura T (1994b) Male-mediated teratogenesis-ionizing radiation/ethylnitrosourea studies. In: Olshan AF, Mattison DR (eds) *Male-mediated Developmental Toxicity*. Plenum Press, New York, pp117-127.

Nomura T (1998) Dose rate effectiveness and repair in radiation-induced mutagenesis, teratogenesis, and carcinogenesis in mice. In: Sharan RN (eds) *Trens in Radiation and Cancer Biology*. Forschungzentrum, Julich, pp149-155.

Nomura T (2000) Transgenerational teratogenesis and carcinogenesis by radiation. In: Sato F, Yamada Y, Onodera J (eds) *Biological Effects of Low Dose Radiation*. Inst Environ Sci, Aomori, pp103-108.

Nomura T, Gotoh H, Namba T (1990) An examination of respiratory distress and chromosomal abnormalities in the offspring of male mice treated with ethylnitrosourea. *Mutat Res*, **229**: 115-122.

Nomura T, Kanzaki T (1977) Induction of urogenital anomalies and some tumors in the progeny of mice receiving diethylstilbestrol during pregnancy. *Cancer Res*, **37**: 1099-1104.

Nomura T, Kurokawa N (1997) Comparative study on germ cell mutation induced by urethane (ethyl carbamate) gas and X-rays in Drosophila melanogaster. *Jpn J Cancer Res*, **88**: 461-467.

Nomura T, Masuda M (1980) Carcinogenic and teratogenic activities of diethylstilbestrol in mice. *Life Sciences*, **26**: 1955-1962.

Nomura T, Nakajima H, Hatanaka T, Kinuta M, Hongyo T (1990) Embryonic mutation as a possible cause of *in utero* carcinogenesis in mice revealed by postnatal treatment with 12-*O*-tetradecanoylphobol-13-acetate. *Cancer Res*, **50**: 2135-2138.

Nomura T, Nakajima H, Li LY, Fukudome Y, Baskar R, Ryo H, Koo JY, Mori K (1999) Concern for the hereditary effects of dioxins. *Environ Mutagen Res*, **21**: 207-211. (Japanese)

Nomura T, Okamoto E (1972) Transplacental carcinogenesis

by urethan in mice: Teratogenesis and carcinogenesis in relation to organogenesis. *Gann*, **63**: 731-742.

Nomura T, Sutoh K, Hongyo T, Yamamoto O, Yokoro K, Nakajima H, Gotoh H, Kawaguchi T, Fukuda K (1993) Somatic mutations and cancer induced by in utero exposure to ^{252}Cf neutron. *J Radiat Res*, **34**: 79. (Abst)

Nomura T, Takebe H, Okamoto E (1973) Long retention of urethan transferred into newborn mice transplacentally; as a possible cause of high carcinogenesis. *Gann*, **64**: 29-40.

Parker L, Pearce MS, Dickinson HO, Aitkin M, Coft AW (1999) Stillbirth among offspring of male radiation workers at Sellafield nuclear reprocessing plant. *Lancet*, **354**: 1407-1414.

Rugh R, Duhamel L, Karedoff L (1966) Relation of embryonic and fetal X-irradiation to life time average weights and tumor incidence in mice. *Proc Soc Ext Biol Med*, **121**: 714-718.

Russell WL, Kelly EM (1982a) Specific-locus mutation frequencies in mouse stem-cell spermatogonia at very low radiation dose rates. *Proc Natl Acad Sci USA*, **79**: 539-541.

Russell WL, Kelly EM (1982b) Mutation frequencies in male mice and the estimation of genetic hazards of radiation in men. *Proc Natl Acad Sci USA*, **79**: 542-544.

Russell WL, Russell LB, Kelly EM (1958) Dependence of mutation rate on radiation intensity. *Science*, **128**: 1546-1550.

Sadamoto S, Suzuki S, Kamiya K, Kominami R, Dohi K, Niwa O (1994) Radiation induction of germline mutation at a hypervariable mouse minisatellite locus. *Intl J Radiat Biol*, **65**: 549-557.

Sasaki S, Kasuga T, Sato T, Kawashima N (1978) Late effects of fetal mice X-irradiated at middle or late intrauterine stage. *Gann*, **69**: 167-177.

Shiono PH, Chang CS, Myrianthopoulos NC (1980) Preconception radiation, intrauterine diagnostic radiation, and childhood neoplasia. *J Natl Cancer Inst*, **65**: 681-686.

Shu XO, Gao YT, Brinton LA, Linet MS, Tu JT, Zheng W, Fraumeni JFJr (1988) A population-based case-control study of childhood leukemia in Shanghai. *Cancer*, **62**: 635-644.

Takahashi T, Watanabe H, Dohi K, Ito A (1992) ^{252}Cf Relative biological effectiveness and inheritable effects of fission neutrons in mouse liver tumorigenesis. *Cancer Res*, **52**: 1948-1953.

Upton AC, Odell TT, Smiffen EP (1960) Influence of age at time of irradiation on induction of leukemia and ovarian tumors in RF mice. *Proc Soc Exp Biol Med*, **104**: 769-772.

Vorobstova IE, Aliyakparova LM, Anisimov VN (1993) Promotion of skin tumors by 12-*O*-tetradecanoyl phorbol-13-acetate in two generations of descendants of male mice exposed to X-ray irradiation. *Mutat Res*, **287**: 207-216.

Vorobstova IE, Kitaev EM (1988) Urethane-induced lung adenomas in the first-generation progeny of irradiated male mice. *Carcinogenesis*, **9**: 1931-1934.

Watanabe H, Okamoto T, Yamada K, Ando Y, Ito A, Hoshi M, Sawada S (1993) Effects of dose rate and energy level on fission neutron (^{252}Cf) tumorigenesis in B6C3F$_1$ mice. *J Radiat Res*, **34**: 235-239.

Yoshimoto Y, Kato H, Schull WJ (1991) Cancer risk among *in utero*-exposed survivors. *J Radiat Res*, **32**: 231-238.

Yoshimoto Y, Mabuchi K (1991) Mortality and cancer risk among the offspring (F$_1$) of atomic bomb survivors. *J Radiat Res*, **32**: 294-300.

Yoshimoto Y, Schull WJ, Kato H, Neel JV (1991) Mortality among the offspring (F$_1$) of atomic bomb survivors, 1946-1985. *J Radiat Res*, **32**: 327-351.

UNSCEAR-Report (1982) (United Nations Scientific Committee on the Effects of Atomic Radiation). *Genetic and Somatic Effects of Ionizing Radiation*. United Nations, New York.