Radiation-Induced Lymphomas in MSM, (BALB/cHeA x MSM) F₁ and (BALB/cHeA x STS/A) F₁ Hybrid Mice

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Abstract: M.MOL-MSM (MSM) mice derived from *Mus musculus molossinus* progenitors showed extreme resistance to the induction of lymphomas following whole-body X-irradiation with four doses of 1.7 Gy. (BALB/cHeA x MSM) F₁ mice between a high lymphoma strain, BALB/cHeA and the MSM showed a high incidence of radiation-induced lymphomas which was quite similar to that in BALB/cHeA mice, but the latent period was prolonged in the hybrids. Susceptibility in incidence was dominant over resistance in these crosses. Incidences of (BALB/cHeA x MSM)F₁ hybrids irradiated with four doses of 2.5 Gy X-rays were 77% in females and 88% in males. F₁ hybrids between BALB/cHeA and another resistant strain STS/A, (BALB/cHeA x STS/A) F₁, also showed a high level of susceptibility, that is, lymphoma incidence was 64% in females and 63% in males. The mean latent period in the (BALB/cHeA x STS/A) F₁ hybrids was similar to that in (BALB/cHeA x MSM) F₁ hybrids. As all cases of tumors developed in F₁ hybrids are informative concerning the detection of the loss of heterozygosity in the loci depending on the combination of two parental strains, the radiation-induced lymphomas obtained from (BALB/cHeA x MSM) F₁ and (BALB/cHeA x STS/A) F₁ hybrids could be useful for fine analysis of the genetic alterations involved in lymphomagenesis.

Key words: LOH (loss of heterozygosity), molossinus, mouse, lymphoma, radiation

Introduction

It is well established in both animal models and humans that normal cells change to malignant cells through multiple steps in which various mutations of genes are

involved, and that these mutations include the activation of oncogenes and inactivation of tumor suppressor genes. In many human tumors, chromosomal deletions containing common regions were found at high frequency. Several important tumor suppressor genes were

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identified in these deleted regions: deletion of p53 gene on chromosome 17p13.1 in many cases of small-cell lung carcinomas, breast cancers, bladder cancers and colorectal cancers [1, 2], and deletion of Rb gene on chromosome 13q14.2 in various human tumors such as retinoblastoma, small-cell lung carcinomas and breast cancers [3, 4]. Although a considerable number of tumor suppressor genes are predicted to exist, only a few such genes have been identified so far. Numerous attempts to detect deletions of common regions in human tumors have been made, but it is often difficult to detect the loss of heterozygosity (LOH) of the same regions in all cases of human tumors because all alleles of these tumor cells do not always show polymorphism.

On the other hand, genetically controlled experimental animals have provided an excellent model system to systematically study the relationship between genetic changes and a disease. Tumors developed in F_1 hybrid mice between two inbred strains with genetically large difference are very suitable materials for the study of various changes in both the structure and expression of each allele because many polymorphic genetic markers are easily obtained in these F_1 hybrid tumors. If tumors are induced in F_1 hybrid mice between two parental strains which differ greatly from each other in their susceptibility to experimental induction of tumors, the characteristics of genome structures involved in the tumorigenesis might be determined by pursuing the behavior of genes derived from resistant and sensitive strains in the tumors.

BALB/cHeA mice are highly susceptible to radiation lymphomagenesis, but STS/A mice are highly resistant [5]. In the present study, we demonstrated that M.MOL-MSM (MSM) mice belonging to *Mus musculus molossinus* were also remarkably resistant, and that (BALB/cHeA $\times$ MSM) F_1 and (BALB/cHeA $\times$ STS/A) F_1 hybrids were highly susceptible to radiation lymphomagenesis. These lymphomas could be excellent materials to analyze genetic alteration including LOH involved in lymphomagenesis.

Materials and Methods

Mice: MSM mice are derived from *Mus musculus molossinus* progenitors trapped in Mishima city, Shizuoka Prefecture, Japan in April, 1978, and have been maintained by K. Moriwaki [6]. The origins of

BALB/cHeA and STS/A mice were described previously [5]. All animals were kept in air-conditioned rooms (temperature, $24 \pm 0.5^\circ\text{C}$ and the relative humidity, $55 \pm 5\%$, with automatic lighting on from 7:00 a.m. to 7:00 p.m.). The mice were given a commercial diet (MF, Oriental Yeast, Co., Tokyo) and sterilized tap water *ad libitum*.

X-irradiation: Mice were exposed to four doses of 1.7 Gy or 2.5 Gy (0.5 Gy/min) of X-rays at weekly intervals starting at 4 weeks of age, as previously described [7].

Examination of developed lymphomas: Moribund mice were sacrificed for diagnosis by histological and cytological examination of their tumors as previously described [7].

Results

1. Resistance to radiation lymphomagenesis in MSM mice

MSM female mice were exposed to four doses of 1.7 Gy of X-rays (Fig. 1, Table 1). Only one mouse developed thymic lymphoma at 373 day of age (= 322 day after the last irradiation). The incidence of lymphoma was very low (3%, 1/30) at 1 year after the last irradiation and was similar to that of resistant strain STS/A [5]. In contrast, many other strains such as BALB/cHeA [5], C57BL/Ka [8], C57BL/10Sn [9] and NFS [10] mice show a high incidence of lymphomas (more than 75%) by 1 year after fractionated irradiations. In these strains there is little difference between sexes in the incidence or latent period. MSM was therefore found to be very resistant to radiation lymphomagenesis.

2. Highly efficient development of radiation-induced lymphomas in (BALB/cHeA $\times$ MSM) F_1 hybrids

Female (BALB/cHeA $\times$ MSM) F_1 mice exposed to 4 doses of 1.7 Gy irradiation developed lymphomas with quite similar incidence (78%: 14/18) to the susceptible parent, BALB/cHeA (Fig. 1, Table 1). The latent period of lymphoma development in the F_1 mice (221 ± 47 days) was, however, significantly longer than that in BALB/cHeA (151 ± 53 days) ($P < 0.001$). Susceptibility in incidence was dominant over resistance in these crosses.

Female and male (BALB/cHeA $\times$ MSM) F_1 mice ex-

Fig. 1. Cumulative incidences of lymphomas in females MSM, BALB/cHeA and (BALB/cHeA $\times$ MSM) F_1 mice exposed to four doses of 1.7 Gy of X-rays. —●—: MSM, —○—: BALB/cHeA, —◐—: (BALB/cHeA $\times$ MSM) F_1 .

posed to four doses of 2.5 Gy of X-rays also developed a very high incidence of lymphomas (Fig. 2, Table 1). The lymphomas in the female and in the male were first observed on day 80 and on day 108 after the last irradiation, respectively. Incidence reached 77% (41/53) in females and 88% (15/17) in males at one year after the last irradiation. Thirty-nine (95%) out of 41 lymphomas in females and all 15 cases in males were of the thymic type. The incidence and average latent period in the females exposed to 4 doses of 2.5Gy irradiation were comparable to those in the females exposed to 4 doses of 1.7 Gy (Table 1).

3. Development of radiation-induced lymphomas in STS/A and (BALB/cHeA $\times$ STS/A) F_1 hybrids

In the previous experiment, we demonstrated that STS/A mice exposed to four doses of 1.7 Gy hardly develop the lymphomas (female: 5/60, 8%; male: 6/68, 9%) within one year after the last irradiation [5]. In the present experiment, STS/A mice were irradiated four times with 2.5 Gy X-rays. This high dose level of X-irradiation (total dose of 10 Gy) was under the upper limit of the sublethal dose for STS/A mice. Nevertheless, a slight increase in the incidence of lymphomas

Table 1. Development of lymphomas in MSM, BALB/cHeA, STS/A and their hybrid mice after fractionated X-irradiation^{a)}

Strain	Sex	Dose	Lymphoma incidence (%)	Latent period (days)		
				Range	Median	Mean $\pm$ SD ^{b)}
MSM	Female	1.7 Gy $\times$ 4	3 (1/30) ^{c)}	322	322	322
BALB/cHeA ^{d)}	Female	1.7 Gy $\times$ 4	77 (33/43)	72–285	142	151 $\pm$ 53
	Male	1.7 Gy $\times$ 4	86 (24/28)	94–365	174	190 $\pm$ 72
(BALB/cHeA $\times$ MSM) F_1	Female	1.7 Gy $\times$ 4	78 (14/18)	133–327	216	221 $\pm$ 47
	Female	2.5 Gy $\times$ 4	77 (41/53)	80–341	180	199 $\pm$ 70
	Male	2.5 Gy $\times$ 4	88 (15/17)	108–364	204	231 $\pm$ 85
STS/A	Female ^{d)}	1.7 Gy $\times$ 4	8 (5/60)	104–292	224	208 $\pm$ 77
	Male ^{d)}	1.7 Gy $\times$ 4	9 (6/68)	87–306	227	216 $\pm$ 82
	Female	2.5 Gy $\times$ 4	23 (9/39)	108–228	201	175 $\pm$ 50
	Male	2.5 Gy $\times$ 4	10 (3/29)	201–330	299	276 $\pm$ 67
(BALB/cHeA $\times$ STS/A) F_1	Female ^{d)}	1.7 Gy $\times$ 4	40 (14/35)	86–365	173	208 $\pm$ 76
	Female	2.5 Gy $\times$ 4	64 (36/56)	72–365	169	179 $\pm$ 60
	Male	2.5 Gy $\times$ 4	63 (15/24)	128–365	194	211 $\pm$ 67

^{a)} Development of lymphomas was observed up to 365 days after the last irradiation. ^{b)} Standard deviation ^{c)} Numbers in parentheses : number of mice which developed lymphomas / number of mice tested ^{d)} From Ref. 5.

Fig. 2. Cumulative incidence of lymphomas in (BALB/cHeA $\times$ MSM) F_1 mice exposed to four doses of 2.5 Gy of X-rays.

Fig. 3. Cumulative incidence of lymphomas in STS/A mice exposed to four doses of 2.5 Gy of X-rays.

was observed only in female mice (9/39, 23%) ($p < 0.05$) as compared with that in females exposed four times to 1.7 Gy (Fig. 3, Table 1). The lymphoma in females was first observed at 108 days and developed frequently from 200 to 230 days after the irradiation. All lymphomas developed in both sexes were of the thymic type.

In contrast, (BALB/cHeA $\times$ STS/A) F_1 hybrids developed a high incidence of lymphomas after four 2.5 Gy doses of X-irradiation (Fig. 4, Table 1). The first lymphoma appeared at 72 days in females and 128 days in males after the last irradiation. The incidence reached 64% (36/56) in females and 63% (15/24) in males at one year after the last irradiation. (BALB/cHeA $\times$ STS/A) F_1 hybrid mice showed a slightly lower incidence than (BALB/cHeA $\times$ MSM) F_1 hybrids, but the difference was insignificant at four 2.5 Gy doses. The mean latent period of lymphoma development in (BALB/cHeA $\times$ STS/A) F_1 hybrids was also similar to that in (BALB/cHeA $\times$ MSM) F_1 hybrids at both four 2.5 Gy and four 1.7 Gy doses. Thirty-five (97%) out of 36

Fig. 4. Cumulative incidence of lymphomas in (BALB/cHeA $\times$ STS/A) F_1 mice exposed to four doses of 2.5 Gy of X-rays.

lymphomas in females and 14 (93%) out of 15 cases in males were of the thymic type.

Discussion

The development of radiation-induced lymphomas is considered to be regulated by genetic factors because the incidence of lymphoma strikingly differs among strains of mice. In order to analyze the genetic factors controlling the susceptibility to radiation lymphomagenesis, mouse strains with remarkably different phenotypes are very valuable. Although many laboratory strains derived mainly from *M. musculus domesticus* are highly susceptible to lymphomagenesis, the STS/A strain is one of the most resistant mice among laboratory strains. The present study demonstrated that MSM mice derived from *M. musculus molossinus* were extremely resistant to radiation-induced lymphomas. These low lymphoma strains are very useful for comparative study with high lymphoma ones on the genetic basis of susceptibility.

Both (BALB/cHeA $\times$ STS) F_1 and (BALB/cHeA $\times$ MSM) F_1 hybrid mice were found to develop a considerably high incidence of lymphomas. It is interesting to examine the LOH of both alleles in all cases of tumors developed in F_1 hybrids derived from parents with distinct susceptibility to lymphoma induction. If relevant genes derived from resistant STS/A or MSM mice were lost in some lymphoid cells of irradiated F_1 mice or if expression of the relevant genes from resistant strains were repressed, the cells may acquire some growth advantage and may proceed through some steps leading to lymphoma. Tumor-related genes or tumor suppressor genes might be detected by analysis of common loss of alleles in F_1 mice.

A number of loci have been identified in mice with polymorphic microsatellite DNA [11, 12], and the number of identified loci is increasing more and more. The typical rate of polymorphism among inbred laboratory strains such as STS/A and BALB/cHeA was about 50%, and the typical rate of polymorphism between an inbred laboratory strain and either *M. musculus castaneus* or *M. spretus* was about 90% [11]. These polymorphic markers are very powerful tools to separately and systematically analyze the structural alterations of both alleles and/or changes in the levels of gene expression.

Most mutations induced by ionizing radiation in

mammalian cells are deletion, and point mutation is rather infrequent [13]. Precise examination of LOH in F_1 mouse strains may therefore be a very good way to analyze the genes involved in radiation carcinogenesis.

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