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Clonal Origin of Skin and Bone Tumors Produced by Repeated Beta-irradiation in Mosaic Cell Mice

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Clonal origin of skin and bone tomors produced by repeated beta-irradiation was determined by using mice with cellular mosaicism created by random X-chromosome inactivation, on the basis of phosphoglycerate kinase-1 (PGK). The backs of female C3H/He (*Pgk-1*^{a}/*Pgk-1*^{b}) mice were exposed to beta rays from ^{90}Sr-^{90}Y at a dose of 3 Gy per exposure 3 times weekly until tumors appeared. The cumulative tumor incidence reached 100% 500 days after the beginning of irradiation, as determined by the Kaplan-Meier method. All 8 tumors examined were of a single PGK phenotype: 5 squamous cell carcinomas and 2 osteosarcomas of A-type, and 1 squamous cell carcinoma of B-type. The absence of double PGK phenotype (AB-type) tumors indicated the monoclonal origin of the tumors produced by repeated irradiation.

Key words: Monoclonal origin — Radiation-induced tumor — Repeated treatment — Phosphoglycerate kinase — Mosaic cell mouse

We have developed an experimental system to produce skin and bone tumors in mice with 100% efficiency by repeated irradiation with 2–11 Gy of beta rays.[1-3] At a dose of 1.5 Gy per exposure or less, the tumor incidence was sharply decreased in a threshold-like manner.[3-5] Repeated treatment with doses higher than this threshold dose are thought to cause many mutations and to recruit a number of cells in the irradiated tissue. Since these cells could be the origin of tumors, we thought it would be interesting to determine whether these tumors produced by the repeated irradiation are of monoclonal origin or of multi-clonal origin.

To test the clonality of tumors, somatic cellular mosaicism based on X-chromosome inactivation is useful.[6] Tumors of various types produced in the host with mosaic cells have exhibited a single phenotype in humans[6] and mice.[7,8] These results indicate the monoclonal origin of tumors, whereas multiple cell origin has been shown in a minority of tumors such as human polyps in Gardner syndrome[9] and mouse tumors produced by a high dose of a chemical carcinogen.[10] Concerning radiation-induced tumors, mouse leukemia produced by 4 repeated whole-body X-irradiations was shown to be monoclonal.[11,12]

Mosaic cell mice were used in the present study to determine the clonal nature of the mouse solid tumors produced by repeated beta irradiation.

MATERIALS AND METHODS

Animals Male C3H/HeHa mice with a mutant X-linked gene *Pgk-1*^{a}[13] were mated with wild female C3H/He (*Pgk-1*^{b}/*Pgk-1*^{b}) mice (Charles River Japan, Kanagawa-ken). Female offspring of their cross (*Pgk-1*^{a}/*Pgk-1*^{b}) were used. The presence of both A-type and B-type PGK[2] was confirmed in the blood and liver of the mice. Forty-two mosaic mice were used for irradiation and 18 were unirradiated controls. Thirty-one ICR female mice (Charles River Japan) were used as positive controls to reproduce our previous results.[1] Mice were given laboratory chow (CE-2, CLEA Japan, Tokyo) and water *ad libitum*.

Beta-irradiation The irradiation methods were described previously.[1] Briefly, PGK mosaic mice aged 8–15 weeks and ICR mice aged 7 weeks at the start, were repeatedly irradiated with 3 Gy of beta rays from ^{90}Sr-^{90}Y 3 times weekly in a circular area 2 cm in diameter on their backs until emergence of a tumor or death of the mice.

Tumor examination Dates of appearance of palpable tumors and death of the mice without tumors were recorded. Cumulative tumor incidence was calculated by the Kaplan-Meier method.[14] Each tumor was divided into two sections, one of which was fixed with 10% formalin, sectioned, and stained with hematoxylin and eosin, and examined histologically; the other was examined for PGK type.

Detection and analysis Determination of the PGK type of the tumors was described previously.[15] Tumor or tissue specimens were homogenized and subjected to electrophoresis in a starch gel. Blood specimens were applied directly on the gel. PGK was detected as non-

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[2] Abbreviation used: PGK, phosphoglycerate kinase.

fluorescent spots after assay reaction.[15] As a confirmatory experiment,[15] a piece of tumor was transplanted into the groin of a mouse with A-type PGK and another piece of the same tumor into a mouse with B-type PGK, and the regrown tumors were examined for PGK type.

RESULTS

The first tumor appeared in the mosaic mice on Day 379 after the beginning of irradiation (Fig. 1), much later than that observed in ICR mice irradiated in the identical manner (positive controls), or in the previous study.[1] From then on, the cumulative tumor incidence computed by the Kaplan-Meier method increased rapidly and reached 100% on Day 483. The death rate of mosaic C3H/He mice without tumors was very high and only 12 tumor-bearing mice were obtained out of the 42 irradiated. Total numbers of tumors obtained were 7 squamous cell carcinomas and 6 osteosarcomas. One mouse had double tumors, i.e., squamous cell carcimoma and osteosarcoma. No fibrosarcoma was found. No spontaneous tumors were found in the backs of the unirradiated mosaic C3H/He mice.

Of the 31 positive control ICR mice, 23 developed 27 tumors, i.e., 7 squamous cell carcinomas, 16 osteosarcomas and 4 fibrosarcomas. The cumulative tumor incidences in ICR mice are included in Fig. 1.

The PGK types of some of the tumors are shown in Table I. Of the 8 tumors for which the PGK type was determined, 5 squamous cell carcinomas and 2 osteosarcomas had A-type PGK, whereas one squamous cell carcinoma had B-type PGK. No double PGK phenotypes were found.

Table I. Histological and PGK Types of Tumors Produced by Repeated Beta Irradiation in PGK Mosaic Cell Mice

Tumor No.	Day of emergence	Histological type	PGK type
1	379	SSC	A
2	390	OS	ND
3	392	OS	ND
4	394	OS	ND
5	408	OS	A
6	420	OS	ND
7	428	SSC	A
8	440	SSC	A
9	440	OS	A
10	444	SSC	ND
11	447	SSC	A
12	469	SSC	A
13	483	SSC	B

SSC: Squamous cell carcinoma. OS: Osteosarcoma.
ND: not determined.

Fig. 2 shows a confirmatory experiment. Blood cells of a PGK mosaic mouse had both types of PGK, whereas those of a *Pgk-1*^{b}*/Pgk-1*^{b} mouse or a *Pgk-1*^{a}*/Y* mouse had

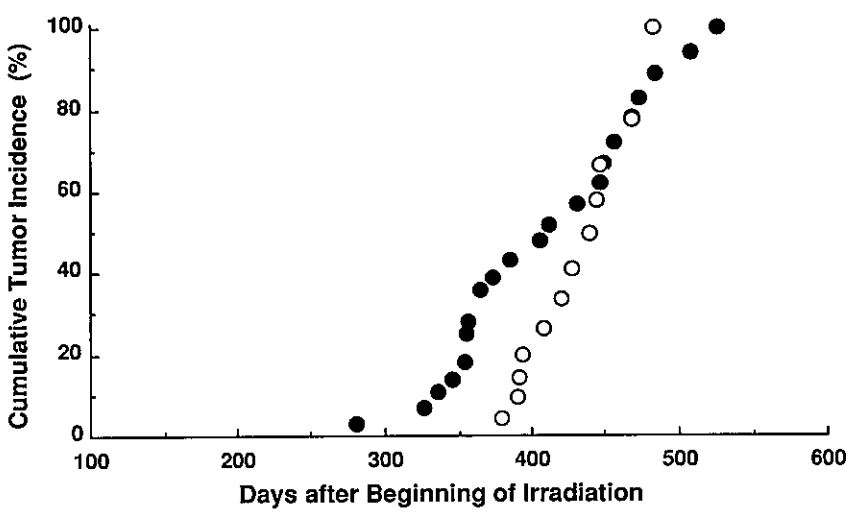

Fig. 1. Time courses of cumulative tumor incidences in PGK mosaic mice (○) and ICR mice (positive control, ●), repeatedly irradiated 3 times weekly with beta rays from ^{90}Sr-^{90}Y until emergence of a tumor or death of the mice. Data were analyzed by the Kaplan-Meier method.

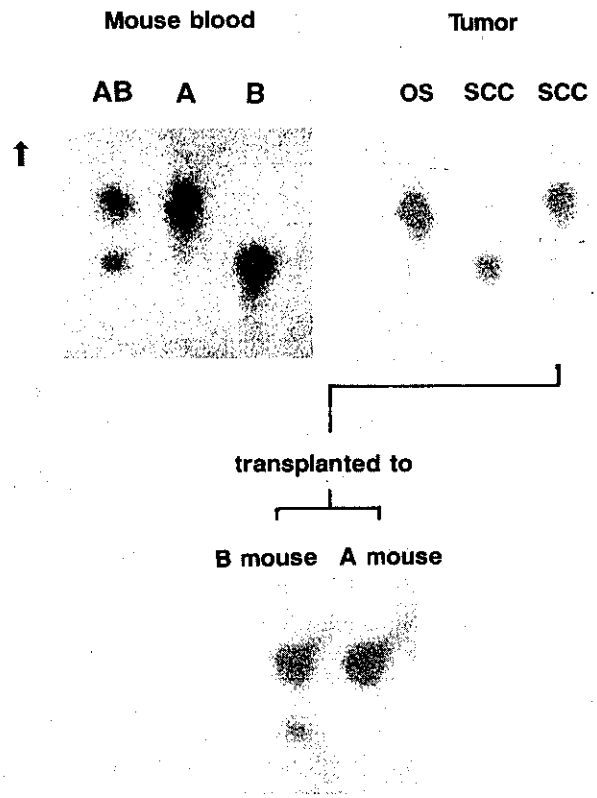

Fig. 2. Electrophoretic patterns of PGK. Upper left: Blood from mosaic cell mouse (AB), PGK A-type mouse (A) and PGK B-type mouse (B). Upper right: osteosarcoma with A-type PGK (OS), squamous cell carcinoma with B-type PGK (middle SCC) and squamous cell carcinoma with A-type PGK (right SCC). Bottom: A squamous cell carcinoma was transplanted into a mouse with B-type PGK (left) or A-type PGK (right), and the regrown tumors were examined for PGK type.

only B-type or A-type PGK, respectively. The 3 tumors shown in the figure exhibit a major spot of A-type or B-type PGK. However, a small spot of the opposite PGK type is seen, which could be due to contamination by supporting tissue or blood, or more importantly, to a small tumor clone with the opposite PGK type present in the tumor tissue.[16] When one of these tumors, squamous cell carcinoma with a major A-type PGK, was transplanted and regrown in a mouse with A-type PGK, the B-type PGK spot in the original specimen disappeared, whereas it still remained in the tumor regrown in the mouse with B-type PGK. It is therefore concluded that this squamous cell carcinoma had a single A-type PGK only.

DISCUSSION

All tumors which were produced by repeated beta-irradiation in PGK-mosaic cell mice exhibited a single PGK phenotype. The composition of the 2 types of cells in the host mouse was confirmed with blood (Fig. 2) and tissue (data not shown). Therefore, repeated irradiation created tumors with a single PGK phenotype on the background of 2 types of somatic cells homogeneously distributed in the host mouse. This result indicates the monoclonal origin of these tumors, after probability considerations.[15] It should be noted that tumors with A-type PGK were predominant (Table I). This tendency has also been observed in fibrosarcomas produced by methylcholanthrene.[10, 15] The A/B ratio of cell populations in the tissue cannot explain the imbalance of the A/B ratio of the tumors, as judged from the A/B PGK phenotypes of the blood and tissue of the host mice. It appears that A-type PGK cells are more susceptible to carcinogenic action of beta rays, or A-type PGK tumor cells grow more rapidly than B-type PGK tumor cells. However, mosaic mice (AB-type) are as sensitive to methylcholanthrene as wild-type mice (B-type) in tumor induction.[17] Moreover, the growth advantage of A-type tumor was not seen after transplantation of a mixture of A-type and B-type tumor cells.[15] This problem of the imbalance in A/B tumors remains unsolved.

Nevertheless, it is interesting to see the indication of monoclonal origin of tumors produced by repeated beta-irradiation. Interaction between the host and tumor clones must be involved in allowing only one clone to develop into a tumor. The monoclonal nature of tumors seems to be a universal phenomenon regardless of whether treatment with a carcinogen is single or repeated, in normal hosts.

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