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Radiation Responses of Subrenally Transplanted Syngeneic and Allogeneic Mouse Fibrosarcomas

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The radiation responses of two tumor types transplantable into C3H/He mice and differing in radiosensitivity, i.e., 3-methylcholanthrene (MCA)-induced fibrosarcoma and NFSa fibrosarcoma, were investigated using the subrenal capsule assay. Subrenal tumor growth was histologically confirmed. Volume regression and regrowth of subrenal capsule-implanted and subcutaneously implanted tumors after X-irradiation were compared. No difference was found in tumor growth speed (volume doubling time: 1.2–1.3 days) between the subrenal transplants into C3H/He mice, syngeneic to the tumor grafts, and those into immuno-incompetent C57BL mice, allogeneic to the tumor grafts. The tumor growth rates for subrenal capsule transplants were 1.7- to 1.8-fold higher than those for subcutaneous transplants of the same tumor type. A marked difference was found in the radiation responses of the two tumor types by the subrenal capsule assay, i.e., tumor regrowth time following 20 Gy irradiation was more than 8 days for MCA-induced fibrosarcoma and 4.5–5.4 days for radioresistant NFSa fibrosarcoma. These values correlate well with the estimated radiation sensitivities of these tumors.

Key words: Subrenal capsule assay — Radiosensitivity — Mouse fibrosarcoma

The radiosensitivity of a primary tumor is usually judged from its organ of origin and its histopathology.[1] However, even in relatively radiosensitive tumors such as early-stage uterine cervical cancer and laryngeal cancer, radioresistant cases are often found, making a complete cure difficult to achieve.[2-3] Predictive assay of the radiocurability of tumors has been devised for clinical application. It is difficult to test the curability of human tumors in experimental systems, and the development of a rapid prediction method, at least, is therefore needed so that radiation can be applied to tumors at an early stage.[4-6]

The subrenal capsule assay developed by Bogden *et al.*[7] has been applied to a rapid test of the response of human tumors to chemotherapeutic drugs. A good correlation between the results of subrenal capsule assay and the tumor response was claimed by Griffin *et al.*[8] However, a limitation of the applicability of this method has been pointed out, i.e., host lymphocyte infiltration into the subrenal tumor transplants during growth is inevitable, so that the assay period is limited.[9-11] More seriously, Cunningham *et al.*[12] criticized the applicability of the subrenal capsule assay, claiming that apparent growth of subrenal transplants of human tumors is an artefact related to infiltration by host lymphocytes. It is now necessary to reassess the applicability of this method.

In the present study, we examined the tumor growth in the subrenal capsule, using syngeneic and allogeneic mouse fibrosarcoma, and confirmed that they did grow in the capsule. Further, two types of mouse fibrosarcomas differing in radiosensitivity were examined. Previously, this test has been successful in judging the efficacy of hyperthermia combined with chemotherapeutic drugs in the treatment of transplantable mouse tumors.[13] The present results again showed the usefulness of this method for the rapid judgement of tumor X-irradiation response.

MATERIALS AND METHODS

Tumor Two types of transplantable C3H/He mouse fibrosarcomas having different radiosensitivities were used, i.e., a fibrosarcoma induced subcutaneously by 3-methylcholanthrene (MCA) with a TCD50/120 of less than 44 Gy (Tanooka and Ootsuyama, unpublished data, 1986; the TCD 50/120 of an MCA-induced fibrosarcoma in an ICR mouse used in a previous study was 44 Gy[14])

and a spontaneously induced NFSa fibrosarcoma with a TCD50/120 of 83 Gy.[15] The latter was chosen as being representative of a radioresistant tumor.

Animals Female C3H/He mice (Charles River Japan, Kanagawa-ken) and C57BL mice (maintained at the National Institute of Genetics, Mishima), aged 7–8 weeks, were used. The mice, five or six to a cage, were kept under specific pathogen-free conditions, and were provided with a laboratory diet (CE-2, Clea Japan, Tokyo) and with sterilized water *ad libitum*.

Subrenal Capsule Implantation The method has been described previously by Bogden *et al.*[7] and in our previous paper.[13] Briefly, the C57BL mice were pretreated with a subcutaneous injection of cyclophosphamide (180 mg/kg body weight) 24 hr prior to tumor implantation. The C3H/He mice, syngeneic to the tumors, were left untreated. Both tumor types were maintained by subcutaneous transplantations in the C3H/He mice and used for subrenal transplantation. Tumors were resected, cut aseptically with scissors into cubes of approximately 1.5 mm, and transplanted with a 18 gauge venula needle under the renal capsule of the mice. Thirty-five mice were used in each group, and implantation day was designated Day 0.

Subcutaneous Transplantation Tumors were minced with scissors under sterile conditions, suspended in phosphate-buffered saline solution, treated with 0.0125% DNase (Sigma Chemical Company, St Louis) and 0.02% pancreatin and 0.2% trypsin (Wako Pure Chemical Industries, Osaka) for 25 min at 37°, and filtered through a no. 200 mesh. Tumor cells in the filtrates were counted with an eosinophil counter (Kayagaki Irika Kogyo Co., Tokyo), and a suspension containing 5×10^5 tumor cells was subcutaneously implanted into the right fat pad of C3H/He mice.

X-Irradiation On Day 1, the mice with subrenal transplants were lightly anesthetized with Nembutal (Abbott Laboratories, North Chicago) and the mouse trunk with the left kidney was locally irradiated with 10 or 20 Gy of 250 kVp X-rays generated by a Maxitron 300 (General Electric Co., Milwaukee) filtered through a 2 mm Cu filter with collimation of 25×20 mm^2 and at a dose rate of 3.3 Gy/min. The dose rate was measured in an ionization chamber (Ionex, type 2500/3; Nuclear Enterprises, Edinburgh) and a Fricke dosimeter. Subcutaneous tumors were irradiated in the same manner, when the tumor volume reached the size of 300 mm^3 (see below).

Measurement of Tumor Size In the subrenal transplantation groups, five mice were killed by cervical dislocation every day following irradiation, so that a total of 35 mice were used to draw one growth curve, for an 8-day period. The left kidney was removed from each mouse. The sizes of the tumor grafts were measured daily from Day 2 to Day 8 using an ocular micrometer; in the subcutaneous transplantation groups, the tumor sizes were measured with a slide caliper. Tumor volumes were calculated as $ab^2/2$ for subrenal transplants, where a and b are the lengths of the long and short axes, and $abc\ \pi/6$ for subcutaneous transplants, where a, b and c are the lengths of the tumor's three-dimensional axes. The relative tumor volumes were given by the ratio of volume of treated tumor on specific days after irradiation to volume of original tumor before irradiation, and the relative volume was plotted against time after irradiation (see Fig. 1). Volume doubling times were estimated from each tumor growth curve, assuming exponential growth. Growth delay times for irradiated tumors were determined from the displacement of the growth curve for the irradiated tumor from that of the unirradiated tumor, assuming parallelism between the two curves. When this parallelism did not hold (Fig. 1a-C and Fig. 1b-C), growth delay times were measured on Day. 8.

Histological Examination Tumor specimens were fixed with 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. The histological sections were microscopically examined in order to judge the effectiveness of irradiation and to confirm the histological type for grown tumors.

Results

The subrenal and subcutaneous transplants of two types of fibrosarcoma were X-irradiated, and the changes in tumor volume after irradiation were followed in three different hosts (Fig. 1). The volume changes of unirradiated transplants were also followed as controls.

The volume doubling time for the unirradiated subrenal transplants was much shorter than for the unirradiated subcutaneous transplants (1.2 days vs. 2.0 days for MCA-induced fibrosarcoma, and 1.3 days vs. 2.4 days for NFSa fibrosarcoma). No difference in volume doubling time for either type of unirradiated tumor was found between the two different subrenal transplantation hosts, i.e., syngeneic C3H/He mice and cyclophosphamide-treated, immuno-incompetent, allogeneic C57BL mice (1.2 days vs. 1.2 days for MCA-induced fibrosarcoma, and 1.3 days vs. 1.3 days for NFSa fibrosarcoma).

The X-irradiation of the tumors residing in the mice suppressed tumor growth. The growth delay kinetics for both types of fibro-

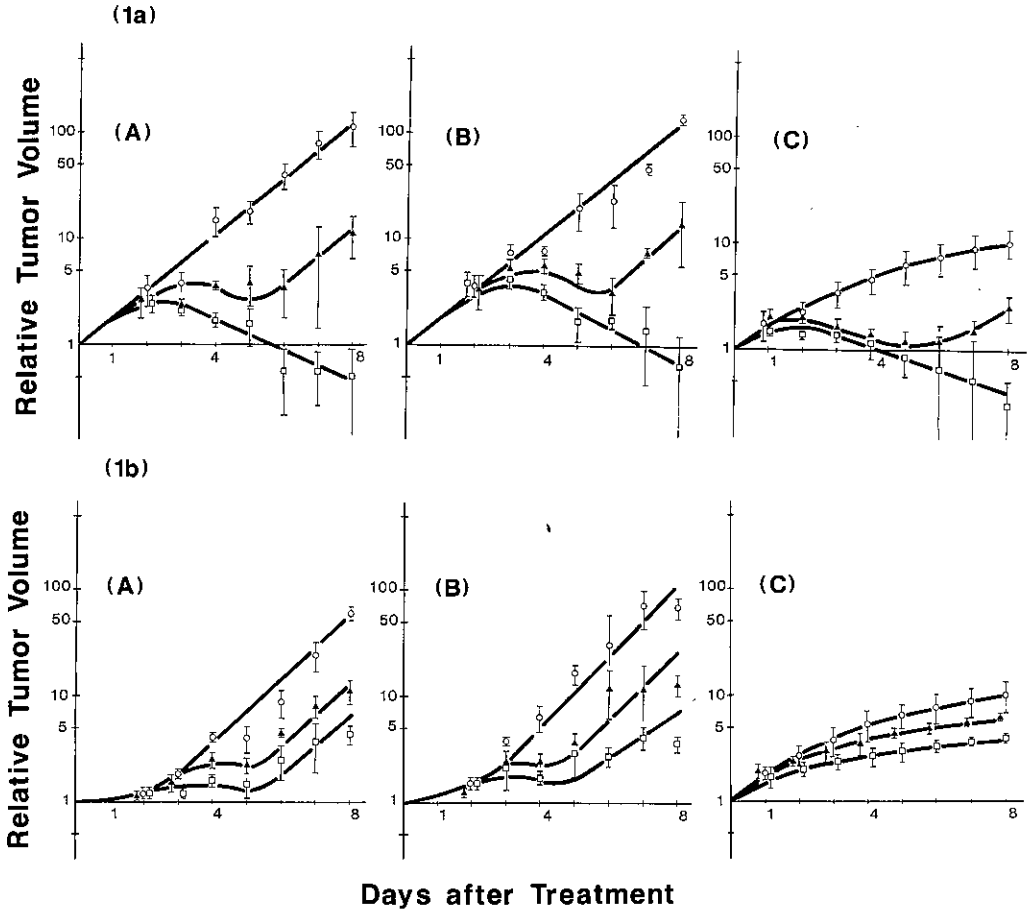

Fig. 1. Growth kinetics following X-irradiation of MCA-induced C3H/He mouse fibrosarcoma transplants (Fig. 1a) and C3H/He mouse NFSa fibrosarcoma (Fig. 1b) in various hosts. (A) Subrenal transplant in syngeneic C3H/He mouse. (B) Subrenal transplant in allogeneic C57BL mouse pretreated with cyclophosphamide. (C) Subcutaneous transplant in syngeneic C3H/He mouse. Radiation doses to tumor: control (○), 10 Gy (▲), 20 Gy (□). Bars indicate SD.

Table I. Growth Delay Times of Two Types of Mouse Transplantable Tumors after X-Irradiation

Fibrosarcoma tested	Dose of X-rays (Gy)	Tumor growth delay times (days)		
		Subrenal transplant into		Subcutaneous transplants into syngeneic C3H/He mice
		Syngeneic C3H/He mice	Allogeneic C57BL mice	
MCA-induced	10	4.0±0.7	3.7±0.9	5.8±0.7
	20	>8	>8	>8
NFSa	10	2.4±0.6	2.9±0.4	3.5±0.1
	20	4.5±0.5	5.4±0.3	5.1±0.3

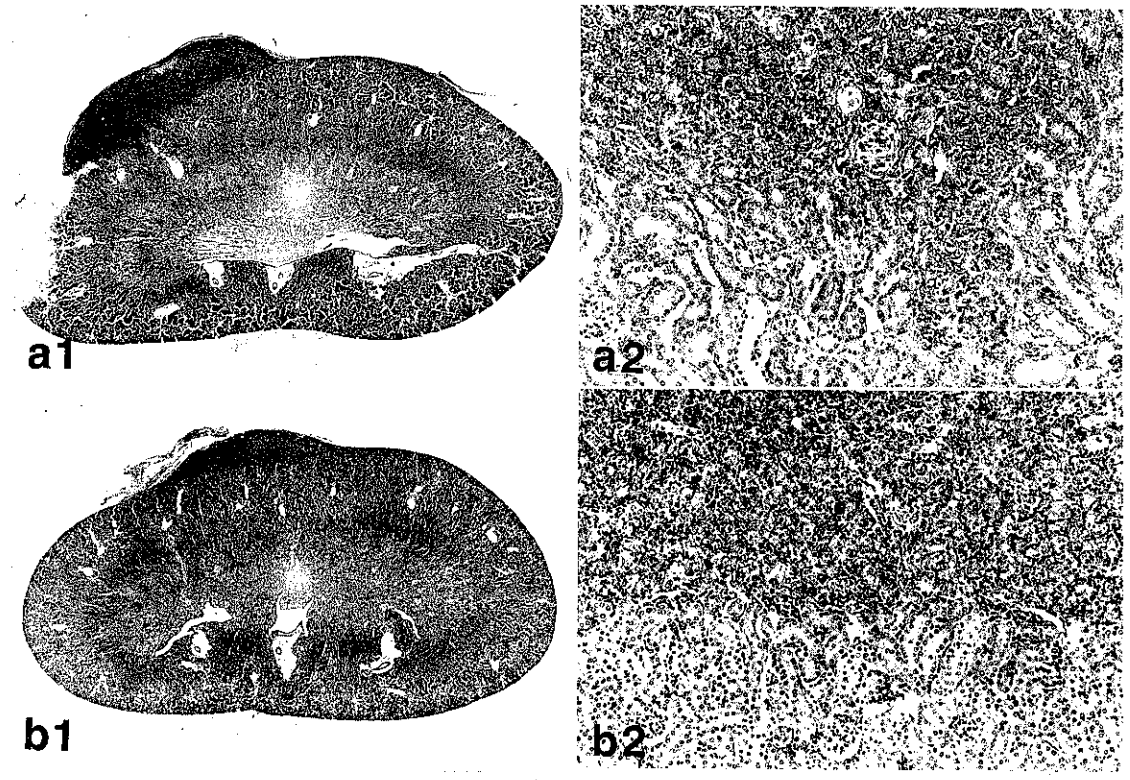

Fig. 2. Photomicrographs of histologic sections of subrenally transplanted MCA-induced C3H/He mouse fibrosarcoma in cyclophosphamide-pretreated allogeneic C57BL mice 8 days after irradiation. (a) Control: (1) Section of kidney with tumor transplant. (2) Magnified at the border of tumor and normal tissue. Note mitosis and direct invasion of tumor cells into the cortex. (b) Regrown tumor after 10 Gy irradiation: (1) Kidney section. Note the effect of radiation on tumor size. (2) Magnified at the border. Note mitosis of tumor cells. No infiltration of lymphocytes were seen in control or irradiated tumor. (c) Complete supression of tumor growth by 20 Gy irradiation.

sarcoma were essentially the same in the syngeneic and allogeneic subrenal transplants. Furthermore, the subrenal transplants showed a more rapid regrowth after irradiation than the subcutaneous transplants. There was a marked difference in tumor response to radiation between the two types of tumors in three different hosts, i.e., MCA-induced fibrosarcoma did not regrow after 20 Gy irradiation (Fig. 1a), while the more radioresistant NFSa fibrosarcoma did regrow after irradiation with the same dose (Fig. 1b). Tumor growth delay times for the irradiated tumors were estimated from the regrowth curves in Fig. 1 and are shown in Table I. It can be clearly seen that radioresistant NFSa fibrosarcoma had shorter regrowth times than those of MCA-induced fibrosarcoma.

Microscopically, histologic sections showed that the subrenal transplant of mouse fibrosarcoma was taken up and did grow in the immuno-incompetent allogeneic host (Fig. 2 a). No obvious evidence for infiltration of host lymphocytes, fibrosis, or granulomatous change at the periphery of the tumor mass was seen. Irradiation of this transplant with 20 Gy caused almost complete suppression of tumor growth during 8 days (Fig. 2c). With 10 Gy irradiation, the allogeneic subrenal transplant exhibited temporary suppression and then regrowth. The histologic section of this regrown tumor again showed no infiltration of host lymphocytes (Fig. 2b). Therefore, the size of the subrenal tumor transplant is considered to represent the true tumor volume.

Death of mice did not occur during the post-irradiation, 8-day observation period.

Discussion

Fertil and Malaise summarized data on the radiosensitivities of various established human tumor cell lines and showed a good correlation between tumor control dose and the mean survival fraction at 2 Gy.[16] In order to obtain a more realistic picture of the effects of radiation therapy, however, it would be desirable to examine the response to radiation of the primary tumor itself. The subrenal capsule assay is a promising method of approach to this problem.

In the present study, we used the subrenal capsule assay to examine the radiosensitivity of two mouse fibrosarcomas, as a preliminary experiment to test the applicability of this method to human tumors that are to be treated with radiation. Growth of mouse tumor transplants under the renal capsule was histologically confirmed (Fig. 2 a). This growth was also confirmed for the tumor transplants regrown after irradiation with 10 Gy (Fig. 2b). Therefore, the possibility of an artefact, i.e., host lymphocyte infiltration causing apparent tumor growth, was excluded. In the mouse subrenal capsule, tumor implants grew rapidly owing to an ample blood supply and adequate temperature, while the tumor growth rate for subcutaneous transplants was much slower. Subrenal transplants, therefore, are superior to subcutaneous transplants from the viewpoint of short assay time. Further, no difference was seen in subrenal transplant growth rate between syngeneic and allogeneic, immuno-incompetent mice. The immuno-incompetent mice can, therefore, be used as allogeneic transplant recipients as well as syngeneic recipients. Upon X-irradiation, subrenal transplants in both syngeneic and allogeneic, immuno-incompetent mice responded in the same manner, this response being more sensitive than that of the subcutaneous transplants, as can be seen in Fig. 1 and Table I. In each assay, both types of fibrosarcoma exhibited a marked difference in response to X-irradiation (Fig. 1). It is clear that NFSa fibrosarcoma, as expected from its higher TCD50/120 value, is more radioresistant than MCA-induced fibrosarcoma; the subrenal assay showed this radiosensitivity difference more clearly than the subcutaneous transplants.

One disadvantage of this method is its inability to measure tumor sizes without sacrificing the animals, making it impossible to follow the tumor growth of the same transplant. Moreover, since mice were made immuno-incompetent for a relatively short period for assaying the allogeneic tumors, it was also impossible to follow tumor curability for a sufficiently long period. To obtain further information on the correlation between tumor radiation response as measured by the subrenal capsule assay, tumor radiosensitivity, and tumor curability, other kinds of experimental tumors with known radiosensitivity are now being studied.

It is hoped that the subrenal capsule test using immuno-incompetent mice can be applied to examine the response of human tumors to radiation.

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