A Study on the Radiation Tumor Control of Microscopic Tumors of the C3Hf/Sed Mouse Spontaneuos Fibrosarcoma

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To answer the question whether last clonogenic cell should be eradicated for the tumor to be controlled, radiation tumor control study was performed using microscopic tumors of variable sizes ranging from 10^1 to 10^5 tumor cells. TCD_{50}'s estimated from experimental data were 14.8, 27. 1, 42.4, 49.9 and 65.5 Gy for 10^1, 10^2, 10^3, 10^4 and 10^5 tumor cells, respectively. Theoretical calculations, assuming that all the clonogenic cells should be inactivated, were 15.65, 28.50, 40. 97, 53.41 and 65.85 Gy.

From this well matched data, it can be concluded that all the clonogenic cells should be eradicated for tumor control, at least in this tumor model.

Key Words: Clonogenic cell, Microscopic, Fibrosarcoma, Mouse

INTRODUCTION

It has long been accepted that tumor cells of small number remaining after anticancer treatment, i. e., surgery, radiotherapy, or chemotherapy, can be controlled by immune function of host[1]. On this basis, when the number of tumor cells is reduced below a certain level, it is believed that tumor would not recur. But there is no sound basis of this theory. All the tumor cells may have to be inactivated for the tumor to be controlled[2].

This study was designed to estimate the doses of radiation needed to control tumors consisting of known numbers of tumor cells and compare them with theoretical estimation.

MATERIALS AND METHODS

Experimental animal and tumor system: Ten to 14 week old C3Hf/Sed mice were used for these experiments. Approximately the same number of male and female mice were used in each assay. These mice were produced and maintained in our defined flora colony[3].

Fifth generation isotransplants of the spontaneous fibrosarcoma, FSa II (a poorly differentiated fibrosarcoma) were used throughout these experiments. FSa II is very weakly immunogenic[3].

Suspensions of single tumor cell were prepared by: 1) mechanical mincing of the tumor tissue into fine pieces, 2) trypsin digestion, 3) addition of calf serum to inactivate trypsin after digestion, 4) viable tumor cell counts based on trypan blue exclusion method, and 5) appropriate dilution for adjustment of cell count. Variable number of trypan blue excluding tumor cells (1×10^0 to 1×10^5) were transplanted into leg muscle in an inoculum volume of 5 μl. Lethally irradiated tumor cells (120 Gy *in vitro*) were mixed with viable tumor cells; the inocula contained a total of 2×10^5 cells.

Radiation: Radiation was performed using a specially designed Cesium-137 irradiator, which provides parallel opposed 3 cm diameter fields[4]. The dose rate was about 7.6 Gy per minute during these experiments. To control the tumor temperature, the mice were positioned on a warm plate, then this was placed into a warmed metal chamber which was immersed in a 37℃ water bath for 15 minutes before irradiation. The irradiator (a massive lead-uranium device) was maintained at 37℃. Radiation was given as a single fraction under sodium pentobarbital anesthesia (50 mg/kg) under hypoxic condition. For hypoxic condition, a clamp was applied at the level of upper thigh for 2 minutes before and during irradiation.

End points: Permanent tumor control is defined as no evidence of tumor at 120 days following treatment.

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Table 1. Tumor Take Probability Along the Number of Tumor Cells Tansplanted

Date of Experiment	Number of Tumor Cells Transplanted								TD_{50} (95% CI)
	1	2	3	5	7	10	15	100	
11/30/84	6/20			18/20		19/20		20/20	1.6 (1.0–2.5)
5/31/85	3/10	7/10	9/10	8/10		10/10			1.4 (0.9–2.2)
6/13/85	4/10	7/10	5/10	9/10	9/10	5/ 5	10/10		1.7 (1.0–2.7)
7/02/85	3/10	3/10	4/10	4/10	9/10	9/10	8/10		3.3 (2.1–5.2)
7/15/85	4/10	7/10	6/10	9/10	10/10	20/20	10/10		1.5 (0.9–2.2)
7/19/85	4/10	3/10	4/10	6/10	6/10	9/10	10/10		2.9 (1.8–4.7)
10/08/85	2/10	6/10	7/10	5/10	9/10	8/10	9/10		2.2 (1.3–3.7)
10/24/85	3/10	9/10	10/10	10/10	10/10	10/10	10/10		1.2 (0.9–1.5)
11/01/85	4/10	7/10	5/10	10/10	10/10	19/19	10/10		1.5 (1.0–2.2)
11/10/85	1/ 5	2/ 5	4/ 5	5/ 5	5/ 5	5/ 5	5/ 5		1.9 (1.3–2.8)
12/16/85	1/ 5	1/ 5	3/ 5	5/ 5	5/ 5	5/ 5	5/ 5		2.3 (1.6–3.4)
1/10/86	2/ 5	4/ 5	4/ 5	4/ 5	5/ 5	5/ 5	5/ 5		1.2 (0.6–2.6)
Pooled	37/115	56/95	61/95	93/115	78/85	124/129	82/85		1.7 (1.5–2.0)

RESULTS

As a basis for quantitative study of radiation response of microscopic tumors of variable sizes, the transplantability of FSa II was examined using two end points: TD_{50} and time for the tumor to reach 500 mm^3 (tumor growth time). As shown in Table 1, the transplantability of the FSa II cell suspensions was reproducible within a narrow range. Tumor growth time also exhibited only a small variation. There was a linear relationship between the log number of tumor cells transplanted and tumor growth time over the range of inoculum size from 10^5 to 10^2 cells (Fig. 1).

To determine the adequate time interval between tumor cell transplantation and irradiation, radiation tumor control study was performed at various interval from 1 to 16 days after transplantation of 10^2 tumor cells (Fig. 2). The estimated TCD_{50} stayed almost constant for first 5 days and then increased relatively rapidly until the tumors reached 4 mm in sizes. Almost same results were obtained after transplantation of 10^3 tumor cells (data not shown). From these results, it could be concluded that the number of tumor cells stays almost constant for first 5 days after transplantation.

Radation tumor control study was performed 3 days after transplantation. The estimated TCD^{50} was 14.8, 27.1, 42.4, 49.9, and 65.5 Gy for

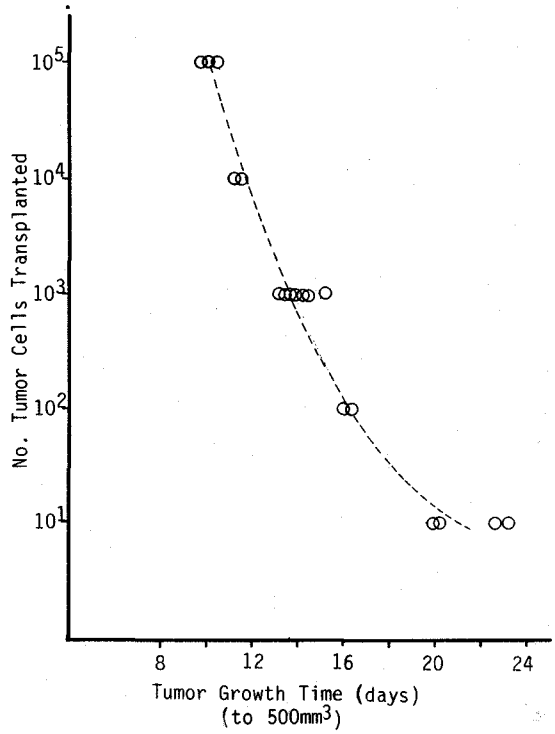

Fig. 1. Tumor growth time to 500 mm^3 after transplantation of 10^1 to 10^5 tumor cells. Estimation of doubling time from the data over the range of 10^2 to 10^5 cells results in 14.8 hours. Each experimental data point indicates mean growth time from one experiment (10 mice).

Table 2. TCD_{50}'s of Microscopic Tumors 3 Days after Transplantation of 10^1 to 10^5 FSa II Tumor Cells

No. of Tumor Cells Transplanted	TCD_{50} (120 days ; Gy)	TCD_{50} (Gy) Theoretical
10^1	14.8 (12.0–18.2)*	15.65
10^2	27.1 (24.7–29.8)	28.50
10^3	42.4 (39.7–45.4)	40.97
10^4	49.9 (47.4–52.6)	53.41
10^5	65.5 (63.8–67.3)	65.85

* 95% confidence interval

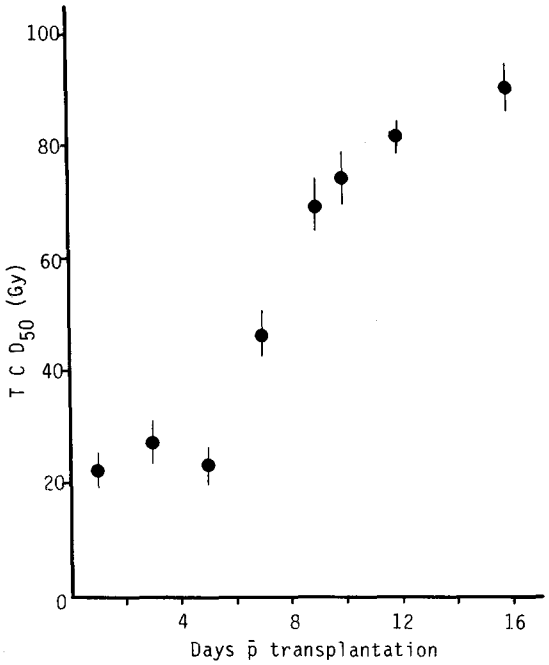

Fig. 2. TCD_{50} under hypoxia after various interval after transplantation of 10^2 tumor cells. TCD_{50} stays almost constant for first 5 days and increases rapidly thereafter, indicating that the tumor cells start to proliferate about 5 days after transplantation. Vertical bars indicate 95% confidence interval (each data from 30 mice).

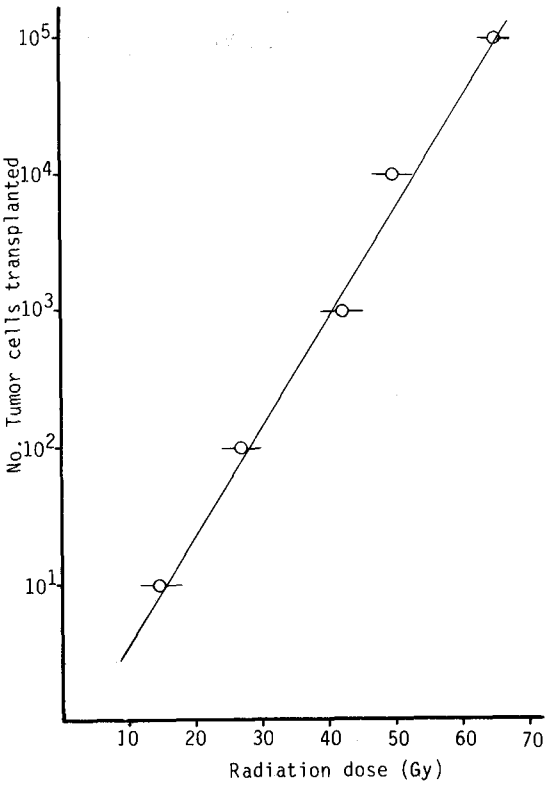

Fig. 3. Comparision of experimental data (open circle with horizontal bars indicating 95% confidence interval) and theoretical calculation. The experimental data matches well with theoretical estimation.

10^1. 10^2, 10^3, 10^4, and 10^5 tumor cells, respectively (Table 2).

DISCUSSION

Assuming that all the clonogenic tumor cells should be inactivated for the tumor to be controlled, the radiation dose needed to control a tumor in 50%, i. e., TCD_{50}, can be estimated when number of clonogenic cells, Do (mean lethal dose) and n (extrapolation number) are known. The clonogenic fraction of the FSa II in cell suspension is 0.338, Do is 5.4 Gy and n is 4.05 as previously reported[5,6].

The relationship among the average number of tumor cells (a), clonogenic fraction (c) and tumor control probability (TCP) can be expressed as follows,

$\ln TCP = -(c \times a) + (c \times a)\ (1 - e^{-D/Do})^n$ see Ref 5

This can be changed as follows,

$(c \times a)\ (1 - e^{-D/Do})^n = (c \times a) + \ln TCP$

$e^{-D/Do} = 1 - \{(c \times a + \ln TCP)/c \times a\}^{1/n}$

$D = -Do \times \ln[1 - \{(c \times a + \ln TCP)/c \times a\}^{1/n}]$

So, $TCD_{50} = -5.4 \times \ln[1 - \{(0.338 \times a - 0.693)/0.338 \times a\}^{1/4.05}]$

From this equation TCD_{50}'s (Gy) are 15.65, 28.50, 40.97, 53.41 and 65.85, for 10^1, 10^2, 10^3, 10^4 and 10^5 cell tumors, respectively. This results are compared with experimental data in Table 2 (Fig. 3).

As the experimental data are almost same as the theoretically estimated TCD_{50}'s, it can be concluded that all the tumor cells had to be eradicated for the tumor to be controlled.

CONCLUSION

To estimate the radiation doses needed to control a tumor of known clonogenic numbers and compare with theoretical calculation, radiation tumor control study was performed using spontaneous fibrosarcoma in mice.

The TCD_{50}'s from experiments matched well with calculated data and so it seems that all the clonogenic cells should be inactivated for the tumor to be controlled at least in this tumor model.

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═ 국문초록 ═

C3Hf/Sed 마우스에 자연발생한 섬유육종의 현미경적 종양을 이용한 방사선조사에 의한 종양치유에 관한 연구

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악성종양 치유에 있어서 증식가능한 종양세포의 완전한 세포사(細胞死) 필요성 여부를 확인하기 위하여 C3Hf 마우스의 섬유육종을 이용하여 실험을 시행하였다. 10^1 내지 10^5개의 종양세포를 근육에 이식한 후 3일 경과후에 방사선조사를 시행하고 120일간 관찰하여 종양치유에 필요한 방사선량을 측정하였다. 평균 50%에서 종양이 치유되는데 필요한 방사선량(TCD_{50})은 10^1, 10^2, 10^3, 10^4 및 10^5개의 종양세포에 대하여 각각 14.8, 27.1, 42.4, 49.9 및 65.5 Gy였다. 종양치유에 증식가능 종양세포의 완전한 세포사가 필요한 것으로 가정하여 이론적으로 계산한 수치는 각각 15.65, 28.50, 40.97, 53.41 및 65.85 Gy로서 이론적 계산수치는 위의 실험결과와 거의 일치하였다. 따라서 최소한 본 실험에 사용한 종양모델에서는 종양치유를 위하여는 모든 증식가능한 종양세포의 세포사가 필요함을 확인할 수 있었다.