研究速報

Analysis of Regrowth Pattern of Irradiated Murine Tumors

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The method to determine tumor regrowth curves after irradiation or chemical agent(s) has been employed in the experimental evaluation of such treatments to tumors[6)7)]. The regrowth curve is preferentially obtained by averaging individual tumor volumes and by plotting them on a semi-logarithmic graph as a function of time of post-treatment days. The experiment, being made easily without deep experience, usually results in with wide variance. In this communication an analytical method of tumor regrowth will be proposed by which precise end-points are possibly determined with smaller variances. The property of tumor growth and regrowth might be elucidated by this method more accurately than by the method of determination of an averaged growth curve.

Materials and Methods

Regrowth studies used for analysis: Regrowth curves of irradiated animal tumors obtained by Tsunemoto et al. were used which have been reported elsewhere[7)], while some pooled data were also employed. Experimental materials and methods were already described[7)] and were briefly as following.

First generation isotransplants of mammary carcinoma arisen spontaneously in C3H/He female mice were used. Transplantation was made into subcutaneous flank tissue of C3H male mice by the trocker technique. Tumors were irradiated when they reached 8–10 mm in average diameter with 200 kvp x-rays or 2 MeV fast neutrons. Three diameters of each tumor were measured by a caliper daily or every other days until tumors grew or regrew to ~15 mm in average diameter. The tumor volume was calculated as an elipsoid, i.e., $^4/_3\,\pi abc$, where a, b and c were each radius respectively. Tumor growth time required for a tumor to regrow to the initial volume (tumor volume at the time when treatment was given) from the treatment day was measured graphically in each individual tumor.

Tumor growth studies: Additional studies were made to determine a growth pattern of non-treated tumors. Single cell suspension was made from second generation isotransplants of a spontaneous mouse mammary carcinoma and 10 μl of the suspension were transplanted into mouse right thigh subcutaneously[8)]. Viable tumor cells were counted in a hemocytometer by the trypan-blue staining method before transplantation. Tumor volume was measured by the same method as mentioned above.

Results

Growth of non-treated tumors: In a large series of experiments for other purpose, the distribution of time for tumor to reach 250 mm^3 after transplantation was examined. Approximately 200–400 mice were served in an experiment. Number of tumors which reached 250 mm^3 was presented as a function of time after transplantation in the left side of Figure 1. The tumor growth time to 250 mm^3 was varied from experiment to experiment, while the pattern of the distribution was consisted with so-called "log-

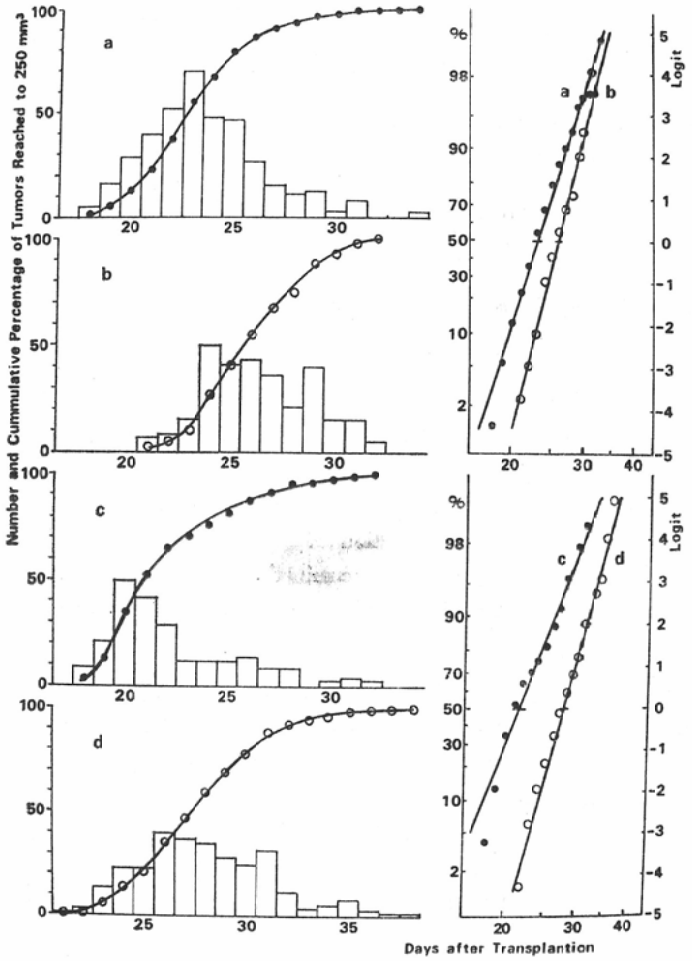

Fig. 1 Distribution of tumor growth time to 250 mm³. Left figures= Number of tumors reached 250 mm³ (8 mm in average diameter) is shown as the histogram after transplantation of 10^5–10^6 tumor cells in 4 duplicated experiments. Solid lines indicate cummulative percentage of tumors reached 250mm³. Right figures=Linear relation is obtained between cummulative percentage of tumor reached 250 mm³ and post-transplantation days on a log-logit graph. 50% tumor growth time to 250mm³ were; a=23.0 (22.9–23.1), b=25.8(25.7–25.9), c=21.4 (21.3–21.6) and d=28.4 (28.3–28.5) days respectively.

normal distribution" in all the studies presented. The log-normal distribution might result in a linear regression line if the cummulative percentages of number of tumors reached 250 mm³ were plotted as a function of logarithm of the time on a *probit* or *logit* graph. Results were shown in the right side of Figure 1, where the linear relation between *logit* of the cummulative percentage and *logarithm* of the time was clearly demonstrated. The slope of the regression line might mean the dispersion in the number of viable tumor cells transplanted. Therefore, it might be reasonable to calculate 50% tumor growth time to reach a fixed volume. It was analysed by logit analysis method and presented in the figure.

The dependence of 50% tumor growth time to 250 mm³ on the number of transplanted tumor cells was examined and the results are tabulated in Table 1, where the averaged growth time is also presented. Note the difference in the variance in two methods. The averaged growth time usually posseses a larger variance than the 50% tumor growth time. In addition, the smaller the number of transplanted tumor cells was, the larger the variance was, in the averaged growth time.

Regrowth of irradiated tumors: The regrowth time distribution of irradiated tumors was analysed by

Table 1. 50% tumor growth time or the time required for 50% of transplants to grow to 250 mm³ and averaged growth time to 250 mm³ after transplantation of different number of viable tumor cells.

Number of tumor cells transplanted	50% tumor growth time (95% confidence limit) = days =	Averaged growth time ±Standard deviation = days =
2.7×10^6	29.6 (29.0–30.1)	30.7 ± 3.3
9.0×10^5	32.5 (31.5–33.5)	33.3 ± 6.4
3.0×10^5	32.8 (31.6–34.0)	33.9 ± 7.2
1.0×10^5	46.7 (44.2–49.4)	—*
3.3×10^4	53.5 (49.4–57.9)	—*

*Averaged growth time was not available, since tumors did not develope in 3–4 animals until the 70th post-transplantation day.

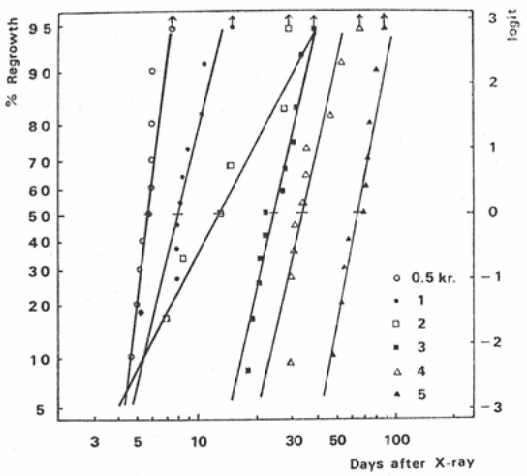

Fig. 2. Regrowth time distribution of tumors irradiated with 200 kVp x-rays under hypoxic condition. Regrowth time or time required for a tumor to regrow to the initial volume (tumor volume at the time when irradiation was given) was analysed by a logit method.

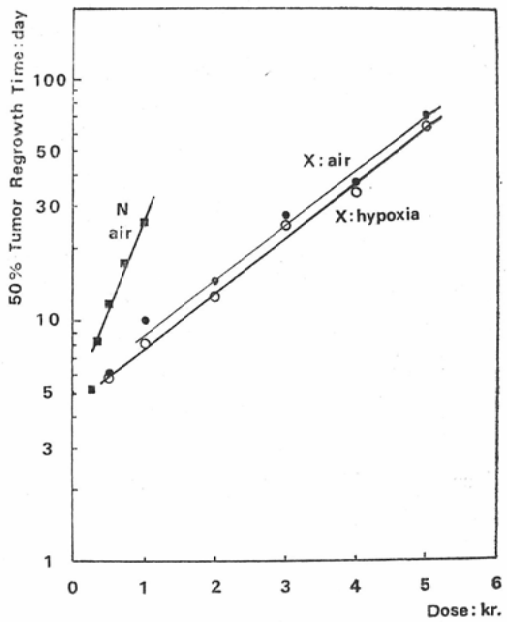

Fig. 3 50% tumor regrowth time (TRT_{50}) as a function of radiation dose. X or N indicates that tumors were irradiated with x-rays or neutrons respectively.

the same method. It is plausible to assume that a fixed radiation dose reduces the cell survival to a fixed fraction with small variances. As example, regrowth time distribution of tumors irradiated under hypoxic condition with x-rays were illustrated in Figure 2. The cummulative percentage of number of regrowth tumors falls on a straight line on a *logit* graph as a function of post-treatment days. The slopes of these lines were similar to each other except for that of tumors received 2000 rads. This exception might be due to the shortness of tumors employed. On the basis of the regrowth time frequency, 50% tumor regrowth time (TRT_{50}) or the time for 50% of the irradiated tumors to reach the initial volume

was calculated by logit analysis method. TRT_{50}, if plotted on a semi-logarithmic graph, was linearlly related with radiation dose within a dose range tested (Figure 3). In the same figure, TRT_{50} of tumors, which were irradiated with x-rays or fast neutrons when animals were breathing normal air, are also presented as a function of radiation dose. That the TRT_{50} of tumors x-irradiated under air-breathing is shorter than that of tumors irradiated under hypoxic condition, could be interpreated by a well-known fact that the tumor is composed of both populations of aerobic and hypoxic tumor cells.

Discussion

The present evidence that the distribution of tumor growth time to 250 mm^3 was log-normal might be due to the facts that tumor cells are multi-component in growth rate and that number of tumor cells transplanted is not technically constant. It is well established that the cell generation time in a mammalian cell line distributes log-normally[5] and that the number of tumor cells transplanted might spread out in the Gaussian distribution. It is pointed out by Koch that, when several variances are piled up in an experimental result, the variance in the result could be expressed as the force of each variance and consequently, experimental points distribute lognormally[2]. In the presenst study, the variance in tumor growth time would be the force of variance in the generation time and that in number of transplanted cells, and, consequently, might result in the log-normal distribution.

Similar variances might take place in the regrowth time of irradiated tumors. If tumors with a fixed volume, i.e., tumors containing a fixed number of tumor cells, were irradiated with a constant dose, number of surviving tumor cells might distribute as the Gaussian distribution. The generation time and division probability[9] of irradiated tumor cells might be varied from tumor to tumor. In addition, tumor volumes at irradiation fluctuate as a technical error. Accordingly, as pointed out by Koch[2] the force of these variances would result in the log-normal distribution of tumor regrowth time.

That TRT_{50} of tumors irradiated under hypoxia was exponentially related with radiation dose implies that the regrowth rate of irradiated tumor cells exponentially decreased with increased dose. This decrease would not only be resulted from increase in the cell generation time, but also from the reduced growth fraction[1] and from tumor bed effect[8].

The exponential relation between TRT_{50} and radiation dose could be disturbed by cellular repair of sublethal damage at doses of lower than 500 rads. At a dose as high as TCD_{50} (radiation dose to yield 50% local control in irradiated tumors), TRT_{50} would be longer than the expected on the TRT_{50} *vs.* dose curve because of very reduced division probability of surviving tumor cells[3].

Summary

A determination method of tumor regrowth time in an experimental animal tumor system was discussed. The time required for a transplant to grow up to a fixed volume of tumor spread out as log-normal distribution. Regrowth time of irradiated tumors, i.e., time required for a tumor to regrow to the initial volume or the volume at treatment time after irradiation, was possibly analysed as the same distribution. Therefore, logit analysis method was fitted and TRT_{50} (50% tumor regrowth time) was calculated. TRT_{50} was exponentially related with radiation dose within a limitted dose range.

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