EFFECT OF THE X-IRRADIATED TUMOR BED ON TUMOR CELLS

Part II.　Effect of Tumor Bed on Cellular Radiation Sensitivity

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移植前照射の腫瘍発育環境におよぼす影響

第2報　腫瘍細胞の放射線感受性におよぼす影響

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　臨床上しばしば認められる如く，以前に放射線治療を受けた部位に再発した悪性腫瘍は，初回治療時よりも放射線に対し抵抗を示す．これを説明するのに，腫瘍細胞自体の変化，即ち，細胞が突然変異をおこすこと，放射線感受性の低い細胞のみが生存しうること，あるいは腫瘍発育床の放射線による変化があげられてきた．

　著者は前報において，腫瘍移植前に腫瘍床がレ線照射された場合，腫瘍の発育が遅延するとともに，担癌動物の生命延長が認められ，また理論的に算出した腫瘍の極大値が対照群に比べ小さいという3つの実験結果を報告した．今回は腫瘍床が腫瘍移植前にレ線照射を受けたとき，それが腫瘍細胞の放射線感受性にどのような影響を与えるかを，ddY系雄性マウスおよび滝沢肉腫を用い検討した．

　マウス右大腿部に2,000radsを照射し，その3日後に滝沢肉腫細胞0.05ml を同部位に移植し，それが 0.7ml に発育したとき，空気中にて種々線量を照射した．その後 100日間，腫瘍の再発の有無を観察し，腫瘍を 50%治癒せしめる線量（TCD-50）を logit を用い算出した．

　その結果，照射後 100日における TCD-50 は，対照群が5526radsであるのに比べ，移植前照射を行つたものでは7367radsであつて，半数の腫瘍を治癒せしめるのに，1.33倍の線量を要することがわかつた．

　Extrapolation Number (N) および37%線量 (D_{37}) を推定すると，対照群では$N=1$，$D_{37}=$ 308rads，と$N=3$，$D_{37}=$ 331rads の間だが，移植前照射をうけたものでは$N=1$，$D_{37}=$ 411rads. と$N=3$，$D_{37}=$ 440rads の間にあり，後者において37%線量がはるかに大きいことを知ることができた．

It is a well known clinical observation that tumors recurring in previouly irradiated regions are more radioresistant than the original tumor.　The several theories designed to explain this phenomenon, can be divided into three groups[6,7,18,20]: (1) the initial sublethal irradition kills off all but a few resistant tumor cells, so that when the recurrences take place, the resistant cells comprise the tumor.　(2) the initial irradiation induces a mutation towards radioresistance.　(3) in addition to a change in radiosensitivity

of the tumor cells themselves, irradiation causes some changes in the host (tumor bed changes), and the affected tumor bed makes the recurring tumor behave as though more resistant to subsequent irradiation.

A previous paper[44] described some tumor bed effects (TBE) with Takizawa-sarcoma in mice. When the tumor beds had been irradiated before tumor transplantation, the growth of the tumor was significantly decreasea, the survival time of the hosts prolonged and the maximum tumor volumes (the estimated final size of tumors causing death of animals) were usually smaller.

In addition to these findings, this paper reports some investigations of the effect of tumor bed (TBE) on the radiosensitivity of malignant tumor cells.

Materials and Methods

Animals

Male mice of the ddY strain supplied by the Kansai Animal Laboratory, Kyoto, were used in all experiments. Their room temperature was kept constant during the experiments. The animals were housed in groups of eight to ten in stainless steal wire cages with wood shavings for their bedding. They were provided with standard purina pellets (made by the Funahashi-nojou Company) and water ad libitum.

Tumor Transplantation

The tumor used was the Takizawa-sarcoma induced by Takizawa[41] (Department of Pathology, Chiba University) by daily injections of 25 per cent fructose solution. A subline of this strain has been propagated in the Department of Radiology, Kyoto Prefectural University of Medicine, since November, 1963. A tumor cell suspension of 0.025 to 0.05 ml. was injected into the subcutaneous tissue of the right thighs of mice weighing 13 to 15 grams. The method of inoculation is described in detail in the previous paper[44].

X-irradiation

(a) X-irradiation of the tumor bed before tumor transplantation: An X-ray machine of the Toshiba KXC-18 type was operated at 120 kvp., 25 mA. with a 1.0 mmAl. filter. The HVL was 2.4 mmAl., and focus-skin distance was 30 cm. The dose rate measured with Shimazu Dose-Reader and No. 422 chamber at the same position as the tumor bed to be irradiated was 145 rads per minute.

(b) X-irradiation of the tumors: Physical factors were 160 kvp. and 25 mA. with 0.3 mmCu and 0.5 mmAl. filter. The HVL was 0.53 mm Cu. The measured dose rate was 197 rads per minute at the same position as the tumor.

In both methods of X-irradiation, the right thighs of eight mice were irradiated in a 10 by 10 cm. field on a parafine plate 10 cm. thick. The animals were anesthetized by ether only to keep them motionless during their fixation.

Theoretical Consideration on 50 per cent tumor control dose

The experiment planned to determine the tumor cure dose took into account the following theoretical considerations.

From the standpoint of cellular radiation biology, Puck and Marcus (1956)[27] established that the lethal response of single mammalian cells to radiation can be described by the maltitarget survival curve given by the formula,

$$S = 1 - (1 - e^{-\frac{D}{D_{37}}})^N$$

where, S is the surviving fraction, N is the extrapolation number, D_{37} is 37 per cent dose, and D is the X-ray dose. When D is much larger than D_{37}, this formula can be simplified as follows;

$$S = Ne^{-\frac{D}{D_{37}}} \quad\ldots\ldots(1)$$

Since their description it has been demonstrated by many investigators that this formula can be applied to the response of various mammalian and human "single" cells to irradiation.

Providing that each cell in "solid" tumors responds to irradiation independently and according to formula (1), in many tumors containing M cells the average number of cells per tumor left surviving after a dose of D rads will be SM. The probability that a tumor is cured is equal to the probability that all cells in a tumor are killed, and expressed as

$$P = e^{-SM}$$

Substituting for S from formulla (1),

$$P = e^{-NM\ e^{-D/D_{37}}} \quad\ldots\ldots(2)$$

(Munro and Gilbert, 1961)[21]

This equation can be transformed to

$$\ln\ln P^{-1} = \ln NM - D/D_{37}$$

In this equation, when P is 1/2, the X-ray dose which will destroy half of the tumors can be calculated from the equation,

$$D/D_{37} = \ln NM - \ln\ln 2 \ldots\ldots(3)$$

Using this equation, Suit (1963[36,37] and 1964[38]) evaluated the radiation response of C3H mouse mammary carcinoma and named the X-ray dose which killed half the tumor "50 per cent local tumor control dose (TCD-50)."

If D is much larger than D_{37}, equation (2) is a similar function as the logit P[3], that is, a straight line transform of logistic function;

$$\text{logit } P = \ln\frac{P}{1-P} = a\log D + b$$

(a and b are constants)

For the bio-assay of X-ray doses which will cure local tumors, the logit analysis was used and TCD-50 was calculated. These experiments were based on these theoretical considerations, and TCD-50 was calculated by logit analysis.

Experiments and Results

50 per cent local tumor control dose

The purpose of this experiment is to investigate the effect of tumor bed alteration induced by X-ray on the 50 per cent local tumor control dose (TCD-50). Three days before tumor transplantation, the tumor beds of the right thighs of 96 mice were irradiated by 2,000 rads. 0.05 ml. of tumor cells were transplaneted to each irradiated area and to thighs of 120 non-irradiated control animals. When the tumors had grown to 0.7 ml., they were irradiated in air with various dose levels as presented in table 1.

After the irradiation of the tuomrs the animals were observed every day. If a mouse died with a recurrent tumor, it was checked as non-controled. If a mouse died without any visible tumor, it was excluded from the results. The TCD-50's of tumors in nonirradiated and in irradiated tumor beds were

calculated every ten days for 100 days after the irradiation.   The results are shown in figure 1, and the TCD-50's evaluated in 50 and 100 days after irradiation are presented in detail in table 1.   These results indicate that 100 days after tumor irradiation is enough time to observe the regrowth of irradiated tumors because the TCD-50's become constant after 60 to 70 days.

By 100 days after tumor-irradiation, 78 of the 120 mice with tumors in normal tumor beds had died with tumor-recurrence.   The great majority (71) had died within 50 days and about half (36) within 30 days after irradiation.   On the other hand, of the 96 mice with tumors in irradiated tumor beds, 57 had died by 100 days, 51 by 50 days and 22 by 30 days after tumor-irradiation.

Without visible tumors, of the 120 mice with tumors in normal beds 5 died within 100 days and 4 of them within 30 days.   Of the 96 mice with tumors in irradiated tumor beds, 6 died without tumor-recurrence within 50 days and 7 within 100 days.   (These animals were excluded from the results.)

The values of the TCD-50's clearly demonstrate tumor bed effect (TBE).   Tumors in irradiated tumor beds were more radioresistant than those in non-irradiated tumor beds.

The TCD-50 in 50 days of tumors in non-irradiated tumorbeds was 5420 ± 110 rads, and that of tumors in irradiated tumor beds was 7054 ± 184 rads.   The TCD-50 in 100 days of tumors in normal and irradiated beds was 5526 ± 84, and 7367 ± 182 rads, respectively.   (Fig. 2).   The ratio of the TCD-50 of tumors in normal and irradiated tumor beds increased gradually with time.   It was1.19 in 30 days after irradiation, 1.30 in 50 days and 1.33 in 100 days.
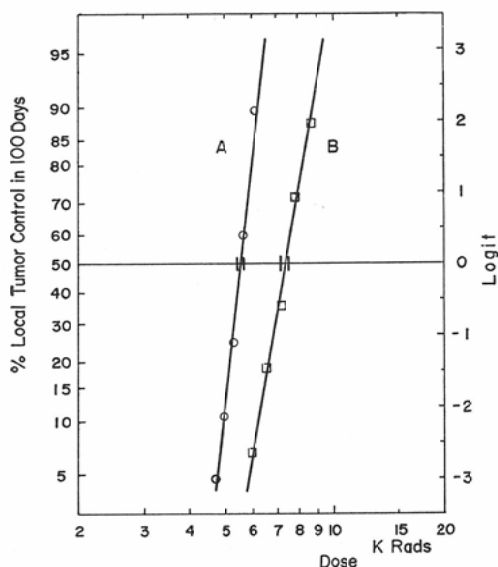

Figure 1.   50% local tumor control doses(TCD-50) of 0.7 ml.   Takizawa-sarcoma growing in right thighs of mice. (They were calculated every ten days after tumor-irradiation)
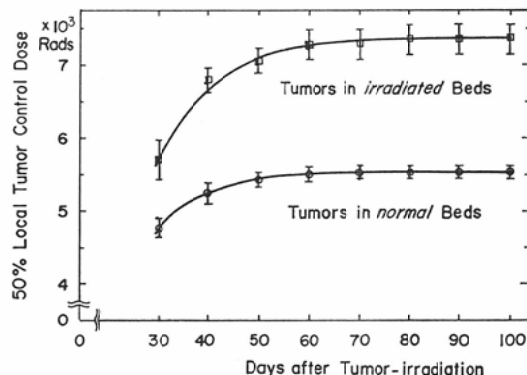
Figure 2.   50 Per cent Local Tumor Control by 100 days after tumor-irradiation Dose
(A) Tumors growing in normal tumor beds
(B) Tumors growing in tumor beds irradiated with 2,000 rads three days before transplantation.

On the other hand, the slopes of the logit-lines in figure 1 are very steep.   However, that of tumors in irradiated beds is slightly less steep than that of tumors in normal beds.   This means that to get a higher percentage of cures, the ratio of the tumor control dose in normal and irradiated beds must increase.

Table 1. Local control of 0.7 ml. mouse Takizawa-sarcomas growing in right thighs, following local single x-irradiation. (Animals dying without recurrent tumor were excluded from the results)

(A) Tumors in normal tumor beds

Tumor dose (rads)	4297	4607	4933	5286	5664	6069	TCD—50
Tumor control in 50 days	1/18 (5.6)	3/20 (15.0)	3/18 (16.7)	6/20 (30.0)	13/20 (65.0)	18/19 (94.7%)	5420± 110
Tumor control in 100 days	0/18 (0)	1/20 (5.0)	2/18 (11.1)	5/20 (25.0)	12/20 (60.0)	17/19 (89.5%)	5526± 84 rads

(B) Tumors in tumor beds irradiated with 2,000 rads, three days before tumor-transplantation

Tumor dose (rads)	5409	5931	6503	7173	7819	8573	TCD—50
Tumor control in 50 days	0/15 (0)	3/15 (20.0)	5/16 (31.3)	7/14 (50.0)	9/13 (69.2)	14/16 (87.5%)	7057± 184 rads
Tumor control in 100 days	0/15 (0)	1/15 (6.7)	4/16 (18.8)	5/14 (35.7)	9/13 (69.2)	14/16 (87.5%)	7367± 187 rads

The 90 per cent local tumor control doses (TCD-90) of tumors in normal beds and in irradiated beds are 6207 and 8784 rads, respectively. The ratio with TCD-90 in 100 days is 1.42, which is higher than the ratio with TCD-50.

From these results alone it is impossible to analyse the radiosensitivity of Takizawa-sarcoma cells, because any data concerned with 37 per cent dose and extrapolation number was not given in this experiment.

Radiosensitivity of Takizawa-sarcoma cells

To analyse the radiosensitivity of Takizawa-sarcoma cells another experiment was planned. When tumors grew up to 0.7 ml. in the right thighs of animals after transplantation, they were irradiated with 500 to 2,000 rads in vivo. Within one hour after irradiation, 0.025 ml. of the irradiated tumor cells were transplanted to 15 normal mice in every dose levels. These tumor volumes were measured every two days and growth curves were calculated according to the formula of logistic curve[24,43] expressed as follows:

$$Vt = \frac{K}{1 + ae^{-bt}}$$

(The method of measuring and calculating the tumor volumes has been described in a previous paper[44]) In this formula $V(t=0)$ reveals the viable tumor cell volume at the time of transplantation providing that tumor cells begin to grow without any initial delay in gorwth or mitosis (Fig. 3). The surviving fraction after irradiation was calculated by comparing the $V(t=0)$ values of every irradiated cells with that of normal cells.

From these results, with the least square method, the extrapolation number (N) was 1.7 (1—3) and the 37 per cent dose (D_{37}) was 320 rads (Fig. 4). These values ignore the initial growth delay and radiation induced mitotic delay. It was demonstrated that mitotic delay and X-ray dose have a linear correlation, using Chinese Hamster Cells (Elkind et al., 1963)[11]. However, it is unknown whether same correlation is observed in Takizawa-sarcoma or not, and it is also unknown what kind of correlaton there is between initial tumor growth delay and radiation-induced mitotic delay. Therefore, the cellular radiosensitivity of Takizawa-sarcoma was analyzed with N=1.7 (1—3) and D_{37}=around 320 rads.
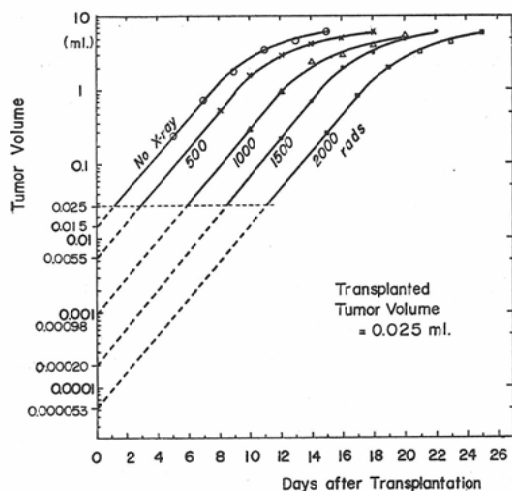
Figure 3. Growth curves of Takizawa-sarcoma transplanted within one hour after in vivo irradiation with various doses. Each value extrapolated on the ordinate reveals surviving tumor volume in 0.025 ml. transplanted tumor volume. (They were estimated measured tumor volumes using logistic function.)
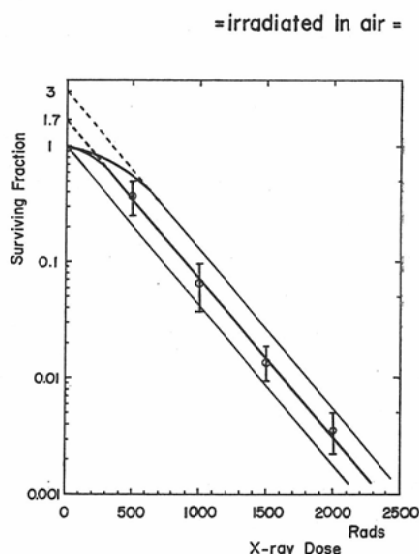

Figure 4. Surviving fraction of Takizawa-sarcoma cells in 0.7 ml. tumors in air. Each point is a ratio of surviving tumor volume after irradiation versus control one.

Discussion

It has been demonstrated that the irradiation of tumor beds before transplantation causes tumor cells to behave as though more radioresistant to subsequent irradiation. The lethal dose for tumors grown in irradiated tumor beds, when evaluated as TCD-50 in 100 days, had to be multiplied by that for tumors in normal beds by a factor of 1.33. In discussing these findings, it is necessary to analyse the data from the aspect of cellular radiation sensitivity.

The extrapolation number and 37 per cent dose for Takizawa-sarcoma cells were roughly estimated as 1.7 (1—3) and around 320 rads, respectively. Substituting these values for N and D_{37} in equation (3), the number of cells in a 0.7 ml tumor, when the TCD-50 is 5526 rads, is calculated as 1.3×10^7. Hemocytometrically 6 (4—9) $\times 10^5$ cells were counted in one cubic milimeter, so the 0.7 ml. tumor used in these experiments would contain 4.2 (2.8—6.3) $\times 10^8$ cells. Between these two values, there is a difference of a factor of 32 (22—48). This is considered that 32 cells is the number of surviving cells required for irradiated tumor to grow again.

In a pilot experiment, various numbers of tumor cells were inoculated into the right thighs of mice. Tumors were able to grow in two of the five mice receiving 10 cells and four of the five receiving 100 cells.

These facts support the hypothesis that 10 to 100 surviving cells are needed for irradiated tumor to recur.

In table 2, 37 per cent doses (D_{37}) were calculated from equation (3) with an extrapolation number (N) of 1 to 3 and the number of cells needed for tumor-regrowth, 10 to 100. The curves of the cellular surviving fractions of Takizawa-sarcoma cells could be drawn between a straight line with N=1 and $D_{37}=$ 306 rads, and a curve with N=3 and $D_{37}=331$ rads, as shown in figure 5.

It was demonstrated that, in air, the TCD-50 in 100 days of 0.7 ml. solid Takizawa-sarcomas growing in beds irradiated with 2,000 rads before transplantation was 7367 rads, and the ratio of TCD-50 in irradiated beds to that in nomal beds was 1.33.　　These results show that it is important to analyze how the extrapolation number and 37 per cent dose changed.

In some stituations, the radiation sensitivity of mammalian cells was demonstrated to be modified at the point of an extrapolation number at other than a 37 per cent dose. It was proved by Dewey (1960)[8] that in the X-ray survival curve of human livercells, not only the 37 per cent dose but also the extrapolation number was higher when they were irradiated in nitrogen than in oxygen. Bagshow (1962)[1], Mohler and Elkind (1963)[19], and Humphrey et al. (1963)[17] showed that 5-bromodeoxyuridine (BUDR) increased the X-ray response of mammalian cells in vitro and decreased the extrapolation number in X-ray survival curves. Moreover, the extrapolation number was modified by different kinds of radiation (Hornsey and Silini, 1963[16] and Berry and Andrews, 1963[5]), by temperature variation and substances in the medium at the time of irradiation (Beer et al., 1963[2]). From these results it might be concluded that, in conditions in which the cellular radiation sensitivity is decreased, the extrapolation number becomes higher.

In the present experiments, it was expected that in the radiation response curve of Takizawa-sarcoma cells grown in irradiated beds, the extrapolation number and 37 per cent dose would increase. However, there is no way of knowing how the extrapolation number was modified. Therefore, the radiosensitivity of these cells was analyzed under the assumption that the extrapolation number did not change in the irradiated tumor beds.

The TCD-50 of Takizawa-sarcoma in beds irradiated before transplantation was 7367 rads in 100 days; i.e. higher than the 5526 rads of tumors in normal tumor beds. It was stated above that 10 to 100 cells are needed to cause an irradiated tumor to recur and that the extrapolation number is between 1 and 3 (average 1.7). Substituting these values in equation (3), 37 per cent doses were calculated and are shown in table 2. The radiation surviving fraction of these cells could be drawn between a straight line that have, N=1 and D^{37}=411 rads and acurve with N=3 and D_{37}=440 rads, as shown in figure 5. The ratio of D_{37} of tumors in irradiated beds to that of tumors in normal beds was 1.33.

In explaining the acquired radioresistance of tumors recurring in previously irradiated regions, three possible mechanisms have been proposed by many authors[6,7,18,20]: selection of tumor cells, induction of mutation towards radioresistance, and tumor bed changes. In other words, it may be considered that

Table 2.　Calculated 37 per cent doses (D_{37}), computing extrapolation number (N) as 1-3 and number of surviving cells neecssary for tumor-regrowth as 10-100, where the 50 per cent local control dose wasassumed to be 5526 rads (tumors in normal tumor beds -A-) or 7376 rads tumors in irradiated tumor beds -B-).

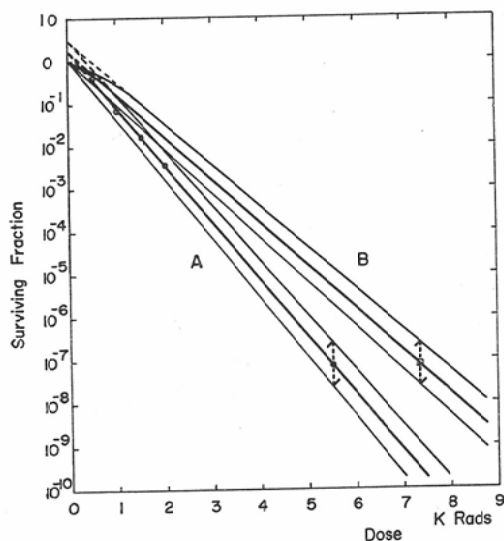| Number of surviving cells | 37 per cent dose (D_{37}) | | | | | | | | D(A)/D(B) |
	N= 1		N= 1.7		N= 2		N= 3		
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	
10	308	411	300	400	297	396	291	387	1.33
100	354	472	342	456	339	452	331	441	

Figure 5. Calculated surviving fraction Takizawa-sarcoma cells
in 0.7 ml. tumors, by estimating that 10 to 100 cells are
necessary for the regrowth of irradiated tumors.

(A) Tumors growing in normal tumor beds.

(B) Tumors growing in tumor beds irradiated with 2,000 rads
three days before transplantation.

this phenomenon depends on some changes occurring in the tumor cells themselves or in tumor beds.

In regard to radiation-induced alterations in the tumor cells themselves, many conflicting results have been reported.

Whitfield an Rixon (1960)[47] observed in cell culture studies that repeatedly irradiated strain L mouse cells were less sensitive than the original ones. However, using many strains of mouse tumor, Pearson (1959)[26] demonstrated that the lethal radiation dose of serialy irradiated tumors was the same as that of nonirradiated tumors. Kaneta and Muta (1960)[18] irradiated Yoshida sarcoma in vivo up to 94 generations (total dose was 87,500 R.) and found that the degree of tumor regression was higher for the preirradiated than the non-irradiated control line.

Rhynas and Newcombe (1960)[30] induced radioresistant lines of strain L mouse fibroblasts by repeated irradiation and showed that the number of chromosomes was a little lower in cells with acquired resistance. Muta's experiments (1964)[23] suported these results. However, Berry (1963)[4] demonstrated anopposite fact that diploid P-388 lymphocytic leukemia was slightly radiosensitive than tetraploid. Till (1961)[42] examined the radiosensitivity of various cell lines whose chromosome number varied from 53 to 109 and concluded that chromosome number was independent of radiosensitivity. Révész et al. (1963)[28] investigated the relationship between chromosome number and radiosensitivity of two lines of Ehrlich ascites carcinoma (ELD and ELT) and serially irradiated lines of ELD and also found no consistent relationship.

Measuring non-protein sulphydryl (NP-SH) levels of these cell lines, Révész et al. (1963)[29] reported that the increased NP-SH level per cell is associated with increased resistance of the cells to anoxic irradiation. Radiosensitivity has been also found to have a correlation with chromosome size (Wakonig-Vaartaja, 1963)[46] or $Q^{N_2}_{CO_2}$ (Schubert, 1954)[33].

From these results, it is not possible to conclude that the changes induced in cells by repeated irradiation have a consistent relationship with the occurrence of acquired radioresistance, although in some cases there is a definite correlation.

Montgomery and Warren (1953, using Gardner mouse lymphosarcoma)[20] and Conger and Luippold (1957, using Ehrlich mouse ascites tumor)[7] failed to obtain radioresistant lines in "in vivo" studies. They concluded that the phenomenon of acquired radioresistance could be explained on the basis of the radiosensitivity of the tumor cells themselves.

Nice (1957)[25] obtained resistant tumors of mouse 1016 lymphatic leukemia. Tumors were irradiated with 500 R. when they were 1 cm. in diameter. Recurrent tumors were irradiated twice more with 500 R. After the third irradiation, the tumors were radioresistant. However, when these tumors were transplanted to non-irradiated mice, they reacted to radiation in the same way as the original tumors. This experiment appears to indicate that the effect of irradiation on the tumor bed is one of the factors operating in acquired local resistance to irradiation, and not only its effect on tumor cell population, i.e. selection or cellular mutation towards radioresistance.

In the present experiments, non-treated tumor cells were transplanted to irradiated tumor beds, and these tumors were less radiosensitive than tumors in normal beds.

The tumor bed effect (TBE) on tumor cells was first noted many years ago (Murphy et al., 1923[22] Russ and Scott, 1927[31] and 1940[32]). The delay of tumor growth and prolongation of life of tumor-bearing animals have been reported to be due to TBE (Stenstrom et al., 1955[34], 1956[35], Vermund et al., 1958[45] and Summers et al., 1964[40]). A smaller theoretical maximum tumor volume was also demonstrated as a TBE (Urano, 1966)[44]. It was discussed in the previous paper[44] that (1) diffusible toxic facrtos, (2) connective tissue changes, (3) retarded capillary formation and (4) changes in vascular function were the main mechanisms of TBE. These factors might cause the tumor cells to be under-nourished, mainly hypoxic. Gray (1957)[13] discussed the role of oxygenation in radiotherapy as follows: "From clinical and histological observation it has been inferred that the tumor bed, and particulary its degree of vascularisation, plays a very important role in successful radiotherapy. Experimentally it has frequently been observed that whether cells are irradiated in vivo or in vitro they suffer considerably less damage if they are anaerobic than if they are aerobic at the time of irradiation. These two streams have made contact from time to time. They meet again today".

In other wards, pretransplantation irradiation of the tumor bed reduces the blood supply to the tumor cells and probably thereby decreases cellular radiation sensitivity. This is considered to be a mechanism similar to the indirect effect of oxygenation in radiotherapy that has been reported by many investigators[14,15,39].

This evidence might offer a different opinion to the concept of dose fractionations. Depending on Elkind's type of recovery[9,10], Fowler and Alper et al. (1963)[12] designated Dq as the dose represented by the interception of the straight portion of a survival curve with an extrapolation number of N on the 100 per cent survival abscissa, and stated that the relationship between a single dose D_1 and n fractioned dose Dn required to obtain the same survival rate as with D_1, if each dose is large enough to bring the survival to the straight part of the cell survival curve, can be expressed as "Dn-D_1%(n-1) Dq". According to this theory the extrapolation number (N) and 37 per cent dose (D^{37}) are constant during the fractionated radiotherapy. However, in the presenst experiments cellular radiation sensitivity was altered even by

tumor bed changes induced by previous irradiation. These findings suggest that, in vivo, D_{37} (and N?) may gradually increase during the fractionated treatment.

Summary

The effect of X-irradiated tumor beds on cellular radiation sensitivity was investigated.

Tumor bed effect (TBE) was clearly demonstrated, when the 50 per cent local tumor control dose (TCD-50) in 100 days of a 0.7 ml. Takizawa-sarcoma was calculated by logit analysis. The TCD-50 in 100 days of tumors in normal tumor beds was 5526 rads, while that of tumors in tumor beds irradiated before transplantation was 7367 rads. The ratio of TCD-50 in irradiated beds to that in normal beds was 1.33.

When it was presumed that 10 to 100 cells are enough to cause irradiated tumors to recur, survival curves of Takizawa-sarcoma cells in a 0.7 ml. tumor could be drawn between a straight line with as extrapolation number (N) of 1 and a 37 per cent dose (D_{37}) of 308 rads and a curve with $N=3$ and $D_{37}=331$ rads. On the other hand, survival curves of tumor cells in irradiated tumor beds could be drawn between a line with $N=1$ and $D_{37}=411$ rads, and a curve with $N=3$ and $D_{37}=440$ rads. The ratio of D_{37} of the tumor in irradiated tumor beds to that of tumor in non-irradiated tum beds was 1.33.

Tumor bed changes due to irradiation, especially changes of vascularisation, are considered to be a main factor of acquired radioresistance of tumors recurring in previously irradiated regions.

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