Comparative Study of Three Methods of Measuring The Responses of Tumours to Irradiation

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放射線照射に対する腫瘍の反応の計測に就いて

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生体内でX線照射を受けたマウスメラノーマB16を照射直後に移植し，その後の反応を腫瘍容積法，^{125}IUdR 生体内標識法，^{3}HUdR 生体内標識法で計測し結果を比較検討した．腫瘍容積法が最も感度が良く ^{125}IUdR 生体内標識法がこれに続いた．腫瘍が皮下に被膜で包まれている事，部分的生体内細胞同調を行つた事等が結果に影響を与えると考えられた．

Responses of tumours to ionizing radiation are primarily reflected in those of the population size of clonogenic cells in tumour. The changes in size are based on the kinetics of cycling or noncycling cells in tumour. While labelled precursors of nucleic acids are used to know the cell kinetics in vitro, the methods to know those in vivo are insufficiently developed. The method using ^{125}IUdR is one of these methods in vivo[1)2)3)], but it has a weekness of low labelling efficiency although various methods to increase the efficiency are reported[4)5)6)]. The data obtained from a method of tumour volume measurement may include somewhat large technical errors, however it is one of the most direct and important methods to know tumour responses to radiation[7)8)]. Though it is neccessary to compare these methods each other, to make clear the characteristics of each method and to find a method suitable to a given tumour in tumour biology or basic radiation therapy, few papers are reported on these problems today. The present experiment was intended to study these problems.

The present paper deals with the three kinds of methods used to measure responses of melanoma B16, namely volume measurement of tumour transplanted to recipient mice, and measurements by counting radioactivities of tumour cells labelled with ^{125}IUdR, or ^{3}HUdR transplanted to recipients after irradiation.

Materials and methods

Tumours and animals: Mouse melanoma B16 which is a radioresistant tumour[9)] and has a mode

number of 42 chromosomes with a few triploid and tetraploid cells was used in the present experiment.

Melanoma is one of tumours which are applicable to a neutron capture therapy by an atomic reactor. Six-week-old C57BL/6j male mice weighing 20–25 g were used in the experiment. Volume measurement: Tumours were irradiated when they grew to about 10 mm in diameter. The tumour grew exponentially from about 6 days to about 14 days after transplantation and did not grow any more actually after 19 days. Tumours of 10 mm in diameter were still exponential phase. Immediately after irradiation, tumour mass were removed from five to seven mice, collected, minced and pipetted through a slim needle; 0.05 ml of the minced tumour mass was injected subcutaneously into the back of recipient C57BL/6j mice. The number of cells with melanin pigments included in 0.05 ml of tumour mass was 3.7×10^5. The cell count seems to be somewhat small. As the tumour mass include comparatively large amount of blood usually, the count is reasonable. Five recipient mice were used for each radiation dose. The three diameters of each tumour were measured with a scale and the tumour volume was calculated from the average diameter, assuming a spherical shape. All measurements were carried out by one person to minimize technical error. The volume was estimated using growth curves of tumours at 9.5 days after transplantation. The time interval between irradiation and removal of the tumours was less than 100 minutes. The volume of non-irradiated tumour measured 9.5 days after transplantation was taken as 1.00 and the relative value at the same time after irradiation was calculated for each dose. No significant difference was observed in the slopes of growth curves between non-irradiated and irradiated tumours in the present dose range.

^{125}IUdR method: Tumour-bearing mice were repeatedly injected intraperitoneally with 3.02 mg of cold thymidine dissolved in 0.1 ml of physiological solution five times with the time interval of one hour. Five hours after the last injection, the same amount of cold thymidine was again injected repeatedly five times. Five hours after the second series of thymidine injections, the mice received intraperitoneal injection repeatedly five times in every one hour with 0.5 μg of colcemid dissolved in 0.1 ml of physiological solution. One hour after the last injection, the mice were injected with 3.7 kBq of ^{125}IUdR dissolved in 0.1 ml of physiological solution intraperitoneally 13 times with the time interval of one hour. The mice were irradiated at one hour after the last injection with ^{125}IUdR for the experiment. After irradiation, tumours were removed, collected, minced, pipetted and transplanted as described above. The transplanted mass was 0.5 ml, which was correspondent to about 3.7×10^6 cells with melanin pigments.

The recipient mice were given drinking water containing 0.1% KI from one week before transplantation to the end of experiment. The number of tumour-bearing mice was five to seven and that of recipients was five at each radiation dose level. The probe of a GM counter (Aloka Model GP-101) was used to measure radioactivity. The mice were fixed in a plastic tube with thin wall for counting and the counting time was five or ten minutes. The background count was substracted from each count after correction using the physical half-life. The value at zero time, that is, immediately after transplantation, was taken as 1.00 and the relative values were calculated for each measurement. Measurements were made in every 24 hours for 10 days. The count per minute used to draw the dose-effect curve was that calculated at 4 days after transplantation, because the count of radioiodine decreased rapidly immediately after transplantation and then became stable at the time.

^{3}HUdR method: The same radioactivity of tritium as that of radioiodine was used, instead of ^{125}IUdR. The recipients were not given KI solution. The transplanted mass was 0.75 ml and the mass was removed at 4 days after transplantation to count radioactivity. The removed mass was about 0.3 ml. The counting was corrected to the count per unit volume of mass. The counting tritium was done as previously described[10]. The data was used to draw the dose-effect curve as described above.

Irradiation: A deep X-ray therapy unit was used for irradiation. The physical conditions were 150 kvp, 24 mA, added filter with 0.5 mm Cu and 1.0 mm Al and 50 cm of distance between tumour and focus. The dose rate was 479 mGy/min. Dosimetry was carried out at each exposure with Radocon dosimeter, and the values of the R unit were converted to the Gy unit with a conversion factor of 0.957×10^{-2}.

Results

1. Tumour volume method: Tumours were exposed to 9.57, 10.53, 11.48, 12.44, 14.36 and 19.14 Gy. The dose-effect curve, shown in Fig. 1, does not include a linear portion in this dose range. The dose-effect curves were drawn with the eye. The D_{37} value was 13 Gy. The dose required to reduce surviving fraction to 0.01, that is, $D_{0.01}$ was 18 Gy.

2. ^{125}IUdR method: The count of radioiodine increased with the number of injections to the third injection and decreased temporarily at the third hour and thereafter increased again from 4 to 12 hours. The increase rate was 70 to 73 cpm at each injection. On the contrary, the rate was 25 to 28 cpm in the untreated mice tumour. After the last injection, the count of the tumour reduced

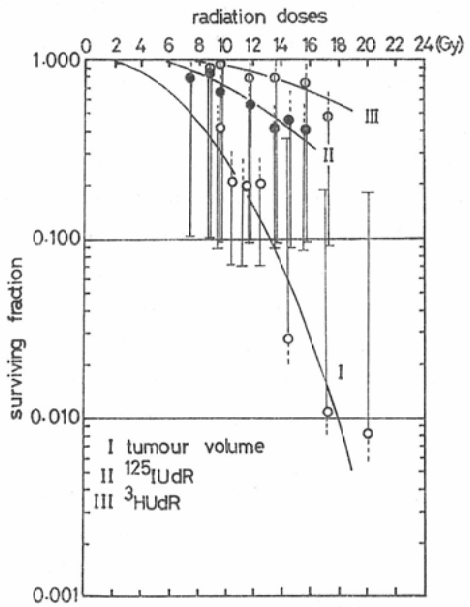

Fig. 1. The dose-effect curve of melanoma B16 to X-rays estimated by three methods. The vertical bars show the 2σvalues to each mean relative value.

but soon showed a stable value while the count in the untreated mice tumour reached to the undetectable level soon. The count of radioiodine in tumour mass heated on 70°C for 30 minutes reduced quickly to be on an undetectable level. The dose-effect curve was shown in Fig. 1. The doses used were 7.65, 8.61, 9.57, 11.48, 13.40, 14.36 and 17.23 Gy. The slope of the curve obtained by this method was less steep than that obtained by the tumour volume method. The $D_{0.8}$ was 8.6 Gy.

3. ^{3}HUdR method: The doses of 8.61, 9.57, 11.48, 13.40, 17.23 and 19.14 Gy were used. The estimated $D_{0.8}$ was 13.5 Gy. The results were shown in Fig. 1. The slope of the dose-effect curve was even less steep than that obtained with the ^{125}IUdR method.

Discussion

The experimental procedures for labelling with ^{125}IUdR or ^{3}HUdR in vivo were decided to synchronize partially the tumour cells to increase the labelling efficiency, with references to the data of other reports[4)5)6)11)]. The technique to know the distribution of melanin using labelled drugs which are incorporated into melanin through metabolic pathways, has been reported[12)].

However, the labelled radioactive elements removed from dead cells to other living cells and the amount of radioactivity in a cell does not depend upon mitotic activity but the activity of melanin formation. These properties make those unsuitable to only as the index of clonal living cells. Though the method was used to increase the labelling efficiency in vivo in the present experiment, it could be also applied to measure an average cell cycle times of clonal cells in tumour mass in vivo or prepare tumour for the radiotherapy based on cell cycle[13)14)].

In all three methods, tumour cells were irradiated in vivo and thereafter transplanted to recipient. For this reason, irradiated tumour cells were not affected by the irradiated tumour-bearing mouse body, only did by the artificial factor of transplantation. The present method is similar to those reported in some authors[15)16)17)], though the comparison of tumour volume size is used as the last procedure of method in the present paper.

The comparison of tumour volume was used for the comparison of delay time in the growth of tumour after irradiation, in the present experiment, because the comparison of tumour size is a method suitable to compare with data of other two methods that radioactivities were measured at a given days after treatment. The hocky stick form of dose-effect curve reported in some reports[17)18)] was not observed in the present experiment with radiation dose more than 7.65 Gy. As the first component of hocky stick form is only observed in the range of comparatively small radiation doses, the present data show possibly the second component alone. As shown in Fig. 1., the tumour cell survial after irradiation estimated by the tumour volume method was somewhat larger when compared with that estimated by the in vitro method in the other papers[7)8)], though the surviving fraction in the present data was similar to those in anoxic tumours in other papers[15)16)] and somewhat larger than those in another data[17)]. The tumours in the last three reports and the present report were different each other but all these tumours were irradiated in vivo before transplantation. It has also been reported that the intercellular contact among tumour cells increases the D_q value[19)].

The surviving fraction calculated by the ^{125}IUdR method was higher than that obtained by the tumour volume method. As tumours were irradiated at 14 hours after the last injection with colcemid,

the responses of tumour might be effected with the cell cycle dependent radiosensitivity of tumour cell to a certain extend[14)20)]. Almost no ^{125}IUdR released from dead cells is reused by living cells or their descendants[1)]. The present data also show that radioactivity decreased rapidly in transplanted tumour mass treated with heat. The reduction of radioactivity in the transplanted tumour mass was slower than other data[2)3)]. The difference in the present and the other data could be due to the difference in inoculation sites. The difference between the data obtained by the ^{125}IUdR method and by the tumour volume method is interesting, since it means that the population sizes of living cells and their descendants with ^{125}IUdR method did not correspond with that needed to induce tumours in the recipients. As there is no significant difference in the slopes of the growth curve of tumour between non-irradiated and irradiated tumours as reported above, it is unlikely that the difference in the slopes shows a higher radiosensitivity through the processes of calculation used to draw the dose-effect curve. Although it is possible that the GM probe counts radioactive iodine in the liver as well as in the tumour, it is neither likely that the radioactivity in the liver would induce unexact results in the ^{125}IUdR method, since a body counting method was usually used in this method to measure radioactivities in the tumour mass in vivo[1)2)3)4)5)6)]. As the thyroid glands had already been blocked with KI in the drinking water, free radioactive iodine did not accumulate there. As the melanoma was located subcutaneously in the present experiment and it became to be enclosed with a relatively thick fibrocytic membrane as the tumour grew, so ^{125}IUdR released from dead cells could be held in the necrotic tumour mass, even if the released ^{125}IUdR was not reused.

Although the dose-effect curve obtained by the ^{3}HUdR method was expected to be the same as that obtained by the ^{125}IUdR method, it was less steep compared to the latter. Hydrogen is not excreted from the tumour mass through the same pathway of catabolism as in iodine. Tritium is bounded with other molecules of biological substances probably through an exchange with hydrogen in biological molecules and stays in the body for a time[21)]. This could be a further reason why the slope of the dose-effect curve in the ^{3}HUdR method was less steep than that in the ^{125}IUdR. As presented in the results, ^{125}IUdR or ^{3}HUdR method was less sensitive method in this type of tumour compared with the tumour volume method. It is neccessary to pay attention to the difference in the structure of the tumour and in the tumour bed as important factors, in comparing the data from these different methods of measuring the responses of tumours irradiated in vivo. We must also pay attention to the effects of partial synchronization in ^{125}IUdR method or ^{3}HUdR method. The method of tumour volume is one of the important and direct methods to observe the responses of tumour to irradiation, instead of the method with relatively large errors in its own technical procedures.

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