特別掲載

Growth Pattern of Subcutaneously Trans-Planted Yoshida Sarcoma

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皮下移殖吉田肉腫の増殖態度

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臨床的に測定し得る患者の皮下腫瘍（原発，転移）を経日的に測定して graph に plot して見ると exponential growth でない事を見出した．そこで吉田腹水肉腫を rat の皮下に移植し4日後から測定し下記の3項目について観察を行つた．

1．移植後4日目から8日目迄の皮下腫瘍を測定し増殖曲線を描いてこれより各時点のdoubling time を算出した．

2．移植4日目から8日目迄の腫瘍を剔出し組織学的に観察した．

3．剔出した腫瘍に対して in vitro labeling (3H-TDR を使用）を行い腫瘍の周辺から中心迄 labeling index を測定した．

腫瘍の増殖は exponential growth ではなくむしろ linear である．autoradiograph 法によつて測定された各時点の doubling time と labeling index から算出された平均の generation-time とを比較するとよく一致した．剔出した腫瘍を組織学的に検索すると腫瘍の周辺は活性細胞が殆んどをしめ中心部は necrosis におちいつている．即ち腫瘍が次第に大きくなると増殖しているのはほとんど周辺のみという事になる．labeling を施行してみると腫瘍の周辺部は labeling Index が高く中心部に移行するに従つて低くなる．中心部は necrosis のため labeling index は0である．腫瘍の増殖が linear と見られるのは中心部は殆んど necrosis となり周辺のみが増殖に関与しているためと解される．

Many reports[2)3)9)10)11)] have been published on the growth pattern of malignant tumor since Collins et al[1)]. observed the X-ray film of lung tumors. According to them, the tumor showed exponential growth to a certain size. But we have rather small chance of observing the exponential growth, since in tumor bearing patients we generally observe the tumors which have already grown to a considerable size, and since the growth rate is slowed down with the enlargement of the tumor. This is so easily understandable pathohistologically that we need not repeat it here. It is also well known that in relatively smaller tumor of the early stage as well as in large one, the constituent cells do not show uniform growth in each part. Therefore the terms of "the exponential growth of tumor" should mean an approximate or average fact concerning a population of cells which have varying

growth rates in different parts. It seems rather meaningless toapply the definite formula to the growth of the tumor which is irregular and dependent on complicated factors such as a variety of tumors, the essential nature, interactions between tumor itself and its host and between constituent cells themselves[1)4)5)6)7)11)12)]. In order to clarify these points authors attempted to observe the mode of growth of Yoshida sarcoma (hereafter referred to as Y.S.) which was transplanted subcutaneously. Then they found that the growth rate was higher in the peripheral thin layer of the tumor, and that the central part, which manifested chiefly necrosis, was inactive. In the basis of this fact and along the line of the Mayneord's consideration authors obtained some findings of interest. The object of this paper is to report on them.

Experimental materials and methods:

1. Growth curve

Wister rats received subcutaneous transplantation of 10^6 cells of Y.S. in the right side of abdomen. Then the daily averages for 3 diameters of the tumor were plotted to obtain the growth curve. The mean generation time $\bar{T}$ at each time point was evaluated from this curve.

2. Histological examination:

The tumors were removed at 4, 5, 6, 7 and 8 days after the subcutaneous transplantation of 10^6 cells, and samples 2 μ—3 μ thick were prepared, and stained with hematoxylin and eosin to make microscopical observation.

3. Autoradiography:

Wister rats received subcutaneous transplantation of 10^6 cells of Y.S. in the right side of abdomen, and 4, 5, 6, 7 and 8 days later the tumors were removed. Their slices were put into the incubating tube of inside diameter 2 cm together with 1 cc of Eagle's medium, added 10% calfserum and 1—3 μ Ci of ^{3}H thymidine (3H-TDR) at a specific activity of 2.5 Ci/mM and the whole was incubated in a water bath at 37.5°C for 1 hour. After the incubation in vitro labeling, the tumor slices were washed with water for 5 minutes, fixed with 10% formalin for 24 hours, and embedded in parraffin, then histological samples of 2 to 3 μ thick were prepared. Fuji ET-2E stripping films were put on the samples, desiccated at room temperature, and after 2 weeks exposure in a dark box at 4°C, the samples were subjected to development and fixation. Then they were washed with water for 12 hours, stained with hematoxylin, embedded with balsam, and microscopically examined. Labeling index (numbers of labeled cells per 100 cells, hereafter referred to as l.i.) was measured of the bandforming part as deep as 100 μ from the surface of the sample at 1 mm intervals. From this value $\bar{T}$ was calculated according to the Mayneord's model[6)]. In the cell count, since histiocytes and fibroblasts were not distinctly discernible, both were totalled as a back ground counting which was 5 grains or less in a cell, those counting above 6 grains were scored as labeled cells.

Results

1. Growth curve (shown in Fig. 1.):

The growth curve for subcutaneously transplanted Y.S. was not exponential but linear as long as on 4 to 8 days after the transplantation. The doubling times (hereafter referred to as d.t.) on 4, 5, 6, 7 and 8 days after the transplantation, measured from the curve[13)], were 29, 34, 39, 45 and 51 hours, respectively, becoming longer with the lapse of days. If the tumor growth were exponential, d.t. would be constant. Since d.t. was not constant but became gradually longer in the present experiment, the growth of the Y.S.

tumor should be considered linear as to the diameter or radius of the tumors.

2. Histological examination:

Microscopical examination of the sample of the subcutaneous Y.S. revealed that with enlargement of the tumor, necrotic focus was produced in its center, and gradually increased in size. It seemed that only the peripheral part which thickness was about 0.1 to 0.15 cm at all the stages continued active growth. But the demarcation between the necrotic and active part was not distinct, and there was a border zone between them where the active cells were scattered among inactive cells.

Table 1 Doubling times of Y.S. tumor transplanted subcutaneously

Days after transplantation	Radius (cm)	$\bar{L}^*$	$\bar{T}^{**}$ (hr)	$\bar{T}^{***}$ (hr)
4	0.35	0.37	31	29
5	0.42	0.33	35	34
6	0.48	0.30	38	39
7	0.55	0.28	42	45
8	0.62	0.25	46	51

* calculated by the equation $\bar{L}=L\times\frac{\{R^3-(R-D)^3\}}{R^3}$

** calculated by the equation $\bar{T}=0.693\times\frac{ts}{\bar{L}}$.

*** estimdted from the Fig. 1.

$\bar{L}$: mean value of a labeling index.

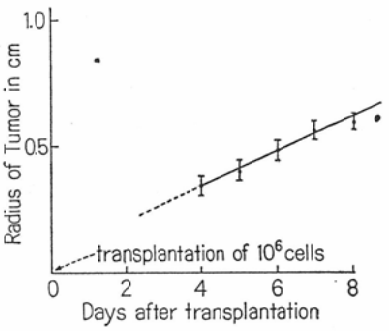
L : measured value of a labeling index.

D : thickness of a peripheral active zone of tumor.

$\bar{T}$: mean generation time or doubling time.

ts : duration of phase S.

Fig. 1. Growth curve of Y.S. tumor



3. Autoradiographic examination:

The autoradiogram of the tumor sample labeled with 3H-TDR in vitro was microscopically examined to obtain l.i.. The value was nearly the same (0.45) in the active peripheral part of all the cases, but the central necrotic part did not show any 3H-TDR uptake. Computation by the Mayneord's model from l.i. (0.45) and the thickness of the active part, D (0.12 cm), gave 31, 35, 38, 42, and 46 hours for the tumors at 4, 5, 6, 7 and 8 days after the transplantation, respectively. They are substantially in agreement with the values estimated from the growth curve.

Discussion

The histological fact that growth activity of the subcutaneously transplanted Y.S. was higher in its peripheral part of a constant thickness and became steeply lower in the central part, gave qualitative evidence in support of the fact that when the daily measured diameters of the tumors were plotted, the obtained growth curve was approximately linear as shown in Fig. 1. This point was further treated quantitatively after Mayneord[6]. By the procedure as described in the section of experimental method, $\bar{L}$'s at various days after the transplantation were obtained from L and then $\bar{T}$'s from $\bar{L}$'s. And these values were compared with d.t.'s obtained from the growth curve representing the measured values. It was to be in good agreement between them. It can be said that these $\bar{T}$'s conversely indicate that the thick-

ness of the active peripheral part of the tumor is almost constant regardless of the lapse of day, and that growth of the cell in this part is asynchrous and exponential. In effect, l.i. of the peripheral part obtained autoradiographically by the use of 3H-TDR was 0.45, which is nearly equal to that of ascites. But, Oehlert et al.[8] found 0.384 for the peripheral part of subcutaneous Ehrlich ascites carcinoma, 0.172 for the central part, and 0.288 for ascites cancer.[12]

Summary

Growth of subcutaneously trasplanted Y.S. was daily observed for 8 days, and it was found that the growth was active in the peripheral part of about 0.1 to 0.15 cm in thickness, that the growth rate was abruptly lower in the inner part, that the growth was not exponential but linear as to the radius of the tumors, and that the model suggested by Mayneord could be well applied to the present experiment.

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