Experiments on Single-Cell Inoculation with the Hirosaki Sarcoma[1)]

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(With 13 Text-figures)

Several experiments have been undertaken to determine the number of cells necessary for the transmission of malignant tumors of mice. It has been generally believed that more than many thousand cells were necessary to transmit mouse carcinomas and sarcomas, prior to the first successful transmission of mouse leukemia with a single living leukemic cells made by Furth and Kahn (1937). Several attempts of the single cell transplantation in the Yoshida sarcoma were made by Ishibashi (1950) and Hosokawa (1950, 1951), demonstrating that the tumor could be transmitted with a single tumor-cell inoculation, and that cell-free material was not capable of introducing the disease. Sato (1952) investigated the chromosomes of the tumor cells in the single-cell tumor derivative produced through isolation, and failed to find out distinct cytological differences between the derivative and the stock tumor. On this basis Sato (1952) and Yoshida (1952) have questioned the presence of the stemline of tumor cells from which the tumor propagates itself.

Based on the cytological studies involving morphological and statistical analyses of chromosomes, Makino and his co-workers (Makino 1952, Makino & Kanô 1953, Makino & Tanaka 1953, Tonomura 1953, Yosida 1954) have developed the concept of stemline-cells in several rat ascites tumors. Here, further confirmation of this concept is needed, and this suggested a re-examination through the single-cell inoculation with comparison of chromosomes in the single-cell clones and the original stock tumor.

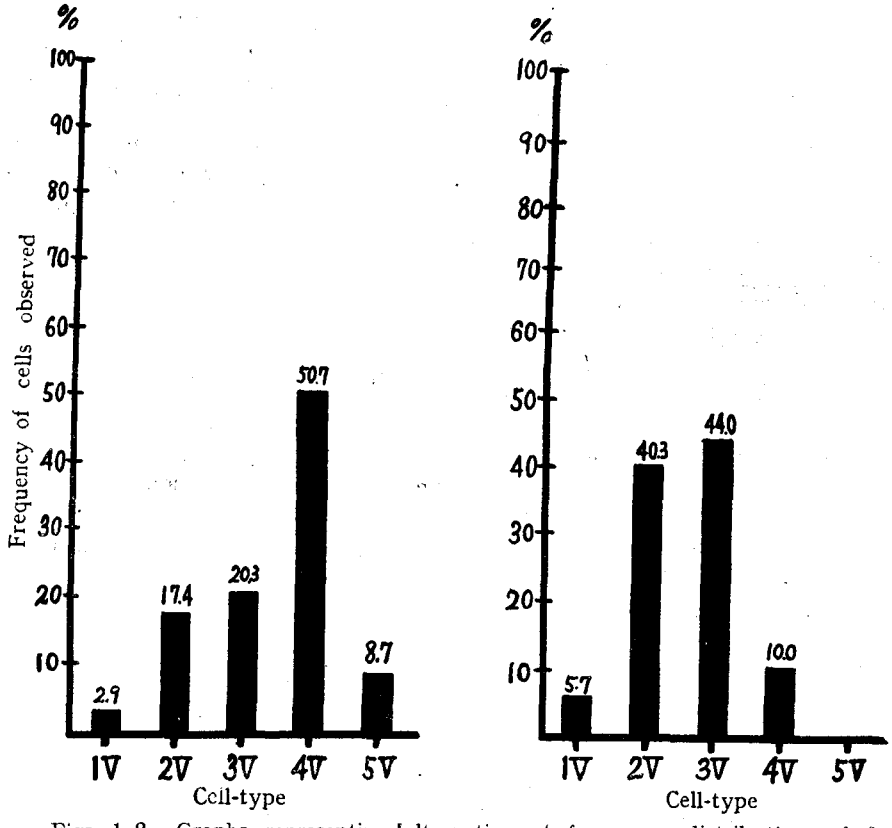
For the critical single-cell isolation in this work, the Hirosaki sarcoma was chosen as material, because it consists of several stemline-cells characterized by different chromosome complexes. Isolation of single cells was performed with the

1) Contribution No. 314 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

With the aid of a grant from the Scientific Research Fund of the Ministry of Education.

aid of the micromanipulator following the technique devised by Ishibashi (1950) and Hosokawa (1950) for the Yoshida sarcoma. After examination under the microscope of the number of cells drawn into the micro-pipette, injection was made in the peritoneal cavity of the rat with this micro-pipette containing known numbers of cells.

It was made clear by Makino & Kanô (1953) that the Hirosaki sarcoma contained five types of stemline-cells characterized by the presence of one, two, three, four and five V-shaped chromosomes of outstandingly large size. In the present study a subline of the Hirosaki sarcoma different from that used in the former study (Makino & Kanô 1953) was employed as material. This subline showed in its early transplant generation the frequency distributions of the stem-cells of five types as shown in Figure 1, while in the later generation they altered



Figs. 1–2. Graphs representing alternation of frequency distribution of five cell-types in the serial transplant generations of the stock Hirosaki sarcoma.
Fig. 1 (right), the 22nd transplant generation. Fig. 2 (left), the 73rd generation.

considerably as seen in Figure 2. At the time of the present experiment the chromosome feature of this subline was as in Figure 2.

Results

Inoculation with single, isolated tumor cells was tried in 51 rats which included derivatives from five different strains. Four out of 51 injected rats showed successful inoculation with single-cells; they developed the ascites sarcoma and died with the disease after from 17 to 25 days. It is interesting that the four rats received successful inoculations were all T-strain rats which specially adapted for the transmission of the original Hirosaki sarcoma.

The percentage of successful inoculations is very low. The difficulty of transmitting with single cells may be due to the fact that the cells used for inoculation were either injured or lost during the process of micro-manipulation.

The ascites tumors developed through the introduction of a single tumor cell in the four rats were capable of transmission from rat to rat by intraperitoneal injection, resulting in the establishment of four tumor sublines. They are tentatively called B-, C-, D-, E-lines, respectively. Generally speaking, the essential nature of these sublines is identical to that of the stock tumor whose cell was introduced.

Table 1. Cytological data of four single-cell sublines

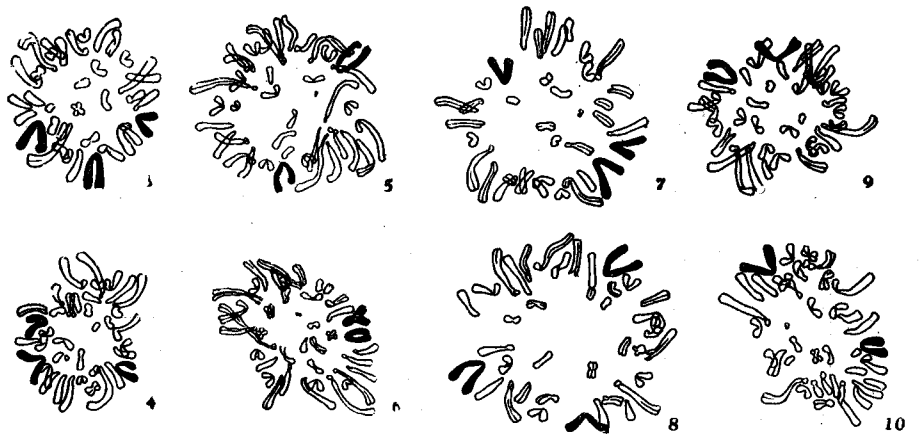
Subline	Cell-type	Chrom. no.	Frequency of cells (%)	Transplant generation	Life days[5] (av.)
B	3V-type	37 (36–39)[1]	3V 79.4 (95)[2] aberrant 20.6 (25)	24	8.7
C	2V-type	39 (37–41)	2V 90.0 (90) aberrant 10.0 (10)[3]	74	9.4
D	3V-type	37 (35–39)	3V 79.2 (67) aberrant 20.8 (18)[4]	40	9.1
E	2V-type	38 (36–40)	2V 81.3 (48) aberrant 18.7 (11)	2	—

1. Numerals in parentheses denote the range of variation in chromosome number.
2. Numerals in parentheses indicate the number of cells observed.
3. 6.5 % out of this was represented by the cells showing extrusion of large V-chromosomes from the equatorial plate.
4. 6.1 % out of this was represented by the cells showing extrusion of large V's from the equatorial plate.
5. Averaged life span of the stock Hirosaki sarcoma was 11.2 days.

Cytological investigations revealed that each tumor subline possessed a distinct population of tumor cells which were characterized by a well-balanced subdiploid complex of chromosomes and occurred in a remarkably high frequency. They multiply with a regular mitotic manner showing usual chromosome behavior.

Considering the evidence observed in other sarcomas, it is evident that these cells with subdiploid chromosomes are primary contributors to the growth of this tumor. The chromosomes of these cells were analysed in comparison with those of the stock tumor, Hirosaki sarcoma. The data concerning these four sublines are outlined in Table 1.

The neoplastic populations of the B- and D-lines were represented by the tumor cells characterized by three prominent V-shaped chromosomes of outstandingly large size, together with certain numbers of rod-, J- and V-shaped elements of varying sizes (Figs. 3–4, 7–8, 11, 13). After a comparison with the chromosomes of the original Hirosaki sarcoma, it is highly evident that the tumor cells of these two sublines correspond to the 3V-type cells of the stock tumor because of the similarity in their chromosome constitution. Thus it is certain that the B- and D-lines are descendants from one original 3V-type cell isolated from the Hirosaki sarcoma. The number of chromosomes varies within a narrow range ranging from 35 to 39, with the most frequent number of 37. The 3V-type cells were predominant in occurrence showing about 80 perecent in appearance. The aberrant cells showing various mitotic abnormalities were found at about 20 percent. Evidently these aberrant cells are all descendants from one original 3V-type cell.

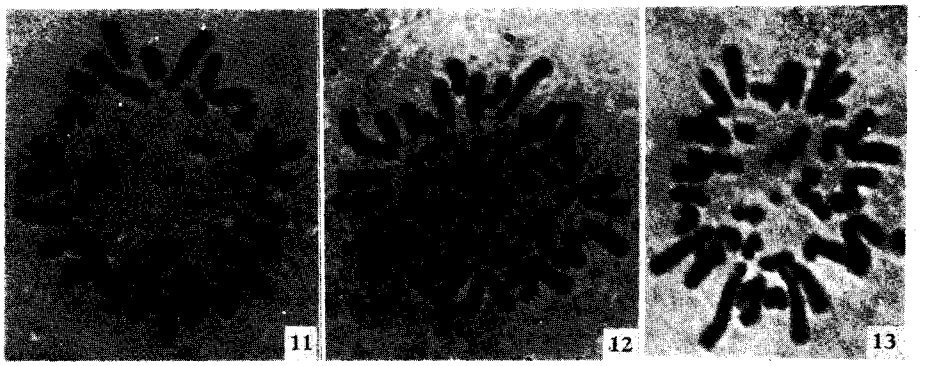


Figs. 3-10. Metaphase plates of four single-cell sublines, ca. ×1300.
3-4, 3V-type cells of the B-line. (3, 36 chromosomes. 4, 36 chromosomes).
5-6, 2V-type cells of the C-line. (5, 40 chromosomes. 6, 39 chromosomes).
7-8, 3V-type cells of the D-line. (7, 37 chromosomes. 8, 37 chromosomes).
9-10, 2V-type cells of the E-line. (9, 38 chromosomes. 10, 38 chromosomes).

The C- and E-lines were characterized by the populations of tumor cells which showed without exception outstandingly large V-shaped chromosomes, always two in number, in addition to certain numbers of rod-, J- and V-shaped

elements of varying sizes (Figs. 5–6, 9–10, 12). A comparison made it clear that these tumor cells correspond to the 2V-type cells of the Hirosaki sarcoma on account of a striking similarity of the chromosomes between these two sublines and the stock tumor. It is concluded therefore that the C- and E-lines are the 2V-type cell clones descended from the progeny of one original 2V-type cell isolated from the stock tumor, Hirosaki sarcoma.

The tumor cells of 2V-type in these two sublines showed a high frequency in occurrence being 90 percent for the C-line and 80 percent for the E-line. The number of chromosomes in these cells was found to vary from 35 to 40, with the modal numbers of 37 and 38 for the C- and E-lines, respectively.



Figs. 11–13. Photomicrographs of metaphasic chromosomes of tumor stem-cells from single-cell clones, × 1800. 11, B-line (3V-type cell clone). 12, C-line (2V-type cell clone). 13, D-line (3V-type cell clone).

Conclusion and summary

The results of a single-cell inoculation undertaken in this study with the Hirosaki sarcomsa were positive in 4 out of 51 trials. It is probable that most of the isolated cells were not viable, that they were lost or injured during the process of manipulation, and that they did not have the capacity as the stem-cells to grow autonomously. It is of noteworthy evience that four successful transplantations were made all in the T-strain rats which particularly adapted for the transmission of the stock tumor.

Cytological observations revealed that the four tumor sublines established through single-cell inoculation were characterized in each by neoplastic populations provided with tumor stem-cells of a pure single-cell type ; two of them were found to be the 3V-type cell clones while the other two were assumed as the 2V-type cell clones. These single-cell clones were comparable to the original stock tumor in malignant growth as well as in the type of the disease.

By way of summary, it can be said that the isolation experiments with the Hirosaki sarcoma were resulted in the production of pure single-cell clones isolated from the original stock tumor of a mixed-cell type. Each clone is characterized by a stemline ideogram which is structurally distinct from those of the other clones and has persisted without visible change during certain transplant generations. All cytological features derived from this study seem to strengthen the concept of stemline-cells from which the tumor develops itself. The stem-cell concept has been supported by Levan and Hauschka (1953) and Hauschka (1953) based on their detailed cytological investigations of mice tumors.

The detailed quantitative data together with the experimental procedures and discussion will be published in Journ. Natl. Cancer Inst., Vol. 15 (1955 April issue) in the joint name with Dr. Makino.

Cooperation of Professor S. Makino with important discussion, and stimulating criticisms given by Mr. T. Tanaka were gratefully acknowledged here.

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