The data at hand derived from some recent investigations of the chromosomes of human tumors have shown that the most frequently occurring tumor cells which have a characteristic number mode (or modes) along with a particular chromosome ideogram (or ideograms) form a stem-line (or -lines) of tumor cells which are the primary progenitors of growing neoplasms, in parallel with the evidence presented by the chromosome analysis of rat and mouse ascites tumors (Hasen-Melander, Kullander & Melander 1956, Levan 1956, Ising & Levan 1957, Manna 1957, Wakabayashi & Ishihara 1958, Makino, Ishihara & Tonomura, 1959). Since the chromosome conditions of human tumors are important in the consideration of the general clinical and pathological properties of neoplasms, the karyological data of human tumors have strikingly aroused a great deal of interest in the medical field.

The present paper reports the results in some detail of a chromosome analysis in tumor cells of a human gastric carcinoma in ascites form, with special regard to certain chromosomal features in relation to the therapeutic data.

The author wishes to tender his expression of sincere gratitude to Professor Masaru Wakabayashi and Professor Sajiro Makino for their direction and valuable advices. Further cordial thanks are offered to Dr. Kouichi Kaneta, Department of Radiology, Sapporo Medical College, for the material with which the present study was carried out.

Method: The ascites material for study was obtained from a patient with a gastric carcinoma in the Hospital of Sapporo Medical College. The tumor cells were obtained by centrifuging the ascites for ten minutes at 700 r.p.m. They were smeared on slides and stained with acetic dahlia according to the water pretreatment method (Makino 1957).

Records of the tumor patient: The patient who provided the present material was a man, 62 years old. On the 20th of July, 1957, he was diagnosed to have a gastric carcinoma, and laparotomy was performed on the 31st. On the 10th of December 1957, metastasis of the tumor was observed on the operated portion. Radiation therapy was carried out with 5400r irradiation, from February 7 to March 9, 1958. In the middle of February the patient showed a considerable amount of ascites. The ascites was taken out by paracentesis on the 8th of March. Microscopical examinations of the ascites showed a large number of dividing tumor cells. On the 17th of March, another metastatic tumor was found in the lower abdomen. The X-ray therapy was done toward the metastatic tumor by spot irradiation with 3400r during a period from
March 24 to May 7. The patient died on the 25th of May. No histological diagnosis of this tumor was made since no autopsy was carried out.

**Observations**

1) **Tumor cells**: The peritoneal fluid contained a large number of tumor cells of various sizes, their shape being round or oval. The mitotic rate of tumor cells was about 2.0 percent. Most of the mitotic cells were characterized by normal mitotic behavior, though a few showed mitotic abnormalities such as stickiness and coalescence or condensation of chromosomes.

2) **Chromosome number**: For the chromosome counting, 201 good metaphase plates which showed clear chromosomes available for counting were selected from three different samplings. The results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Chromosome number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56</td>
</tr>
<tr>
<td>(I) March 7</td>
<td>1 1 4 8 7 11 23 5 1 3 2 1 2 2</td>
</tr>
<tr>
<td>(II) March 24</td>
<td>1 1 1 6 28 3 5 9 4 1 1 1</td>
</tr>
<tr>
<td>(III) May 10</td>
<td>2 2 7 2 2 1</td>
</tr>
</tbody>
</table>

Table 1. Chromosome number distributions in three different samplings in a patient with a gastric carcinoma.

In the first sampling made on March 7 (1958), the chromosome number exhibited a wide range of variation from 35 to 94, showing two definite modes: one modal range fell between 41 and 48, the modal number being 45 (28 percent), while the other between 80 and 84 (16.0 percent).

In the second sampling on March 24, the chromosome number varied from 26 to 95, showing two modes as in the first sample. In this sampling, however, the tumor cells with 45 chromosomes (45–cells) which ranked first in the first sample, showed remarkable decrease (10.8 percent), whereas the tumor cells with 42 chromosomes (42–cells) were observed most frequently (34.1 percent). The tumor cells with 80–84 chromosomes (80–84 cells) were observed to number about the same as in the first sampling (13.09 percent).

In the third sampling on May 10, the chromosome number varied from 40 to 60. As in the second sample, 42–cells occurred with the highest frequency (31.2 percent), while the 45–cells appeared with such a low frequency as 10.7 percent.
On the basis of the above results, it is possible to state that there are in this tumor three cell-populations which form three cell-lines, two at near-diploid and one in the range from hypertriploid to hypotetraploid.

3) **Chromosome ideogram**: The chromosomes were morphologically classified into three groups according to Tjio and Levan (1956) as follows: M (the chromosome with a median-submedian centromere), S (the one with a subterminal centromere) and T (the one with a terminal centromere). The individual chromosomes in each group were arranged in order of size.

Ideogram analyses were made in eight ideal metaphase plates of 42 and of 45 cells, and in five of 80-84 cells.

**42-cell**: The length of chromosomes of 42-cells fell mostly between 2 and 11 µ, the largest being 15 µ. The 42-cell was composed of approximately 17 M, 21 S and 4 T chromosomes in a formula, $17M + 21S + 4T$ (Figs. 1, 2, 9, 10, 11). Every 42-cell in three different samplings showed quite identical chromosome patterns.

**45-cell**: In the 45-cell plates chromosomes varied from 1.9 µ to 13.5 µ in length. Chromosomes from No. 1 to No. 4 were rather large over 10 µ long. The ideogram was made up of approximately 21 M, 18 S and 6 T chromosomes showing a formula, $21M + 18S + 6T$ (Figs. 3, 4, 12, 13, 14).

**80-84-cell**: The 80-84-cell was constituted by about 34 M-chromosomes, 40-42 S-chromosomes and 8 T-chromosomes having a formula of $34M + 40-42S + 8T$. It is evident that the 80-84-cell has almost twice as many chromosomes as those of the 42-cell (Figs. 5, 6, 15, 16).

Thus it was found that each stem-line had its own characteristic chromosomal complex clearly different from each of the others. None, of course, had any similarity.
to human normal chromosomes in number and morphology, which, according to Tjio and Levan (1956) and Makino and Sasaki (1959), are 46 in number and consist of 20 M, 20 S and 6 T chromosomes.

An interesting feature was presented that in some cells the chromosomes showed a sign of endoreduplication (Figs. 7, 8). Such cells were observed in about 2 to 3 percent of mitotic cells under study. In reference to the fact that the 80-84-cell has a constitution which is approximately double that of the 42-cell, it seems very probable that the former might have been produced by means of endoreduplication from the 42-cell. Levan and Hauschka (1953) reported in several mouse ascites tumors that some hyperploid tumor cells were formed by endoreduplication of chromosomes.

**DISCUSSION**

The results of the present observations have shown clearly that the gastric carcinoma here considered is characteristic in having three populations of tumor cells, two at near-diploid and one in the range from hypertriploid to hypotetraploid; they show a high frequency in occurrence and possess a particular chromosome pattern in each. In parallel with the conclusions derived from rat and mouse tumors, it seems likely that the three populations of tumor cells represent three stem cell lines.

Figs. 15-16. Serial alignment of chromosomes in 80-84-cell line of a human gastric carcinoma. Fig. 15; 84-cell. Fig. 16; 82-cell.
in separate existence which serve as primary progenitors in growth of this tumor. It seems probable that two cell-lines consisting of 42-cells and 45-cells are the principal lines. The line of 80-84-cells which is less remarkable than the above two is regarded as a supplementary stem-line, since the cells with 80-84 chromosomes seem to be produced through endoreduplication of chromosomes in the 42-cell.

It is of great interest to find that the two cell-lines, 42-cells and 45-cells, showed different frequencies in three samplings: 45-cells occurred with the highest frequency in the first sampling, whereas in the second and third samplings, 42-cells appeared most frequently. The 42-cells which appeared in the second and third samples seem not to be new products, since they were quite the same in chromosome constitution in all three samplings. The evidence presented may be reasonably explained as due to adaptability of the two cell-types to the physiological conditions of the patient: the 42-cells are probably more adaptable than the 45-cells to a new condition of the patient caused by the X-ray therapy.

In the Hirosaki sarcoma Makino and Kanô (1953) have reported the occurrence of five types of tumor cells which vary in frequency with the increase of transfer generations. Watanabe and Tonomura (1955) have found in the Watanabe ascites hepatoma that there are three stem-lines which show a change of frequency during transfer generations. Further, Hasen-Melander, Kullander and Melander (1956) working on a human ovarian cytorecacinoma have reported a change of frequency in two types of stem-line cells in different samplings.

It is interesting that both the 42-cells and 45-cells are characterized by some large chromosomes, 10 to 15μ in length: especially, No. 1 to No. 4 chromosomes of 45-cells measure over 10μ in length. The largest chromosomes of the human normal cell was reported to be 8 to 10μ by both Tjio and Levan (1956) and by Chu and Giles (1958).

In addition to the difference in length of the chromosomes, three types of stem-cells greatly differ in both chromosome number and constitution from normal somatic cells. Each type possesses its own characteristic constitution of M-, S- and T-chromosomes completely different from each other. Thus it appears that, together with the numerical changes, considerable structural and mutational changes might have taken place in the tumor chromosomes in relation to the development of the tumor. Such a cytological situation as observed in this gastric carcinoma is comparable to that found in certain transplantable animal tumors (Makino 1957).

**SUMMARY**

The present paper describes the results of a chromosome analysis in tumor cells of a human gastric carcinoma. The samples for study were obtained from the peritoneal fluid of a patient.
It was found that there were present in this tumor three populations of tumor cells which were characterized by the chromosome numbers of 42, 45 and 80-84, each showing a particular chromosome pattern and a high frequency in occurrence. They are regarded as stem-lines of tumor cells which contribute principally to the growth of this tumor: the lines formed by 42- and 45-cells are considered to be principal lines and the line of 80-84 cells is supplementary.

Evidence was obtained that the frequency in occurrence of the two principal stem-lines varied in three samplings made on different dates. The variation seems explicable as due to the adaptability of the cell-types to certain physiological conditions of the patient.

References


Explanation of Plate XXVIII

Figs. 1-6. Microphotographs of chromosomes of tumor cells in a human gastric carcinoma. Figs. 1-2; 42-cell line. Figs. 3-4; 45-cell line. ×1600. Figs. 5-6; 84-cell line. ×1150.

Figs. 7-8. Microphotographs indicating endoreduplication of chromosomes. ×1600.