THE CYTOLOGICAL EFFECT OF CHEMICALS ON TUMORS.\(^1\)

VIII. OBSERVATIONS ON CHROMOSOMES IN A GASTRIC CARCINOMA TREATED WITH CARZINOPHILIN\(^2\)

(Plate I)

AKIRA TONOMURA
(Zoological Institute, Hokkaido University, Sapporo)

Cytological investigation inquiring into the action of anti-tumor agents upon tumor cells is one of the important challenges in an analysis of the fundamental problems of cancer chemotherapy. Many ascites tumors of rats and mice have been used for this purpose with interesting results for understanding of the mechanism of tumor-cell damage.

Recently several anti-tumor agents have been in practical use for tumor patients. Many clinical and chemotherapeutic reports have been published by medical investigators, but there are few from the cytological viewpoint on the effect of the anti-tumor agents in human tumors, due probably to the technical difficulties in handling of human tumor cells.

Current studies of chromosomes in several human tumors have made possible the morphological analysis of chromosomes to a considerable extent (Ising and Levan 1957, Makino, Ishihara and Tonomura 1959). Carzinophilin, one of the antibiotics (Hata, Koga, et al. 1954) was found to be rather effective for certain human tumors. The present author has an opportunity to study the cytological effect of Carzinophilin on a gastric carcinoma with special reference to the effect on the chromosomes of the tumor cells.

The author wishes to express his cordial thanks to Professor Sajiro Makino for his kind direction and for improvement of the manuscript for publication. The author is also indebted to Dr. Y. Okuda, Department of Surgery, Hokkaido University Hospital, for the collection of the material for study.

Material and Methods: The patient bearing a gastric carcinoma was a woman, 46 years old. The tumor was first found in her stomach by the clinical examination

---

\(^1\) Beginning with this article and hereafter, "tumors" will be used in the main title of this series of studies in order to include various kinds of neoplasm, because the term "ascites sarcomas" previously used indicates tumors of limited sorts.

\(^2\) Contribution No. 458 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Supported by a grant-in-aid to Dr. S. Makino from the Scientific Research Fund of the Ministry of Education.
at the Hokkaido University Hospital in January 1959. The tumor growth was rather active, showing metastasis to many parts of the stomach, and producing a rich serous fluid in the patient’s peritoneal cavity. Histological examination of the tumor proved it to be a typical carcinoma simplex. During the period from the 16th of February to the 9th of March, the patient was treated with 2,000 to 5,000 units of Carzinophilin, every day or every other day. Finally she received 99,000 units of Carzinophilin in total amount. Before the first sampling for this study which was made on 2nd March, this patient had received 55,000 units of Carzinophilin. The 2nd sampling was made on the 14th March, being 5 days after the final treatment with Carzinophilin. The 3rd sampling was made on the 19th March and the 4th on the 1st April. The patient died on the 10th April. The details of sampling data are given in Table 1.

<table>
<thead>
<tr>
<th>Data</th>
<th>Carzinophilin treatment</th>
<th>Total amount of Carzinophilin</th>
<th>Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 11</td>
<td></td>
<td></td>
<td>(operation)</td>
</tr>
<tr>
<td>Feb. 16</td>
<td>2,000</td>
<td>2,000</td>
<td>1st sampling</td>
</tr>
<tr>
<td></td>
<td>:</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td>March 2</td>
<td>5,000</td>
<td>55,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>:</td>
<td>:</td>
<td>2nd sampling</td>
</tr>
<tr>
<td>March 9</td>
<td>5,000</td>
<td>99,000</td>
<td>3rd sampling</td>
</tr>
<tr>
<td>March 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 10</td>
<td></td>
<td></td>
<td>(died)</td>
</tr>
</tbody>
</table>

The peritoneal fluid with suspended tumor cells drawn directly from the patient into small bottles was centrifuged for 4 minutes at 1,200 rpm. in order to collect tumor cells. The preparation for the chromosome study was made according to a water pre-treatment squash method with acetic dahlia staining. For details one may refer to the paper published by Makino, Ishihara and Tonomura (1959).

**Observations**

1. Frequency of the tumor cells below and above tetraploidy.

The observations revealed that the chromosome number varied within a considerably wide range from diploidy to over octoploidy in the material from earlier samplings (Fig. 6). Frequencies of the cells with less and more than 92 chromosomes were observed and summarized in Table 2. The cells with chromosome numbers over tetraploidy occurred at 57.1 per cent in the first sampling (Fig. 7), with those below tetraploidy occurring at 42.9 per cent. The 2nd and 3rd samplings gradually decreased in cell-populations. In the 4th sampling the cells over tetraploidy disappeared in the peritoneal fluid, while in striking contrast, those below tetraploidy showed a gradual increase. The data are given in summary in Table 2. The distribution of
chromosome numbers in the cells below tetraploidy is to be discussed in some detail below.

2. Abnormalities in the chromosomes.

Morphological response of the tumor cells to Carzinophilin resulted in the production of many kinds of chromosomal abnormalities. These abnormalities were observed rather frequently in highly ploid cells. Frequently the chromosomes showed irregular elongation into atypical chromonemata (Fig. 8). In some chromosomes, the elongation appeared in a part near their centromere. Chromosomes with a bead-like appearance were also found in many highly ploid cells (Fig. 9). Stickiness and coalescence of chromosomes and chromatid breaks were rather common in the tumor cells of various types. Further, chromosomes showing somatic translocation of various types were observed in the first sample (Figs. 10-11).

It is interesting to note that similar types of chromosomal abnormalities were found by Hori and Sasaki (1958) to occur in normal and neoplastic cells in vitro following treatment with Carzinophilin, and by Awa (1958) in the MTK-sarcoma III of rats after Carzinophilin treatment.

3. Chromosome-number distribution in the cells below tetraploidy.

Exact counting of chromosome numbers was made in the cells with chromosomes less than 92 in number which were derived from four samplings: 39 cells were counted in the 1st sampling, 16 cells in the 2nd, 29 cells in the 3rd, and 34 cells in the 4th. Cells under study were 118 in total.

As given in Figure 1, the chromosome number scattered within a wide range from 39 to 90, with the most frequent value lying in near-triploid regions (Figs. 12-15). In every sample, the chromosome-number distribution was characterized without exception by a marked peak at 62. As already mentioned, the 1st sample was taken on the 2nd of March from the patient while Carzinophilin was being applied, whereas the 2nd to 4th samplings were made after the stoppage of the treatment. All the samples here examined showed tumor cells remarkable for their showing of a distinct stem-line number of 62. The situation here presented is sufficient to indicate that the stemline chromosome-number has persisted without change during the period when

<table>
<thead>
<tr>
<th></th>
<th>&gt; ±4 n</th>
<th>&lt; ±4 n</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st sampling</td>
<td>39 (42.9%)</td>
<td>52 (57.1%)</td>
<td>91</td>
</tr>
<tr>
<td>2nd *</td>
<td>16 (59.3%)</td>
<td>11 (40.7%)</td>
<td>27</td>
</tr>
<tr>
<td>3rd *</td>
<td>29 (76.3%)</td>
<td>9 (23.7%)</td>
<td>38</td>
</tr>
<tr>
<td>4th *</td>
<td>34 (100%)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>72</td>
<td>190</td>
</tr>
</tbody>
</table>
the four samplings were made.


As stated above, the distribution of the chromosome-number of this tumor was characterized by a hypotriploid cell with 62 chromosomes in each sampling. Idiogram analysis was made in seven cells having distinctly 62 chromosomes. The idiograms analysed are presented in Figures 2 and 3, and in Figures 4 to 5. According to Tjio and Levan (1956), the chromosome complements were classified into three groups as follows; M type with median or submedian centromere, S type with a subterminal centromere, and T type with a terminal centromere. In each type, the individual chromosomes were arranged in order of their decreasing sizes. Idiogram analysis indicated that every cell with 62 chromosomes contained 29 M-type chromosomes decreasing from 6\(\mu\) to 1\(\mu\) in length, 26 S-type chromosomes ranging from 5\(\mu\) to 1\(\mu\), except the longest one which was about 7\(\mu\) in average length, and the remaining 7 T-type chromosomes varied in length from 3\(\mu\) to 1\(\mu\). The longest S type chromosome shows a ratio of long arm to short arm at 3 to 1. It is evident that the stemline idiogram of this tumor was represented by a formula, 29 (M)+26 (S)+7 (T).

The stemline idiogram of this tumor was marked specially by the presence of an outstandingly long S-type chromosome. The existence of a similar S-type chromosome...
has been noted in a mammary carcinoma (Wakabayashi and Ishihara 1958) and in a gastric carcinoma (Makino, Ishihara and Tonomura 1959).

DISCUSSION

The present investigation after the application of Carzinophilin showed the chromo-
some-number distribution to cover wide range of variation from diploidy to high polyploidy in earlier samplings. Unfortunately the chromosomes were not observed in the samples before the Carzinophilin-treatment, but it is most probable that the drug-application caused the production of mitotic irregularities of various types, together with the appearance of highly ploid cells. Carzinophilin-treatment in normal and neoplastic cells in tissue culture by Hori and Sasaki (1959), and in the MTK-sarcoma III of rats by Awa (1958) was observed to produce mitotic abnormalities of a similar type.

Working on the effect of podophillin on rat ascites tumors, Makino and Tanaka (1953a) reported that some of the tumor cells remained unaffected and formed a source of a renewed malignant growth after their proliferation. A similar situation was found to occur in several other rat ascites tumors following the application of anti-tumor agents (Makino and Tanaka 1953b, Tanaka, Kano, et al. 1955, Sasaki 1956, Awa 1959).

In the present study, exact counting of the chromosome numbers particularly in tumor cells below tetraploidy showed a variation ranging from 39 to 90, with a modal ploidy in the near-triploid region. In every sample herein examined, the modal chromosome number was characterized by a distinct stemline number of 62. This indicates that the stemline cells of this tumor have persisted without change during four successive samplings here undertaken. Further, the stem-cells characterized by 62 chromosomes gradually increased in number in the 3rd and 4th samples which were obtained after the stoppage of Carzinophilin-treatment. The evidence here presented implies that the stemline cells of this tumor have remained unaffected by the drug and formed a cause of tumor growth through their proliferation.

**SUMMARY**

Chromosomal conditions in a human gastric carcinoma treated with Carzinophilin were observed in the present study. Distribution of chromosome numbers in four successive samplings showed a wide range of variation from hypodiploidy to above octoploidy. Various chromosome abnormalities were produced, especially in highly ploid cells.

In every sample observed here, the chromosome-number distribution was characterized by a marked mode at 62. It seems probable from the above evidence that the stemline cells with 62 chromosomes have remained unaffected by the drug, maintaining their individuality through the Carzinophilin-treatment.

**REFERENCES**


———. 1953b. The cytological effect of chemicals on ascites sarcomas, II. Selective damage to tumor cells by CaCl₂, AlCl₃ and H₂O₂. Gann 44: 39-46.


EXPLANATION OF PLATE I

All figures are photomicrographs of the chromosomes in a human gastric carcinoma.

(Figures 1~5 in the text).

Fig. 6. Tumor cells with various ploidy in the peritoneal fluid. ×140.

Fig. 7. Tumor cells with over 100 chromosomes. ×350.

Fig. 8. Chromosomes showing irregular elongation of the chromonemata. ×1200.

Fig. 9. Chromosomes with a bead-like appearance. ×1200.

Fig. 10. Somatic translocation of chromosomes. ×2500.

Fig. 11. Traces of Fig. 10.

Figs. 12-15. Metaphase chromosomes. ×1000.
