CHROMOSOMAL ALTERATION AND DEVELOPMENT OF TUMORS
II. CHROMOSOME CONDITIONS AND INVASIVENESS OF
THE YOSHIDA SARCOMA CELLS1)

TOSIHIDE H. YOSIDA
(National Institute of Genetics, Misima)

A new subline of the Yoshida sarcoma which is characterized by the presence of
three strikingly large chromosomes arose spontaneously in the course of transplanta-
tion (Yosida 1957, 1958 a, b). The cells of the Yoshida stock tumor have 40 chromo-
somes among which two large V-shaped elements are remarkable (Fig. 1), while the
new subline possessing also 40 chromosomes, differs from the stock tumor by the
existence of a conspicuously large J-shaped element in addition to two large V's
(Fig. 2). On the basis of the karyological analysis, it is assumed that the new
caryotype was originated by reciprocal translocation (or translocations) between two
chromosomes of the original tumor (Yosida 1959).

The tumor cells of the new karyotype increased in number with the increase of

1) Contribution from the National Institute of Genetics, Japan, No. 279.
transfer generations. After about the twentieth transfer generation, almost all tumor cells showed the new karyotype (Table 1). At the ninth transplant generation of this sarcoma, the ratio of cells having the new karyotype to the total number of tumor cells was 79.9 per cent in the ascites form.

It is well-known that malignant tumor cells generally invade various organs of the tumor-bearing host. If both kinds of tumor cells, those with the new as well as those with the original karyotype, invaded the organs in the same ratio as that observed in the ascites form, their frequency should be similar. The results of observations showed that the ratio of both karyotypes in the organs was quite different from that in ascites form and strikingly varied accordingly to the organs examined.

The present paper deals with the results of some cytological observations with special reference to the invading ability of tumor cells with different chromosome constitutions, and describes the technique for study of tumor cells invading various organs.

The author wishes to acknowledge his indebtedness to professor Sajiro Makino, Hokkaido University, for his keen interest in this investigation and his kind revision of the manuscript.

TECHNIQUE

The stock line of the Yoshida sarcoma from which the new subline was derived was obtained from the Institute for Infectious Diseases, Tokyo. The tumor was transferred serially from rat to rat in the National Institute of Genetics, Misima (for details, see Yosida 1959).

To observe the chromosomes in invading tumor cells is difficult because of the small number of tumor cells. Since a large number of mitotic cells is needed for their statistical study, small pieces of various organs containing tumor cells were removed from tumor-bearing rats and inserted into peritoneal cavities of new hosts. This method made possible a proliferation of tumor cells in the peritoneal cavity forming a new cell-population in ascites form. In this way, three ascites populations in the tenth transfer generation were obtained from inoculations of liver, lung and spleen of a tumor-bearing rat in the ninth transfer generation (Fig. 3). On the other hand, a tumor mass of the Yoshida sarcoma has developed in the peritoneal cavity of the same tumor-bearing rat mentioned above. By inoculation of small pieces of the tumor mass into the peritoneal cavities of new hosts a cell population of ascites form newly developed. As control experiment, ascites tumor cells were transferred into intraperitoneal cavities of new hosts by usual transfer technique.

A practical method for organ transplantation may be given as follows: Small pieces of liver, lung, spleen and a tumor mass of a tumor-bearing rat were washed three or four times with normal saline containing peniciline and streptomycin in order to wash down the tumor cells adherent to the surface of the organs as completely as
possible. Then small pieces of the organs were inserted into the peritoneal cavities of non-tumorous animals with the aid of a transplantation needle.

Ascites tumor cells were observed by acetic orcein squash technique after treatment with hypotonic solution.

RESULTS OF OBSERVATIONS

1) Control (observations in the ascites tumor cells developed by usual transfer technique): Observations at the ninth transfer generation of the ascites tumor of a usual form showed that ratio of the new karytype cells to the total number of tumor cells was 82.6 per cent. The data shown in Table 1 and Fig. 3 indicate that the ratio of the new tumor cells gradually increased with the increase of transfer generations. This seems to imply that the tumor cells of the new karyotype are superior in adaptability to the original cells.

2) Observation in ascites tumors obtained from the inoculation of a solid tumor: A small piece of tumor mass which developed in the tumor-animal was inserted into peritoneal cavities of new rats. Out of three rats thus treated, two developed an ascites tumor in their peritoneal cavities. The karyological examinations of these tumors showed that 95 per cent of tumor cells under study represented the new karyotype. The results show that the vast majority of tumor cells forming the solid tumor used for inoculation were of the new karyotype.

3) Observations of ascites tumors induced by liver inoculation: Two or three pieces of liver tissue derived from a tumor-bearing animal were inoculated into peritoneal cavities of three non-tumorous rats, one of which developed an ascites tumor. It is most probable that the ascites tumor thus produced arose by proliferation of
tumor cells which invaded the liver. 90 per cent cells in this tumor showed the new karyotype, while about 10 per cent were of the original karyotype.

Table 1. Occurrence of the new karyotype in several transfer generations of the Yoshida sarcoma.

<table>
<thead>
<tr>
<th>Transfer gener.</th>
<th>No. of cells of new karyotype</th>
<th>No. of cells of original karyotype</th>
<th>No. of cells observed</th>
<th>% of new karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>25</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>62.5</td>
</tr>
<tr>
<td>VI</td>
<td>12</td>
<td>17</td>
<td>29</td>
<td>41.4</td>
</tr>
<tr>
<td>VII</td>
<td>37</td>
<td>24</td>
<td>61</td>
<td>60.7</td>
</tr>
<tr>
<td>IX</td>
<td>119</td>
<td>25</td>
<td>144</td>
<td>82.6</td>
</tr>
<tr>
<td>X</td>
<td>96</td>
<td>12</td>
<td>108</td>
<td>88.9</td>
</tr>
<tr>
<td>XI</td>
<td>106</td>
<td>30</td>
<td>136</td>
<td>77.9</td>
</tr>
<tr>
<td>XIII</td>
<td>61</td>
<td>7</td>
<td>68</td>
<td>89.7</td>
</tr>
<tr>
<td>XV</td>
<td>99</td>
<td>11</td>
<td>110</td>
<td>90.0</td>
</tr>
<tr>
<td>XX</td>
<td>185</td>
<td>0</td>
<td>185</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>720</td>
<td>154</td>
<td>874</td>
<td></td>
</tr>
</tbody>
</table>

tumor cells which invaded the liver. 90 per cent cells in this tumor showed the new karyotype, while about 10 per cent were of the original karyotype.

Table 2. Frequency of tumor cells having the new karyotype in ascites tumor which developed by organ transplantation.

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of new tumor cells</th>
<th>No. of original tumor cells</th>
<th>Total no. of cells observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid tumor</td>
<td>38(95%)</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Lung</td>
<td>15(33.3%)</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Spleen</td>
<td>1(2.6%)</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Liver</td>
<td>36(90%)</td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

4) Observations of ascites tumor induced by lung-and spleen-inoculation: Small pieces of lung and spleen tissues were inoculated separately into peritoneal cavities of 5 rats. One rat with the lung-inoculation and another with the spleen-inoculation developed ascites tumors. The karyological study of cells of those tumors produced the surprising result that 33.3 per cent of tumor cells from lung-inoculation, and 2.6 per cent from spleen-inoculation were of the new karyotype. Based on these results, it is reasonable to conclude that the majority of tumor cells which invaded the lung and the spleen had the original karyotype.

Remarks

Invasion of organs of tumor-bearing animals by tumor cells is a subject of primary importance in cancer cytology. It was found that malignant cells can invade any place in the body through transportation by the blood system. Yoshida (1949) reported that the Yoshida sarcoma infiltrates every organ of the tumor-bearing rat.
Observations of the chromosome condition of the invading tumor cells indicate that the success of inoculation seems to differ according to their chromosomal pattern. It seems that tumor cells with the new karyotype show a predominant peritoneal proliferation in an ascites or a solid form and a particular adaptability to liver tissue, while those having the original karyotype are well-adapted to spleen and lung tissues.

In the chromosomal studies of human cancers Yosida and Tabata (1958) and Tabata (1959) reported that the majority of the tumor cells of the primary stomach cancer had hypotriploid and hypopentaploid chromosome constitutions, whereas the tumor cells of the maxillary tumor which was based on a metastasis of stomach cancer showed a hypodiploid chromosome constitution. The evidence of the difference in chromosome pattern between the maxillary and the primary stomach cancer indicates that a selective adaptation takes place in metastatic cells. A similar concept of selective adaptation of tumor cells could be applied in the case of the Yoshida sarcoma involving the difference in adaptability of tumor cells to particular tissues.

The relationship between the chromosomal pattern of tumor cells to their infiltration-ability may be important in connection with the problem of metastasis.

**SUMMARY**

The results of observations of chromosomes in tumor cells invading various organs of a Yoshida sarcoma rat were described in the present paper.

New tumor cells of the Yoshida sarcoma which possess a prominently large J-shaped element have developed in the course of transplantations at Misima. These tumor cells gradually increased in number during the course of ensuing generations. In the process of establishing the new tumor subline two types of cells, with the new and the original karyotypes, were observed. Using this tumor material, the facility of invasion of various organs by the tumor cells with two kinds of karyotypes, the new and the original, was examined. The results of observations indicate that infiltration-ability of tumor cells into tissue differs according to their chromosomal pattern. The tumor cells with the new karyotype showed a predominant peritoneal proliferation in an ascites or solid form and a particular adaptability to liver tissue, while those having the original karyotype were well-adapted to spleen and lung tissues.

**LITERATURE**

— 1958 a. Idiogram of a new subline of the Yoshida sarcoma and some its properties. Ibid. 8: 24-25.
— 1958 b. A karyological study on the Yoshida sarcoma cells infiltrated into organs of a
tumor-bearing rat. Ibid. 8: 25.