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Transformation of a rat hepatoma into an ascites tumor

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In a broad sense it can be said that many of the questions underlying cancer problems are connected, directly or indirectly, with anything relating to the mechanism of cell division in their fundamental aspects. Especially the question of the origin of cancer may be interpreted to a considerable extent by inquiring into the behaviour or the karyological changes occurring in cells during the course of carcinogenesis, though the origin of cancer cannot be sought in a single cause. However, cytological knowledge of cancer is meagre at present. Cytological study of the tumor is thus primarily required before one can undertake the causal analysis of many problems remaining unexplored.

With this thought in mind, the senior author, Tanaka, has engaged during recent years in the study of the mechanism of chromosomal changes taking place in rat liver cells during the course of carcinogenic experiments. This has been done by applying o-Aminoazotoluene. It is of prime importance to study how change takes place in hepatic cells during carcinogenesis. For this purpose, solid tumor tissue such as hepatoma is not fully suitable due chiefly to technical difficulties in handling. It would be of great value if the solid hepatoma could be transformed into a fluid tumor. Accordingly, several attempts have been made by the authors to transfer the rat hepatoma into a fluid form, and finally success was attained in producing an ascites hepatoma growing in the peritoneal cavity of white rats. The present paper describes the procedure of experiments together with the general features of the ascites hepatoma thus produced.

Here it is the authors' pleasant duty sincerely to acknowledge their great indebtedness to Professor Sajiro Makino for his kind guidance and suggestions given during the course of this work.

Procedure of experiments and the outline of development

Fifty white rats (Rattus norvegicus), descendants from the Wistar strain, were administrated with p-Dimethylaminoazobenzene for 100–
120 days according to the method advised by Harada et al. (1937). By 100 days after beginning of treatment, a remarkable development of malignant hepatoma was induced in the liver of each of the animals. In the course of the development a part of the dividing tumor cells was found removed from the liver into the peritoneal cavity (Figs. 1–2). This situation suggests the possibility of the transformation of the solid hepatoma into an ascites-tumor of fluid form. In view of this possibility the following experiments were made with these hepatoma as material.

A part of hepatoma was removed from the rat and cut fine with scissors. Then about 0.5–0.7 cc of the hashed tissue thus made was inoculated in the peritoneal cavities of each of ten healthy rats. After inoculation, microscopical examinations of the peritoneal fluid were made by smear at one day intervals to see the developmental condition of hepatoma cells after injection. At about one day after injection, a hemorrhagic exudation was formed in all specimens here treated. The exudation, about 0.5 cc in volume, contained a number of inflammatory cells. They could be distinctly differentiated from cells of the peritoneal cavity by application of acetic-gentianviolet (Tanaka 1951), acetic-dahlia¹ and acetic-orcein. Many polymorphonuclear leucocytes, lymphocytes, histocytes and mesothelial cells were observed (Fig. 4). Intermingled with the inflammatory cells, tumor cells forming islands, provided with many mitotic figures are detected in the preparations (Fig. 3). By three days after inoculation, the cellular activity in the peritoneal fluid had become gradually inconspicuous. Afterward, two out of ten animals here treated showed a hemorrhagic exudation in their body fluid. On the 28th day after inoculation, there appeared solid masses of tumor cells with hemorrhagic fluid in the body cavities of these two animals.

Then, about 0.5 cc of the hemorrhagic fluid taken from these animals was injected into each of seven healthy animals. Two out of such treated specimens again developed solid masses of tumor cells in their peritoneal cavities. In their body cavities they showed again the formation of exudation, quite similar in visible nature to that observed in the first transplant experiment. The peritoneal cavities of these animals were provided with a considerable amount of hemorrhagic ascites in which were present a number of tumor cells of characteristic shape and size. These tumor cells were clearly distinguishable from other peritoneal cells by their staining reaction and by the presence of peculiar nucleoli.

The hepatoma islands suspended in the ascites grew larger in size with time representing active proliferation of tumor cells. On the 17th

¹ Acetic Dahlia solution is prepared as follows: Dissolve 0.75 gr of dahlia in 100 cc of hot 30% acetic acid, then leave till cold and filter.
day after inoculation, one of the two rats died; there had occurred remarkable hepatoma islands in the hemorrhagic ascites of this rat. A small amount of the hemorrhagic ascites taken from this specimen was inoculated into the peritoneal cavities of six new rats. These specimens in each without exception produced a typical ascites tumor containing a number of characteristic tumor cells.

The ascites tumor thus developed has been successively transmitted without changing its characteristic nature from rat to rat. At present the successive transplantation has been continued for 26 generations. The authors call this strain of the tumor "Ascites hepatoma I" in the following.

The rat which has been subjected to peritoneal injection of this tumor dies in a period of from 15 to 20 days, showing striking proliferation of tumor cells in the ascites with its remarkable accumulation. In general features this tumor bears many points of analogy to the Yoshida sarcoma as well as to the MTK-sarcoma I and II (Tanaka and Kanô 1951). The tumor ascites is of milky or hemorrhagic appearance; 1 cmm of it contains approximately one million tumor cells showing active multiplication. It is noticeable that the tumor cells tend to form characteristic islands. The hepatoma islands were mostly composed of cancerous parenchyma cells, sometimes containing various sorts of exudate cells of normal appearance. By multiplication and crowding together of neoplastic cells suspended in the ascites fluid the hepatoma islands increase in number and size (Figs. 5-6). The tumor cells are very prominent by their larger size as compared with any other cells observable in the peritoneal cavity. The nuclei are of oval shape.

Table 1. Daily frequency of mitotic cells in a tumor rat. The percentage of dividing cells was calculated on the basis of 2000 cells per day in the observation through a transplant generation

<table>
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<tr>
<th>Days after transplantation</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of dividing cells</td>
<td>1.9</td>
<td>2.6</td>
<td>2.8</td>
<td>3.0</td>
<td>4.0</td>
<td>4.3</td>
<td>3.6</td>
<td>2.9</td>
<td>3.1</td>
<td>1.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.5</td>
</tr>
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</table>

Conspicuous nucleoli, generally one or two in number, are visible within the nucleus. Daily observations on the mitotic rate in hepatoma cells were carried out through the whole life span of certain tumor rats: namely two thousand tumor cells were observed every day through a transplant generation (15 days), and daily frequencies of all dividing cells in late prophase, metaphase, anaphase and telophase were calculated in percentage. The results are given in Table 1. Generally speaking, the number of dividing cells strikingly increases during the early part of a transplant generation, while the mitotic rate is seen at
Figs. 1-11. 1. A group of hepatoma cells containing a number of different normal exudate cells in the peritoneal fluid of the hepatoma bearing rat ($\times$400). 2-3. Mitotic figures of hepatoma cells in the peritoneal cavity of the hepatoma bearing rat ($\times$1000).
4. Inflammatory exudation and hepatoma cells into the peritoneal fluid, one day after inoculation ($\times$500). 5. Hepatoma islands, from the 5th transplant generation ($\times$400).
the highest frequency in the middle part, that is on the 5th or 6th day after transplantation. Then, it decreases gradually towards the latter part of the animal’s life. Various mitotic abnormalities such as reported for the Yoshida sarcoma and also for the MTK-sarcomas (Makino and Yosida 1951, Tanaka and Kanō 1951), were observable in this ascites hepatoma too. They represent stickiness and coalescence of chromosomes, lagging and non-disjunction of chromosomes, chromosome bridges, multipolar mitoses, variation of chromosome numbers and so on.

In the present ascites hepatoma the whole life span, extending from the first day of transplantation of tumor to the death of the host animal, is considerably longer than in cases of the Yoshida sarcoma and the MTK-sarcomas. Most of the tumor rats die at about 15 to 20 days after transplantation. There was no difference in susceptibility respecting either age or sex.

Chromosome observations were made in this ascites hepatoma. As was clearly demonstrated in the Yoshida sarcoma and the MTK-sarcomas (Makino 1951, 1952, Makino and Kanō 1951), the presence of a strain of tumor cells was established in this case also; the strain cells are characterized by their specific chromosome constitution composed of a certain number of both rod- and V-elements. Thus the chromosome set of this tumor differs greatly from the normal set of the host, white rats (Figs. 7–8). The results of cytological study of this tumor now in progress will be reported elsewhere in the near future together with other data involving general characters of this tumor.

Remarks

From the descriptions above presented, it is evident that the ascites hepatoma here described originates mainly from the hepatoma cells which had developed by the application of azo dye. Therefore, it is needless to say that the tumor cells multiplying in the ascites of the diseased rat are derivatives from the hepatoma. Generally, the hepatoma has proved unfavorable for cytological study because of its hard and though texture, which makes it difficult to mash or to separate into the constituent cells. Now the ascites hepatoma here established provides hepatoma cells which are freely suspended in the fluid; this condition serves to make the material advantageous for the study of cytology, especially for chromosome research.

In early development stages of this ascites hepatoma, many hepatoma islands, each represented by tumor cell masses, appeared in the

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Figs. 1-4 and 7, from acetic gentianviolet preparations. Figs. 5-6 and 9, from acetic dahlia preparations. Figs. 8, 10 and 11, from acetic orcein preparations.
peritoneal fluid of the host which had received injection. Through the repeated injections of them from rat to rat, the massive tissue of hepatoma becomes gradually separated into its component cells. They become active in their multiplication with successive transmissions from host to host, surpassing in activity all other cells existing in the peritoneal cavity.

The hepatoma island seems to consist of the neoplastic liver cells and endothelial cells of the liver. Yosida (1944, 1949) has presented the view that the Yoshida sarcoma which originally developed in a white rat arose from liver cells, since it appeared in the course of hepatoma-producing experiments with some carcinogenic chemicals. But, a question is now left open among pathologists whether the Yoshida sarcoma has been produced spontaneously or induced by carcinogens. From his histological study of hepatoma, Yoshida (1951) concluded that the Yoshida sarcoma may take their origin in the endothelial cell of the liver. This view of Yoshida is very interesting in connection with the formation of the ascites hepatoma here under consideration. In the present experiment, the hashed hepatoma was first inoculated in the peritoneal cavity of the rat and this leads finally to the formation of the fluid tumor. Thus the situation is very important in that it stands in close relation to the problem of the origin of the Yoshida sarcoma now in question.

**Summary**

With the purpose to study the transformation of the solid azo hepatoma of the rat into an ascites tumor in fluid form, the present experiment was undertaken. The procedure is as follows: The hepatoma tissue was cut fine with sharp scissors. The hashed tissue thus prepared was injected in the peritoneal cavities of some healthy rats. At about one day following treatment, the formation of a hemorrhagic exudation occurred in all specimens treated. The exudation contained a number of inflammatory cells. Intermingled with these inflammatory cells, there were formed many islands of cells due to crowding of tumor cells together with the accumulation of hemorrhagic ascites. The hemorrhagic fluid taken from these animals was injected into the body cavities of seven other rats. In two out of such treated specimens, there occurred again the formation of an exudation in their body cavities. In the peritoneal cavities of these animals there was found a considerable amount of hemorrhagic ascites in which are formed many islands consisting of hepatoma cells. A small amount of the hemorrhagic ascites removed from these specimens was inoculated into the peritoneal cavities of six rats. These specimens produced in each case a typical ascites tumor which contains a number of characteristic tumor cells.
The ascites tumor thus induced was called "Ascites hepatoma I". It is capable of successive transmission by intraperitoneal injection of the tumor ascites. The rat which has received the injection of this tumor dies at 15 to 20 days. In general characters this tumor shows many points of analogy to the Yoshida sarcoma as well as to the MTK-sarcomas.

References


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