NOTE

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DNA CONTENT OF RAT ASCITES HEPATOMA CELLS WITH HYPO- AND HYPER-DIPLOID NUMBER OF CHROMOSOMES*1,*2

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Comparative studies on the chromosomal features of 24 different lines of Yoshida ascites hepatoma in the rat demonstrated that they had aneuploid modal number of chromosomes and ideograms differing from each other, line to line. The chromosome pattern of each ascites hepatoma differed considerably from the somatic ideogram of a rat. The difference was due to numerical and structural changes of chromosome in tumor cells which might be associated with changed chemical structure and/or amount of chromosome substance such as deoxyribonucleic acid (DNA), in which the author had a particular interest.

A number of cytophotometric studies on the amount of DNA in individual cell nuclei were made, using Feulgen-stained materials of cancerous and normal tissues. The data of these studies indicated that the amount of DNA per nucleus in normal tissues was directly proportional to the chromosome number and the amount of DNA in each of cancerous and normal cells varied according to their growth cycle or metabolic difference. It was also shown that the DNA content per nucleus in cancer tissue was larger than in normal tissues. However, there are still little informations on the subject of whether any correlation exists between the amount of DNA per nucleus and chromosomal constitution in tumors. The present study deals with the modal chromosome number and DNA content of individual metaphasic nuclei in six different hypo- and hyper-diploid ascites hepatomas of the rat.

MATERIALS AND METHODS

Six different lines of the rat ascites hepatoma, including five Yoshida ascites hepatomas, AH-13, AH-130, AH-39, AH-414, and AH-7974, which are maintained by serial transfers in Donryu rats, and a line of the ascites form of Morris hepatoma in ACI/N rats, AH-3683, were used as materials.

For the determination of DNA amount in individual cell nuclei, Feulgen-stained smears were made with tumor ascites of each line and submitted to microspectrophotometric examination of Feulgen-stained nuclei. The specimens, most suitable to this Feulgen-microspectrophotometry, were prepared in the following manner.

1. Ascitic fluid was taken from animals bearing the ascites hepatoma 4~9 days after intraperitoneal transplantation with about 10⁷ tumor cells, smeared on a cover glass (0.13 mm in thickness), and air-dried.
2. Fixed in fresh Carnoy's solution (absolute ethanol: glacial acetic acid = 1:3) for 60 mins.
3. Washed in distilled water.
4. Hydrolysed with 1N HCl at 60° for 7 mins.

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*2 Major points of this paper were presented in the Symposium on Hepatomas held in Philadelphia, U.S.A., May 19~20, 1967.

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(5) Stained in glycine-buffered Schiff’s reagent at 18° for 4 hrs.
(6) Bleached with a mixture of 10% K$_2$S$_2$O$_5$ solution, 1N HCl, and distilled water (5:5:95), three times at 18° for 10 mins.
(7) Rinsed in distilled water.
(8) Dehydrated in graded alcohol series.
(9) Cleared in xylene and mounted in synthetic resin (Bioleit).
(10) Covered with a 0.13-mm thick coverslip.

Each smear of the ascites was kept together in a holder and submitted to each of these procedures simultaneously, in order to make each preparation under the same condition. For cytophotometry, a microspectrophotometer (Olympus, modified Type-IV) was employed. This model is characterized by the illuminating system which makes possible to illuminate an area of specimen only to be measured at each wave-length of monochromatic light, but the illumination is limited to a round area ranging from 0.9 to 89.5 µ in diameter. After measuring the transmission at each wave-length through a homogeneously stained part of interphasic nuclei, an absorption curve for Feulgen-dye was made, in order to evaluate a wave-length of the maximum absorbance and a wave-length corresponding to one-half the value of the maximum absorbance. Thus, two wave-lengths, 570 and 505 mµ, were evaluated. Mitotic nuclei of tumor cells in prometa- and meta-phase were measured exclusively after the two-wavelength method of Patau at 570 and 505 mµ. Computation of the amount of DNA in arbitrary unit was made by the use of Mendelsohn’s table.

For examination of chromosomes, tumor ascites was harvested from the ascites hepatoma-bearing animal 3 hrs. after an intraperitoneal injection of colchicine in a dose of 0.035 mg/kg. Then it was mixed with 0.03M saline solution and aceto-orcein stained squash preparations were made. Metaphase chromosomes were examined in each tumor line.

RESULTS AND COMMENTS

Table I indicates the mean value of one-half DNA content of individual mitotic nuclei in arbitrary unit in the six lines of ascites hepatoma. Mean value of DNA content of polymorphic leucocytes in the same specimens is also shown as a control. This table was made by taking into consideration the following two points. First, it is accepted that prometa- and meta-phase nuclei have twice the amount of DNA, compared to the DNA of G$_1$-phase nuclei. Therefore, one-half of DNA content of the mitotic nuclei was calculated and then a mean value was computed. Second, it is possible that nuclei of polymorphic leucocytes cannot enter the S-phase. Therefore, their DNA value was indicated as a control for the G$_1$-phasic diploid cell. The results demonstrate that each of the six different ascites hepatomas has a larger amount of DNA, compared to the leucocyte.

Fig. 1 shows the chromosome number of the six ascites hepatomas. Distinct mode is marked in each tumor line and modal number of chromosomes is 37 in AH-3683, 38 in AH-13, 43 in AH-130, 44 in AH-39, and 47 each in AH-414 and AH-7974, different hypo- and

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Amount of DNA (arbitrary unit)</th>
<th>No. of nuclei measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH-3683</td>
<td>1.46±0.48</td>
<td>94</td>
</tr>
<tr>
<td>AH-13</td>
<td>1.53±0.28</td>
<td>103</td>
</tr>
<tr>
<td>AH-130</td>
<td>1.30±0.27</td>
<td>105</td>
</tr>
<tr>
<td>AH-39</td>
<td>1.46±0.20</td>
<td>106</td>
</tr>
<tr>
<td>AH-414</td>
<td>1.39±0.26</td>
<td>109</td>
</tr>
<tr>
<td>AH-7974</td>
<td>1.46±0.25</td>
<td>107</td>
</tr>
<tr>
<td>Polymorphic leucocytes</td>
<td>1.26±0.13</td>
<td>103</td>
</tr>
</tbody>
</table>

576
hyper-diploids. Tumor cells containing double or almost double the number of modal chromosomes are found in about 9% in AH-3683 and less than 5% in the remaining five tumors. Karyotype analyses revealed a great deal of dissimilarities among ideograms of the six tumors.

Histograms in Fig. 2 indicate frequency distribution of the one-half value of DNA amount of mitotic nuclei in the tumors. Mean amount of the leucocytic DNA is represented by a line, symbolized with "2c", and the range of its DNA content by dotted lines. It is noted that even cells of AH-3683 or AH-13 with hypodiploid number of chromosomes, 37 or 38, have a similar or larger amount of DNA, when compared with modal peaks of DNA content in the leucocytes and four hyperdiploid lines. An exact parallelism cannot be detected between the chromosome number and DNA content.

Fig. 1. Distribution of chromosome number in six lines of rat ascites hepatoma

Number of tumor cells examined is shown in parentheses.
There are found several reports dealing with both chromosome number and DNA content per nucleus in cancer cell population. Makino\(^4\) described venereal tumors of dogs with similar chromosomal patterns and the same amounts of DNA per cell. Freed and Hungerford\(^2\) and Utsumi\(^7\) observed in sublines of Ehrlich ascites carcinoma of the mouse, derivations of the same tumor, that the amount of DNA in nuclei was proportional to the chromosome number. In different carcinomas of the human uterus or stomach, however, such a parallelism between the modal chromosome number and DNA content was not present, as shown by Ojima et al.\(^5,6\). Recently, Atkin et al.\(^1\) reported that the modal DNA content and the chromo-

Fig. 2. Frequency distribution of the one-half value of DNA content of metaphase nuclei in six lines of the rat ascites hepatoma.

![Diagram showing frequency distribution of the one-half value of DNA content of metaphase nuclei in six lines of the rat ascites hepatoma.](image)

Mean value of DNA amount of polymorphic leucocytes in the rat is shown by "2c" and distribution range of amount of the leucocytic DNA per nucleus is indicated by dotted lines. Number in parentheses shows modal chromosome number of each tumor.

Mean value of DNA amount of polymorphic leucocytes in the rat is shown by "2c" and distribution range of amount of the leucocytic DNA per nucleus is indicated by dotted lines. Number in parentheses shows modal chromosome number of each tumor.
some number of 49 human malignant tumors were in fairly close mutual agreement, but there was frequently a small discrepancy. Summarizing the results of the present study, there is no exact parallelism between the chromosome number and DNA content per nucleus in malignant tumors, when studied with different tumors, though they are derivations from a common normal ancestry, the liver cell of rats. Ascites hepatoma cells, even those with hypodiploid numbers of chromosomes, have a larger amount of DNA, when compared with normal cells.

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REFERENCES