DNA CONTENT OF HUMAN TUMOR CELL NUCLEUS: A
STUDY ON FIBROADENOMA AND CARCINOMA
OF THE BREAST

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Synopsis
A microspectrophotometric evaluation of the DNA content of tumor cell nuclei was undertaken in 24 cases of fibroadenomas and 30 cases of carcinomas of the human breast. There was a general trend that fibroadenomas of the breast showed the DNA values lying in a diploid range, with limited deviation among the cells, while in carcinomas of the breast, the DNA values were slightly or definitely higher than the basic diploid value with a larger scatter from cell to cell. The modal DNA value from malignant tumors was distributed from hypodiploid to hypotetraploid ranges. Further, there was no direct correlation between the DNA content and the histopathological pattern of the tumor. There was also no tumor case which had a large amount of DNA such as tetraploidy or over tetraploidy in the breast tumors so far examined here.

INTRODUCTION
Quantitative cytochemistry has been widely used to study the DNA content of malignant tumors. These data showed that DNA content in cell nucleus of malignant tissues was higher in value and larger in variability than those of normal tissues.1-5, 6, 13) In many of malignant tumors, there was aneuploid mode or modes which have been referred to as chromosomal stemline.11) Stich15) found that malignant tissues of the human breast showed a much larger scatter of DNA value from cell to cell than the normal tissue, and many cases of breast tumor examined had a DNA value lying in over tetraploidy. Further, from the measurement of DNA in the benign adenoma of a lung and of a malignant pulmonary adenomatosis, Seidel14) reported that the adenoma had a diploid pattern; the DNA distribution pattern was the same as that of normal cells. In contrast, the malignant pulmonary adenomatosis was characterized by a stem-line of DNA values which deviated from the normal (aneuploidy). It is thus evident that there are varied reports on DNA studies of malignant tumors, but knowledge on non-malignant pathological tissues or benign tumors is still meager at present, so far as the breast tissues are concerned.

In the present paper is reported the result of DNA studies in benign tumors and carcinomas of the human breast, with special reference to their histological features.

MATERIALS AND METHODS
In the present experiment, DNA content was examined in 22 cases of benign fibroadenoma, 30 cases of adenocarcinoma of the breast, and two specimens of a normal mammary gland from patients with fibrosis of the breast. These materials were removed

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from the patient by biopsy or surgical operation. Some pathological data of these tumors are listed in Table I.

The tissues, immediately after removal, were fixed in acetic acid-ethanol solution (1:3) for exactly 30 mins., embedded in paraffin, and cut into 8~10μ thickness required for microspectrophotometry. For the measurement of the DNA content, the sections of different tumors were placed on one and the same slide and subjected to Feulgen reaction as described in the previous report. A microspectrophotometer (Olympus IV type) was used for the photometric determination of DNA by the single wavelength method at 555 mμ.

For histological examinations, the sections were prepared according to the usual paraffin method and stained with Hematoxylin and Eosin.

### RESULTS

**Fibroadenoma of the Breast** The results of DNA measurement in individual nuclei of 11 cases of fibroadenoma pericanaliculare of the breast and cells from a normal mammary gland are shown in Fig. 1. The results of this experiment revealed that DNA content of normal material in somatic cell is markedly constant with a limited deviation from cell to cell, since the average of DNA is approximately 1,800 to 2,000 at arbitrary units and the values of about 55% of the cells are scattered in this region. The mean amount of DNA in normal somatic cells was 1802.4 at arbitrary units; on this basis, this value is considered in all probability to be a basic diploid value of the human normal cell.

The modal peaks from DNA measurement of individual nuclei in 11 cases of the adenoma are also at 1,800 to 2,000 units. In 5 out of 11 cases of the material (Ma–30, 53, 58, 63, and 65*2), over 60% of the cells had this basic value, and the pattern of distribution of these cells was rather similar to that of the control. There was a narrow deviation from cell to cell, but the mean amount of DNA from total cells was slightly higher (3.4%) than that of the control, though this increase of DNA was not a statistically significant difference. On the other hand, 6 cases (Ma–37, 38, 43, 48, 52, and 97) showed rather a scatter from cell to cell around the mode, the modal peaks also being rather

### Table I. 57 Cases of Human Breast Tissues with Some Pathological Data

<table>
<thead>
<tr>
<th>Breast tissues examined</th>
<th>Pathological pattern</th>
<th>No. of cases</th>
<th>Method of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroadenoma</td>
<td>Pericanaliculare</td>
<td>11</td>
<td>Biopsy</td>
</tr>
<tr>
<td></td>
<td>Intracanalicular</td>
<td>11</td>
<td>&quot;</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Papillotubulare</td>
<td>5</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Papillotubulare</td>
<td>7</td>
<td>Surgery</td>
</tr>
<tr>
<td></td>
<td>Scirrhosum</td>
<td>2</td>
<td>Biopsy</td>
</tr>
<tr>
<td></td>
<td>Scirrhosum</td>
<td>12</td>
<td>Surgery</td>
</tr>
<tr>
<td></td>
<td>Mucoïd carcinoma</td>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Lobular carcinoma</td>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>Normal mammary gland</td>
<td>Fibrosis</td>
<td>2</td>
<td>Biopsy</td>
</tr>
</tbody>
</table>

*2 Symbol “Ma” is used for material No. in this experiment.
lower than the former. About 40% of the cells ranged in the modal basic value of DNA, and the mean amount of DNA form these cases was 7.3% higher than that of the control. Such increase of DNA occurring in fibroadenoma pericanaliculare, however, was not essentially different from that of the normal breast tissues statistically.

The results of the DNA measurement of individual nuclei in 11 cases of fibroadenoma intracanaliculare and of normal mammary tissues are shown in Fig. 2. Similar to the above evidence, the mean amount of DNA from the tumor cells showed the main mode in basic diploid value; 40~60% of the cells were in this region, with a fairly narrow range of deviation from cell to cell, except for one tumor (Ma–68), whose distributed pattern of DNA contents was similar to that of the control. The mean amount of DNA from the total cells was 6.4% higher than that of normal tissues. In tumor Ma–68, the modal peak is at 35%, with a rather wide range of deviation from cells to cell, but there were no cells lying over the tetraploid range. Only in one case (Ma–72), the cells had an over tetraploid DNA content, though the mean amount of DNA was slightly higher than that of control, and there was no essential difference in DNA amount between the tumor cases and the normal mammary gland tissues. In the light of such results, the DNA value of fibroadenoma of the breast is essentially the same as that of the control breast tissues.
Fig. 2. Histograms indicating relative amount of DNA in one specimen of normal tissue and 11 cases of fibroadenoma intracanaliculare of the breast

Fig. 3. Relationship between the mean amount of DNA and histological types in 30 cases of the human breast carcinoma
Adenocarcinoma of the Breast  The plots and histograms provided by the present study are represented in Fig. 3, illustrating the results of the mean amount of DNA from each tumor case (dots) and the mean amount of DNA from total cells (histograms). Table II represents data on the correlation between the DNA content and histopathological patterns of breast tumor.

Measurements indicated that the cells from the control tissue have a mean DNA value at about 1650 arbitrary units; this value may, therefore, be accepted as the probable basic value of normal human tissues in this examination. It has been found from this study that the mean value of DNA from each tumor case was in the hyperdiploid to hypotetraploid regions and that 73% of the tumors (22 out of 30 cases) had a mean DNA value from hypodiploid to hypotetraploid region, and further, that 6 cases of tumors had DNA values in the hypodiploid range. It is seen that among the 30 cases of the tumor examined here, 17 cases were in diploidy in DNA value, 12 cases in triploidy, and only one case in hypotetraploid range. There was no tumor which has a DNA value over tetraploidy.

Twelve cases of adenocarcinoma papillotubulare showed the mean amount of DNA from total cells measured lying in hyperdiploid range, though the mean value of DNA from each tumor case (shown in dots in Fig. 3) was distributed from hypodiploid to hypertriploid regions. Five out of 12 cases in this tumor type had the mean DNA value lying in near- or hyper-diploid regions, 4 cases in hypotriploidy, 2 cases in hypodiploid regions, and only one case showed DNA content in hypotetraploid range. There was no tumor which has a DNA value over tetraploidy.

Fourteen cases of adenocarcinoma scirrhosum also exhibited the mean amount of DNA from total cells lying in the hyperdiploid range. It is evident that the value from this group is slightly higher than that of the former, but the mean amount of DNA from each tumor case scatters from hypodiploid to hypertriploid ranges. Among the 14 cases, 8 cases had DNA content in hypotri- to hyper-diploid and especially 3 cases were in the hypodiploid ranges. Among the remaining 6 cases, 3 cases had a DNA value occurring in the hypotriplody and others in the hypertriploidy.

<table>
<thead>
<tr>
<th>Histological feature of tumors</th>
<th>Papillotubulare</th>
<th>Scirrhosum</th>
<th>Adenocarcinoma Mucoid carcinoma</th>
<th>Lobular carcinoma</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA amount</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower ploidy</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Hyper-2n</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Hypo-3n</td>
<td>4</td>
<td>3</td>
<td>—</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Near-3n</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Hyper-3n</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Hypo-4n</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
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<tr>
<td>Near-4n</td>
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<tr>
<td>Hyper-4n</td>
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<tr>
<td>Over-4n</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

Adenocarcinoma of the Breast
Two cases of tumors were diagnosed as mucoid carcinoma. The mean amount of DNA from total cells lies in near diploid range, though 1 case is in hypodiploidy and the remaining case in hyperdiploid regions.

Two cases of lobular carcinoma of the breast showed the mean amount of DNA from total cells in the hyperdiploid range, though the mean value of DNA in one case was hyperdiploidy, and another case in the hypotriploidy.

The results of DNA measurement of individual nuclei in the random samples picked from 4 cases of adenocarcinoma papillotubulare, 4 cases of the scirrhosum, 2 cases of mucoid carcinoma, and 2 cases of lobular carcinoma of the breast, and those of normal mammary gland cells are arranged in Fig. 4. It is evident from the results in this graph that there is a remarkable constancy of DNA content in somatic cells from normal tissue and that the major mode is represented at approximately 60% in basic value with rather small scatters. In carcinoma papillotubulare, the DNA value is slightly higher than...
that of the controls, the modal peak of the tumor lying in the hyperdiploid range (20\%) with a very wide deviation from cell to cell. Studies on adenocarcinoma scirrhosum showed that the modal peak is located in the near-diploid range, being at about 22\%, and the second peak occurs in the hyperdiploid range, with a fairly large deviation from cell to cell. The mean amount of DNA from total cells is 30\% higher than that of the control tissues. In two cases of mucoid carcinoma, the modal peak of DNA was in hypotriplaid range with a fairly large deviation, and the mean amount of DNA was about 40\% higher than that of the control. Two cases of lobular carcinoma of the breast have two modal peaks in hyperdiploid (18\%) and near-triploid ranges with a fairly wide distribution. The mean amount of DNA from these tumor cells is about 35\% higher than that of the control.

These results show that the mean DNA content of cell nuclei in breast tumor is slightly higher than that of the controls. Further, there was no tumor case with a large amount of DNA such as tetraploidy or over tetraploidy in breast carcinomas so far studied here. There is also no direct correlation between the DNA content and histopathological patterns of breast tumor.

DISCUSSION

Cytophotometric data have shown that there is a constancy in the amount of DNA per nucleus in parallel with the number of chromosomes. Working with the DNA measurements of tissues in rats, frogs, and other animals, many workers showed that the amount of DNA per cell nucleus was approximately the same in the liver, pancreas, lymphocytes, and some parts of the body.\(^4,12,16\) On the contrary, there are some others who have presented data which suggest a variation of the DNA amount in the nucleus under the influence of pathological disturbances. For example, in malignant neoplasm, DNA content of cell nuclei was usually higher than that of normal cells and the deviation from cell to cell was also wider than that of normal tissue. There is, however, no report except that of Seidel et al.\(^14\) on the DNA content of benign tumor. They measured the DNA content of benign adenoma of lung and stated that one case of cystic adenoma had a modal peak in the diploid range with relatively limited variation, but the distribution pattern of DNA was in a normal range.

The data presented in the present invastigation have shown that 22 cases of benign tumors of the breast have the mean amount of DNA slightly increased to that of the normal mammary gland cells. However, the mode of DNA value from these tumors was in a diploid range. The mean DNA amount from 22 cases of fibroadenoma of the breast was 5 to 6\% higher than that of the control tissues, showing rather a large but limited deviation from cell to cell. Statistical treatment indicated that the increase in the amount of DNA occurring in fibroadenoma of the breast was not significantly different from that of the control somatic cells. On the basis of these results, the conclusion may be allowed that the DNA value of benign tumor is essentially the same as the DNA content of normal tissues.

Stich and Steel\(^15\) concluded from the measurement of the DNA content of breast tumor that the cancers had aneuploid DNA content lying in tri- and penta-ploid regions. Further, chromosome studies on breast carcinomas by Ishihara et al.\(^7,8\) and by Makino et al.\(^9\) provided evidences that 10 out of 22 cases of breast tumors had
hypo- to hyper-diploid chromosome number; two cases had triploid and the remaining ones had tetraploid numbers, leaving only 2 cases which showed no modal number of chromosomes. These results agreed with the fact that most of the malignant cells in man were characterized by an increased or decreased amount of DNA and by change of chromosome numbers.

The data provided by the present study have shown that 18 out of 30 cases of breast tumors had the mean DNA value occurring in the diploid range, and that there is no tumor showing over tetraploid DNA contents. Further, there is no direct correlation between DNA content and histopathological feature of the tumor.

In reference to the above results, the comment may be made that human breast tumors have a relatively higher DNA value which seems to be suitable for the tumor growth.

The author wishes to express his sincere gratitude to Dr. Shozo Takayama for his critical reading of this manuscript. The author is also indebted to Drs. Haruo Sugano and Kyoichi Nakamura who permitted the use of unpublished pathological data and for valuable advices.

(Received February 10, 1967)

References