Quantitative Cytological Analysis of Bilateral Breast Cancer

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Abstract

From a quantitative cytological point of view, the authors analyzed the nuclear DNA content of six bilateral breast cancer cases and attempted to determine whether it is possible to distinguish between primary and metastatic lesions in terms of DNA.

In 3 of 6 cases the DNA distribution pattern of the first and the second tumors were different and had different main peaks. The overlap rates of the two distributions were under 65% in each case. These second tumors were regarded as primary cancers clinically. In the other 3 cases, the DNA distribution pattern of the first tumors were similar to those of the corresponding second tumors. The overlap rates were over 65% in each case. The second tumors were regarded as metastases clinically.

This study suggests that different DNA distribution pattern indicate double primary breast cancers.

Introduction

Breast cancer cases are increasing annually in Japan. Breast cancer will become the most common cancer in terms of prevalence among female malignant diseases by the year 2000 in Japan. Because of the early detection of breast cancer and progress in adjuvant therapy, the numbers of second breast cancers on the contralateral side, i.e. bilateral breast cancers, are also increasing1. When bilateral breast cancer is detected, the question of whether the second cancer is a metastatic lesion or a double primary cancer arises. Since the therapeutic approach in these two instances is different, it is important to distinguish between them. The authors analyzed the nuclear DNA content of the bilateral breast cancer cases and attempted to determine whether it is possible to distinguish between primary and metastatic lesions in terms of DNA distribution pattern. The utility of DNA analysis is discussed.

Materials and Methods

Among resected bilateral breast cancer cases at Tokyo Medical College Hospital, six cases, in which there was no therapy before operation were used for this study. The cases, in which the second cancer was treated within 12 months after adjuvant therapy for the first cancer, were excluded in order to deny the effect of the adjuvant therapy (table 1, 2). From those cases sufficient materials for analysis were obtained. If the interval between the first and the second operation was less than 6 months, the lesions...
Table 1. Bilateral Breast Cancer Cases (1)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at first operation</th>
<th>Location</th>
<th>Histologic type (WHO)</th>
<th>Histologic type (JPN)</th>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>53</td>
<td>l-E</td>
<td>r-C</td>
<td>inv</td>
</tr>
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<td>28</td>
<td>r-C</td>
<td>l-A</td>
<td>inv</td>
</tr>
<tr>
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<td>47</td>
<td>r-A</td>
<td>l-C</td>
<td>inv</td>
</tr>
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<td>inv</td>
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<td>39</td>
<td>l-E</td>
<td>r-A</td>
<td>inv</td>
</tr>
<tr>
<td>6</td>
<td>55</td>
<td>l-A</td>
<td>r-C</td>
<td>inv</td>
</tr>
</tbody>
</table>

inv: invasive ductal carcinoma  
pap: papillotubular carcinoma  
sol: solid-tubular carcinoma  
sci: scirrhous carcinoma

Table 2. Bilateral Breast Cancer Cases (2)

<table>
<thead>
<tr>
<th>Case</th>
<th>Pathological stage</th>
<th>Interval</th>
<th>Synchronous or metachronous</th>
<th>Prognosis</th>
<th>DNA histogram</th>
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<td></td>
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<td>2</td>
<td></td>
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<td>III</td>
<td>t2n2m0</td>
<td>116M</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>t2n1m0</td>
<td>IV</td>
<td>t3n2m1</td>
<td>19M</td>
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<tr>
<td>5</td>
<td>II</td>
<td>t2n1m0</td>
<td>I</td>
<td>t1n0m0</td>
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<td>6</td>
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<td>t1n1m0</td>
<td>IV</td>
<td>t1n1m1</td>
<td>24M</td>
</tr>
</tbody>
</table>

were regarded as “synchronous” and if not, were regarded as “metachronous”.

Preparation of single cell suspensions from paraffin-embedded material (Fig. 1): The tumor location was confirmed beforehand. Three sections of 50 μm slices of paraffin-embedded materials were cut using a microtome. The sections were dewaxed using two changes of xylene for more than 3 hours, and then rehydrated in a sequence of 100, 95, 70, 50, 30% ethanol for 5 min each at room temperature, and washed in distilled water. The tumors in the sections were enucleated and minced with scissors. The materials were resuspended in 2.0% 2 Na-EDTA (pH 7.3) at 37°C for one hour and washed twice in saline solution, then treated with collagenase (Sigma, St. Louis, 20 mg/l) at 37°C for one hour and washed twice in saline solution. In addition the materials were isolated with an ultrasonic generator and filtered with a nylon mesh. The specimens were obtained from isolated cells using an automatic cytosedimentation machine. Feulgen stains were made of each specimen.

Measurement of nuclear DNA content: Generally, one hundred or more cancer cells were randomly measured with a microspectrophotometer (MSP) at 547 nm. Twenty or more small lymphocytes on the same slide were used as control cells. The MSP use for this study was an original model developed in our department. Taking the DNA content of lymphocytes to be 2 C, i.e. normal diploid DNA content, the DNA contents of cancer cells were calculated and histograms were drawn. The histograms of the first
and second tumors were then compared.

If the distribution pattern was similar and the main peak of the histogram was the same and distributions overlapped by 65% or more, the two histograms were regarded as “similar”, and if not, regarded as “different”.

**Results**

In cases 1, 3 and 5, the DNA distribution pattern of the first and second tumors were different and they had different main peaks. The overlap rates of the two histogram were under 65% each. In case 5 the overlap rate was relatively high (59.2%), the main peak of the first tumor was 3 C but the histogram of the second tumor showed broad distribution from 2 to 3 C. The two histograms in each of cases 1, 3 and 5 were regarded as “different” (Fig. 2). Of these two cases were metachronous and the other was synchronous. In two of these three cases, the histological types of each pair of tumors were different. One of these showed intraductal carcinoma. The remaining case showed invasive ductal carcinoma.

In cases 2, 4 and 6, the DNA distribution pattern of the first tumors were similar to that of the respective second tumors. Furthermore the overlap rate of the two histograms was over 65% each. Each pair of histograms in cases 2, 4 and 6 were regarded as “similar”. Of these two cases were metachronous and the other was synchronous. Pathologically all of the tumor in these cases showed invasive ductal carcinoma.

**Discussion**

Many authors have proposed criteria for bilateral primary breast cancer34). Giving a summary of these, (1) curative resection must be possible for both. (2) At the time of resection of the second tumor, there must be no evidence of local recurrence or distant metastasis of the first tumor. (3) There is no evidence of early local recurrence or early distant metastasis after resection of the second tumor. Furthermore histologically (1) The histological types of both sides are clearly different. (2) At least one of the tumors is intraductal carcinoma.

According to “General Rules For Clinical And Pathological Recording Of Breast Cancer” published by the Japanese Breast Cancer Society5) invasive ductal carcinoma is classified into three groups: papillotubular carcinoma, solid-tubular carcinoma and scirrhous carcinoma, if more than two types were
observed, the histological type should be considered to be that which is the predominant type, and if it is difficult to decide which is dominant, the more poorly differentiated type should be the classification. As a result, tumors classified as different histological subtypes do not necessarily mean different tumors and could conceivably have a primary-metastatic relationship. Except for the invasive lobular carcinoma of case 1 and intraductal carcinoma of case 5, all of our cases were invasive ductal carcinoma. Partial histological similarity between the lesions on both sides was observed. Therefore it was difficult to distinguish between primary or metastatic lesions only by histological subtype when the first and second tumors were both invasive ductal carcinoma.

Moreover metastases or local recurrence may occur soon after operation even in unilateral breast cancer cases. Metastases were also detected ten or more years after operation in some cases also. Therefore contralateral breast cancer cannot be decided to be a primary or metastatic lesion on the basis of clinical findings, Robbins and Berg proposed that contiguous in situ carcinomas are evidence of primary tumors and this opinion has been generally accepted. However this method requires further pathological study. Generally a tumor is initially evaluated clinically as a primary tumor before operation, followed by postoperative pathological classification.

The authors proposed preoperatively distinguishing primary and metastatic tumors quantitatively using aspiration cytological material. Quantitative cytological analysis was performed in a basic experi-
ment using single cell suspensions of paraffin-embedded materials. Auer et al.\(^{10}\) reported aspiration cytological materials from 18 cases of primary breast cancers and 44 metastatic lesions analyzed with an MSP. Of the 44 metastases, 39 showed the same type of DNA histogram as the corresponding primary tumors. In only one patient did the DNA histogram change in the metastatic lesion. Furthermore the DNA pattern remained unchanged even in patients with survival periods exceeding 10 years. Therefore, conversely speaking, if left and right tumors of bilateral breast cancer show the same DNA pattern, the possibility that one of the tumors is metastatic is naturally high. The histogram of another tumor detected during treatment of the primary lesion would naturally be likely to be affected by any such therapy. Therefore cases treated before operation were excluded from this study. The cases, in which the second cancer was treated within 12 months after the adjuvant therapy for the first tumor, were also excluded.

In the present study the cases in which the DNA pattern differed were alive 44 to 119 months after operation without any distant metastases and were regarded as double primary bilateral breast cancer. On the other hand the cases in which the DNA pattern was similar had metastasis to other organs. One case was alive with local recurrence and metastasis and death occurred in two cases within 3 years. Therefore in these cases, it appeared clinically that one of the tumors was metastatic from the contralateral side.

In a cytophotometrical study of the nuclear DNA content of primary lung cancer and its metastatic
lymph nodes\textsuperscript{11}, the stem line of adenocarcinoma showed no change between the primary and metastatic lesions while the stem line of squamous cell carcinoma showed some change. Therefore this method to distinguish primary and metastatic tumors using microspectrophotometric DNA measurement is useful in adenocarcinoma cases. Further more study using this method might be necessary concerning the squamous breast cancer cases.

Concerning the carcinogenesis of breast cancer, environmental factors (e.g. food) and internal factors (e.g. heredity, hormones) are important. Since these factors affect both breasts, the possibility of multicentric breast cancer cannot be denied. Therefore it was still difficult to clarify whether the second tumors were primaries or metastases in the cases which showed similar DNA distribution pattern. However the present study shows that different DNA histograms indicate double primary breast cancers, if the adjuvant therapy for the first tumor appears to be unlikely to have affected the second tumor. This method should be useful to distinguish primary and metastatic lesions using preoperative needle aspiration materials.

References

両側乳癌の定量細胞化学的検討

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三浦 弘之 小中 千守* 平良 修
内 田 修 加藤 治文*

両側乳癌を定量細胞化学的に解析し、第二癌が他方からの転移か、原発か鑑別を試みた。
東京医科大学外科で両側手術の行われた女性乳癌 6 例を対象に用いた。同時性 3 例、異時性 3 例であった。
方法は、パラフィン包埋材料から 50 μm 切片 5 枚を切り出し、酵素処理を行い、単離細胞を作製した。オートスメアでスライドガラス上に塗抹し、Feulgen 染色を施した。測定には顕微分光測光機 (定量細胞診断 TV カメラシステム) を使用し、癌細胞 100 個と、対照となる同一標本上の小リンパ球 20 個を無作為に抽出し測光した。対照の核 DNA 量を 2 C といた時の癌細胞核 DNA 量の相対値を算出し、比較検討した。
同時性の 2 例、異時性の 1 例で、左右の DNA ヒストグラムの主ピークが異なり、ヒストグラムの overlap rate は各々 65% 以下であったため、異なる DNA 型と判定した。他の 3 例は、左右とも同一の主ピークを有し、ヒストグラムの overlap rate も各々 65% 以上を示したため、同一の DNA 型と判定した。
臨床的にヒストグラムの異なった症例は、再発なく長期生存しており、第二癌は原発性と考えられた。一方同じヒストグラムを呈した 3 例は、他臓器にも転移を認め、第二癌は他側からの転移と考えられた。

乳癌は発癌の内外環境因子により、multi focal に同性格を持つ癌が発現しても矛盾はない。しかし、左右で治療因子に差の無い状態で測定した場合、組織型にかかわらず、DNA の異なる症例は二重癌の可能性が高いたと考えられた。