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Comparison of DNA Distributions in Primary Human Breast Cancers and Their Metastases

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Abstract—*Quantitative measurements of nuclear DNA in individual cells were performed on fine-needle aspirates from* 22 *primary breast tumors and their metastases, found either at operation or as much as* 126 months *afterwards. The DNA distribution patterns of each tumor differed widely. However, the primary tumor and its metastasis always showed similar distribution patterns, irrespective of location or time of occurrence of the metastasis. These results indicate a monoclonal genesis of breast carcinoma. It is suggested that the primary tumor and its metastases are made up of similar cell populations with comparable proliferative activities, which may be information of great importance for diagnosis and treatment of secondary breast malignancies.*

INTRODUCTION

THE gravest sign in the treatment of breast cancer patients is the presence or subsequent appearance of metastases [1]. The usual origin of metastases is spread from the original primary tumor. The metastasis may originate from a population comparable to that in the primary tumor or may be the result of the dissemination of a more malignant subpopulation. Occasionally, metastasis occurs in a patient who has previously been known to have breast cancer, but the recurrence derives from an entirely different primary tumor. Such a possibility may become more frequent due to treatment of the initial tumor with radiation and cytostatic agents known to be carcinogenic. With the advent of increasingly individualized therapy for breast cancer it would be expedient to be able to differentiate between these origins in order that therapy may be directed towards the proper histopathological type of tissue. While this can often be done by a simple histological identification of the tissue in the metastasis, there is sometimes difficulty in the less differentiated types of tumors. Fine-needle aspiration biopsy techniques [2] allow sampling of tissues without the necessity of surgical biopsy and apart from accuracy, speed and patient acceptability, can provide individual cells for the study of neoplasia. A further advantage of fine-needle aspiration is that the technique insures representative sampling of the entire tumor cell population since the aspirating needle passes through the entire tumor mass and collects cells from all parts of the tumor [3]. In this study, we have compared Feulgen–DNA values in individual cancer cells taken from fine-needle aspirations of primary mammary tumors and metastases. The study was begun in order to determine whether distribution of nuclear DNA measurements could be used to associate primary tumors with subsequent metastases, and whether the distribution of DNA contained in the tumor cell population changed during the months or years between the time when the tumor was first diagnosed and metastases appeared.

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MATERIALS AND METHODS

Patient material

Patients for the prospective studies were selected consecutively as they appeared in the Department of Cytopathology or were selected from the surgical material from the Department of Surgery. Immediately after excision of the surgical specimen, fine-needle

aspiration biopsy was performed on the breast tumor(s) or on the metastatic tissue. Where retrospective studies were done, the archives of the Department of Cytopathology were searched for patients whose original diagnostic slides accompanied by subsequent aspiration slides of metastases were available, which constituted the sole basis for selection.

Fine-needle aspiration biopsy

Fine-needle aspiration biopsy has been extensively used for diagnostic biopsies of tissues, and especially of suspicious breast masses [2, 4]. The technique consists of inserting a thin (0.5–0.7 mm o.d.) needle into the tumor mass and aspirating cellular material into the lumen of the needle by negative pressure produced by drawing back the plunger of a 10 ml syringe attached to the needle. During sampling, the needle is moved back and forth several times through the tumor mass, usually at different angles to the original angle of insertion. The technique is relatively painless and requires no local anaesthesia. After aspiration, the cellular material is smeared on a glass slide and fixed in buffered 4% formaldehyde (1 hr, 25°C), or allowed to air dry. The cellular preparations consist of single cells or clumps of cells that are a representative sample of the entire tumor mass and are considered to be more representative than the cell distribution in histological sections. Fine-needle aspirations in this study were from both operative and diagnostic material.

Cytophotometry

Cytophotometric measurements were made of Feulgen stained nuclei. Feulgen stained preparations were prepared from formaldehyde-fixed slides or, in the case of archival material that had been Giemsa stained, the cover-slips were removed, the cells destained in methanol, refixed in 4% formaldehyde and then Feulgen stained. Previous studies [5] showed that hydrolysis for 1 hr at room temperature in 5 M HCl gave optimal staining intensities. Diploid amounts of DNA were determined by using normal mammary epithelium and lymphocytes adventitiously present in all aspirates as internal controls. The discrepancies between DNA values of small lymphocytes with condensed chromatin were partially corrected for by measuring at the "off-peak" wavelength of 610 nm. Usually 10, but occasionally 50 control cells were measured. Tumor cells could be readily distinguished from normal cells, either by their nuclear morphology, or by the additional use of phase contrast. Measurements were made with a rapid, scanning-integrating microscpectrophotometer developed by Professor Caspersson [6]. All Feulgen–DNA values were expressed in relation to their corresponding staining control, which was given the value 2c, denoting the normal diploid DNA content. All DNA values were expressed in such relative units. Figure 1 illustrates that the DNA-values of non-growing normal mammary epithelium shows variation between 1.5c and 2.5c. The value of 2.5c was therefore used as the upper limit of the normal diploid DNA content and indicated by broken lines.

RESULTS

A comparison between the distribution of nuclear DNA in primary breast cancers and axillary metastases removed at the same time as the original tumor are shown in Figs. 1 and 2. DNA distributions for both cells from the primary tumor and from the axillary metastases show comparable distributions in nuclei with diploid or increased amounts of DNA. In case 1, for example, both the original tumor cells and cells from the metastasis had a diploid distribution, while in case 4, there was a tetraploid distribution on both sites. Cases 5–7 showed greater variations in amounts of DNA, but similar distributions when the cell

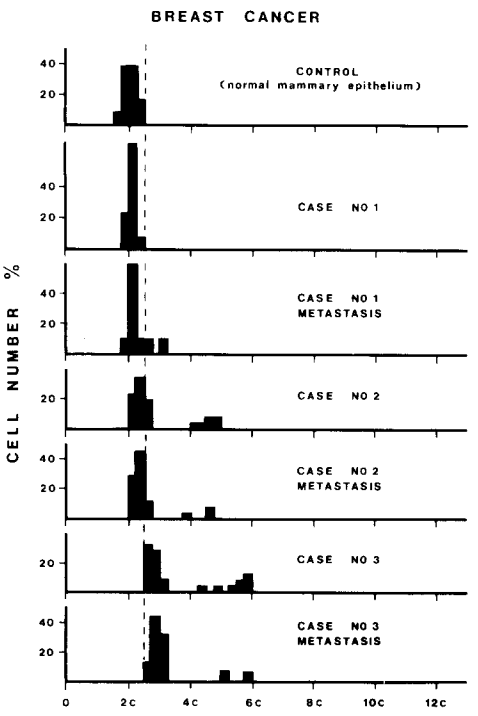

Fig. 1. Amounts of DNA in primary breast carcinoma cells and in cells from corresponding axillary metastases.

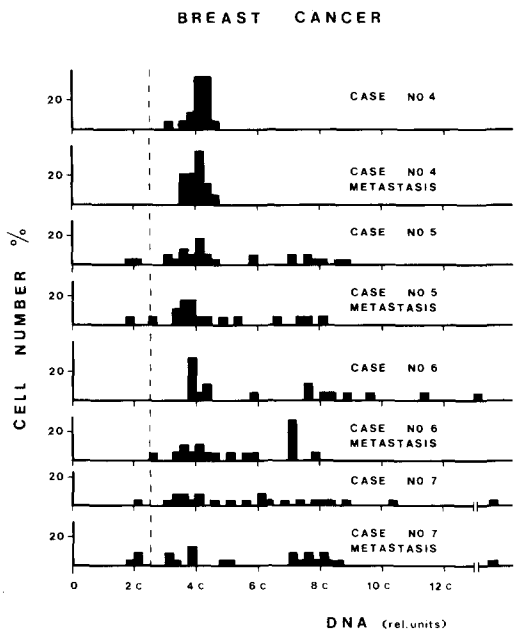

Fig. 2. Primary breast carcinomas compared with their axillary metastases as in Fig. 1.

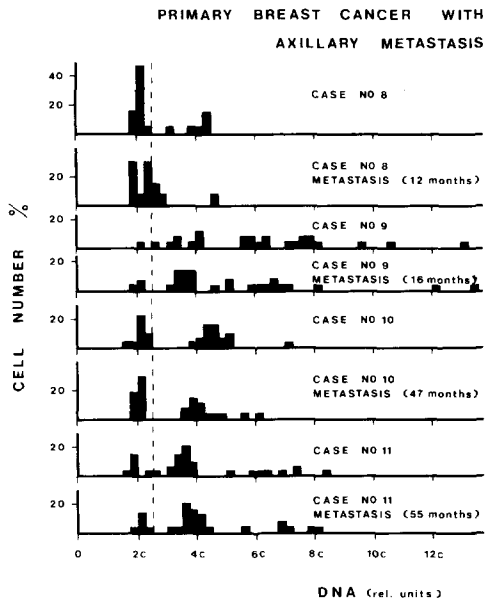

Fig. 3. Amount of DNA in primary breast carcinoma cells and in cells from axillary metastases subsequently appearing. Both preparations were made from archival slides used for diagnosis.

populations in the primary tumors and the metastases are compared. Cases 8–11 (Fig. 3) were studied retrospectively when axillary metastases, occurring between 12 and 55 months after the original tumor, had been resected. None of the these patients had received radiation or chemotherapy between time of operation and appearance of metastases. A similar situation occurred in cases 12–14 (Fig. 4) who, subsequent to mastectomy, developed local recurrence at the incision site.

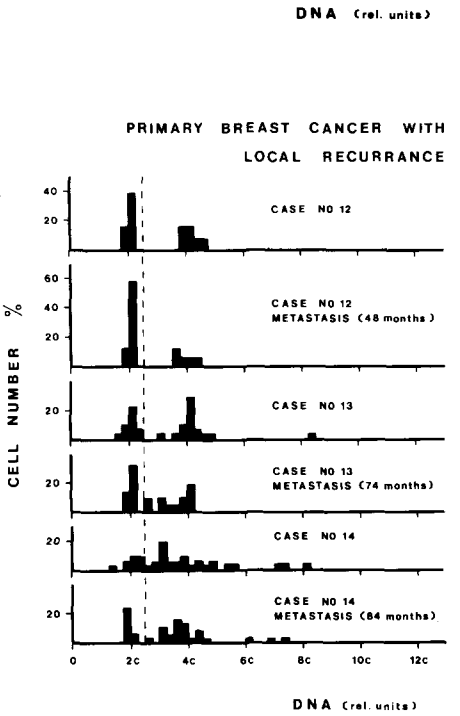

Fig. 4. Amounts of nuclear DNA in primary breast carcinoma cells and in cells from local recurrence.

In these patients, from 48–84 months had elapsed between the time of operation and aspiration biopsy of the recurrence. In each case (8–14) there was a resemblance between the DNA distribution patterns of the tumors.

Figure 5 shows aspiration biopsy comparisons of DNA distribution in metastases to

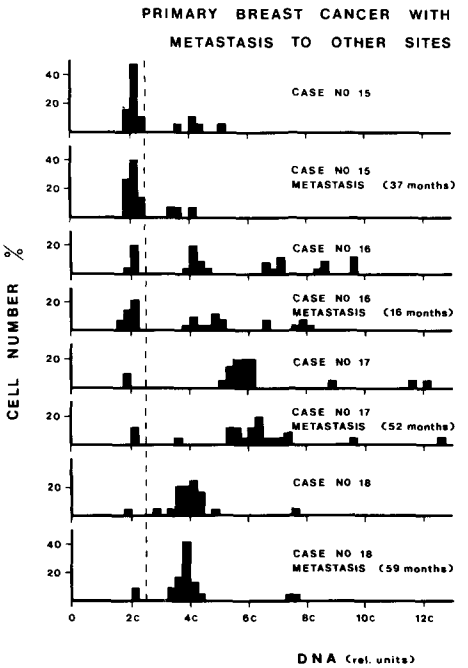

Fig. 5. Comparison of cellular DNA values in patients subsequently developing distant metastases. Case 15 developed a bone marrow metastasis, cases 16 and 17 liver metastases and case 18 a lung metastasis.

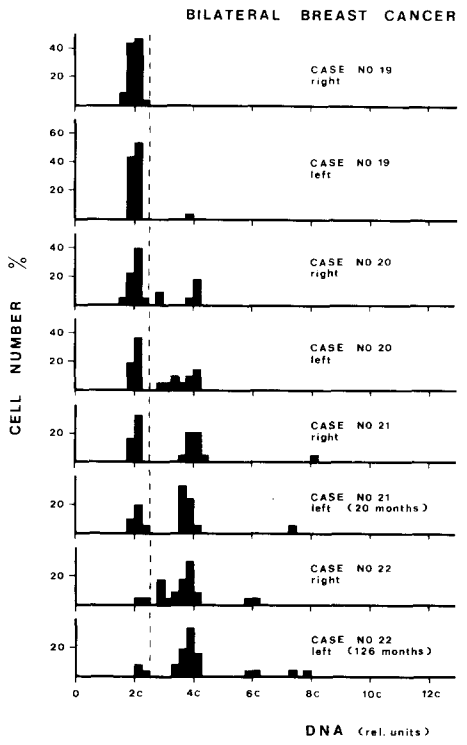

Fig. 6. Comparison of DNA values in patients with simultaneous (cases 19, 20) or subsequent (cases 21, 22) bilateral breast cancer.

distant sites including lung, (cases 15, 16) liver, (case 17) and bone marrow (case 18). As before, distribution patterns are similar in both sites. We found four cases (Fig. 6) where there was either simultaneous occurrence of cancer in both breasts (cases 19 and 20) or subsequent occurrence in the other breast (cases 21 and 22). All of these fell in the diploid-tetraploid range and presented some difficulties in interpretation.

DISCUSSION

The nuclear DNA distribution pattern in mammary carcinomas may show broad differences in spite of histological similarity [7]. In some tumors nearly all the malignant cells exhibit DNA values located in the diploid or tetraploid region of normal mammary epithelial cells, whereas in other tumors the DNA values are highly scattered and exceed the tetraploid region [7, 8]. The biological and clinical significance of these findings are still unknown. However, evidence has been obtained that breast cancers with a homogeneous diploid distribution pattern have a better prognosis than those showing a heterogeneous type of DNA distribution including cells exceeding the tetraploid region [7]. Since the clinical presence or occurrence of metastases has been demonstrated to be correlated with an impaired prognosis [1], it could be assumed that secondary breast malignancies are in general made up of cell populations with heterogeneous DNA distribution patterns. This in turn would imply that in primary tumors with for example, a diploid type of DNA distribution for metastasis to occur, either a change in the genotype of the cell populations takes place, or that metastases arise from pre-existing small subpopulations with augmented malignant properties.

The data presented in this report show that surprising similarities in DNA-distributions exist between primary breast cancers and their metastases even after many months, and regardless of the location of the secondary malignancies. These results are in agreement with previous findings by Meek [9] who observed that when cells in histological sections were measured, the distribution of DNA tended to be similar in both the primary tumor and its axillary metastases [9]. In contrast to our data, Meek described a few exceptional cases with differences of the DNA-distribution pattern betweeen primary tumor and its metastases by emphasis on those cells containing greater amounts of DNA [9]. The total number of cases investigated both in our and in Meek's work is still small and for that reason it is impossible to make any conclusive statements at present. However, the data clearly indicate that in mammary carcinomas the primary tumor and its metastases are in general made up of cell populations with identical genotypes, in turn suggesting a monoclonal development of breast cancer. The monoclonal concept has received further support from a recent study [3] using computerized nuclear morphometry of gallocyanin-stained fine-needle aspirates in which primary breast cancers were found to be "geometrically monoclonal" using pattern recognition techniques.

The four cases of recurrence or simultaneous occurrence of cancer in the other breast are difficult to relate to our other findings. All of the tumors were either diploid or tetraploid which could indicate either that the tumors arose independently, or were the result of metastasis to a target tissue such as has been shown by Kinsey [10]. This latter possibility is not unlikely, as is demonstrated by the rather high incidence of simultaneous or subsequent appearance of breast cancer in the other breast which has been estimated to occur in between 10 and 20% of breast cancer patients.

Application of photometric DNA-

distributions to actual diagnostic situations might best be done where, for example, there is a question of whether a tumor represents a recurrence of a pre-existing cancer or is the manifestation of a new disease. DNA-determinations might also provide an additional and objective guide for the mode of treatment of primary and secondary breast carcinomas. This is of particular interest when patients with estrogen-sensitive tumors are being treated.

The use of archival cytological material for quantitative measurements of DNA in breast carcinomas is a novel and potentially useful method. It is especially relevant to this study as it allows a retrospective study including metastases which occurred a long time period after the diagnosis of the primary tumor.

Fine-needle aspiration biopsy [2, 4] for obtaining tumor cells has some decisive advantages over surgical biopsy. The technique ensures representative sampling of the entire tumor cell population since the aspirating needle passes through the entire tumor mass thereby collecting cells from all parts of the tumor.

Apart from this, fine-needle biopsy is minimally traumatic and allows repeated sampling for example during cytostatic or endocrine treatment of inoperable tumors. We believe, therefore, that fine-needle aspirates provide information useful to the clinician and that fine-needle aspirates of tissues removed at operation can be a valuable reference in the event of subsequent recurrence of cancer in the patient.

Similarities between DNA distribution patterns of primary tumors and their metastases might be interpreted to reflect similar patterns of cellular proliferation. While this may be the case, it is also possible that only a portion of the cell population is in a state of active proliferation, and that the inequities of DNA distribution measured by the Feulgen measurements (which are relatively crude estimates of proliferative activity) do not reflect the proliferative activity of the neoplastic tissue. In analogy with Reed–Sternberg cells in Hodgkin's disease, cells with highly aneuploid DNA values may not replicate, but instead, may be in the G_0 phase of the cell cycle. It is also difficult to determine the proliferative activity of cells in the 2–4c populations since some may be in G_0 or G_1 phase and 4c cells may or may not have undergone S phase. We are attempting to further resolve these questions using ^{3}H-thymidine incorporation in cells of known DNA content from living cell populations (obtained by needle biopsy) to determine rates of cell proliferation in cell populations with different DNA values, and by determining endogenous RNA-polymerase activity to establish the numbers of cells between G_1 and G_0 in different tumor cell populations.

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