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DNA Ploidy in Intraductal Breast Carcinomas

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Cellular DNA-ploidy in 74 clinically detected intraductal breast carcinomas (IDCs) was analysed by flow cytometry. The histograms were classified as either diploid or aneuploid, and the DNA ploidy pattern compared with that of invasive breast carcinomas and normal breast tissue. All normal breast tissues were diploid while 28 (38%) of the IDCs were aneuploid, the DNA indices ranging from 1.32 to 2.00. The frequency of aneuploidy in invasive ductal carcinomas (73%) was significantly higher ($P = 0.003$), DNA index ranging from 1.34 to 2.92, compared with that in IDCs. Retrospectively, 14.5% of the patients had invasive breast cancer 16–166 months after the diagnosis of IDC. Neither DNA ploidy nor histopathological classification alone predicted clinical outcome, but patients with DNA diploid non-comedo IDC had a more favourable course.
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INTRODUCTION

THE INCIDENCE of intraductal carcinoma (IDC) is not known but among the tumours reported to the Norwegian Cancer Registry between 1976–1986, IDC acounted for only 2.4% of all breast cancer. However, because of screening, an increasing number of IDC cases are being detected, accounting for 15–40% of all breast malignancies [1, 2]. Retrospective studies indicate that 25–50% of IDC is likely to progress to invasive cancer [3, 4]. Histological differentiation and nuclear grading [2] and growth patterns [5] may be related to the clinical outcome of IDC.

Analysis of DNA content in some solid tumours has revealed that recurrence and survival is adversely affected by increasing DNA abnormality [6]. In breast cancer DNA ploidy may be an independent prognostic factor [7, 8] or associated with other more powerful prognostic factors [9–11]. Limited information is available on DNA ploidy in IDC based on flow cytometry [12], while static cytometry from a few screening detected lesions has indicated that aneuploidy may be of value in predicting the most biologically aggressive pre-invasive lesions [13].

Our aim was to compare DNA ploidy in pre-invasive and invasive ductal breast carcinomas and to examine the prognostic value of DNA ploidy in IDC.

PATIENTS AND METHODS

Breast tissues

Formalin-fixed and paraffin-embedded breast tissues, histologically classified as IDC, from 106 women with tumours clinically detected during 1965–1983, samples from 30 invasive carcinomas and from 30 cases with morphologically normal breast tissue were retrieved from the files of the Department of Pathology, Regionsykehuset, University of Trondheim, and the Department of Pathology, Fylkessjukehuset in Molde, Norway. On pathological review of the material classified as IDC, 11 cases (10%) were excluded because of probable foci of invasion. 1 case (1%) showing axillary lymph-node metastases was also excluded. 6 cases (6%) with diagnosed invasive carcinoma in the ipsilateral breast less than 12 months after biopsy were excluded and 4 cases (4%) were reclassified as atypical hyperplasia. Thus, 84 cases were included in the study.

Histological classification

IDC with areas of advanced cytological atypia and necrotic cellular debris in ductal spaces was classified as comedo type [14]. All other IDCs were classified as non-comedo type, since they often contained a mixture of histological patterns. Among the cases accepted as IDC the median number of blocks examined was 7 (range 2–38) per patient.

Clinical information

Due to failure in reporting 5 cases of IDCs, complete clinical information was available in 69 cases analysed by flow cytometry, including reports from the Norwegian Cancer Registry and death certificates from the National Bureau of Statistics.

The cut-off date for recurrence-free survival analysis was 31 December 1987. The median observation period for the 69 patients with IDC was 75 months (range 1–273, mean 88). The following were recorded: (a) invasive recurrences in the ipsilateral breast; (b) metastases with no evidence of a new primary tumour; and (c) patients officially classified as dead from mammary carcinoma with no mammary malignancy other than the IDC.

14 patients (20%), had been treated with biopsy or wide local excision only. 55 (80%) underwent some type of mastectomy. 10 of these patients had mastectomy with no previous biopsy,

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and 3 of these cases contained more than one focus of IDC. Additional foci or residual elements of IDC were found in 28 (61%) of the breasts removed after a primary biopsy. Unfortunately, data on the size of the lesions were incomplete, but information on residual or additional foci were used as an indirect measurement of the extension of the IDC.

The mean age of patients with intraductal and invasive breast carcinomas was not significantly different at the time of diagnosis (60.4 [SD 15.0] vs. 59.9 [15.6] years respectively, *t* test. The normal breast tissue controls were significantly younger (40.4 [13.1] years ($P < 0.001$).

Preparation of nuclear suspensions

Nuclear suspensions were prepared from 1–2 50 μm paraffin sections as described by Hedley *et al.* [15], with some modifications. Briefly, sections were dewaxed, rehydrated, digested with 0.5% pepsin (Sigma) in 0.9% NaCl at pH 1.5 for 90 min at 37°C, centrifuged at 400 *g* for 5 min, resuspended in 1.5 ml RNase-solution (sodium citrate with 30 U/ml RNase) for 10 min at 37°C, washed in 1.5 ml phosphate-buffered saline (PBS) and the solution filtered through (45 μm) nylon gauze. The pellet was stained with propidium iodide (10 mg/ml) at 4°C overnight and kept in the dark. The solution was spun down, the pellet resuspended in 1 ml PBS and passed twice through a 25 G needle immediately before analysis.

5 μm sections were cut immediately adjacent to the 50 μm sections and stained with haematoxylin–eosin to confirm the representativeness of the sample.

DNA flow cytometry

Nuclear DNA content was measured in a FAC/Scan flow cytometer (Becton Dickinson) equipped with an air-cooled, 15 mW argon laser and a Hewlett Packard model 310 computer for DNA analysis. The fluorescense signals from 10 000 nuclei were plotted on a 1024-channel linear scale and displayed as a frequency histogram.

Interpretation of DNA histograms

To be accepted as evaluable the histograms had to fulfil the following criteria: (a) be composed of 10 000 nuclei; (b) contain at least two clearly recognizable peaks; and (c) histograms showing a diploid pattern (i.e. one large $G_0/1$ peak, and a small G_2/M peak at about twice the channel number of the first peak) had to have a coefficient of variance (CV) less than 10%—otherwise the possibility of aneuploid near-diploid cell populations could not be excluded. The CV is the standard deviation divided by the mean, calculated from the DNA diploid $G_0/1$ peak. In cases with obvious aneuploid peaks, no such limit was set on the CV. In all cases markers identifying the respective peaks were set manually. The first distinct peak of the histogram was considered diploid, and assigned a DNA index of 1.00, with the known but small risk of missing a hypoploid cell population [16]. Furthermore, microscopy confirmed that every sample contained a sufficient number of non-neoplastic cells to identify a diploid $G_0/1$ peak [17]. Aneuploidy was defined as the presence of additional $G_0/1$ peaks. Histograms displaying more than 13.4% of the nuclei in the diploid G_2/m region were regarded as a near-tetraploid variant of DNA aneuploidy (DNA index 1.90–2.10). This upper limit for the second peak of an apparently diploid cell population is the mean plus 3 S.D. of the G2/M-peaks of the 29 samples of normal breast tissue. No attempt was made to evaluate the fraction of the values corresponding to S-phase cells.

Table 1. DNA ploidy of normal breast tissue, IDCs and invasive breast carcinomas

	No. of cases	DNA ploidy: Diploid (DI = 1.00)	Aneuploid (DI = 1.10–1.90 or DI > 2.10)	Aneuploid (DI = 1.90–2.10)
Normal breast tissue	29	29 (100%)	–	–
IDC	74	46 (62%)	19 (26%)	9 (12%)
Invasive carcinoma	26	7 (27%)	15 (58%)	4 (15%)

DI = DNA index.

Statistics

Student's *t* test was used to test differences between means and Fisher's exact probability test was used to detect differences between proportions. Recurrence-free survival was defined as the period from the time of the diagnosis to recurrence of invasive cancer as stated above. Calculations of recurrence-free survival were based on Kaplan–Meier estimates on the recurrence curves within the actual patient group. Multivariate analysis was done with a Cox proportional hazards model.

RESULTS

DNA ploidy

DNA histograms were successfully obtained in 129 cases (92.8%) (Table 1). The CV of the diploid $G_0/1$ peak was 8.3 [2.0]%. In all 29 cases of normal breast tissue the histograms revealed a single large narrow peak containing on average 91.8 [3.6]% of the nuclei counted. A small second peak regularly occurred at twice the channel number of the first peak. This second peak contained on average 5.6 [2.5]% of the nuclei counted. Thus, all histograms of normal breast tissue were obviously diploid.

In the IDC group (Table 1) 62% of the tumours were diploid and 38% were aneuploid, with DNA indices ranging from 1.32 to 2.00. Among the invasive ductal carcinomas 27% were diploid. The remaining 73% of the tumours were aneuploid, with DNA indices ranging from 1.34 to 2.92. Only a small and nearly equal proportion of the intraductal (12%) and invasive carcinomas (15%) were near tetraploid (DNA indices 1.90–2.10). The difference in distribution of DNA ploidy patterns between intraductal and invasive carcinomas was significant ($P = 0.003$).

DNA ploidy and histopathology

Most non-comedo IDC were DNA diploid, and most of the comedo type IDC were non-diploid (Table 2). This difference in ploidy distribution was significant ($P = 0.02$).

Table 2. DNA ploidy and histopathological classification of IDCs

Histopathological classification	No.	DNA ploidy: Diploid	Aneuploid
Non-comedo	45	33 (73%)	12 (27%)
Comedo	29	13 (45%)	16 (55%)

Table 3. DNA ploidy and age in 74 IDCs and 26 invasive breast carcinomas

DNA ploidy	No. (%)	Mean age (S.D.)
IDC		
Diploid	46 (62%)	58.9 16.0
Aneuploid	28 (38%)	62.8 13.1
Invasive carcinoma		
Diploid	7 (27%)	61.0 14.2
Aneuploid	19 (73%)	59.6 16.5

Table 4. Surgical treatment in 74 patients with IDC in relation to DNA ploidy and histopathological classification

		Surgical procedure		
DNA ploidy	Histopathological classification	Local excision	Mastectomy	Total
Diploid	Non-comedo	9	23	32
	Comedo	1	12	13
Aneuploid	Non-comedo	2	11	13
	Comedo	3	13	16
Total		15	59	74

DNA ploidy and age

There was no correlation between DNA ploidy and age of the patients, either in the IDC group or in the invasive carcinoma group (Table 3). Nor was there any significant correlation between histopathological classification and age among patients with IDC.

Surgical treatment

The surgical procedures in patients with IDC are shown in Table 4. There was no correlation between the selected surgical procedures and DNA ploidy or histopathological classification. The mean ages of patients treated with local excision and mastectomy were 60.1 [18.8] and 59.8 [14.0] years, respectively (not significant).

Tumour recurrence

10 patients (14.5%) had recurrences. The mean time between biopsy and recurrence was 62.6 months (range 16–166, median 37). The mean age of the patients with (63.4 [11.8]) and without (59.2 [15.9]) recurrences was not significantly different. The mean follow-up was 90.3 months (22–166, median 78).

The recurrence rate was 9.5% among patients with DNA diploid IDC and 22.2% among those with aneuploid tumours (Table 5). Among the patients with non-comedo and comedo

Table 5. Number of recurrences in 69 patients with IDC in relation to DNA ploidy and histopathological classification

	Histopathological classification		
DNA ploidy	Non-comedo	Comedo	Total
Diploid	1/31*	3/11	4/42
Aneuploid	4/12	2/15	6/27
Total	5/43	5/26	10/69

*No. with recurrence/no. in group.

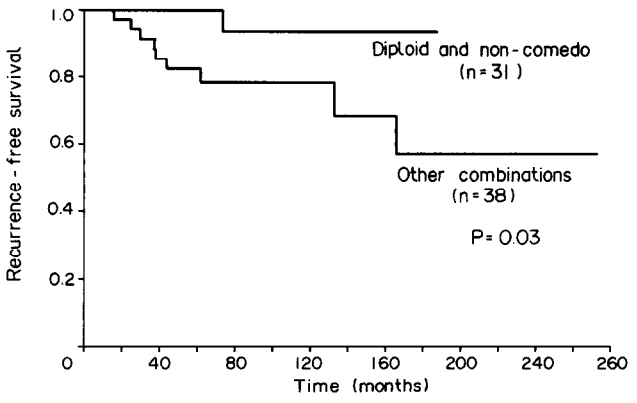

Fig. 1. Recurrence-free survival in patients with intraductal breast carcinomas grouped according to combinations of DNA ploidy and histopathological classification.

IDC, the recurrence rates were 11.6% and 19.2%, respectively. 14% of the patients treated with local excision and 14.5% of those treated with mastectomy had recurrences. 5 of the 31 patients whose mastectomy specimens contained residual elements or multiple foci of IDC had a recurrence, while among the 25 patients without additional IDC in the mastectomy specimens, 4 had a recurrence.

Univariate analysis showed that none of the variables could predict the clinical outcome. On the other hand, a combination of DNA ploidy and histopathological classification indicated that patients with DNA diploid, non-comedo IDC may have a more favourable prognosis ($P = 0.03$) (Fig. 1). However, when other variables were accounted for in the Cox multivariate anaylsis, the predictive value of this combination only reached borderline statistical significance ($P = 0.06$).

DISCUSSION

Flow cytometry has some technical limitations, and a normal DNA index by this technique does not exlude the possibility of an abnormal karyotype [11]. Furthermore, from paraffin embedded material, histograms are of variable quality and therefore we did not do S-phase studies. However, there is, in general, good agreement between results in fresh and paraffin-embedded tissue [15, 18]. We successfully obtained interpretable DNA histograms in 93% of cases studied. Failures were mainly due to very broad $G_0/1$ peaks and in some cases there were too few cells in the suspensions.

All normal breast biopsy specimens and most (62%) of IDC samples were DNA diploid. The frequency of diploidy in invasive carcinomas was 27%, which is in agreement with previous studies in invasive mammary carcinomas [7, 8, 17]. We could not confirm any relation between DNA ploidy and age, as suggested by Taylor *et al.* [19]. Weiss *et al.* [12] reported aneuploidy in 1 of 3 IDCs analysed by flow cytometry, and Carpenter *et al.* [13] using static cytophotometry found that 8 of 12 IDCs (67%) were diploid [13].

We found a correlation between DNA ploidy and histopathology of IDC: comedo type tumours were preferentially associated with aneuploid histograms. This observation accords with studies reporting DNA ploidy to be linked to cellular immaturity in invasive breast cancer [7, 11]. Certain histological types of IDC are said to be more aggressive than others [2]. However, on follow-up, there was no significant relation between histopathology and clinical outcome in our series. The histological complexity of IDC might make it difficult to define clearly pure subgroups.

The recurrence rate was 14.5%, which is lower than 25–50% reported [3, 4]. No obvious reasons explain this discrepancy, but shorter observation and a high number of mastectomies might be involved. However, there was no difference in the recurrence rate between patients treated with mastectomy or local excision. In all the 6 women treated with mastectomy and followed by local invasive recurrences, we found areas of normal breast tissue adjacent to foci of invasive carcinoma. Thus, the surgical procedures had evidently not been as radical as intended. Whether the invasive cancer evolved from a remnant of the primary lesion, or from another focus remains undecided. Furthermore, in retrospective studies the question of adequate histopathological examination of the original lesion may also be raised. In the present study, 6 patients in whom ipsilateral invasive carcinoma was diagnosed within a year of the IDC, were excluded from the follow-up study since the possibility of sampling error could not be excluded.

Additional foci or residual elements of IDC were found in 55% of the mastectomy specimens. Rosen *et al.* [20] demonstrated residual non-invasive carcinoma in 60% of their mastectomy specimens. Such residual foci of IDC could be the main determinant for a later invasive tumour. Furthermore, it is probable that only some IDC are true precursors of invasive cancer. Foci of IDC are detected in the contralateral breast of up to 48% of women with invasive breast carcinomas [21], yet the cumulative risk of opposite-breast cancer 20 years after diagnosis of initial tumour is about 12% [22]. Necropsy series report a prevalence of IDC in up to 15% of adult women [23]. These findings support the concept that not all IDC will progress to invasion.

IDC is recognised as a pre-invasive lesion of the mammary gland. Theoretically one could expect true precursors of invasive cancer to have a ploidy status similar to that of the invasive carcinomas, and that the diploid IDC perhaps more often represents what has been named temporarily proliferative conditions in the ductal epithelium [21]. Alternatively, the significant difference in DNA ploidy between IDCs and invasive carcinomas that we saw may indicate a possible instability in the ploidy status of IDCs as they evolve to invasive carcinoma. Using static cytometry, Carpenter *et al.* [13] compared the DNA ploidy of the "true" IDC with that of the IDC adjacent to invasive carcinoma, and found a significanlty higher proportion of DNA aneuploidy in the latter. They concluded that DNA aneuploidy may be of value in predicting the most biologically aggressive pre-invasive lesions.

In our study DNA ploidy alone could not separate IDC into significantly different prognostic groups, although there was a tendency for aneuploid tumours to be more aggressive. Moreover, a combination of DNA ploidy and histopathological classification was a possible predictor for clinical outcome. However, no firm conclusions should be drawn from this retrospective study on a limited number of cases.

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