

Cytogenetic analysis of breast carcinoma

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ABSTRACT

Cytogenetic analysis was performed on six breast carcinomas. All cases had 46 as a modal chromosome number and all but one had clonal numerical alterations. The most frequent numerical alterations were the clonal monosomy of chromosomes 18 and 16, present in four and three of the six cases, respectively. The only clonal structural alteration found in one case was del(X)(q24). In one of the cases it was possible to propose the sequence of clonal evolution.

INTRODUCTION

By studying cytogenetic alterations in breast carcinoma, important starting points can be obtained for further more detailed analysis involving the cloning of genes (Van de Vijver, 1993). The literature on cytogenetic aberrations in primary breast tumors is limited and often contradictory. Fewer than 300 breast tumors have been fully karyotyped to date and no characteristic cytogenetic abnormality has been observed among them (Devilee and Cornelisse, 1994).

Most primary breast cancer tumor cell lines and metastases have abnormal karyotypes which include structural marker chromosomes, numerical alterations, homogeneously staining regions (HSRs), and double minutes (DMs) (Callahan, 1987). According to the data gathered by Mitelman (1991), all chromosomes, with the exception of the Y, have numerical and/or structural alterations in breast cancer.

MATERIAL AND METHODS

Six untreated patients were either submitted to surgery or to biopsy and the material was sent for histopathological study and for tissue culture and cytogenetic analysis.

Tissue culture and cytogenetics

Fragments of surgical specimens received under sterile conditions were cut into very small pieces, treated with 0.8% collagenase IV (Sigma) and plated in sterile bottles containing HAM-F10 medium (Sigma) supplemented with 20% fetal calf serum and antibiotics. Cells were grown at 37°C and fed twice a week. Table I presents the length of time that the cells were in culture. For cytogenetic analysis, cells from primary cultures in the exponential growth phase were submitted to cell synchronization (Yunis, 1981), collected by trypsin treatment (0.05%), treated with hypotonic 0.075 KCl for about 20 min at 37°C, and fixed with methanol:acetic acid (3:1). Metaphases were submitted to standard Giemsa staining and banded with trypsin-Giemsa (G-banding).

The description of chromosome aberrations was based on the ISCN (1991).

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Table I - Length of time of the cultures.

Case (Code)	Time (days)
1 (MLPG)	11
2 (FMA)	09
3 (DPC)	11
4 (TSS)	12
5 (LS)	12
6 (MHS)	10

RESULTS

Diagnosis

Table II presents the clinical data of all cases.

Chromosome counts

The chromosome counts for the six karyotyped cases are summarized in Table III. In all cases the modal chromosome number was diploid or near diploid, but there was a wide range of chromosome numbers: case

1 was mostly triploid-tetraploid and most of the other cases were essentially diploid (Table III).

Karyotypes

The only clonal structural alteration was the deletion del(X)(q24) (18.8% of the cells) in case 3 (Figure 1). This case also had the telomeric association *tas* (4;5)(p16;p15.3) in one cell (Figure 2).

All cases except case 5 had clonal numerical chromosome alterations. No numerical alteration was shared by all cases, although monosomies of chromosomes 18 (cases 2, 3, 4 and 6; Figures 1, 3 and 5) and 16 (cases 3, 4 and 5; Figures 3 and 4) were the most frequent. Two cases had monosomy of chromosome 6 (cases 3 and 4; Figures 1 and 3), 15 (cases 4 and 6; Figure 5), and 19 (cases 2 and 4; Figure 5). One case each had trisomies of chromosomes 4 (case 4), 8 (case 4; Figures 5 and 6), 11 (case 2), 19 (case 6) and 20 (case 5; Figure 4).

DISCUSSION

Breast cancer is the most common neoplasia among Western women and the major cause of death

Table II - Clinical data.

Case (Code)	Histopathological diagnosis	Lymph node involvement	Breast	Age (years)	Menopausal status	Receptors Er/Pr	Obstetric antecedents	Recurrence
1	Infiltrative ductal carcinoma	Negative	Right	49	Post	Not known	P1/B1	Not known
2	Infiltrative ductal carcinoma	Positive	Left	66	Post	-/-	P2/B2	No
3	Infiltrative ductal carcinoma	Negative	Right	40	Pre	Not known	P1/B1	Not known
4	Infiltrative ductal carcinoma	Positive	Right	55	Post	Not known	P2/B2	Not known
5	Intraductal cibriform and comedocarcinoma	Positive	Left	42	Pre	-/-	P3/B3	Yes
6	Infiltrative lobular carcinoma	Negative	Left	39	Pre	-/-	P4/B4	Yes (lymph node)

ER = Estrogen receptor.

PR = Progesterone receptor.

P = Pregnancy.

B = Birth.

Table III - Distribution of chromosome number.

Case	Range	Haploidy 23-34 (%)	Diploidy 35-57 (%)	Triploidy 58-80 (%)	Tetraploidy 81-103 (%)	> 104 (%)	Modal No.	No. of cells with modal No.	Total of cells
1	25-173/195	12 (6.42)	76 (40.64)	36 (19.25)	59 (31.55)	4 (2.14)	46	26 (13.9)	187
2	35-51	---	72 (100)	---	---	---	46	32 (44.44)	72
3	25-100/150	4 (3.54)	89 (74.34)	---	19 (16.81)	1 (0.88)	46	24 (18.50)	113
4	26-81/91	9 (8.49)	94 (88.68)	2 (1.89)	1 (0.94)	---	46	34 (32.08)	106
5	26-47	3 (2.97)	98 (97.03)	---	---	---	44	21 (20.79)	101
6	28-81/103	9 (8.82)	86 (84.31)	3 (2.94)	4 (3.92)	---	46	24 (23.53)	102

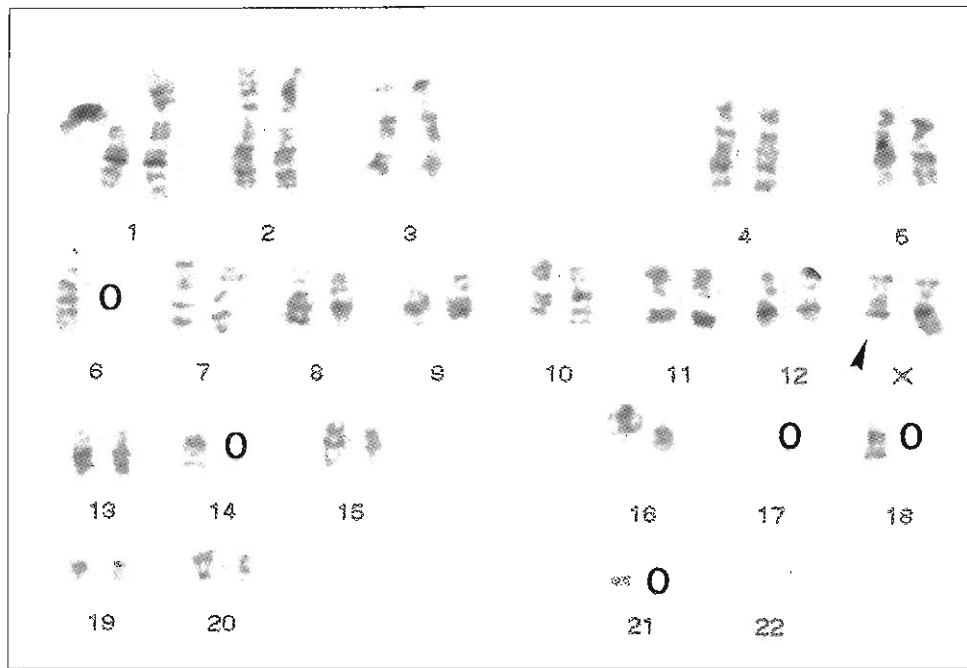


Figure 1 - G-banded karyotype, 41,X, del(X)(q24), -6, -14, -17, -18, -21 (case No. 3).

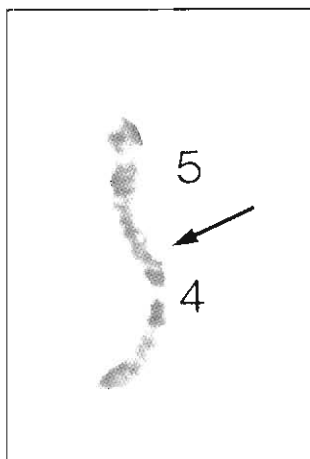


Figure 2 - Partial G-banded karyotype of *tas(4;5)(p16;p15.3)* (case No. 3).

between 35 and 54 years of age (Leonessa *et al.*, 1992). Although the incidence of breast cancer is very high, the number of cytogenetic studies carried out on these neoplasias is relatively small (Sandberg, 1990).

All cases analyzed had a diploid modal number although wide variation existed around this number. Lópes-Guines *et al.* (1991) have described the difficulties involved in determining the modal number in breast tumor cells, due to the presence of several cytogenetically different populations especially involving polyploid cells, a fact that hampers the interpretation of the karyotypes. Case 1 presented the highest degree of polyploidy, followed by cases 3, 4 and 6. Cases 2 and 5 did not present this characteristic.

Del(X)(q24) (case 3) was the only clonal structural chromosome alteration found in the present sample; however, nonclonal structural alterations were also found in cases 3, 4 and 5. Del(X)(q24) has been previously reported in human breast cancer (Rodgers *et al.*, 1984; Hill *et al.*, 1987), but the involvement of the Xq24 breakpoint in chromosome aberrations of breast cancer is infrequent (Mitelman, 1991). No gene related to malignant processes has been detected thus far in this region (HGM 11, 1991). The telomeric association *tas(4;5)(p16;p15.3)* was also observed in case 3, an abnormality that has been reported in other types of human neoplasias and which may be a characteristic of neoplastic cell chromosomes (Pathak *et al.*, 1988).

Cytogenetical clonal deletions are common in human cancer and have led to the discovery of tumor suppressor genes (Frei III, 1993). Substantial evidence suggests that inactivation of tumor suppressor genes play an important role in pathogenesis of human cancer. Inactivation of a tumor suppressor gene often involves mutation of one allele and loss or replacement of a chromosomal segment containing the other allele (Ponder, 1988; Sager, 1989). The chromosomal regions where allelic losses have been detected are thought to include specific target genes whose inactivation either is essential for transformation or provides a selective advantage associated with progression (Chen *et al.*, 1992). Loss of genetic material, identified by loss of heterozygosity (LOH), is the most frequent genetic alteration found in solid tumors, and breast cancer is no exception to this rule (Negrini *et al.*, 1995). Cytogenetic

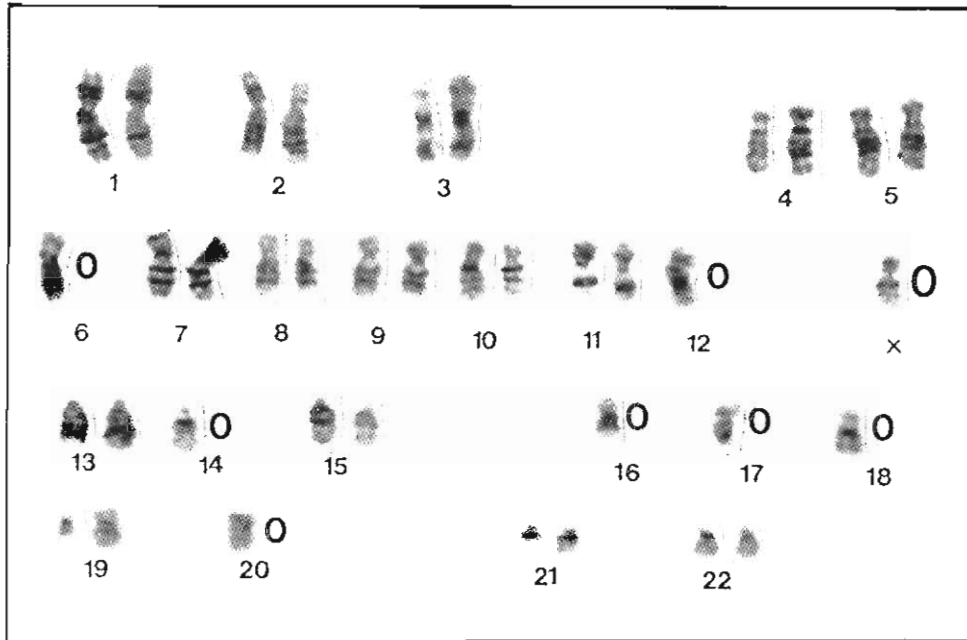


Figure 3 - G-banded karyotype, 38, -X, -6, -12, -14, -16, -17, -18, -20 (case No. 3).

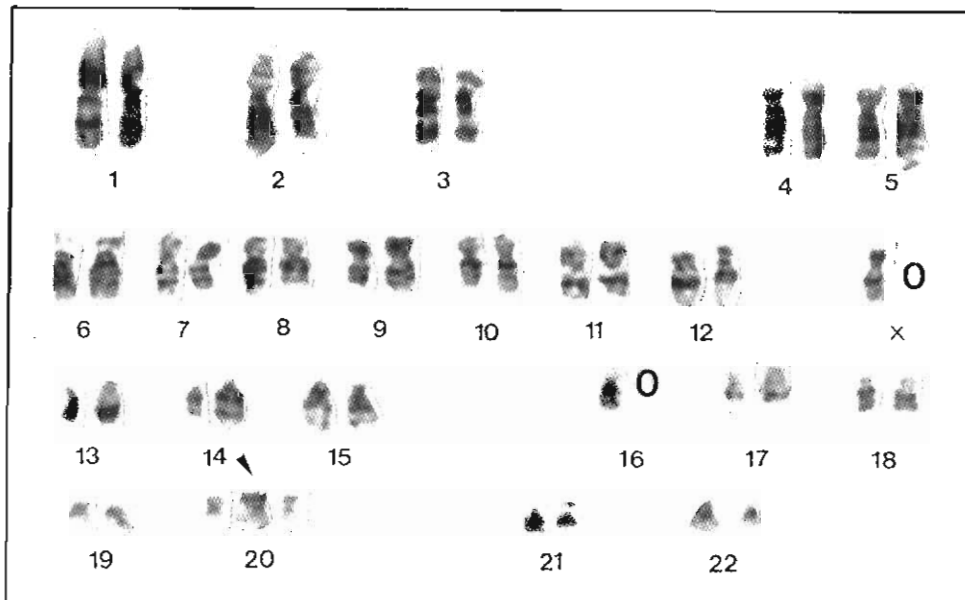


Figure 4 - G-banded karyotype, 45, X, -X, -16, +20 (case No. 5).

analysis shows that breast cancer cells are always aneuploid (Li, 1993). In the present sample the loss of chromosomes 18 and 16 were the most frequent finding, found in four and three of the six cases, respectively. The site of DCC locus is at 18q21.3. LOH at the DCC locus has been demonstrated mainly in sporadic colorectal cancers (Vogelstein *et al.*, 1988; Fearon *et al.*, 1990) and was also identified in sporadic breast cancer (Thompson *et al.*, 1993). Thus, loss of DCC may confer a growth advantage on evolving tumor cells (Weinberg, 1991). Cleton-Jansen *et al.* (1994) have described LOH on

the long arm of chromosome 16 and suggest that this chromosome contains at least two different tumor suppressor genes. Monosomies of chromosomes 7 (case 4), 17 (case 3), and 22 (case 4) may also represent losses of tumor suppressor genes since the loss of allele heterozygosity has been reported for these chromosomes (Chen *et al.*, 1992; Van Vlijver, 1993). Besides the monosomies related to LOH, our sample showed clonal losses of other chromosomes (Table IV) which may also be related to mammary tumorigenesis or to proliferation. In addition, all the chromosomes found in

increased numbers (four and eight in case 4, 11 in case 2, 19 in case 6, and 20 in case 5) had proto-oncogenes and/or growth factors (HGM 11, 1991), which may have been involved in the proliferative and malignant process in the breast tumors.

Evaluation of cytogenetic data indicates that no type of numerical alteration is specific to breast cancer (Hainsworth and Garson, 1990), demonstrating the wide cytogenetic heterogeneity characterizing human breast tumors (Wolman, 1986). In the present study, no clonal numerical alteration was repeated in all cases. This fact supports the hypothesis that rapidly growing tumor cells may lose chromosomes at random (Seizinger et al., 1991). Thus, many monosomies and nullisomies reported in breast tumors may not have significant effects on the etiology or progression of the tumor. Trisomies may also be due to nondisjunctions provoked by a process of accelerated cell division. These alterations may subsequently have been favored because they carried genes presenting advantages for the tumor cell.

The development of sporadic human breast cancer is associated with an accumulation of genetic alterations in several chromosomes (Hampton et al., 1994). There was a high frequency of some alterations in case 4. Twenty of 25 cells analyzed had a loss of chromosome X and 15 were trisomic for chromosome 8 (Figures 5 and 6). The first event in the development of this tumor was probably the loss of one X chromosome; subsequently there was the gain of one chromosome 8 and then a loss of chromosomes 3 and 21.

We suppose that the first event was a loss of the X chromosome because 45, X, -X cells were observed. Trisomy of chromosome 8 was always shared with other alterations. The evolution of this tumor may have occurred in this way:

- 1 - 46, XX
- 2 - 45, X, -X
- 3 - 46, *idem*, +8
- 4 - 44, *idem*, -3, -21

The final formula of the tumor would be:

$n-39-46, X, -X[20], -3[7], +4[2], -6[4], -7[3], +8[15], -15[3], -16[3], -18[3] -19[3] -20[4] -21[6] -22[3]$ [cp 25].

The order of occurrence of chromosome aberrations may not be precise since the number of times an abnormality is repeated is not an indicator of an initiation or progression event. Bullerdiek et al. (1993) found trisomy 8 as a recurrent clonal abnormality in two out of 15 breast tumors. They believe that this trisomy

is an early genetic change and may be associated with a particular subgroup of ductal carcinomas.

Breast cancer is a heterogeneous disease and, not surprisingly, multiple genetic loci are implicated in predisposition to breast cancer (Goddard and Solomon, 1993). These concepts lead to a multifactorial onset of cancer that may arise by the evolution of multiple stages involving progressive chromosome changes and clonal expansion (Weinstein, 1991).

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RESUMO

Análise citogenética foi realizada em 6 carcinomas mamários. Todos os casos têm 46 cromossomos como número modal. Todos os casos, com exceção de um, apresentaram alterações numéricas clonais. As monossomias dos cromossomos 18 e 16 foram as alterações numéricas mais frequentes, apresentando-se em quatro e três dos seis casos, respectivamente. Somente uma alteração estrutural clonal foi detectada, a del(X)(q24). Em um caso foi possível propor a seqüência de sua evolução clonal.

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