Extra copies of chromosomes 16 and X in invasive breast carcinomas are related to aggressive phenotype and poor prognosis

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Background: Breast cancer is a genetically complex disease, which involves the accumulation of various structural and numerical chromosomal aberrations.

Aim: To assess the numerical status of chromosomes 16 and X by interphase cytogenetics, in 114 women with primary invasive breast carcinomas, in relation to clinicopathological parameters, patients' overall survival and indices of cell growth (c-erbB-2, topoisomerase II α (topoll α)) and cell survival (caspase-3, bcl-2).

Experimental design: Chromogenic in situ hybridisation with pericentromeric probes was performed for molecular analysis, while oestrogen and progesterone receptors, cerbB-2, topoll α , caspase-3 and bcl-2 expression was immunohistochemically detected (ABC/HRP). The results were statistically assessed by univariate and multivariate analyses.

Results: Polysomy of chromosomes 16 and X was detected as the predominant aberration (73.7% and 57.9%, respectively). Gain of chromosome 16 copies was associated with high nuclear grade ($p=0.009$), increased tumour size ($p=0.041$), advanced stage ($p=0.002$), the expression of topoll α ($p=0.005$) and worse overall survival by multivariate analysis ($p=0.032$). Chromosome X polysomy was increased in ductal carcinomas of high histological grade ($p=0.008$), in high nuclear grade tumours ($p=0.001$), and was associated with the expression of topoll α ($p=0.005$), loss of caspase-3 ($p=0.036$) and impaired prognosis of ductal carcinomas ($p=0.041$).

Conclusions: Polysomy of chromosomes 16 and X was reported as the predominant alteration in phenotypically aggressive breast tumours, characterised by poor differentiation, increased growth potential and impaired prognosis, whereas gain of chromosome X in particular is probably implicated in cell survival.

Numerical aberrations, including aneusomies (monosomies or polysomies) and changes in ploidy (hypodiploidy or hyperploidy), are frequently found in tumours, including in breast carcinomas, and they are believed to constitute a manifestation of genetic instability.¹

Chromosomes 16 and X have been reported to be frequently affected by structural and/or numerical alterations in several forms of malignancies. Chromosomal arm 16q is a region of common loss of heterozygosity (LOH), observed in in situ and invasive tumours of the breast.^{2–3} This particular region harbours several candidate tumour-suppressor genes, such as CDH1 (16q22.1), encoding for the E-cadherin adhesion molecule, which is implicated in the development of invasive lobular carcinomas, and the WWOX/FOR gene (16q23.2–24.1), of which the expression strongly inhibits anchorage-independent growth in vitro.^{4–5} Moreover, loss or gain of the entire chromosome 16 has been reported in high-grade invasive ductal breast carcinomas.⁶

As far as chromosome X is concerned, several loci of common LOH have been recognised, involving both Xq and Xp,⁷ and Xq, in particular, is usually lost in breast carcinomas related to oestrogen receptor negativity.² In addition, over-representation of chromosome X has been related to lymph node involvement in breast cancer⁸ and constitutes a recurrent change in non-Hodgkin's lymphoma and in meningioma tumours in both female and male patients,^{9–10} whereas loss of the entire X chromosome is observed in invasive ovarian carcinomas.¹¹

Interphase chromogenic in situ hybridisation using pericentromeric probes can be used for chromosome enumeration, as it

is generally accepted that gaining or losing of the centromeric region of a chromosome indicates the gain or loss of the entire chromosome.¹² Furthermore, due to retention of the tissue architecture, it allows an optimal correlation of the cellular-level alterations with the phenotypic characteristics, and bypasses the possible problems of dark-field microscopy.

The purpose of this study was to evaluate the numerical aberrations affecting chromosomes 16 and X in invasive breast cancer, in relation to clinicopathological parameters and patients' recurrence-free and overall survival. Several biological indices were also included to assess the possible implication of both chromosomes in tumour growth and cell survival.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed paraffin-wax-embedded tissues were obtained from 114 women who had had surgery for primary infiltrating breast cancer. None of them had received radiation or chemotherapy before surgery. The patients' age ranged from 25 to 87 years (mean 56.15 years, median 56 years) and they have been followed-up at 6-month intervals, for a median period of 92 months, presenting a mean (range) recurrence-free and overall survival period of 74.73 (2–114) and 81.99 (5–114) months, respectively. Determination of tissues' pathological features was performed as described previously.^{13–14}

Abbreviations: LOH, loss of heterozygosity; topoll α , topoisomerase II α .

Figure 1 (A) Multiple copies of chromosome 16 in neoplastic nuclei ($\times 300$, in situ hybridisation, ISH); (B) neoplastic nuclei with one (monosomy) or two (disomy) hybridisation signals for chromosome 16 ($\times 200$, ISH); and (C) breast cancer cell nuclei, monosomic or disomic for chromosome X ($\times 300$, ISH).

In situ hybridisation

Sections (4 μm thick) were deparaffinised in xylene and pretreated with 30% sodium bisulphate in 2 $\times$ standard saline citrate. Exposure of the target centromeric DNA sequences was achieved by digestion with proteinase K, followed by a brief rinse in 2 $\times$ standard saline citrate and dehydration in graded alcohols. Hybridisation with digoxigenin-labelled pericentromeric probes specific for chromosomes 16 and X (D16Z2 and DXZ1, respectively; Appligene Oncor, Chester-Le-Street, Durham, UK) was performed overnight in a humidified chamber, after simultaneous denaturation of chromosomal and probe DNA. Posthybridisation stringent washings were followed by enzymatic detection, using a peroxidase-conjugated

streptavidin antibody, and amplification according to the manufacturer's instructions.

Evaluation of hybridisation signals for each chromosome per neoplastic nucleus was performed by two independent observers using light microscopy with a $\times 100$ planar objective, and was limited to section areas with intact cell morphology and hybridisation signals in at least 80% of cells. For each probe, the counts were expressed as the percentage of nuclei with >2 signals (trisomy, polysomy), with 2 signals (disomy) and with <2 signals (monosomy), resulting from the enumeration of approximately 300 non-overlapping nuclei per individual tumour. Normal epithelial tissue adjacent to the neoplastic tissue was used as a control for hybridisation sensitivity.

Based on the published data^{8 12-14} and on the evaluation of the signal profile of normal epithelial cells adjacent to the neoplastic tissue (mean value of the control specimens plus three times the SD: nuclei with 1 signal 11% and 9% SD; 2 signals 22% and 15% SD; and ≥ 3 signals 8% and 3.5% SD), we concluded that signal loss in $>40\%$ of cancer cell nuclei could be safely characterised as monosomy, whereas the presence of $>20\%$ of nuclei with signal gain constitutes a reasonable evidence of polysomy.

Immunohistochemistry

The detection of oestrogen and progesterone receptors (ER and PR), c-erbB-2, topoisomerase II α (topoII α), caspase-3 and bcl-2 proteins was performed using a standard avidin-peroxidase technique. Sections (4 μm thick) were incubated overnight at 4°C with the following antibodies: anti-ER clone ID5 and anti-PR clone 1A6 (Dako, Glostrup, Denmark), anti-c-erbB-2 clone CB11 (Biogenex, San Ramon, California, USA), anti-topoII α clone JH2.7 (Biocare Medical, Walnut Creek, California, USA), anti-caspase-3 polyclonal antibody (Santa Cruz Biotechnology, California, USA) and anti-bcl-2 clone 124 (DAKO, Glostrup, Denmark) in dilutions 1:450, 1:150, 1:150, 1:100, 1:80 and 1:100, respectively.

The cut-off values used for the evaluation of the expression levels for each protein have already been mentioned elsewhere.¹⁵⁻¹⁷

Statistical analysis

The possible associations between the percentage of copies of both chromosomes 16 and X and the other assessed parameters were investigated with the Kruskal-Wallis test (histological and nuclear grade, tumour size, stage), the Mann-Whitney U test (menopausal status, histological type, ER, PR, c-erbB-2, topoII α , caspase-3, bcl-2) and by analysis of variance. The impact of the numerical status of both chromosomes on postoperative recurrence-free and overall survival rates was evaluated by univariate (log rank) and multivariate (stepwise forward Cox's proportional hazard regression model) analyses. $p \leq 0.05$ was considered as significant. The models of multivariate survival analysis included menopausal status, histological type, histological and nuclear grade, tumour size, stage and ER, PR and c-erbB-2.

RESULTS

Breast carcinoma tissues demonstrated a heterogeneous ploidy pattern consisting of disomic, monosomic and polysomic nuclei, whereas normal epithelium adjacent to the neoplastic compartment contained mainly diploid nuclei populations.

Chromosome 16

Among the 114 cases studied, polysomy of chromosome 16 (fig 1A) was the more frequently found numerical aberration, predominantly expressed in 73.7% (84/114) of the cases, whereas the remaining 21.1% (24/114) and 5.3% (6/114) of

Table 1 Distribution of chromosome 16 copies in relation to clinicopathological parameters and biological indices (Kruskal–Wallis test (histological and nuclear grade, tumour size, stage), Mann–Whitney U test (menopausal status, histological type, oestrogen and progesterone receptors, c-erbB-2, topoll α , caspase-3, bcl-2) and by analysis of variance)

Patients characteristics	n	Disomy Mean	SD	p Value	Monosomy Mean	SD	p Value	Polysomy Mean	SD	p Value
Menopausal status										
Pre	41	21.39	6.52		27.98	10.33		50.41	13.65	
Post	73	22.79	8.83		25.14	11.16		52.18	15.41	
Histological type										
Ductal	85	21.61	7.55		25.56	11.00		52.85	13.80	
Lobular	29	24.28	9.32		27.90	10.62		47.72	16.98	
Histological grade										
1	13	25.85	12.55		29.54	11.22		45.38	15.70	
2	50	21.42	6.06		24.94	11.35		53.48	13.81	
3	22	19.55	6.03		24.64	10.01		55.82	11.47	
Nuclear grade										
1	42	26.00	9.71		28.95	12.12		45.05	16.91	
2	37	20.19	5.94	0.002	25.24	10.73		54.62	12.72	0.009
3	35	20.06	6.22		23.77	8.95		56.09	11.16	
Tumour size										
<2 cm	27	23.41	7.87		31.37	13.65		45.11	18.91	
2–5 cm	72	22.21	8.64		25.13	9.11	0.016	52.75	12.57	0.041
>5 cm	15	20.67	5.26		21.73	10.73		57.33	13.06	
LN										
Non-infiltrated	41	22.10	6.28		27.20	12.99		50.76	16.14	
1–3 infiltrated	29	23.66	9.41		25.41	11.10		50.93	15.25	
≥4 infiltrated	44	21.57	8.67		25.68	8.60		52.68	13.30	
Stage										
1	17	25.82	10.58		34.06	14.20		39.94	19.20	
2	79	22.01	7.76		25.29	9.32	0.011	52.76	12.51	0.002
3	18	20.17	5.85		22.50	11.08		57.17	14.46	
ER										
Negative	52	21.17	8.39		25.31	9.14		53.37	12.60	
Positive	62	23.23	7.74		26.87	12.23		50.02	16.31	
PR										
Negative	57	21.26	8.50		24.16	10.70		54.44	14.67	
Positive	57	23.32	7.56		28.16	10.84		48.65	14.41	
c-erbB-2										
<10%	47	22.23	6.94		23.34	10.36		54.32	13.28	
≥10%	67	22.33	8.83		28.13	10.93	0.012	49.60	15.52	
Topoll α										
<10%	77	23.21	8.46		27.83	10.78		48.92	14.47	
≥10%	36	20.39	7.03		22.94	10.51	0.016	56.72	14.13	0.005
Caspase-3										
<10%	30	21.70	6.49		27.07	11.86		51.13	15.60	
≥10%	84	22.50	8.59		25.83	10.61		51.69	14.55	
bcl-2										
<10%	48	21.35	6.18		24.02	9.14		54.73	11.54	
≥10%	66	22.97	9.20		27.71	11.86		49.23	16.42	

ER, oestrogen receptor; LN, lymph node; PR, progesterone receptor.

the cases were characterised mainly by monosomy (fig 1B) and disomy, respectively. The percentage of chromosome 16 polysomy was significantly associated with nuclear dedifferentiation ($p = 0.009$; table 1, fig 2A), increased tumour size ($p = 0.041$; table 1, fig 2B), advanced stage of the disease ($p = 0.002$; table 1, fig 2C) and the expression of the topoll α proliferation index ($p = 0.005$; table 1, fig 2D). Loss of chromosome 16 copies was correlated with decreased tumour size ($p = 0.016$; table 1, fig 2B), lower stage ($p = 0.011$; table 1, fig 2C), loss of topoll α expression ($p = 0.016$; table 1, fig 2D) and, moreover, with positive c-erbB-2 protein levels ($p = 0.012$; table 1, fig 2E). The only significant association observed for chromosome 16 disomy was with tumours of low nuclear grade ($p = 0.002$; table 1, fig 2F), whereas aneusomy, in general, was related to increased nuclear grade of breast carcinomas ($p = 0.003$, fig 2F).

Chromosome X

Polysomy of chromosome X was predominantly found in 57.9% (66/114) of the specimen, while monosomic (fig 1C) and disomic cases represented 35.9% (41/114) and 6.2% (7/114) of the breast cancer tumours studied, respectively. Invasive ductal

as well as high nuclear grade carcinomas presented increased levels of chromosome X aneusomy ($p = 0.006$, fig 3A and $p < 0.001$, fig 3B, respectively; table 2). Furthermore, aneusomy was significantly associated with the expression of c-erbB-2 oncoprotein ($p = 0.016$; table 2, fig 3C) and with the loss of the apoptosis-related proteins caspase-3 and bcl-2 ($p = 0.001$, fig 3D and $p = 0.006$, fig 3E, respectively; table 2). In contrast, the levels of disomy were increased in invasive lobular carcinomas ($p = 0.006$; table 2, fig 3A) and low nuclear grade carcinomas ($p < 0.001$; table 2, fig 3B) and were further related to negative c-erbB-2 ($p = 0.016$; table 2, fig 3C) and with positive caspase-3 and bcl-2 expression ($p = 0.001$, fig 3D and $p = 0.006$, fig 3E, respectively, table 2). The levels of chromosome X polysomy, in particular, were elevated in ductal carcinomas of high histological grade ($p = 0.008$; table 2, fig 3F) and high nuclear grade tumours ($p = 0.001$; table 2, fig 3G). In addition, the percentage of copy gain was related to the expression of topoll α ($p = 0.005$; table 2, fig 3H) and to the loss of caspase-3 protein ($p = 0.036$; table 2, fig 3I). High levels of monosomy for chromosome X were observed in ductal tumours of low histological grade ($p = 0.020$; table 2, fig 3F), in low nuclear grade cases ($p = 0.041$; table 2, fig 3G) and coincided with

Figure 2 Boxplots of the relationships observed between the number of chromosome 16 copies and nuclear grade (A, F), tumour size (B), stage (C), topol α (D) and c-erbB-2 (E) proteins.

absence of topol α and c-erbB-2 ($p = 0.031$, fig 3H and $p = 0.026$, fig 3J respectively; table 2).

Patients' survival

The cases presenting, as the predominant alteration, gain of chromosome 16 copies had the worse overall survival, assessed by univariate ($p = 0.017$, fig 4A) and multivariate ($p = 0.032$, hazard ratio (HR) 3.2321, 95% CI 1.1080 to 9.4286) analyses. Similarly, invasive ductal carcinomas with prevalence of chromosome X polysomy presented an impaired outcome, compared with the monosomic cases, assessed by both univariate ($p = 0.019$, fig 4B) and multivariate ($p = 0.041$, HR 3.0842, 95% CI 1.0450 to 9.1025) analyses. Patients with invasive lobular carcinoma had no significant difference in their overall survival regarding the number of chromosome X copies ($p = 0.312$; fig 4C). Among the other parameters included in both multivariate analyses models, only stage was a predictor of impaired prognosis ($p < 0.001$; HR 4.5030, 95% CI 2.3785 to 8.5252 and $p < 0.001$, HR 3.9790, 95% CI 1.8000 to 8.7956, respectively). There was no relationship between the

numerical status of either chromosome and recurrence-free survival of the patients.

DISCUSSION

In the present study, we evaluated the numerical status of chromosomes 16 and X in a group of 114 women with primary invasive breast carcinomas, using an interphase cytogenetic approach. Aneusomy, and more specifically polysomy, of both chromosomes was the predominant ploidy pattern in most cases. A similar pattern for chromosome 16 copies has been reported in invasive ductal carcinomas of the breast,⁶ whereas chromosome X polysomy was the most frequently found aberration in invasive carcinomas of the breast and of the cervix uteri^{8, 18} and in non-Hodgkin's lymphomas.⁹

Deviations of both chromosomes from the normal disomic complement were associated with aggressive phenotypic features of breast tumours such as advanced stage, larger tumour size, high nuclear and histological grade, as has already been mentioned for in situ and invasive ductal carcinomas of the breast^{8, 19} and cervical intraepithelial neoplasia,¹⁸ probably

Figure 3 Boxplots representing the distribution of chromosome X numerical status in relation to histological type (A), histological grade (F), nuclear grade (B, G), and c-erbB-2 (C, J), topoll α (H), caspase-3 (D, I) and bcl-2 (E) proteins.

reflecting groups of breast cancers with different cytogenetics and biologies.² The above-mentioned findings indicate an implication of both chromosomes with breast cancer progression and they may reflect a more widespread accumulation of genetic changes, due to increased genomic destabilisation inherent to a more aggressive form of malignancy.

A novel observation of this study is the statistically independent relationship with survival of impaired patients. Prevalence of chromosome 16 polysomy was found to be a

predictor of shortened overall survival irrespective of the histology of the tumour, whereas chromosome X polysomy was a factor of adverse prognosis specifically for ductal carcinomas. This restriction of the prognostic significance of chromosome X to the ductal breast tumours could be attributed to the observed relationship between aneusomy of this chromosome with this specific histological subtype, whereas the lobular carcinomas included in our study consisted mainly of disomic nuclei populations, probably reflecting the presence

Figure 4 (A) Graphical representation of patients' overall survival according to chromosome 16 numerical status (log rank, Kaplan-Meier); (B) overall survival curves of patients with ductal invasive breast carcinomas according to chromosome X aneusomy (log rank, Kaplan-Meier); (C) overall survival curves of patients with lobular invasive breast carcinomas according to chromosome X aneusomy (log rank, Kaplan-Meier).

of distinct oncogenetic pathways between tumours of the ductal and lobular histological type.

The numerical status of both chromosomes seems to have a direct implication on cell growth, as suggested by the relationships with the expression of topoII α and c-erbB-2 proteins, a finding mentioned for the first time, to our knowledge. Gain of centromeric signals for both chromosomes predominated in topoII α -expressing cells, whereas the levels of chromosome X aneusomy were also associated with the expression of c-erbB-2 protein. Interestingly, chromosome 16 monosomy presented a parallel relationship with c-erbB-2 and an inverse one with topoII α protein. TopoII α is a key regulatory protein in DNA replication and is considered as an indicator of tumour cell proliferation, as it is mainly expressed during the S phase of the cell cycle²⁰ and in phenotypically aggressive breast tumours.¹⁵ Overexpression of c-erbB-2 may result in a shortening of the G1 phase of the cell cycle and early entry to S phase, which leads to hyperproliferation in breast luminal epithelial cells.²¹ Furthermore, due to the physical proximity of topoII α and c-erbB-2 gene loci on chromosome 17, it has been reported, in certain cases, to be co-amplified along with the highly recurrent 17q gain, which results in the overexpression of c-erbB-2 oncoprotein,^{20, 22} observed in most breast carcinomas with increased malignant potential.¹⁶ According to Jarvinen *et al*,²² a physical deletion of topoII α gene also occurs, even before hyperploidy of the tumour in c-erbB-2-amplified breast cancer cell lines, and is associated with decreased levels of topoII α protein expression. Our suggestion regarding a probable relationship of chromosomes 16 and X with cellular proliferation derives further support from the fact that both chromosomes harbour genes implicated in tumour growth, including genes CTCF, SEN16 and Glypican 3.²³⁻²⁵

Of special interest is the observation of a possible involvement of chromosome X aneusomy with apoptosis. The inverse relationship with caspase-3 may be partly supported by the presence on chromosome X of the gene encoding the X-linked inhibitor of apoptosis protein, which specifically binds and inhibits the function of caspase-3, 7 and 9, and is found overexpressed in cancer cell lines, providing a survival advantage.²⁶ The absence of expression of bcl-2 protein in tumours with increased levels of chromosome X gain at first may seem controversial due to the fact that caspase-3 is an inducer of apoptosis, whereas bcl-2 is an antiapoptotic protein which acts as a regulator of caspase-3. Despite that, Fujita *et al*²⁷ report that caspase-3 has the ability, at least in the case of VP-16-induced U937 cell apoptosis, to modulate the function of bcl-2 by cleaving it to a shorter truncated form, which is proapoptotic in contrast with the longer anti-apoptotic form.

In our study, monosomy coincided with rather less aggressive features. This finding is probably unsustainable, given the extensive lack of genetic material, which leads to alterations, or even silencing of numerous genes. Several studies focusing on structural chromosomal aberrations propose that certain losses of genetic segments may in fact correlate with more favourable prognostic features. In a cytogenetic and DNA flow cytometric analysis of a series of breast carcinomas, the loss of the long arm of chromosome 16 was significantly associated with low S phase fraction.²⁸ LOH of 16q in breast cancer has been reported to be present in low-grade non-invasive and invasive breast carcinomas.²⁹ In addition, allelic loss of 16q23.2-24.2 has been reported to be an independent predictor of good disease-free and overall prognosis in primary breast cancer.²⁹ The reports concerning structural alterations of chromosome X provide compelling results, which are further complicated due to the random inactivation of one X chromosome in every cell in females³⁰ and the fact that up to 30 genes are known to escape X chromosome inactivation.³¹ Although

Table 2 Distribution of chromosome X copies in relation to clinicopathological parameters and biological indices (Kruskal–Wallis test (histological and nuclear grade, tumour size, stage), Mann–Whitney U test (menopausal status, histological type, oestrogen and progesterone receptors, c-erbB-2, topoll α , caspase-3, bcl-2) and by analysis of variance)

Patients characteristics	n	Disomy			Aneusomy			Monosomy			Polysomy		
		Mean	SD	p Value	Mean	SD	p Value	Mean	SD	p Value	Mean	SD	p Value
Menopausal status													
Pre	41	26.66	6.78		73.34	6.78		31.63	12.83		41.71	14.34	
Post	73	28.90	8.17		71.11	8.17		31.03	14.16		40.08	16.41	
Histological type													
Ductal	85	27.02	7.31	0.006	72.98	7.31	0.006	31.21	14.03		41.76	15.51	
Lobular	29	31.21	8.27		68.79	8.27		31.34	12.65		37.45	15.91	
Histological grade													
1	13	28.15	11.52	<0.001	71.85	11.52		37.00	15.70	0.020	34.85	16.06	0.008
2	50	27.30	6.51		72.70	6.51		32.28	13.90		40.42	14.88	
3	22	25.73	6.03		74.27	6.03		25.36	11.71		48.91	14.49	
Nuclear grade													
1	42	31.88	7.59	<0.001	68.12	7.59	<0.001	34.48	15.46	0.041	33.64	16.95	0.001
2	37	25.59	7.77		74.41	7.77		32.16	12.66		42.24	14.00	
3	35	26.17	6.13		73.83	6.13		26.40	11.07		47.43	12.23	
Tumour size													
<2 cm	27	28.33	6.96		71.67	6.96		33.56	14.20		38.11	14.14	
2–5 cm	72	27.68	8.29		72.32	8.29		30.67	13.47		41.65	15.86	
>5 cm	15	29.60	6.52		70.40	6.52		29.87	13.91		40.53	17.66	
LN													
Non-infiltrated	41	25.29	7.43		74.71	7.43		34.98	15.92		39.73	17.42	
1–3 infiltrated	29	28.90	6.67		71.10	6.67		29.86	12.43		41.24	14.68	
≥4 infiltrated	44	30.16	8.06		69.84	8.06		28.68	11.45		41.16	14.83	
Stage													
1	17	27.65	8.69		72.35	8.69		37.06	15.78		35.29	15.28	
2	79	28.23	7.69		71.77	7.69		30.46	13.10		41.32	15.20	
3	18	27.89	7.50		72.11	7.50		29.22	13.20		42.89	17.68	
ER													
Negative	52	27.37	7.19		72.63	7.19		29.02	12.05		43.62	14.01	
Positive	62	28.69	8.19		71.31	8.19		33.11	14.68		38.19	16.62	
PR													
Negative	57	27.51	7.20		72.49	7.20		31.96	14.31		40.53	15.83	
Positive	57	28.67	8.28		71.33	8.28		30.53	13.02		40.81	15.61	
c-erbB-2													
<10%	47	26.04	8.59	0.016	73.96	8.59	0.016	33.70	13.69	0.026	40.26	16.30	
≥10%	67	29.52	6.79		70.48	6.79		29.52	13.44		40.96	15.30	
Topoll α													
<10%	77	29.00	8.20		71.00	8.20		33.52	14.68	0.031	37.48	16.34	0.005
≥10%	36	26.28	6.46		73.72	6.46		26.67	9.86		47.06	11.83	
Caspase-3													
<10%	77	24.60	7.04	0.001	75.40	7.04	0.001	30.93	15.18		44.47	17.14	0.036
≥10%	36	29.33	7.64		70.67	7.64		31.36	13.15		39.31	14.96	
bcl-2													
<10%	48	25.77	6.19	0.006	74.23	6.19	0.006	30.92	13.69		43.31	15.02	
≥10%	66	29.77	8.35		70.23	8.35		31.48	13.71		38.74	15.93	

ER, oestrogen receptor; LN, lymph node; PR, progesterone receptor.

LOH in the proximal portion of the inactive Xq, overlapping the AR locus (Xq11–12), it has been described to be important for the control of ovarian tumours of low malignant potential,¹¹ an Xq25 loss of the inactive X chromosome in breast carcinomas

has been related to increased tumour size, lymph nodal involvement and poor differentiation.³²

In the present study, polysomy of chromosomes 16 and X was present as the predominant alteration in phenotypically aggressive breast cancer tumours, characterised by poor differentiation, increased growth potential and impaired outcome, whereas gain of chromosome X, in particular, is probably implicated in cell survival. In contrast, monosomy especially in the case of chromosome 16 was related to a prognostically more favourable phenotype, indicating that loss of chromosome 16 copies may constitute an early event in breast carcinogenesis.

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Take-home messages

- Numerical chromosome aberrations (monosomies, polysomies) are frequently found in breast carcinomas by conventional karyotypic analysis and interface in situ hybridisation technique.
- Polysomy of chromosome 16 was the predominant alteration in phenotypically aggressive breast tumours, affecting patients' survival negatively, while monosomy correlated with tumours of less-aggressive features.
- Polysomy of chromosome X was more frequently detected in less-differentiated invasive ductal carcinomas exerting unfavourable impact on prognosis.

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