ORIGINAL ARTICLE

Frequent 7q gains in flow cytometric multiploid/hypertetraploid breast carcinomas: a study of chromosome imbalances by comparative genomic hybridisation

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Objective: To investigate underlying genetic events associated with complex DNA ploidy breast carcinomas.

Methods: Screening for chromosome imbalances was carried out using comparative genomic hybridisation (CGH) in 14 frozen samples of tumour from a series of 13 breast cancer patients with multiploid (n=11) and hypertetraploid (n=2) tumours. They had previously been analysed by DNA flow cytometry and also assessed immunohistochemically for p53 tissue expression. Ploidy status was determined on frozen samples using the Multicyc software program.

Results: The total number of copy gains (n=242) was significantly greater than the number of copy losses (n=51). The mean (SD) number of gains per sample was 17.3 (5.7), and of losses, 3.6 (4.2) (p=0.0001). Gains of chromosomal regions at 1q (14/14; 100%), 7q (12/14; 85.7%), and 3q (11/14; 78.6%), as well as 1p, 2q, 5p, 8q, and 13q (10/14; 71.4%) were the most frequent aberrations in this series. Losses were most commonly found on 17p (5/14; 35.7%). Three patients dying of the disease had tumours with high level amplifications at 1q12-qter, 3q22-q25, and 8q22-q23 regions. Six cases had p53 overexpression, of whom four showed 12q gains and two showed 17p losses.

Conclusions: There is a very high incidence of genetic aberrations, mainly related to chromosomal gains, in this subgroup of aneuploid breast cancer patients, associated with a poor clinical outcome. The 7q locus, not previously reported as showing frequent changes in breast cancer, was found to be a potential site for some candidate oncogenes.

Prognostic and predictive factors in breast cancer are of great importance in making decisions about adjuvant treatments. In breast cancer patients, cytometric DNA aneuploidy is generally regarded as a marker of an unfavourable clinical outcome in a particular stage of the disease.^{1–3} Among the categories of DNA aneuploidy, the multiploid/hypertetraploid tumours, which account for 5–10% of total cases,^{2,4} have been associated with a worse prognosis.^{3–7} However, DNA ploidy analysis does not provide refined characterisation measurement of the nuclear DNA cancer alterations—that is, the chromosomal sites of genomic imbalances. Comparative genomic hybridisation (CGH) is a molecular cytogenetic technique which enables the detection and mapping of chromosomal gains and losses on the whole genome, thus providing new insights into the biological significance of tumour DNA ploidy.

There is increasing interest in the complex biological functions of the p53 tumour suppressor gene protein; p53 is highly mutated in many different cancers and its regulatory role in carcinogenesis appears more intricate than previously believed.⁸ The main purpose of our study was to screen chromosome imbalances using CGH in a series of multiploid/hypertetraploid breast carcinomas, and additionally, to compare these with p53 tissue expression to achieve better characterisation of the potential underlying genetic events involved in the pathogenesis of these tumours.

METHODS

Clinical and pathological data

The series investigated was retrieved from a larger data file of 740 breast cancer patients previously analysed for DNA flow cytometry (FCM) on a single frozen sampling basis at our department between January 1993 and December 2002. Fifty

four patients (7.3%) were considered to have multiploid/hypertetraploid tumours, but after the FCM study only 14 samples from 13 patients (one patient had two samples) were still suitable for reliable CGH analysis. Of this series, 11 tumours were classified as DNA multiploid and three as DNA hypertetraploid; eight hypertetraploid clones were also present among the multiploid cases. The mean age of the patients was 58.6 years (range 38 to 78). Table 1 summarises the clinical and pathological characteristics of the series. Histological type and pathological stage were evaluated according to the TNM-UICC system.⁹ Tumour differentiation was defined following the Nottingham grading criteria.¹⁰ The patients were submitted either to modified radical mastectomy (nine cases) or tumorectomy (four cases) as the primary surgical treatment. Axillary dissection was carried out in all the patients, with further histological study of the lymph nodes. Seven patients received adjuvant radiotherapy, five received chemotherapy, and one had hormonal treatment. At the end of follow up period (mean 72 months; range 24 to 126), eight patients were alive without evidence of recurrence, three had died from the disease, one had died from another cause, and one was lost to follow up.

DNA flow cytometry

Flow cytometric analysis was undertaken on representative frozen samples of breast carcinoma obtained at surgical resection, according to a previously reported procedure.³ After mechanical disaggregation to obtain monodispersed cell suspensions, the tissue samples were treated with ribonu-

Abbreviations: CGH, comparative genomic hybridisation; DI, DNA index; FCM, flow cytometry; TNM, "tumour, node, metastasis" classification; UICC, International Union Against Cancer

Table 1 Clinicopathological characteristics of the series

Case	Age (years)	Histological type	Pathological stage*	Tumour grade	Oestrogen receptors	Status
1	62	Ductal Ca	IIA	3	Neg	DOD
2	42	Ductal Ca	IIA	3	Neg	AWR
3	76	Ductal Ca	IIB	2	Neg	DOC
4	41	Ductal Ca	IIA	3	Neg	AWR
5	76	Lobular Ca	I	2	Neg	DOD
6	61	Ductal Ca	I	2	Pos	LFF
7	57	Ductal Ca	I	3	Neg	AWR
8	38	Ductal Ca	I	2	Pos	AWR
9	47	Ductal Ca	IIB	3	Neg	DOD
10	72	Ductal Ca	IIA	3	Neg	AWR
11	55	Ductal Ca	I	1	Pos	AWR
12	78	Ductal Ca	I	2	Neg	AWR
13	57	Ductal Ca	IIB	3	Neg	AWR

*Grade I, well differentiated tumour; grade II, moderately differentiated tumour; grade III, poorly differentiated tumour.

AWR, alive without recurrence; Ca, carcinoma; DOC, dead from another cause; DOD, died of the disease; LFF, lost to follow up; neg, negative; pos, positive.

clease (Sigma, St Louis, Missouri, USA) and Nonidet P40 non-ionic detergent (Sigma), stained with 50 µg/ml propidium iodide (Sigma) in Tris-MgCl₂ buffer for one hour in the dark at 4°C, and analysed on an Epics Profile II flow cytometer (Coulter Electronics, Miami, Florida, USA). Fluorescent microspheres were used to calibrate the instrument.

DNA histograms were analysed using the Multicycle program (Phoenix Flow Systems, San Diego, California, USA): software which includes a multiple option cell cycle fitting that automatically determines the DNA index (DI) and cell cycle phase fractions. Mixed "non-malignant" diploid cells from the same tumour sample were used as an internal standard. A tumour showing a single peak was classified as DNA diploid; if an additional peak was present, the tumour was considered DNA aneuploid. The DNA tetraploid range

was previously defined in our own laboratory by estimating the ratio between the mean channel number of the G₂M peak and that of the respective G₀/G₁ peak of 100 consecutive diploid breast tumours.³ The mean (SD) value was 1.98 (0.06), and thus peaks within the DI range of 1.92 to 2.04 were regarded as tetraploid populations if they accounted for >15% of the total histogram events in the presence of a recognisable G₂M peak. A tumour with DI >2.04 was classified as DNA hypertetraploid. If more than one aneuploid peak was observed the tumour was classified as DNA multiploid (fig 1).

CGH analysis

Tumour samples were stored in liquid nitrogen, and microscopically examined for the presence of tumour cells

Figure 1 DNA flow cytometric histogram of a multiploid breast carcinoma with DI = 1.65/2.45. Note that the third peak to the right corresponds to a hypertetraploid clone. DI, DNA index.

Table 2 DNA ploidy and CGH imbalances in multiploid/hypertetraploid breast carcinomas

Cases	DNA index	CGH gains	CGH losses	HLA regions
1	1.47/1.67	1p36.1-p21.1,q22-q32,2p21-p13,2q14.2-q33.1,3p24-p22,3p12,3q10-q27,4p11-q34,5p15.2-p14,6p24-p23,6p12-p10,6q11-q23,7q31-q32,8q11.2-q24.2,10pter-p11.2,10q11.2-q21,12q15-q21,14q11.2-q21	19q13.2-q13.3	8q22-q23
2	2.17/2.43	1q21-qter,2p25-p12,3q25,4p15.2,4q33,5p15.1-p13.1,5q22,6p21.3,6q21,7q11-q31,8p23-q24.1,9p23-p12,9q32,10pter-q11.2,11q13.5-q14.1,12q15-q21,13q31.3-q33,Xq13-q27		
3	2.08	1p32-p21.1,q25-q32,2q14-q34,3q25-q27,5p14,6p21.1-q25,7p14-p11.1,7q11.2-q22,8q21-q23,10q10-q21.2,12q14-q22,13q21-q32,14q13-q22,18q12-q21		7p13.2
4	1.34/1.90	1p34.2-p22.1,q21-qter,2q22-q24,3p12-q13.3,3q23-qter,5pter-q12,6p22-q22.1,7q31,8q13-q24,10p14-p11.2,11pter-p10,12p13-q13,13q14.3-q22,14q13-q21,15q25-qter,17q23-q24,20p13-p11.2,21q21,Xp21.3	17pter-p13,18pter-p11.3,19p13.3-p13.1	3q24-q28;5pter-p14;11pter-p10
5	2.11/2.45	1q12-qter,2p24-p12,2q14,2q21,2q24-q31,4p16-p15,4q21.3-q25,+5,+7,+8,9pter-p12,9q12-q34,10pter-p12,11pter-p12,11q13,13q32,16p13.2-p11.2,17q10-q24,18p11-q23,20p12-qter,Xp21.3	11q23-qter,13q14-q21,16q22-qter,17pter-p13	1q12-qter
6	1.50/1.80/2.10	1q22-qter,16p13.1	7q21,7q31.3,7q36-qter	
7	1.76/3.43	1p35-p13,1q21-q41,2pter-p11.2,2q10-q14.3,2q24-q37.1,3p25-p22,3q10-q13.2,3q25,3q26.3-q28,4p13-q22,5p15.2-p15.1,6pter-q15,6q22.3-q24,7p22-q34,8p22-p12,8q21.3-qter,9pter-p21,10pter-p14,10q25,13q22-qter,14q22-qter,15q14-qter,18q10-qter,20p13-p11.2,20q11.2-q13,Xp22.1-p11.2,Xq25-26	4pter-p16,6q25-qter,11q23-qter,12pter-p13,12p12,12q12-q13,12q23-qter,17pter-p11.2,17q11.2-q21.2,18pter-p11.3	1q24-q31;2p23-p16;4q13-q21;7q11.2-31;8q22-q24.3;9pter-p21
8A	2.05	1p32-p31,1q25,2q21-q22,2q24-32,3p21-p13,3q13.2-q24,3q26.2,4q22-q27,4q28.3-q32,6p21.3-21.1,6q14-q23,7q11.23-7q21.1,8q13-q23,9q31-33,11q13-q23,14q13-q21		
8B	2.07	1p36.1-p31,1q21-q41,2p22-p12,2q11.2-q36,3p23-p21.3,2p21.1-p12,3q13.1-3q26.3,4q21-q32,5q13-q31.1,5q31.3-q34,6q14-q22,7q11.2-q32,8q13-q23,13q14-q32,15q22.3-q23,18q12-q21,18q12-q21,Xp22.1-p11.4,Xq21		
9	1.65/2.45	1p32-p31.3,1q21-qter,2p23-p10,2q11.2,2q21-q24,2q33-q37,3pter-p21,3q13.1-qter,4q10-q31.2,5p15.2-p13,6q14-q21,6q22-q25,7q21-qter,8q11.2-qter,10pter-p10,11p14-p11.2,11q12,11q13-qter,12p13-q22,14q11.2-q32,16q12.1-q22,+20,Xp21.3	4pter-p16,4p14-13,9q21-q22,15q21-q25,17pter-p13,17q25-qter	3q22-q25
10	1.46/1.59	1p31-p22,1q24-q31,2p23-p12,3p12.3,3q12-q21,3q23-q27,5q15-q22,6p21.1-q23,7p21,7q21.1-q21.2,7q22.2-q31,3,8q11.2-q24.1,9p23-q21,10p14-p12,13q21.3-q22,14q11.2-q22,Xp22.1-p11.3,Xq13		
11	1.75/2.02	1p31-p22,1q31-q32,2q22-q31.1,3p14.2-p12.3,3q10-q13.3,3q24-q26.3,5p15.2-p13.3,5q11.1-q23,7q21-q31,9p21.3,12q15-q21,13q21-q32,14q13-q21,16p13.2-p13.1,Xp21.2,Xq13.3-q25	8pter-p23.3,8p22-p21,10q26-qter,16q22-qter,19q13.2,20q13.1-qter,21q22-qter,22q12-qter	
12	1.43/1.70	1q24-q41,2q24-q33,5pter-p13,6pter-6q23,7q11.2-qter,8p11.2-qter,10pter-p11.2,12p13.1-q12,13q21.1-q31,16q12.1-q21,18p11-q11.2,21p11-q21	1pter-p36.3,3p22-p21.3,5q31-qter,9q34-qter,10q24-qter,11q23.3-qter,12q24.3-qter,14q32-8q10-qter	
13	1.34/2.29	qter,17,19q13.2,22q13.1,Xpter-q12,Xq25-qter 1p36.1-p21,1q21-q42,2p25-q37,3q25-q26.1,5pter-q13,5q31-q34,6p23-q22,+7,8q13-q22,9p13,9q12-q21,9q31-q34,10q11.2-q25,12q13-q24.1,13q21.3-q22,13q33-qter,16p13.2-p11.2,21q10-q22,Xp11-q26	6q26-qter,8pter-p23,13q33-qter	

HLA, high level amplification.

(at least 70% per sample) before analysis. DNA was isolated by using a standard phenol based method.¹¹

CGH was carried out according to the method of Kallioniemi *et al.*,¹² as follows: tumour DNA was labelled with biotin 16-dUTP (Enzo-Roche, Mannheim, Germany) and normal reference DNA with digoxigenin-11-dUTP (Enzo-Roche) in a standard nick translation reaction. Equal amounts (400 ng) of labelled tumour DNA and labelled reference DNA were co-precipitated with 15 µg of Cot-1-DNA (Invitrogen, Carlsbad, California, USA) in ethanol. After a three day hybridisation period, fluorescent detection of the

biotin and digoxigenin labelled DNAs was accomplished using avidin-FITC (Jackson Immunossearch, West Grove, Pennsylvania, USA) and antidigoxigenin rhodamine (Enzo-Roche) antibodies, respectively. Samples were counterstained in DAPI in antifade solution (Vector Laboratories, Burlingame, California, USA).

For image acquisition, an epifluorescence microscope (Zeiss Axioplan II; Zeiss, Jena, Germany) equipped with a cooled CCD camera and a triple band beam splitter and emission filters was used. For each tumour, three colour images (blue, red, and green) were acquired from at least 10

Figure 2 Overview of chromosomal gains and losses detected by CGH in multiploid/hypertetraploid breast cancer. Gains are represented by lines to the right of the chromosomes, and losses by lines to the left. Each line corresponds to genetic aberrations seen in one tumour. High level amplifications are represented by solid bars.

metaphase cells. Image analysis was undertaken using CGH analysis software from a CytoVision System (version 2.51; Applied Imaging, Sunderland, Tyne and Wear, UK).

Chromosomal regions were interpreted as gained when the red/green profile ratio exceeded 1.25; as highly amplified when the ratio exceeded 1.5; and as underrepresented when the ratio was less than 0.75. Heterochromatic regions in chromosomes 1, 9, 16, and Y, and the p arms of the acrocentric chromosomes, were discarded from the analysis.

Immunohistochemistry assays

Immunocytochemical analyses of p53 expression and oestrogen receptor status were carried out on formalin fixed, paraffin embedded material using the streptavidin-biotin complex peroxidase technique.¹³ Pretreatment procedures included endogenous peroxidase blocking and antigen retrieval employing a pressure cooker (one minute for p53, and six minutes for oestrogen receptors). The primary

Table 3 p53 tissue expression and related CGH 12q gains and 17p losses in the series

Case	p53 expression	12q gains	17p losses
1	Negative	12q15-q21	–
2	Positive 100%	12q15-q21	–
3	Positive 80%	12q14-q22	–
4	Positive 100%	–	17pter-p13
5	Negative	–	17pter-p13
6	Negative	–	–
7	Negative	–	17pter-p11.2
8	Negative	–	–
9	Negative	–	17pter-p13
10	Negative	–	–
11	Positive 100%	12q15-q21	–
12	Positive 100%	–	17
13	Positive 100%	12q13-q24	–

CGH, comparative genomic hybridisation.

monoclonal antibodies used comprised anti-p53 clone DO-7 (Novocastra Laboratories, Newcastle upon Tyne, UK; 30 minutes, 1:50 dilution, at room temperature) and anti NCL-oestrogen receptor 6F11 clone (Novocastra Laboratories; 30 minutes, 1:10 dilution, at room temperature). Reference tissues with previously known positivity (accumulated mutant p53; presence of oestrogen receptors) were used as positive controls for all assays. A 10% cut-off point previously reported^{3, 14} was used for discriminating positive and negative cases.

Statistical analysis

Statistical differences between chromosomal gains and losses in the whole series were analysed using the Wilcoxon Mann-Whitney test.

RESULTS

Table 2 and fig 2 show an overview of the results of the CGH analysis in this series of multiploid/hypertetraploid breast carcinomas. All the samples showed genomic changes. The mean number of events per sample was 20.9 (range 5 to 37). The total number of gains ($n = 242$) was significantly higher than of losses ($n = 51$). The mean (SD) number of gains per sample was 17.3 (5.7) (range 2 to 27) and of losses, 3.6 (4.2) (0 to 13) ($p = 0.0001$). Five tumour samples had gains with no deletions. The most frequent chromosomal gains were of 1q (14/14; 100%), 7q (12/14; 85.7%) (fig 3), and 3q (11/14; 78.6%), as well as 1p, 2q, 5p, 8q, and 13q (10/14; 71.4%). Losses of 17p (5/14; 35.7%) were the most commonly observed. Simultaneous gains of 1q and 8q occurred in 10 of 14 samples. In the three patients who died of the disease, high level amplifications at 1q12-qter, 3q22-q25, and 8q22-q23 regions were found.

Table 3 summarises p53 tissue expression and related genetic abnormalities (12q gains and 17p losses) in the series. Six cases presented p53 overexpression, of which four showed 12q gains and two 17p losses. The remaining cases lacked p53 overexpression, of which three showed 17p losses and one 12q gains. The simultaneous occurrence of both anomalies was not detected in any case.

DISCUSSION

Despite the considerable number of CGH studies reported on breast cancer in recent years, to our best knowledge there are no previous CGH investigations aiming to characterise the chromosomal alterations in the DNA flow cytometric multiploid/hypertetraploid subgroup of breast carcinomas. The high DNA ploidy changes in these tumours appear an interesting biological feature for deeper molecular investigation of breast cancer pathogenesis and tumour evolution. The small size of this preliminary study stems from the overall low incidence of these FCM tumours and the small number of frozen samples available for CGH, together with the well known limitations of the quality of CGH analysis on paraffin embedded tissue. Furthermore, one should take into account the eventual underestimation of DNA multiploid tumours, because, in accordance with data of Beerman *et al*,¹⁵ most breast cancers can harbour two or more distinct clones when more extensively sampled. There was a predominance of moderately or poorly differentiated oestrogen receptor negative tumours in the series, which is in keeping with the normally unfavourable clinical outcome.

Our data confirmed the most consistent chromosomal aberrations generally reported in breast cancer, namely 1q and 8q amplifications and 17p deletions.¹⁶⁻¹⁸ Tirkkonen *et al*,¹⁹ in their study of 55 unselected primary breast carcinomas, found gains of 1q and 8q, or both, as the most frequent aberrations, which is in accordance with the simultaneous occurrence of these alterations in 10 of 14 samples in our

Figure 3 Ideogram of case 7 CGH result showing DNA amplification at 7q.

series. Most chromosomal alterations identified in our aneuploid subgroup of tumours have also been reported by others^{20, 21} in DNA diploid breast carcinomas. The most frequent finding in the study of Ried *et al*,²⁰ similar to ours, was the overrepresentation of 1q chromosomal arm in nine of 10 diploid cases. Moreover, Rennstam *et al*²¹ found no different structural chromosomal changes between diploid and non-diploid breast tumours. The findings favour the hypothesis that both diploid and aneuploid breast tumours share similar genetic changes in the early stages of their evolution.

The larger number of copy gains compared with the copy number losses, although relatively expected, confirms previously published studies in breast cancer that found gains of genetic material to be slightly more common than losses.^{22, 23} Some studies^{19, 20} also showed that aneuploid tumours contain more copy number aberrations than diploid tumours. The more striking finding in our study was the very large mean number of gains per sample (17.6), which is significantly different from the values reported in previous unselected breast cancer studies (ranging from 3.5 to 11.0). This may be explained in part by the high representation of poorly differentiated oestrogen receptor negative tumours in the present series. Richard *et al*,²⁴ comparing well differentiated with poorly differentiated breast carcinomas, as well as positive *v* negative oestrogen receptor tumours, reported statistically significant differences of numerical aberrations in both groups. In contrast, the mean number of losses per sample (3.6) was similar to that in the literature. Although a larger number of genomic aberrations per tumour has generally been associated with advanced pathological stages, our study shows that they also occur in early stages of the disease (stage I and II). The greater number of genetic abnormalities and complex ploidy changes, reflected as DNA multiploidy/hypertetraploidy, appear to be related more to the intrinsic biological aggressiveness of the tumour than to evolution of the disease. In this regard, Jones *et al*²⁵ showed recently that CGH profiles could help identify different prognostic subgroups of patients with grade III invasive ductal breast carcinomas. This could open interesting possibilities of changing non-specific clinical strategies into improved risk assessment and an individual therapeutic approach.

In their large study of 305 unselected primary breast cancers, Rennstam *et al*²¹ found 7q amplifications in 9.2% of cases. In contrast, it is noteworthy that we found 7q gains (fig 3) in 12 of 14 samples, mainly in the subregions of 7q11.2, 7q21-22, and 7q31. Gains at 7q were described by Fozoran *et al*²⁶ in breast cancer cell lines as a frequent phenomenon (64% of their samples), and amplification sites were also observed at 7q21-22 and 7q31. In the latter two locations some candidate genes were found to be amplified—namely *P-glycoprotein* and *met* proto-oncogene—which have been related to unfavourable clinical indices, including high

histological grade tumours.^{27, 28} *P-glycoprotein* has been linked to multidrug resistant tumour cells, and *met* proto-oncogene to the prediction of invasive/metastatic potential in breast cancer. The molecular relevance of 7q gains has not been highlighted previously in breast cancer and deserves further investigation.

In our study, cases 5 and 11 presented simultaneously a loss of 16q and gains of 1q and 16p, which can be related to the unbalanced translocation between the long arms of chromosomes 1 and 16 previously described in breast cancer.^{29, 30} No candidate breast cancer genes have yet been identified on 1q and 16p, but the 16q chromosomal arm contains the *E-cadherin* gene, commonly associated with mechanisms of cellular adhesion and motility. Genetic losses affecting the 16q region have often been found in lobular breast carcinomas,^{23, 24} and the sole example of this histological type of breast carcinoma in our series (case 5) also showed this genetic aberration. The inactivation of *E-cadherin*—irrespective of the molecular mechanism implicated: truncating mutation, hypermethylation, or loss of heterozygosity at the *CDH1* locus—is known to be a powerful tool for distinguishing lobular from ductal carcinoma of the breast.

The 17p13 loss, which is commonly related to mutation of p53 tumour suppressor gene, was the most common aberration found on chromosome 17, being present in two of three patients who died of the disease. However, this aberration was only detected in two of six cases who showed p53 tissue overexpression (table 3). It is known that p53 protein can accumulate by forming complexes with other molecules—either viral or cellular protein products such as MDM2, a negative feedback regulator of p53 tumour suppressor activity.³¹ The remaining p53 positive cases showed 12q gains in the subregions where the *MDM2* gene is mapped, which raises interest in exploring their putative relationship. Three cases with 17p13 losses lacked p53 overexpression, which might indicate that mutations did not lead to protein stabilisation or that the antibodies used did not detect all mutant proteins.

The 8q gain has been associated with breast cancer progression.³² A high level amplification of 8q22–q23, which maps the *c-myc* oncogene (8q24), was found in one patient who died of the disease, supporting the conclusion by Hislop *et al*³³ that *c-myc* may not be the sole proto-oncogene involved in the 8q region. The other two patients who died of the disease showed high level amplifications at 1q12–qter and 3q22–q25, where no specific breast cancer genes have been identified, suggesting that these regions deserve to be more deeply investigated as targets for poor outcome genetic events. Further studies with a larger number of cases are warranted to confirm the present data and to assess the CGH profiles of other DNA ploidy subclasses of tumours affecting disease prognosis.

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REFERENCES

- 1 Clark GM, Dressler LG, Owens MA, *et al*. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. *N Engl J Med* 1989;**320**:627–33.
- 2 Hedley DW, Clark GM, Cornelisse CJ, *et al*. Consensus review of the clinical utility of DNA cytometry in carcinoma of the breast. *Cytometry* 1993;**14**:482–5.

- 3 Pinto AE, André S, Soares J. Short term significance of DNA ploidy and cell proliferation in breast carcinoma: A multivariate analysis of prognostic markers in a series of 308 patients. *J Clin Pathol* 1999;**52**:604–11.
- 4 Frierson HF. Ploidy analysis and S-phase fraction determination by flow cytometry of invasive adenocarcinomas of the breast. *Am J Surg Pathol* 1991;**15**:358–67.
- 5 Beerman H, Kluin PM, Hermans J, *et al*. Prognostic significance of DNA-ploidy in a series of 690 primary breast cancer patients. *Int J Cancer* 1990;**45**:34–9.
- 6 Pinto AE, André S, Nogueira M, *et al*. Flow cytometric DNA hypertriploidy is associated with unfavourable prognostic features in breast cancer. *J Clin Pathol* 1997;**50**:591–5.
- 7 Michels J-J, Duigou F, Marnay J, *et al*. Flow cytometry in primary breast carcinomas: Prognostic impact of multiploidy and hypoploidy. *Cytometry B Clin Cytom* 2003;**55**:37–45.
- 8 Bode AM, Dong Z. Post-translational modification of p53 in tumorigenesis. *Nat Rev Cancer* 2004;**4**:793–805.
- 9 Sobin LH, Wittekind C, editors. *UICC TNM classification of malignant tumours*, 6th edition. New York: John Wiley and Sons, 2002.
- 10 Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;**19**:403–10.
- 11 Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*, 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
- 12 Kallioniemi OP, Kallioniemi A, Piper J, *et al*. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. *Genes Chromosomes Cancer* 1994b;**10**:231–43.
- 13 Hsu SM, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase techniques. *Am J Clin Pathol* 1981;**75**:816–21.
- 14 André S, Pereira H, Nogueira M, *et al*. p53 overexpression is a prognostic indicator in poorly differentiated, node negative and T1/T2 invasive ductal breast cancer patients. *Breast* 1997;**6**:194–201.
- 15 Beerman H, Smit VT, Kluin PM, *et al*. Flow cytometric analysis of DNA stemline heterogeneity in primary and metastatic breast cancer. *Cytometry* 1991;**12**:147–54.
- 16 Kallioniemi A, Kallioniemi O-P, Piper J, *et al*. Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc Natl Acad Sci USA* 1994;**91**:2156–60.
- 17 Pandis N, Heim S, Bardi G, *et al*. Improved technique for short-term culture and cytogenetic analysis of human breast cancer. *Genes Chromosomes Cancer* 1992;**5**:14–20.
- 18 Tanner MM, Karhu RA, Nupponen NN, *et al*. Genetic aberrations in hypodiploid breast cancer: Frequent loss of chromosome 4 and amplification of cyclin D1 oncogene. *Am J Pathol* 1998;**153**:191–9.
- 19 Tirkkonen M, Tanner M, Karhu R, *et al*. Molecular cytogenetics of primary breast cancer by CGH. *Genes Chromosomes Cancer* 1998;**21**:177–84.
- 20 Ried T, Just KE, Holtgreve-Grez H, *et al*. Comparative genomic hybridization of formalin-fixed, paraffin-embedded breast tumors reveals different patterns of chromosomal gains and losses in fibroadenomas and diploid and aneuploid carcinomas. *Cancer Res* 1995;**55**:5415–23.
- 21 Rennstam K, Ahlstedt-Soini M, Baldetorp B, *et al*. Patterns of chromosomal imbalances defines subgroups of breast cancer with distinct clinical features and prognosis. A study of 305 tumors by comparative genomic hybridization. *Cancer Res* 2003;**63**:8861–8.
- 22 Persson K, Pandis N, Mertens F, *et al*. Chromosomal aberrations in breast cancer: a comparison between cytogenetics and comparative genomic hybridization. *Genes Chromosomes Cancer* 1999;**25**:115–22.
- 23 Loveday RL, Greenman J, Simcox DL, *et al*. Genetic changes in breast cancer detected by comparative genomic hybridisation. *Int J Cancer* 2000;**86**:494–500.
- 24 Richard F, Pacyna-Gengelbach M, Schluns K, *et al*. Patterns of chromosomal imbalances in invasive breast cancer. *Int J Cancer (Pred Oncol)* 2000;**89**:305–10.
- 25 Jones C, Ford E, Gillett C, *et al*. Molecular cytogenetic identification of subgroups of grade III invasive ductal breast carcinomas with different clinical outcomes. *Clin Cancer Res* 2004;**10**:5988–97.
- 26 Fozzaran F, Veldman R, Ammerman CA, *et al*. Molecular cytogenetic analysis of 11 new breast cancer cell lines. *Br J Cancer* 1999;**81**:1328–34.
- 27 Larkin A, O'Driscoll L, Kennedy S, *et al*. Investigation of MRP-1 protein and MDR-1 P-glycoprotein expression in invasive breast cancer: a prognostic study. *Int J Cancer* 2004;**112**:286–94.
- 28 Bevilacqua L, Matsumoto K, Lin CS, *et al*. Expression of the c-Met/HGF receptor in human breast carcinoma: correlation with tumor progression. *Int J Cancer* 1997;**74**:301–9.
- 29 Flagiello D, Gerbeau-Seureau M, Sastre-Garau X, *et al*. Highly recurrent der(1;16)(q10;p10) and other 16q arm alterations in lobular breast cancer. *Genes Chromosomes Cancer* 1998;**23**:300–6.
- 30 Pandis N, Heim S, Bardi G, *et al*. Whole-arm t(1;16) and i(1q) as sole anomalies identify gain of 1q as a primary chromosomal abnormality in breast cancer. *Genes Chromosomes Cancer* 1992;**5**:235–8.
- 31 Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature* 1997;**387**:299–303.
- 32 Isola JJ, Kallioniemi O-P, Chu LW, *et al*. Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. *Am J Pathol* 1995;**147**:905–11.
- 33 Hislop RG, Pratt N, Stocks SC, *et al*. Karyotypic aberrations of chromosomes 16 and 17 are related to survival in patients with breast cancer. *Br J Surg* 2002;**89**:1581–6.