

Chromosomal genotype in breast cancer progression: Comparison of primary and secondary manifestations

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Abstract. The purpose of this study was to compare the chromosomal genotype between breast cancers with and without secondary manifestations and between primary tumors and their secondary manifestations. Eighty six breast cancers, twenty lymph node metastases, ten distant metastases and ten local recurrences were analyzed by comparative genomic hybridization. Tumors with local recurrences showed significant more frequent losses at 2q32 than the tumors without recurrences. Lymph node positive cases showed significant more frequent losses at 9p21 than node negative cases. Lymph node metastases exhibited significant more frequent losses at 7q11, 14q24.3–q31 and 17q22–q24 than their primary tumors. In cases with distant metastases, losses at 5q23 were more frequent than in those without, but not reaching the significance level. The distant metastases showed significant more frequent losses at 5p15, 12q24 and 17q22–q24 than the primary tumors. These results reveal strong evidence that the potential for progression is determined in the primary tumor and that different ways of the development of local recurrences, lymph node and distant metastases exist. After confirmation of the results by interphase FISH on tissue micro arrays, the detection of these specific chromosomal imbalances may contribute to a more individual prediction of prognosis in breast cancer.

Keywords: Breast cancer, local recurrences, metastases, comparative genomic hybridization

1. Introduction

The progression of breast cancer disease is characterized by either local recurrences or lymph node and distant metastases. The development of local recurrences seems to depend on factors like positive margins after surgery, multifocal or multicentric disease and extensive intraductal component of the primary tumor [4, 8, 11, 23, 26], whereas the development of metastases requires the capability to growth, detachment from the neighboring cells, neoangiogenesis, entrance to circulation, survival in circulation and invasion into the stroma of host organ [7, 10, 28]. Many mechanisms necessary for the development of secondary manifestations are still unknown, including the onset of metas-

tases and local recurrences and the role of tumor heterogeneity.

In any case, the occurrence of local recurrences, lymph node and distant metastases are signs of tumor progression, which is associated with accumulation of aberrations in the genome. Comparative genomic hybridization (CGH) screens the whole genome for chromosomal gains and losses [13, 15]. The use of genomic DNA isolated from archived, paraffin embedded material makes CGH an ideal tool for the analysis of specimens from different points of tumor progression. Only few studies analyzed chromosomal changes in the metastatic process in breast cancer. These studies were focused on specific aspects, e.g. lymph node negative cases [5] or modifications of the CGH method [17] and considered either primary tumors with and without secondary manifestations or primary tumors and their corresponding secondary manifestations [1, 5, 17]. Some CGH studies and gene expression studies describe also consistent chromosomal imbalances or

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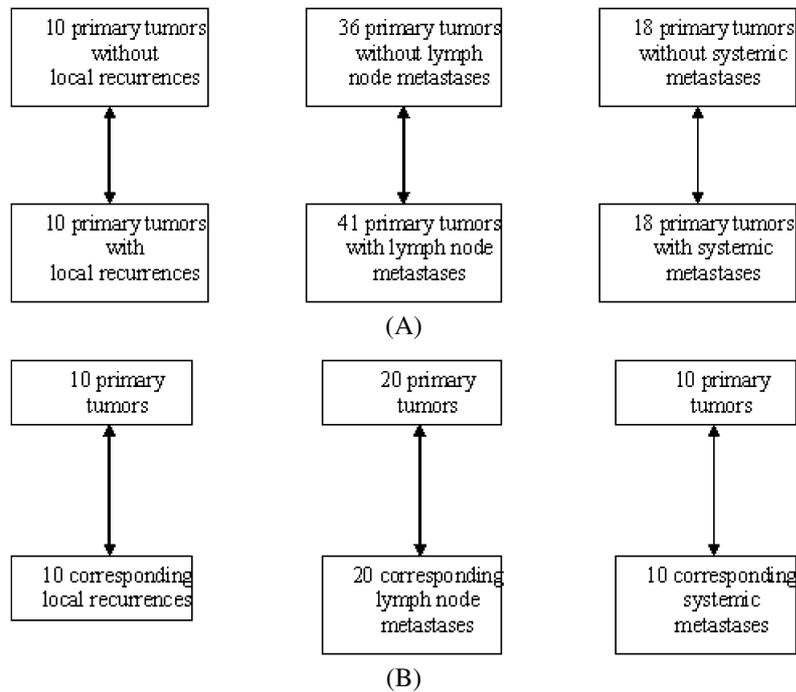


Fig. 1. Study design: (A) Comparison of primary tumors without and with secondary manifestations; (B) Comparison of primary tumors and their corresponding secondary manifestations.

gene expression patterns between primary tumors and their metastases [2,3,29]. These consistent features in primary tumors and their metastases refer to the existence of markers in primary tumors, which are associated with the occurrence of metastases.

Thus, the purpose of this study was to determine differences in the chromosomal genotype between breast cancers with and without secondary manifestations and between primary tumors and their corresponding local recurrences or metastases (Fig. 1). The results may provide new prognostic markers and contribute to a better understanding of the role of genomic events in breast cancer progression.

2. Materials and methods

Eighty six breast cancers (81 invasive ductal carcinomas, four invasive lobular carcinomas, and one papillary carcinoma) were examined by CGH. Table 1 summarizes the clinico-pathological characteristics of the primary tumors. Follow up data were available for all patients. The follow up time ranged from 4 to 118 months (mean follow up 54.7 month). Thirty two patients died of breast cancer between 4 and 110 month after first surgery (mean survival time 47 months). Dis-

tant metastases were found in 18 patients. The mean time from first surgery to occurrence of distant metastases was 26.1 months (between 1 and 83 months). Ten patients developed a local recurrence. The mean time from first surgery to recurrence was 21.7 months (between 7 and 43 months). The lymph node status at the time of first surgery determined the selection of primary tumors for the comparison of lymph node positive and negative cases.

To minimize influences of histological type, tumor stage, lymph node stage or grade in the comparison between cases with and without local recurrences or distant metastases, cases were selected for the best concordance of histological type, tumor stage and differentiation grade. Some cases are part of more than one subgroup.

The formalin fixed, paraffin embedded tissues of corresponding secondary manifestations (twenty lymph node metastases, ten distant metastases and ten local recurrences) were also subjected to CGH analysis (Fig. 1).

The DNA was isolated from archived frozen tumor samples or from routinely processed, paraffin embedded tissue specimens by a standard phenol extraction protocol. Each tissue specimen was reviewed by HE stained sections to ensure a minimum of at least 80%

Table 1
Clinico-pathological characteristics and follow up times of the studied breast cancers

Cases	Histology		pT		pN		Grade			Mean follow up in months (range)	Material
	IDC	Other	pT1	pT2-4	pN0	pN1-3	1	2	3		
With local recurrence	9	1	3	7	4	6	1	3	6	41.1 (12–118)	frozen 8 paraffin 2
Without local recurrence	9	1	3	7	4	6	1	2	7	54.3 (16–93)	frozen 8 paraffin 2
With lymph node metastases	39	2	14	27	0	41	4	11	26	54.1 (4–118)	frozen 32 paraffin 9
Without lymph node metastases	35	1	19	17	36	0	9	18	9	60.3 (5–118)	frozen 30 paraffin 6
With distant metastases	16	2	3	15	4	14	0	2	16	39.4 (5–91)	frozen 11 paraffin 7
Without distant metastases	17	1	5	13	4	14	0	6	12	57.4 (12–104)	frozen 18 paraffin 0
All cases	81	5*	33	53	38	48	13	33	40	54.7 (4–118)	frozen 77 paraffin 19

*Four invasive lobular and one invasive papillary breast cancer.

of tumor cells in each sample. The CGH was performed as previously described [9]. In brief, one microgram of the tumor DNA (nick labeled with biotin-16-dUTP) and normal female DNA (nick labeled with digoxigenin-11-dUTP) and 25 µg Cot-1-DNA were co-hybridized on metaphases with normal female karyotype. The hybridization result was detected by fluorescein isothiocyanate avidin (FITC) for tumor DNA and by anti-digoxigenin conjugated with tetramethylrhodamine isothiocyanate (TRITC). Counterstaining with 4'6-diamidino-2-phenylindol (DAPI) was performed for identification of chromosomes. For control purposes, each hybridization procedure includes at least one cohybridization of normal female with normal male DNA as well as one hybridization of a case with known aberrations, usually the breast cancer cell line SKBR3. The FITC-, TRITC- and DAPI images were captured with a cytometry workstation for fluorescence image acquisition [9]. At least 10 metaphases per case were evaluated with a custom-made CGH analysis program (IBSB, Germany) [19,22] based on AMBA digital image analysis software. The software displayed besides the ratio profile also the 95% confidence interval, which was used for control purpose. The software included a component for assessing chromosomal gains and losses based on a *t*-test (according to Student; $p \leq 0.05$) and a further component for the comparison of chromosomal imbalances in tumor subgroups based on chi-square test ($p \leq 0.05$). To exclude random effects arising from the multitude of

possible aberrations caused by band by band comparison, the chi-square test was performed twice with a randomly selected learn and test set. The chromosomal imbalances with significant differences were checked in the ratio profiles (including the confidence intervals) of each involved case of the subgroup. Finally, the significant differences were confirmed by chi-squared test by the software EXCEL with Bonferroni adjustment of *p*-value for a more stringent analysis of the imbalances detected by chi-square test of learn and test set. According to the principle of Bonferroni adjustment, the significance level desired for the actual discrimination ($p < 0.05$) has to be divided by appropriate degree of freedom. The degree of freedom is equal to the number of uncorrelated variables. If the number of variables exceeds the number of cases, then the number of cases corresponds with the degree of freedom. The regions 1p, 16p, 19p and 22 were not considered for the comparison of different subgroups.

There was no component for detection of amplifications in the used software. Thus gains with a ratio of more than 1.5 were regarded as pronounced chromosomal gains (amplifications).

The number of chromosomal imbalances was analyzed as averaged number of copy alterations (ANCA) [21] in general and additional separately as gains (+) and losses (–) by the *t*-test (according to Student; $p \leq 0.05$).

The study was performed with ethics committee approval.

3. Results

Table 2 gives an overview of the most common chromosomal imbalances and the amplifications for all primary tumors and for the different subgroups. Results of comparison of the different subgroups are summarized in Table 3 and in histograms in the supplementary ma-

terial (http://www.qub.ac.uk/isco/ISCOWEB/CO_06_541.htm).

4. Local recurrences

Ten primary tumors with local recurrences were compared with ten primary tumors without local recur-

Table 2
Most frequent imbalances in all primary tumors and in the analysed subgroups

	Most frequent gains	Number	Most frequent loss	Number	Amplifications	Number
All primary tumors (<i>n</i> = 86)	1q22–q23	47 (55%)	13q21	56 (65%)		20
	1q32–q41	47 (55%)	13q31	56 (65%)	Most frequent at	20 (23%)
	16p12	43 (50%)	18q22	47 (55%)	8q24	8 (9%)
	20q12	43 (50%)			17q21	5 (6%)
				20q13	4 (5%)	
Primary tumors without local recurrence (<i>n</i> = 10)	1q25–q31	7 (70%)	3p21	6 (60%)		1 (10%)
	8q21	6 (60%)	5q31	6 (60%)	With 8q21 and	
	8q24.1	6 (60%)	16q23	8 (80%)	8q24 amplification	
Primary tumors with local recurrence (<i>n</i> = 10)	1q23	8 (80%)	2q32	7 (70%)		2 (20%)
	16p12	6 (60%)	4q26	8 (80%)	Both with	
	20q12	6 (60%)	5q21	7 (70%)	8q22 and	
			5q23	7 (70%)	8q24	
			9p21	7 (70%)	amplification	
			11q23	7 (70%)		
			12p12	7 (70%)		
			13q21	7 (70%)		
			13q31	7 (70%)		
			16q21	7 (70%)		
			18q	7 (70%)		
Local recurrences (<i>n</i> = 10)	1q22	4 (40%)	3p14	7 (70%)	No case with	0 (0%)
	8q22	7 (70%)	8p21	7 (70%)	amplification	
	8q24.1	7 (70%)	13q22	7 (70%)		
			14q21	7 (70%)		
			15q14	7 (70%)		
Primary tumors without lymph node metastases (<i>n</i> = 36)	1q22	24 (67%)	2q32	20 (55%)		8 (22%)
	1q32	22 (61%)	5q21	18 (50%)	Most frequent	
	16p12	20 (56%)	8p22	18 (50%)	at 8q24	2 (6%)
	20q12	20 (56%)	13q21–qter	21 (58%)		
		Xq21	18 (50%)			
Primary tumors with lymph node metastases (<i>n</i> = 41)	1q24	23 (56%)	4q26	28 (68%)		13 (32%)
	1q43	22 (54%)	9p21	29 (71%)	Most frequent	
	8q23–q24.1	23 (56%)	13q31	28 (68%)	at 8q24	4 (10%)
				17q21	4 (10%)	

Table 2
(Continued)

	Most frequent gains	Number	Most frequent loss	Number	Amplifications	Number
Lymph node metastases (n = 20)	8q22 8q23 8q24.1	8 (40%) 9 (45%) 8 (40%)	4q28 4q32 13q31 18q12–q21 Xq21	16 (80%) 16 (80%) 17 (85%) 16 (80%) 16 (80%)	Most frequent at 8q24	4 (25%) 2 (10%)
Primary tumors without haematogenous metastases (n = 18)	1q32 1q42 8q24.1 16p12 20q12	12 (67%) 12 (67%) 11 (61%) 11 (61%) 11 (61%)	4q32 8p21 11q22 16q21 Xq21	9 (50%) 9 (50%) 9 (50%) 9 (59%) 10 (56%)	At 8q24 17q21 20q13	3 (17%) 1 (6%) 1 (6%) 1 (6%)
Primary tumors with haematogenous metastases (n = 18)	1q41 8q22 8q24.1	10 (56%) 10 (56%) 9 (50%)	5q23 9p21 13q31 18q22	13 (72%) 12 (67%) 12 (67%) 12 (67%)	Most frequent at 8q24 17q21	7 (39%) 2 (11%) 2 (11%)
Haematogenous metastases (n = 10)	1q41–q42 1q24 8q13 8q22	4 (40%) 4 (40%) 5 (50%) 7 (70%)	5p15 8p12–p22 11q23 12q24 13q31 17p13 Xq27	8 (80%) 10 (100%) 8 (80%) 8 (80%) 8 (80%) 8 (80%) 8 (80%)	No case with amplification	0 (0%)

Table 3

Results: Significant differences in recurrent chromosomal imbalances between the compared subgroups

	Comparison of		p-value
	Primary tumor without local recurrence	Primary tumor with local recurrence	
Loss at 2q32	0 (0%)	7 (70%)	0.001
Loss at 9p21–p23	Primary tumors without lymph node metastases	Primary tumors with lymph node metastases	<0.001
	11 (31%)	29 (71%)	
Loss at 7q11	Primary tumor	Lymph node metastases	<0.001
	0 (0%)	10 (50%)	
Loss at 14q24.3–q31	4 (20%)	14 (70%)	0.001
Loss at 17q22–q24	0 (0%)	10 (50%)	<0.001
Loss at 5p15	Primary tumor	Distant metastase	<0.001
	0 (0%)	8 (80%)	
Loss at 12q24	0 (0%)	8 (80%)	<0.001
Loss at 17q22–q24	0 (0%)	7 (70%)	0.001

rences.

The primary tumors without local recurrences showed the most frequent gains at 1q25–q31 (70%), at 8q21 (60%) and at 8q24.1 (60%). The most frequent losses in this subgroup were located at 3p21 (60%), at 5q31 (60%) and at 16q23 (80%). Two amplifications were observed in one case of the local recurrence-free group at 8q21 and at 8q24.

The primary tumors with local recurrences showed the most frequent gains at 1q23 (80%), at 16p12 (60%) and 20q12 (60%). The most frequent losses were detected at 4q26 and at Xq21 (both in 80%), at 2q32, at 5q21, at 5q23, at 9p21, at 11q23, at 12p12, at 13q21, at 13q31, at 18q and at Xq25 (all in 70%). Amplifications occurred in two of ten cases with local recurrence, both with amplification at 8q22 and 8q24 (Table 2).

Primary tumors with local recurrences showed a higher number of imbalances than tumors without recurrences (16.2 versus 11.8; $p = 0.05$) (Fig. 2). The primary tumors with local recurrences exhibited significant more frequent losses at 2q32 than the tumors without recurrences ($p = 0.001$) (Table 3).

The ten primary tumors with local recurrences were compared with their local recurrences.

The local recurrences showed the most frequent gains at 1q22 (40%), at 8q22 (70%) and at 8q24.1

(70%). The most frequent losses in local recurrences were detected at 3p14, at 8p21, at 13q22 and at 15q14 (all in 70%).

No amplification was detected in local recurrences (Table 2).

The local recurrences harbored a significantly higher number of chromosomal imbalances than their corresponding primary tumors (22.6 versus 16.2; $p = 0.02$). The number of chromosomal losses was significant higher in local recurrences than in the primary tumors (8.9 versus 14.7; $p = 0.006$), whereas the number of gains was nearly equal in primary tumors and local recurrences (Fig. 3). However, the comparison of primary tumors and their local recurrences did not detect any recurrent imbalances in the local recurrences. A loss at 16q21 occurred more frequent in the primary tumors than in the local recurrences (70% versus 20%; $p = 0.025$), but not reaching the significance level after Bonferroni adjustment.

5. Lymph node metastases

Forty one primary tumors with lymph node metastases and 36 primary tumors without lymph node metastases were analysed.

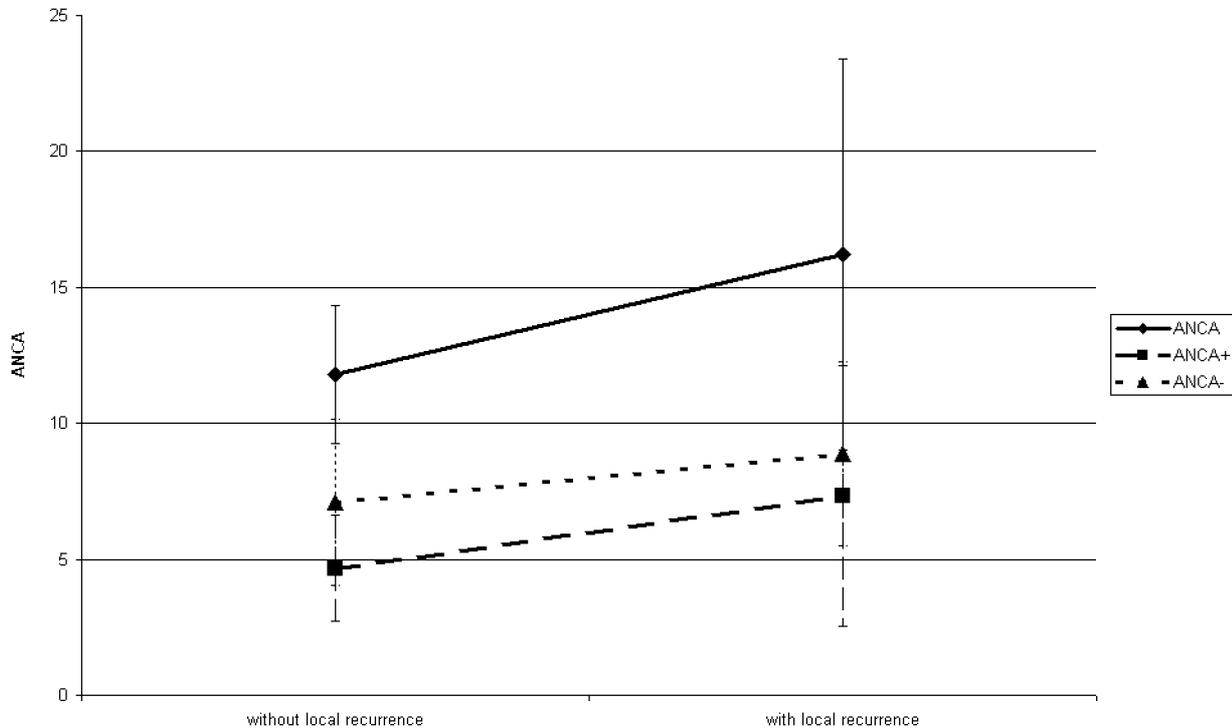


Fig. 2. Averaged number of copy alterations (ANCA) for the comparison of primary tumors with and without local recurrences. The vertical lines represent the standard deviations.

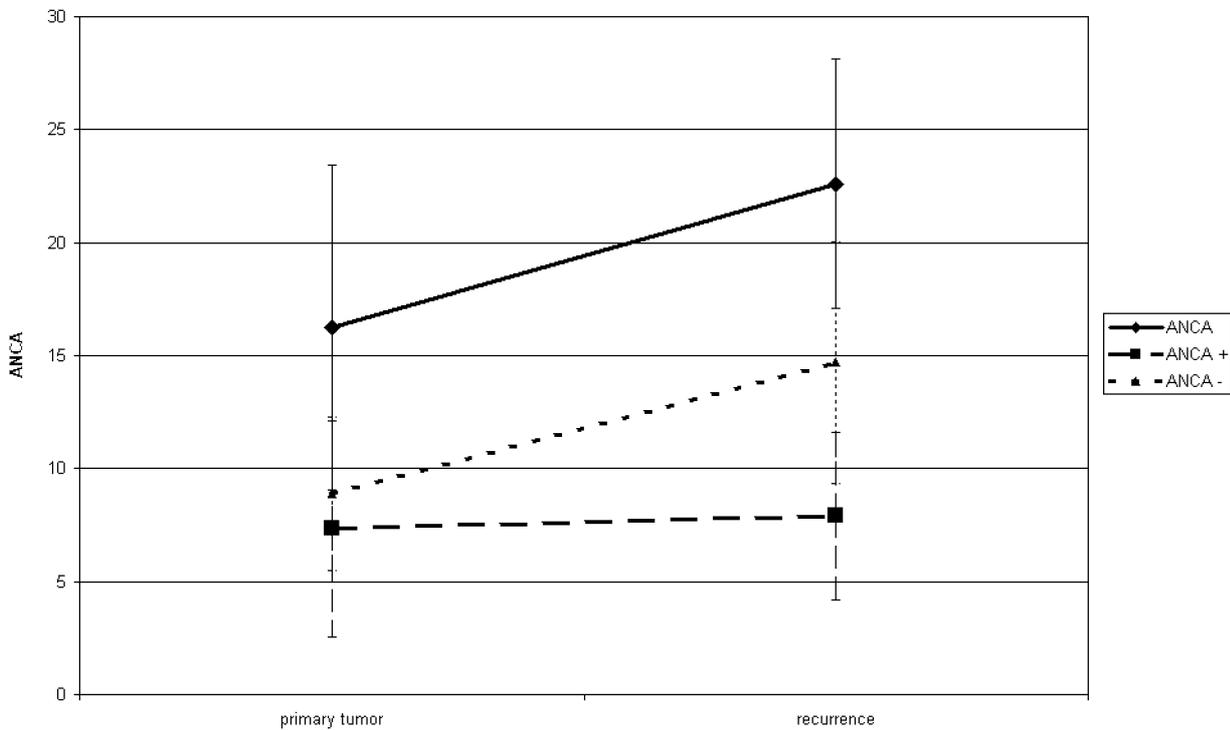


Fig. 3. Averaged number of copy alterations (ANCA) for the comparison of primary tumors and their corresponding local recurrences. The vertical lines represent the standard deviations.

The tumors without lymph node metastases showed the most frequent gains at 1q22 (67%), at 1q32 (61%), at 16p12 (56%) and at 20q12 (56%) and the most frequent losses at 2q32 (55%), at 5q21 (50%), at 8p22 (50%), at 13q21-qter (58%) and at Xq21 (50%). Eight cases of 36 node negative cases (22%) showed amplifications, most frequently at 8q24.

The tumors with lymph node metastases exhibited the most frequent gains at 1q24 (56%), at 1q43 (54%) and at 8q23–q24.1 (56%) and the most frequent losses at 4q26 (68%), 9p21 (71%) and at 13q31 (68%). Thirteen of 41 cases (32%) with lymph node metastases exhibited amplifications, most frequently involving 17q21, 8q24 and 20q13 (Table 2).

There was no difference in the number of chromosomal imbalances between breast cancers with and without lymph node metastases. However, the primary tumors with lymph node metastases exhibited significant more frequent losses at 9p21–p23 ($p < 0.001$) than the tumors without lymph node metastases (Table 3).

Twenty primary tumors were compared with their corresponding lymph node metastases.

The most frequent gains of lymph node metastases were located at 8q22 (40%), at 8q23 (45%) and at 8q24.1 (40%). The most frequent losses of lymph node

metastases were located at 4q28 (80%), at 4q32 (80%), at 13q31 (85%), at 18q12–q21 (80%) and at Xq21 (80%).

Four of 20 metastases harboured amplifications, most frequently at 8q24 (Table 2).

The number of chromosomal imbalances was significantly higher in the lymph node metastases than in the primary tumors (21.5 versus 18; $p = 0.03$). The metastases showed more frequently losses (17.8 versus 10.5; $p < 0.001$) than the primary tumors, whereas the primary tumors displayed more frequently gains (7.5 versus 3.7; $p < 0.001$) (Fig. 4).

The lymph node metastases showed significant more losses at 7q11, at 17q22–q24 ($p < 0.001$ for both) and at 14q24.3–q31 ($p = 0.001$) than their corresponding primary tumors (Table 3).

6. Distant metastases

18 primary tumors with and without distant metastases were compared.

The primary tumors without distant metastases showed the most frequent gains at 1q32 (67%), at 1q42 (67%), at 8q24.1 (61%), at 16p12 (61%) and at

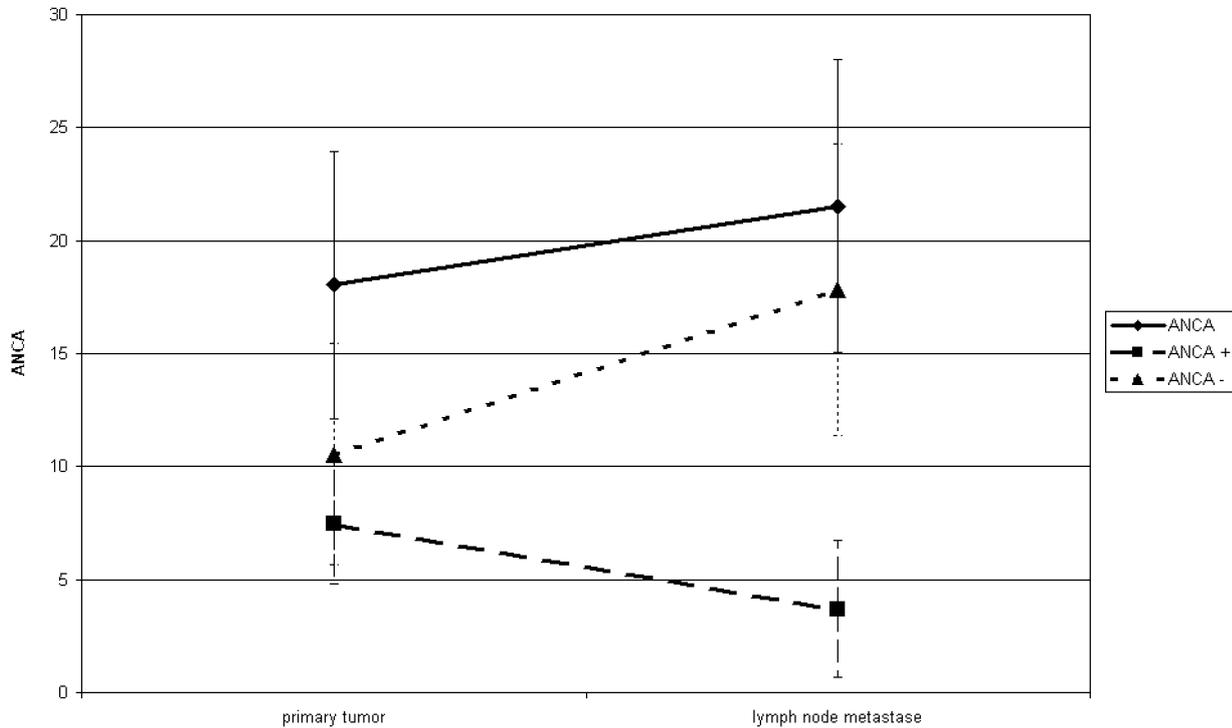


Fig. 4. Averaged number of copy alterations (ANCA) for the comparison of primary tumors and their corresponding lymph node metastases. The vertical lines represent the standard deviations.

20q12 (61%). The most frequent losses in primary tumors without distant metastases were detected at 4q32 (50%), 8p21 (50%), at 11q22 (50%) and at Xq21 (56%). Three of the 18 primary tumors without metastases showed amplifications, involving 8q24, 17q21 or 20q13.

The primary tumors with distant metastases exhibited the most frequent gains at 1q41 (56%), at 8q22 (56%) and at 8q24.1 (50%). The most frequent losses in the group of primary tumor with metastases were detected at 5q23 (72%), at 9p21 (67%), at 13q31 (67%) and at 18q22 (67%). Seven of 18 cases with metastases showed amplifications most frequently at 8q24.1 and at 17q21 (Table 2).

The number of chromosomal imbalances did not differ significantly between the 18 breast cancers with and the 18 breast cancers without distant metastases. The comparison of primary tumors with and without distant metastases revealed no significant differences for recurrent chromosomal imbalances by use of Bonferroni adjustment for significance level. The greatest differences between primary tumors with and without distant metastases were losses at 5q23 (more frequent in primary tumors with metastases, 72% versus 28%; $p = 0.008$), and gains at 16p13–p12 (more frequent in

primary tumors without metastases, 61% versus 11%; $p = 0.002$).

Ten primary tumors were compared with ten corresponding metastases.

The metastases showed the most frequent gains at 1q24 (40%), at 1q41–q42 (40%), at 8q13 (50%) and at 8q22 (70%). The most frequent losses in the metastases were detected at 5p15, at 8p12–p22 (100%), at 11q23 (80%), at 12q24 (80%), at 13q31 (80%), at 17p13 (80%) and at Xq27 (80%). No amplifications were found in the metastases (Table 2).

The number of chromosomal imbalances was significantly higher in the ten metastases than in the ten primary tumors (28.1 versus 22.4; $p = 0.04$). Especially, the losses of chromosomal material contributed to this difference (23.2 versus 15.9; $p = 0.02$) (Fig. 5).

The metastases displayed significant more frequent losses at 5p15 ($p < 0.001$), 12q24 ($p < 0.001$) and 17q22–q24 ($p = 0.001$) than their primary tumors (Table 3).

7. Discussion

The presented study detected differences in the chromosomal genotype between primary tumors with and

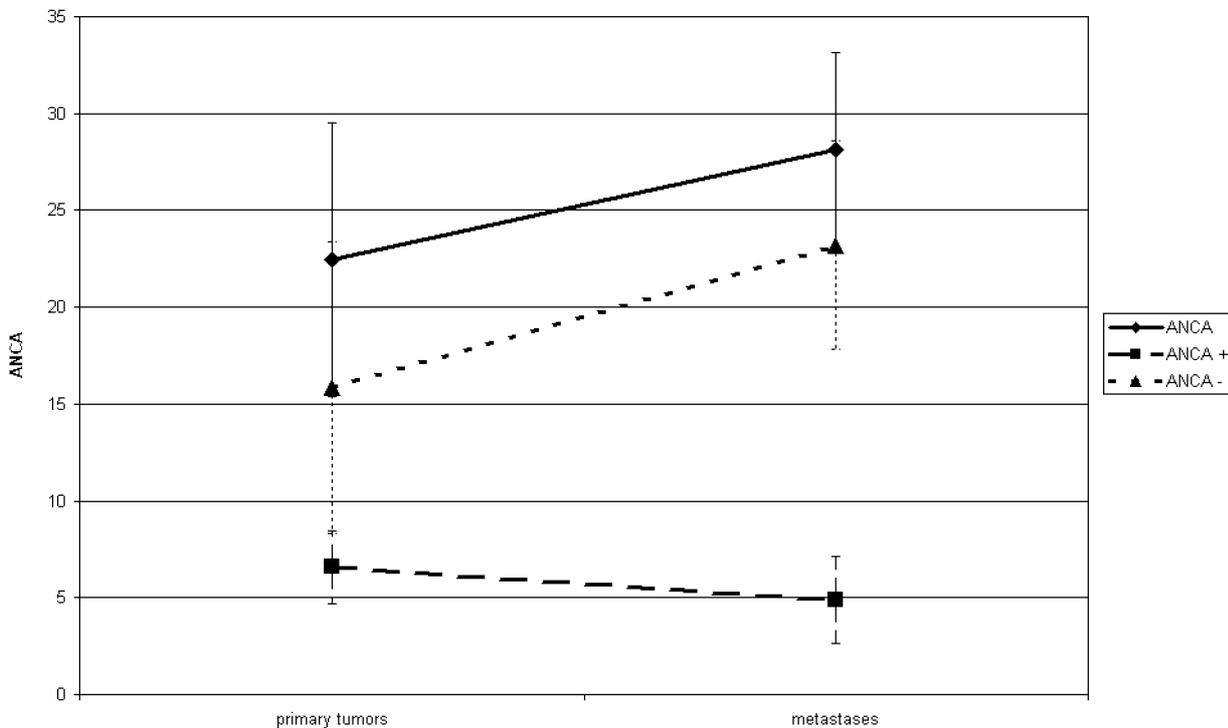


Fig. 5. Averaged number of copy alterations (ANCA) for the comparison of primary tumors and their corresponding distant metastases. The vertical lines represent the standard deviations.

without local recurrences as well as between primary tumors with and without lymph node metastases. The comparison of primary tumors with and without distant metastases revealed a higher frequency of losses at 5q23 in cases with metastases than in metastase-free cases, but not reaching the significance level after Bonferroni adjustment.

The common assumption for the development of local recurrence is the persistence of tumor cells after treatment. However, the difference in the genome of primary tumors with and without local recurrences indicates that the capability to recur already exists in the primary tumor. These differences in the genome may be associated with other risk factors for local recurrence, e.g. an extensive intraductal component or multiple tumors, which render the complete removal of the tumor more difficult. Even though our study did not include cases with positive margins after surgery or with an extensive intraductal component the mean time from first surgery to recurrence was 21.7 months (between 7 and 43 months) suggesting a recurrence arising from residual tumor cells after surgery to be more likely than a de novo development. The progression of residual tumor cells is almost certainly accompanied by acquisition of further genomic changes reflected by

the higher number of imbalances in local recurrences as compared to their primary tumors. However, local recurrences did not show any additional recurrent chromosomal aberrations.

In addition to the development of recurrences from residual tumor cells, a de-novo carcinogenesis from a "field" of increased susceptibility is also being discussed. Studies detecting loss of heterozygosity (LOH) not only in breast cancer tissue but also in phenotypically normal breast epithelium [6,14] support this interpretation. Li et al. [14] describe a higher number of local recurrences in cases with a LOH at 3p in morphologically normal terminal ductal lobular units adjacent to carcinoma than in cases without such an LOH. The time to recurrence was longer in cases with 3p LOH in normal tissue than in cases without the 3p LOH (62.2 months versus 38.6 months).

In contrast to the local recurrences, the metastases showed additional recurrent chromosomal imbalances, which may be associated with the specific prerequisites for survival and growth of tumor cells in different tissue environments.

The comparison of primary tumors and their corresponding lymph node or distant metastases revealed different chromosomal imbalances for the lymphatic

and distant metastases. Different functional factors in these two progression pathways, e.g. the time of occurrence, the way of spread [30] and different microenvironment in the different host organs [7] may be reflected in these genomic differences. The fact that not all patients with lymph node metastases develop distant metastases, but a part of node negative patients suffer from distant metastases may also be attributed to these genomic differences. Supporting this fact, Delias et al. [5] described a higher number of chromosomal imbalances in node negative breast cancers with recurrence compared with node negative cases without recurrence.

The only chromosomal aberration present in both, lymph node and distant metastases, was the loss at 17q22–q24, indicating that the chromosomal region 17q22–q24 may harbor important metastasis suppressing genes. Little is known about this chromosomal loss. Niederacher et al. [16] detected LOH in this chromosomal region in breast cancers, but failed to detect a correlation with any clinico-pathological feature, including lymph node stage. Pandis et al. [18] describe aberrations which may predispose to disease spread, including losses of chromosome 17, but mostly losses of the whole chromosome in their cytogenetic study.

In our study, the losses at 17q22–q24 reflect the change of a minimal overlapping region, which include also metastases with losses of the whole q-arm of chromosome 17 or of the whole chromosome 17.

The primary tumors with and without lymph node metastases differed significantly in imbalances at 9p21–p23. The comparison of primary tumors without and with distant metastases revealed at least a tendency for a higher number of losses at 5q23 in primary tumors with metastases than in primary tumors without metastases. Gene expression studies of breast cancers revealed differences between primary breast cancers with and without metastases [20,25,26,28,29,31]. Van't Veer et al. [24] describe a 70 gene expression profile for prediction of early occurrence of metastases in node-negative breast cancer patients younger than 55 years. Wang et al. [28] reported a similar result also for subsets of pre- and postmenopausal node negative breast cancers patients as well as for node negative tumors with a size between 10 and 20 mm. Woelfle et al. [31] were able to detect a different gene expression pattern in primary breast cancers with bone marrow micrometastasis compared with breast cancers without such micrometastases. Like the results of our study, all these studies refer to the hypothesis that the capability to metastasize is acquired relatively early in tumor progression.

As expected, the metastases differ from their primary tumors in a higher number of imbalances, especially in a higher number of chromosomal losses. The loss of chromosomal material corresponds with a more progressed, aggressive phenotype in breast cancer, as shown for breast cancers with poor outcome or DNA aneuploidy [5,9,12].

The differences in the chromosomal genotype between primary tumors and metastases may be caused by new acquisition of these imbalances in the metastases or may be a result of tumor heterogeneity by a small cell clone of the primary tumor with the capability to metastasize, which is not detectable by CGH in the whole primary tumor. Nishizaki et al. [17] used a modified CGH technique to compare primary breast cancers and their secondary manifestations and showed quantitative differences in the degree of chromosomal imbalances between the primary and secondary manifestations, probably resulting from tumor heterogeneity. A cytogenetic study of Pandis et al. [18] supports this hypothesis by detection of a higher frequency of karyotypic unrelated clones in primary tumors than in metastasis, indicating a higher degree of genetic heterogeneity in the primary tumor than in the metastasis. The question if the differences between primary tumors and metastases are caused by a small metastasizing cell clone in the primary tumor or by acquisition of new chromosomal imbalances in the metastases can be addressed by an interphase-FISH study of primary tumors and their corresponding metastases allowing a cell-by-cell analysis of chromosomal changes.

By use of Bonferroni adjustment of the significance level, the analysis did not reveal significant differences for each comparison. The analysis of a multitude of markers in a limited number of cases requires a strict adjustment of significance level to avoid haphazardly significant results. Such an adjustment is the Bonferroni principle, which considers the degree of freedom (or in other words the number of attempts) by division of significance level by the appropriate degree of freedom. On the other hand, this adjustment is very stringent, because each attempt increases the significance level. The use of such a rigorous adjustment reduces the detection of markers without real significance and may focus the interest on markers with high impact. The risk to fail markers with relevance by such stringent adjustment can not be completely excluded. In such situations, the high through put analysis with fluorescence in-situ hybridisation on tissue micro arrays may clarify the prognostic relevance of chromosomal imbalances with doubtful results in CGH.

In conclusion, this study reveals strong evidence that the occurrence of secondary manifestations is determined in the primary tumor. The crucial difference between local recurrences and metastases is the occurrence of recurrent chromosomal imbalances in metastases but not in local recurrences, reflecting different mechanism in their evolution. The different recurrent chromosomal imbalances for lymph node and distant metastases reflect the different mechanisms of lymphatic and haematogenous spread. After confirmation of the results by interphase FISH, the detection of these recurrent chromosomal imbalances may contribute to a more individual prediction of prognosis in breast cancer.

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