Virchows Arch (1997) 430:365–372 © Springer-Verlag 1997

ORIGINAL ARTICLE

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Amplification units and translocation at chromosome 17q and c-*erb*B-2 overexpression in the pathogenesis of breast cancer

Received: 23 July 1996 / Accepted: 9 December 1996

Abstract Hyperplasia without and with atypia is considered to be a precursor lesion for certain breast carcinomas. The cytogenetic events and the molecular pathology involved in the multistep process from normal to invasive carcinoma are unknown. To characterise the sequence of early genetic abnormalities of chromosome 17q and their biological consequences in the pathogenesis of breast cancer, we performed immunohistochemistry on 451 breast tissues including 180 normal breast specimens, 28 hyperplastic lesions without atypia and 44 with atypia, 100 cases of ductal carcinoma in situ (DCIS) and 99 cases of invasive ductal carcinoma. We correlated the overexpression of the c-*Erb*B-2 protein, the histological and the recently proposed differentiation classification of DCIS with the extent of DCIS. For fluorescence in situ hybridisation (FISH) analysis, different probes spanning the 17q region including the c-*erb*B-2 gene locus and those which are found adjacent, were used. Reverse painting and comparative genomic hybridisation (CGH) were performed on several breast cancer cell lines. c-*Erb*B-2 overexpression was observed in only 29% of DCIS and 23% of invasive carcinomas, but not in hyperplastic and normal tissue. c-*Erb*B-2 overexpression is correlated with poor differentiation in DCIS but not in invasive carcinoma. In DCIS, there was no correlation with the histological subtype classification. The average extent of DCIS is significantly increased from 13.81 mm in c-*Erb*B-2 negative cases to 29.37 mm in c-*Erb*B-2 positive cases. The increase was considered to be a possible consequence of the overexpression and is probably due to the previously described motility enhancing effect of the c-*Erb*B-2 protein. The histological and differentiation classification of DCIS did not correlate with the extent of disease. Using FISH, amplified genes at 17q12, always including the c-*erb*B-2 gene, were detected in all cases of DCIS and invasive carcinoma with c-*Erb*B-2 overexpression. The centromeric region and the NF1 locus, which is located between the centromere and c-*erb*B-2, were not amplified in any of the DCIS and invasive breast carcinomas, but co-amplification of the myeloperoxidase gene was detected in 3/5 DCIS and 1/5 invasive carcinomas with c-*Erb*B-2 overexpression. In contrast to c-*erb*B-2, immunohistochemical overexpression of their respective gene products was not observed. FISH, reverse painting and CGH show similar amplified genes with amplified c-*erb*B-2 in c-*Erb*B-2 overexpressing SK-BR-3 and BT474 human breast cancer cells. The amplified genes are part of two different amplicons. Extensive modifications of the 17q chromosomal region, caused by translocation, were also observed in these cell lines. It is concluded that the modifications of chromosome 17q, inducing overexpression of c-*Erb*B-2 protein, occur at the level of transition from hyperplasia to DCIS. They are preserved in invasive carcinoma with overexpression of c-*Erb*B-2 protein. This had led to the hypothesis that these modifications at 17q may lead to a larger extent of DCIS.

Key words Breast cancer · Fluorescence in situ hybridisation · c-*erb*B-2/*neu*/HER-oncogene · Ductal carcinoma in situ · Translocation · Amplification

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Introduction

The pathogenesis of breast cancer is considered to be a multistep process, with hyperplasia with and without atypia as the initial step, followed by ductal carcinoma in situ (DCIS) and eventually invasive breast cancer [11, 26]. The precursor lesions represent a good model to

study the earliest detectable alterations of the human genome in the onset of breast cancer. Therefore, hyperplasia with and without atypia was thoroughly studied and compared with DCIS and invasive breast cancer.

We focused our study on changes of chromosome 17q during the evolution from normal tissue to invasive carcinoma. The c-*erb*B-2 (HER-2 or *neu*) gene is located on the long arm of chromosome 17 [12], and its protein is overexpressed in approximately 20% of invasive ductal carcinomas and in 50% of DCIS [1, 8, 16, 24, 31]. The c-*erb*B-2 oncogene is a proto-oncogene, which codes for a transmembrane protein belonging to the *erb*B proto-oncogene family of tyrosine kinase receptors. From the first reports, linking this protein with poor prognosis and due to its similarity to epidermal growth factor receptor, the protein was considered as a receptor for a growth factor [9, 27]. Several investigations and experiments supported this view, but the protein was also shown to play an important role in cell motility and in the spread of tumour cells during the process of metastasis [4, 5, 36]. The c-*Erb*B-2 protein is overexpressed in almost all cases of Paget's disease of the breast [21], and due to its motility enhancing effect, it plays a key role in the pathogenesis of the disease [7].

The aim of this study was to characterise changes of chromosome 17q early in the multistep process of breast cancer, these changes being eventually associated with c-*Erb*B-2/*neu*/HER-2 overexpression, and to study their effect on the biological behaviour and classification of DCIS by measuring the extent of the disease. There is evidence that the extent of the lesion is one of the most important determinants of local recurrence of DCIS after resection.

For this purpose, immunohistochemical tests were performed to investigate overexpression of the c-*Erb*B-2, myeloperoxidase (MPO) and *Erb*A proteins, all of which are coded on 17q. Fluorescence in situ hybridisation (FISH) with probes for these and other genes located at 17q was carried out on tissue sections from hyperplastic tissue and from DCIS and invasive ductal carcinoma, with and without overexpression of the c-*Erb*B-2 protein. These results were compared with the results of FISH, reverse painting and comparative genomic hybridisation (CGH) obtained from two human cell lines derived from invasive breast carcinomas, SK-BR-3 and BT474, both overexpressing the c-*Erb*B-2 protein, in order to investigate whether the amplified genes were part of one or more amplicons. The demonstration of amplicons in routinely processed biopsy specimens is not possible; only by reverse painting and CGH using cell lines, these amplicons can be visualised.

Materials and methods

Normal breast tissue (n=180), hyperplasia with (n=44) and without (n=28) atypia, DCIS (n=100) and invasive ductal carcinoma (n=99) of the breast were selected (Table 1). The 100 cases of DCIS were not associated with an invasive carcinoma. These patients had no invasive tumour in the same breast at the time of the biopsy, nor in the past. The biopsy specimens were fixed in 10% formalin and embedded in paraffin at 53° C following routine procedures. In this study, two distinct classifications were used. The histological classification, as described by Page and Anderson and by Sloane, discerns five different growth patterns: comedo, cribriform, papillary and micropapillary [25, 28]. The second classification applied was the differentiation classification, proposed by Holland et al., which is primarily based on cytonuclear differentiation, but also includes architectural differentiation or cellular polarisation [17]. This classification is subdivided into three classes: well, moderately and poorly differentiated. In the cases of DCIS, the extent of the lesion was determined in the following way. Whenever possible, the extent of disease was measured microscopically. In small lesions, the DCIS was completely surrounded by normal tissue in all slides. When the DCIS was too large to be incorporated on one slide, the maximal diameter was determined from combined macroscopical and microscopical data, as previously described [8]. The intact specimen was measured, oriented and sliced at 3 mm intervals after specimen radiography, and the slides were numbered consecutively. The lesion was then reconstructed by combining the individual positive tissue blocks and the largest diameter of the lesion estimated, with only completely resected cases of DCIS considered. The assessment of margins in local excision specimens was performed using pigment inks [8]. To calculate the relationship between c-*Erb*B-2 overexpression and the extent of disease Student's *t*-test was performed.

The stock culture medium used for the maintenance of the SK-BR-3 cells and the BT474 cells, was Rega 3 minimal essential medium containing non-essential amino acids (Gibco BRL, Gent, Belgium) supplemented with 10% fetal calf serum, 20 mM Hepes, 14.3 mM sodium bicarbonate, 50 IU penicillin/ml, 50 IU streptomycin/ml and 2 mM L-glutamine (Gibco BRL).

For immunohistochemistry the avidin-biotin complex technique was applied as follows: 5 μm sections from formalin fixed paraffin embedded tissues were cut and deparaffinised. For the detection of the expression of the c-*Erb*B-2 protein (Oncogene Science, Uniondale, NY), MPO (Dako, Glostrup, Denmark) and the *Erb*A protein (Santa Cruz Biotechnology, Santa Cruz, Calif.), the slides were incubated with the primary antibodies for 1 h at a concentration of 1 μg/ml in phosphate-buffered saline (PBS) pH 7.6 at room temperature in a moist chamber. They were then incubated with biotinylated rabbit-anti-mouse immunoglobulins (Dako; 1:400 in PBS) for 30 min followed by addition of the avidin-biotin-peroxidase complex (Dako) and the incubation continued according to the manufacturer's instructions for 30 min. The chromogen used was 3-amino-9-ethylcarbazole and 0.01% hydrogen peroxide in acetate buffer pH 5.2 was used as the substrate, the slides being incubated for 10 min. Between the steps the slides were washed in PBS. All sections were counterstained with Mayer's haematoxylin and mounted in Aquatex (Merck, Darmstadt, Germany). Negative control sections were prepared by omission of the primary antibody.

In order to investigate the long arm of chromosome 17 by FISH analysis, probes localising the following genes, starting from the centromeric region to the telomeric region, were tested: centromeric probe (pl7H8) [34], NF1 (neurofibromatosis gene 1; probe 7G4) [33], HER-2/*neu* (Oncor, Gaithersburg, Md.) *erb*A (THRA1 Y397F9, GDB 1 d GOO-195-323, NIH Human Genome Center, University of Michigan), RARA (Oncor), MPO (Oncor) and telomeric probe (TEL17; Oncor). MPO is an enzyme found in polymorphonucleocytes and is involved in the destruction of microorganisms and debris [23]. NF1, mapped at 17q11.2-12 proximal to the c-*erb*B-2-locus [3], is classified as a tumour suppressor gene [22]. The *erb*A gene encodes a thyroid hormone receptor [35]. The RARA gene encodes the retinoic acid receptor alpha and has been mapped between *erb*A and MPO [3].

The pl7H8, 7G6 and *erb*A probes were biotinylated (biotin-16-dUTP, 0.4 mM, Boehringer Mannheim, Germany) and/or digoxygeninated (digoxygenin-11-dUTP, 0.4 mM, Boehringer Mannheim) by standard nick-translation.

Five randomly selected examples of each lesion were subjected to FISH. Five micrometre sections were deparaffinised according

to routine procedures and were air-dried after immersion in 96% ethanol. The slides were pretreated with a solution consisting of a powder (Oncor) dissolved in 2× saline sodium phosphate ethylenediaminetetracetic acid-buffer (SSPE). They were rinsed with fresh 2×SSPE and subjected to protein digestion for 45 min with proteinase K (Merck) at 45° C.

For the digoxygenin labelled probes, the slides were hybridised with 10 µl/2 cm^2 tissue digoxigenin labelled probe mix (Oncor), which was first preheated to 37° C for 5 min. They were then heated to 90° C for 12 min and further incubated in a moist chamber at 37° C. After post-hybridisation washes, single detection and amplification of the digoxigenin modified probes was achieved by subsequent incubations with mouse-anti-dig (Sigma, St. Louis, Mo.; 1:500 in 0.5% blocking reagent), sheep-anti-mouse-dig F(ab′)2 (Boehringer Mannheim; 1:100 in blocking reagent) and sheep-anti-dig fluorescein isothiocyanate (FITC) F(ab′)2 (Boehringer Mannheim; 1:100 in blocking reagent), respectively. For the biotin labelled probes, the slides were hybridised with 10 µl/2 cm^2 tissue biotin labelled probe mix. The probes were mixed with Cot-I-DNA (Gibco BRL), sodium acetate and 100% ethanol and put on ice for 30 min. They were then centrifuged at 14000 rpm at 4° C. The pellet was dissolved in 50% formamide/12.5% dextransulphate/2×SSPE at 37° C for 30 min.

After post-hybridisation washes, single detection and amplification of the biotin modified probes was achieved by subsequent incubations with Neutralite-avidin-FITC (Eurogentec, Liège, Belgium; 1:200 in % non fat dry milk), and biotinylated goat-anti-avidin (Vector Laboratories, Brussels, Belgium; 1:200 in 5% non fat dry milk), respectively. The slides were mounted in antifade solution and counterstained with 0.5 µg/ml propidium iodide. Slides were observed with a standard epifluorescence microscope equipped with a 100 W lamp. The SK-BR-3 and BT474 cell lines were also subjected to FISH analysis, using the probes described above.

Reverse painting on normal human lymphocyte metaphase spreads using the DNA of SK-BR-3 cells was done according to Joos et al. [19], with minor modifications [32]. CGH analysis of BT474 cells was performed [10, 20] using the ISIS, digital imaging system and CGH software (Metasystems, Heidelberg, Germany).

Table 1 c-*Erb*B-2 overexpression in breast tissues (*DCIS* ductal carcinoma in situ, + only a staining pattern on the cytoplasmic membrane is considered positive, – no staining pattern on the cytoplasmic membrane)

Lesion (number of cases)	Results	
	+	–
DCIS (100)	29	71
Invasive ductal carcinoma (99)	23	76
Normal tissue (180)	0	180
Hyperplasia without atypia (28)	0	28
Hyperplasia with atypia (44)	0	44

Table 2 c-*Erb*B-2 overexpression, histological classification, differentiation classification and extent of disease for DCIS

Feature	Mean diameter (mm)	standard deviation	number of cases
c-*erb*B-2 overexpression*			
Present	29.37	6.52	29
Absent	13.81	2.36	71
Histological classification**			
Solid	10.77	3.30	24
Papillary	11.00	6.45	4
Comedo	22.89	5.97	34
Micropapillary	26.01	8.42	17
Cribriform	14.74	4.14	21
Total	18.32	16.29	100
Differentiation classification**			
Poorly	21.63	4.11	47
Intermediate	16.12	3.69	41
Well	12.86	4.97	12

* $P<0.001$
** $P>0.05$

Results

The results for the breast lesions stained for c-*Erb*B-2 overexpression are summarised in Table 1. Normal and hyperplastic tissue with and without atypia all proved to be negative. Of the 100 cases of DCIS, 29 showed overexpression for c-*Erb*B-2 whereas 71 were negative.

The 100 cases of DCIS were categorised into the two different classifications described above. The results for both classifications and their correlation with the average extent of disease of each category are summarised in Table 2. The mean diameter of the c-*Erb*B-2 positive cases was 29.37 mm and of the c-*Erb*B-2 negative cases 13.81 mm. There was a significant correlation between c-*Erb*B-2 overexpression and the extent of DCIS ($P<0.001$). For the histological classification, 24 tumours were classified as solid, 4 as papillary, 34 as comedo, 17 as micropapillary and 21 as cribriform. There was no significant correlation between the subtypes of DCIS and c-*Erb*B-2 overexpression ($P>0.05$; data not shown). There was no significant correlation between the histological subtype classification and extent of disease ($P>0.05$; Table 2). The largest lesions were noted in the micropapillary subgroup, but they were not significant because of the high standard deviation. With the differentiation classification, the highest diameter was noted for the poorly differentiated subgroup and the lowest for the well differentiated subgroup (Table 2), but this difference was not significant ($P>0.05$). However, there was a significant correlation between the poorly differentiated subtype and c-*Erb*B-2 overexpression ($P<0.01$; data not shown).

For the invasive carcinomas, 23% overexpressed c-*Erb*B-2 (Table 1). Several invasive carcinomas contained a DCIS component, which always showed the same expression pattern for c-*Erb*B-2 protein as its invasive counterpart. Although poorly differentiated invasive carcinomas tended to be more positive for c-*Erb*B-2 overexpression, the difference was not significant ($P>0.05$; Table 3).

MPO and the *Erb*A-protein were not expressed in DCIS and in invasive carcinomas. From this series of cases, five c-*Erb*B-2 negative and five positive cases of each lesion were randomly selected for the FISH study.

The chromosomal localisation of the genes investigated is presented in Fig. 1. The results of the FISH experiments are summarised in Table 4. Normal cells and cells from hyperplastic lesions showed only 1 or 2 fluorescent

Table 3 c-*Erb*B-2 overexpression invasive ductal carcinoma ($P>0.05$)

Grading	Results	
	+	–
Well differentiated	0	4
Moderately differentiated	5	29
Poorly differentiated	18	43

Table 4 Results of the various breast lesions investigated for amplification of genes as the long arm of the chromosome 17. The order of the genes is from centromere to telomere (– no amplification, + amplification, +/– amplification in some but not all tumour cells, *CEN17* centromeric region, *NF1* neurofibromatosis gene, *c-erbB-2* HER-2 or *neu* gene, *erbA* THRA gene, *MPO* myeloperoxidase gene, *RARA* retinoic acid receptor α gene, *TEL17* telomeric region)

	CEN17	NF1	c-*erb*B-2	*erb*A	RARA	MPO	TEL17
DCIS/with c-*Erb*B-2 overexpression							
1	–	–	+	–	–	–	+
2	–	–	+	–	–	+	–
3	–	–	+	–	–	–	–
4	–	–	+	–	–	+	–
5	–	–	+	–	–	+	–
DCIS/no c-*Erb*B-2 overexpression							
6	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–
8	–	–	–	–	–	–	+
9	–	–	–	–	–	–	–
10	–	–	–	–	–	–	+
Invasive ductal carcinoma/with c-*Erb*B-2 overexpression							
11	–	–	+	–	–	–	–
12	–	–	+	–	–	–	–
13	–	–	+	+	–	–	–
14	–	–	+	–	–	–	–
15	–	–	+	–	+/–	+/–	–
Invasive ductal carcinoma/no c-*Erb*B-2 overexpression							
16	–	–	–	–	–	–	–
17	–	–	–	–	–	–	–
18	–	–	–	–	–	–	–
19	–	–	–	–	–	–	–
20	–	–	–	–	–	–	–
Human breast cancer cell lines							
BT474	–	–	+	+	+	+	–
SK-BR-3	–	–	+	+	–	–	–

spots (Fig. 2A), whereas cells with multiple bright spots were considered to contain amplified sequences. Amplified genes were usually seen as two or more clusters in the nucleus, each cluster containing 10–20 spots (Fig. 2B). However, tumour cells contained 3 or 4 isolated spots in rare cases. This was not considered as amplification but could be due to aneuploidy.

No amplification was observed in the nuclei of DCIS and of the invasive carcinoma after hybridisation with the p17H8 centromere specific and NF1 probes.

In all cases with overexpression of c-*Erb*B-2, amplification of the c-*erb*B-2 gene took place. All cases without overexpression of the c-*Erb*B-2 protein showed only 1 or 2 spots, as did the normal epithelial and mesenchymal cells. In one c-*Erb*B-2 overexpressing invasive carcinoma (case number 13) and in the SK-BR-3 and BT474 cell lines, we detected co-amplification of the c-*erb*B-2 gene with the *erb*A gene.

In three of five cases of DCIS with overexpression of c-*Erb*B-2, amplification of the MPO gene was also detected. None of the DCIS cases without c-*Erb*B-2 overexpression showed amplification of the MPO gene. For the invasive carcinomas, there was amplification for MPO as well as for RARA in one c-*Erb*B-2 overexpressing case (number 15). Both genes were amplified in most but not all tumour cells. The invasive carcinomas without overexpression of c-*Erb*B-2 protein never showed any amplification of the MPO gene. In the interphase nuclei of BT474 cells, the RARA and MPO genes co-amplified with c-*erb*B-2.

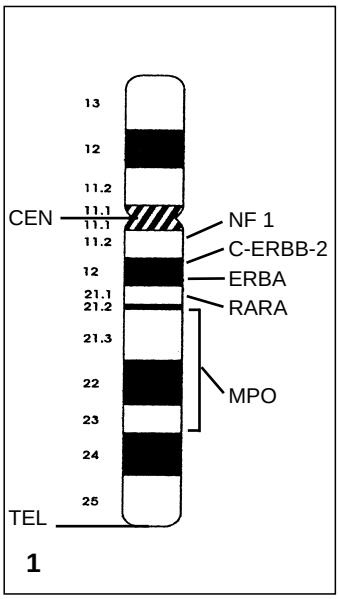
In three cases of DCIS, the specific telomeric region detected by TEL17 probe was shown to be amplified. One case also showed overexpression of c-*Erb*B-2, the two others were negative. All other cases of DCIS and of invasive carcinoma failed to show amplification.

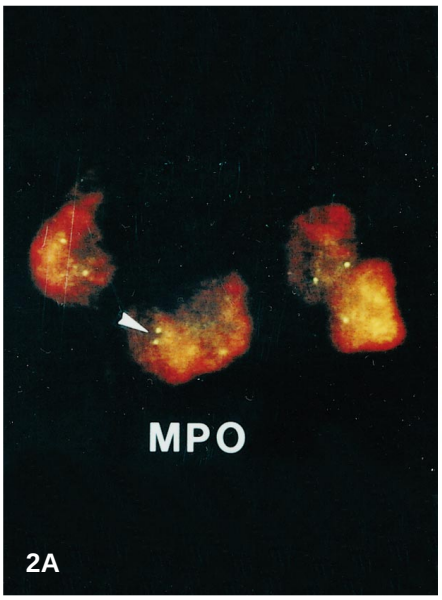
The FISH analysis performed on metaphase chromosomes of BT474 and SK-BR-3 cells gave multiple bright spots on chromosome 17 after hybridisation for the c-*erb*B-2 gene as a consequence of amplification. However, the amplified sequences were also spread over several other chromosomes (Fig. 3). This is attributed to translocations of particular parts of chromosome 17q. Similar amplification patterns were observed for *erb*A in SK-BR-3 cells and for *erb*A, RARA and MPO in BT474 cells. The other genes were not amplified. Double minute chromosomes were not observed.

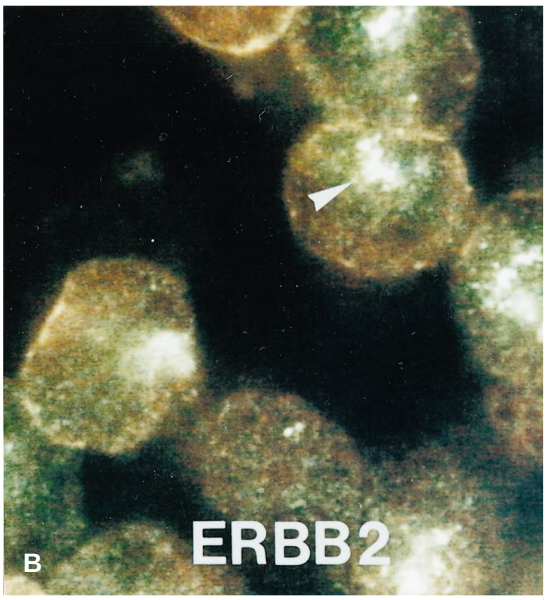
In summary, our results show translocation in both cell lines and amplification in the cell lines and biopsies, of a series of genes located on 17q (Table 2). The amplified genes of 17q12 always including c-*erb*B-2, are highly correlated with c-*Erb*B-2 overexpression and are already present in DCIS but not in hyperplasia. The amplification of the RARA and *erb*A genes is rare. Similar results were obtained in invasive carcinomas.

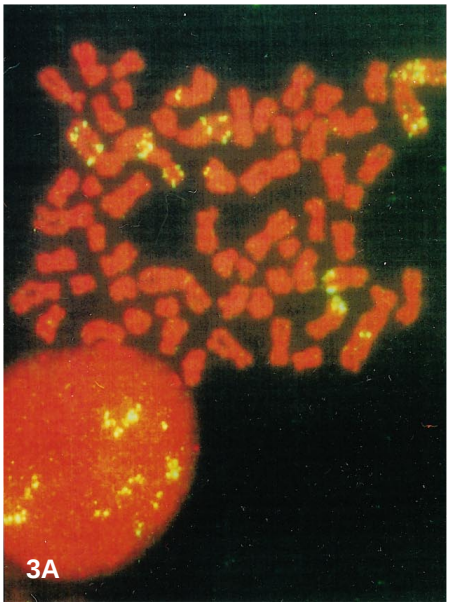
Reverse painting with SK-BR-3 DNA revealed two distinct amplified regions on 17q (Fig. 4). The amplified region starting at 17q12 corresponds with the amplification pattern of the c-*erb*B-2 and *erb*A genes as visualised by FISH analysis on metaphase spreads. The second amplified region at 17q might correspond with the amplification of a series of genes located between the non-amplified MPO gene and the telomere.

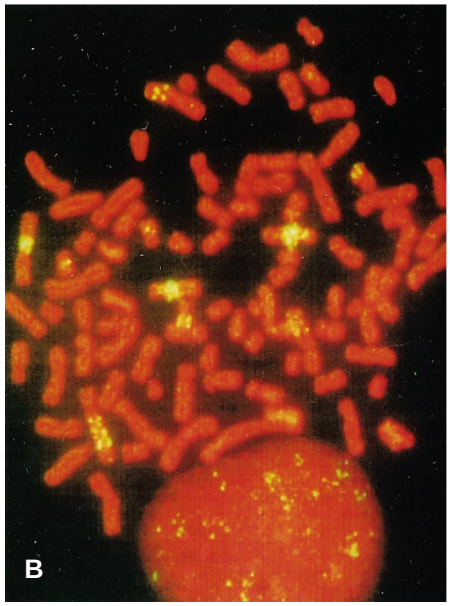
CGH analysis of BT474 cells showed amplification of two regions on 17q (17q12 and 17q22–q24) on individual ratio profiles. The averaged profile of 16 metaphases showed only one large amplified region of 17q (Fig. 5). Again, the amplified regions correspond with the amplification pattern of the different genes on chromosome 17q as visualised by FISH analysis on metaphase spreads of BT474 cells. The first amplified region corresponds











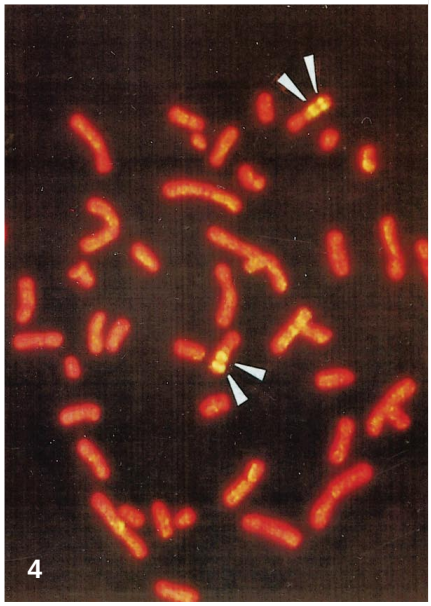


Fig. 1 Chromosomal localisation of the genes on chromosome 17 investigated by fluorescence in situ hybridisation (FISH)

Fig. 2A Interphase nuclei after FISH with an MPO probe: 2 single spots per nucleus are visible (*arrowhead*). There is no amplification. **B** Interphase nuclei after FISH with HER2/*neu* probe on a c-*Erb*B-2 overexpressing ductal carcinoma in situ (DCIS) showing amplification of c-*erb*B-2 (*arrowhead*)

Fig. 3A, B Metaphase spreads from BT474 cells (**A**) and SK-BR-3 cells (**B**) hybridised with HER-2/*neu* probe, showing multiple bright spots representing amplified and non-amplified translocated and non-translocated c-*erb*B-2 gene copies on different chromosomes

Fig. 4 Reverse painting with DNA from SK-BR-3 cells on normal human lymphocytes showing two distinct amplified regions on 17q (*arrowheads*)

with the amplification of c-*erb*B-2 and *erb*A. The second amplicon is located more distally and encompasses the MPO gene.

Discussion

This study describes a sequence of changes at the chromosomal level in the development of breast cancer from normal tissue. The long arm of the chromosome 17 does not show any particular amplification of the genes investigated in normal tissues and in hyperplasia with and without atypia. However, at the level of transition to DCIS, amplifications at 17q take place. These amplifications, which include the c-*erb*B-2 gene, are always associated with overexpression of its protein but not of the co-amplified genes. Reverse painting and CGH studies on SK-BR-3 and BT474 cells show that the amplified

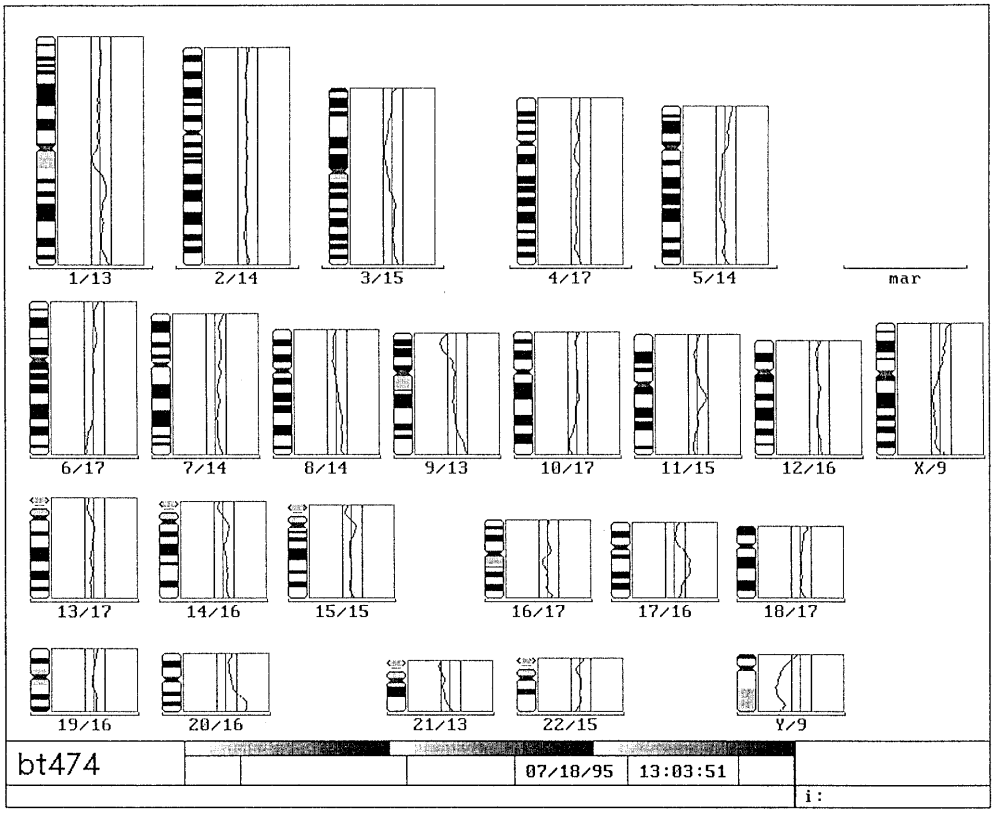


Fig. 5 Comparative genomic hybridisation profile of BT474 cells with high level copy-number increases of chromosomal subregions at 17q and 20q. The number of investigated chromosomes is indicated for each particular chromosome

genes are part of large amplicons located on chromosome 17q. From the FISH study on metaphase spreads of both cell lines, it can be concluded that these amplicons are accompanied by complex translocations of the amplified regions to other chromosomes. The first of these amplicons starts in the region between 17q11 and 17q12 and always contains c-*erb*B-2. In BT474 cells, a second amplicon is located distal to c-*erb*B-2 containing co-amplified MPO.

The amplification at the transition level to DCIS is not sufficient to explain c-*Erb*B-2 overexpression. Not all amplified genes on chromosome 17q are overexpressed; MPO and *erb*A are not. Other investigations have been unable to detect overexpression of *Erb*A and *Int*-2 in breast carcinoma with amplification of these genes [29, 30]. Therefore, other mechanisms than amplification may be involved, such as specific transcription factors which enhance the transcription of the c-*erb*B-2 gene [2, 13, 18]. Even these cannot fully explain why overexpression of c-*Erb*B-2 is usually correlated with amplification and the possibility exists that one or a few translocated c-*erb*B-2 genes might have been rearranged with overexpression of c-*Erb*B-2 as a result. This hypothesis needs further investigation.

The immunohistochemical investigation also shows that overexpression of c-*Erb*B-2 is specific for malignant lesions in the breast and confirms previous results on a large series of patients [6, 14, 17]. The overexpression does not occur in the cases of hyperplasia studied here. From a clinical point of view, the "17q translocation – 17q amplification – c-*Erb*B-2 overexpression" sequence may lead to an extension of DCIS and subdivision of this lesion into two relevant subgroups: DCIS with overexpressed c-*Erb*B-2, usually with a large diameter, and DCIS without overexpression, usually with a smaller diameter. When related to recent publications on the motility function of c-*Erb*B-2 and its role in cell movement, the increased diameter of c-*Erb*B-2 overexpressing DCIS can be regarded as a consequence of the enhanced motility of c-*Erb*B-2 overexpressing carcinoma cells [4, 5, 8]. Increased spread of carcinoma cells throughout the ducts, leading to more extensive disease, would be the result of this increased motility of c-*Erb*B-2 overexpressing tumour cells and might be regarded as an unfavourable factor in considering breast conserving therapy [8].

This study also shows that no significant relationship exists between any specific histological subtype of DCIS and c-*Erb*B-2, although comedo type DCIS tends to be more positive and the solid DCIS usually tends to be negative [8]. Neither the histological classification nor the classification based on differentiation, proposed by Holland et al. [17], correlates with the extent of disease. c-*Erb*B-2 overexpression alone is correlated significantly with the variable extent of disease. It is reasonable to consider whether new classifications for DCIS should not contain c-*Erb*B-2 overexpression as an objective criterion in predicting recurrence of disease.

In conclusion, this study shows a sequence of alterations at chromosome 17q, these being translocation and amplification which then result in c-*Erb*B-2 overexpression. These early chromosomal aberrations in the pathogenesis of breast cancer occur at the transition level to

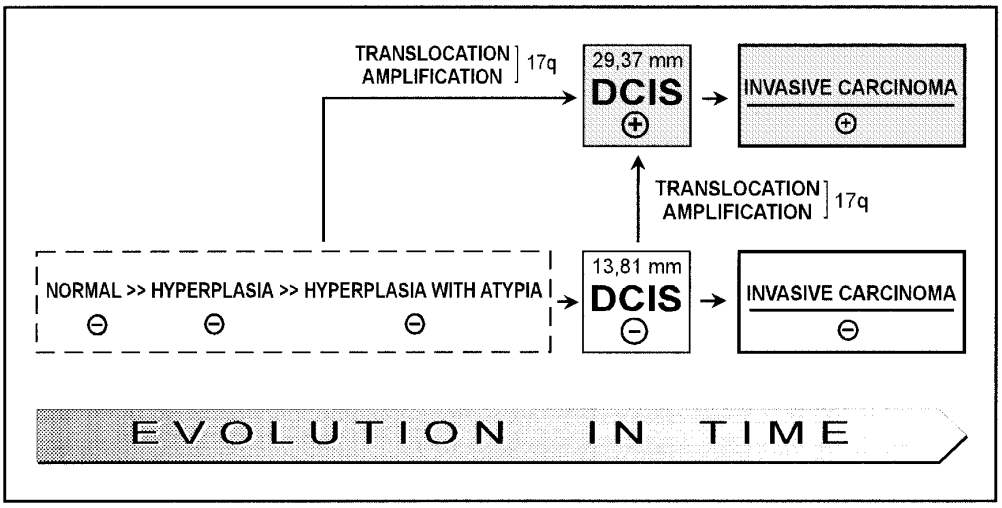


Fig. 6 Schematic representation of the sequence of events on chromosome 17q in the pathogenesis of breast cancer involving translocation, amplification and c-*Erb*B-2 overexpression. The average diameter is indicated for DCIS. – no c-*Erb*B-2 overexpression, + c-*erb*B-2 overexpression

DCIS. They lead to a particular subtype of DCIS with overexpression of c-*Erb*B-2 which has a larger diameter compared with its c-*Erb*B-2 negative counterpart. The chromosomal alterations are preserved in invasive carcinomas with overexpression of c-*Erb*B-2. The involvement of these 17q modifications in the pathogenesis of breast cancer is schematically represented by Fig. 6.

Acknowledgements N.V.R. and F.S. are supported by "Centrum voor Studie en Behandeling van Gezwelziekten" and by "Vereniging voor Kankerbestrijding". E.D.C. receives from "Intern Onderzoeksfonds" of the University of Ghent. R.W. is "chercheur qualifiée of FNRS", M.G. is a recipient of a Télévie grant from the FNRS.

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