Virchows Archiv A Pathol Anat (1992) 420:433–440

Virchows Archiv A
Pathological Anatomy and Histopathology

Expression of the c-*erb*B-2 proto-oncogene product and nuclear DNA content in benign and malignant human breast parenchyma

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Received September 28, 1991 / Received after revision November 27, 1991 / Accepted November 28, 1991

Summary. The expression of the c-*erb*B-2 proto-oncogene product was investigated immunohistochemically in 474 formalin-fixed and paraffin-embedded human breast tissue samples. The series included 32 benign and 26 hyperplastic lesions, 32 carcinomas in situ and 384 invasive breast carcinomas, 107 of which were less than 1 cm in diameter. Cytometric DNA assessments were performed on histopathologically or cytodiagnostically identified cell nuclei, using image analysis. C-*erb*B-2 immunoreactivity was not seen in normal parenchyma or in benign and hyperplastic lesions. Mammary carcinomas in situ were more frequently immunoreactive (59%) than invasive neoplasms (23%). Invasive tumours more than 1 cm in diameter immunoreacted more often (26%) than small invasive carcinomas (16%). C-*erb*B-2 expression in regional lymph node metastases was the same as in the corresponding primary tumours. Significant differences were observed between the c-*erb*B-2 expression in DNA diploid and aneuploid lesions; for carcinomas in situ the figures were 40% and 72%, respectively. Invasive carcinomas of DNA diploid type rarely showed c-*erb*-B-2 expression, irrespective of tumour size and nodal status (7–11%). DNA aneuploid tumours were more frequently immunoreactive with increasing levels during progression (32–41%). Our data indicate that genetically stable invasive mammary tumours seem rarely to express the c-*erb*B-2 protein, even during progression, whereas genetically unstable invasive neoplasms frequently show c-*erb*B-2 immunoreactivity which increases during tumour progression.

Key words: Oncogene – Breast neoplasm – Image analysis – DNA content – Immunohistochemistry

Introduction

The clinical course of human breast cancer is often unpredictable. Conventional clinical staging and histopathological grading systems do not always predict the course of individual tumour development satisfactorily (Saccani Jotti 1989). Additional variables are needed to give supplementary information that can lead to a better understanding of the tumour biology of mammary carcinoma and give a cell biological basis for the choice of adequate treatment.

It has been shown that complex DNA damage is involved in tumour development and progression. Quantitative assessment of nuclear DNA ploidy has gained an important role in assessment of the proliferative potential of various human neoplasms. As a rule, patients with genetically stable tumours, that is to say, where the tumour cell nuclei exhibit a DNA diploid or tetraploid profile, have a better prognosis in general than patients with genetically unstable tumours with aneuploid histogram types (Auer et al. 1980). Previous investigations have shown a significant correlation between certain DNA distribution patterns (DNA histogram types) and the clinical outcome of the neoplastic disease in breast cancer patients (Auer et al. 1984a, b; Fallenius et al. 1988a, b).

In recent years evidence has grown, demonstrating the importance of proto-oncogenes in the pathogenesis of various human neoplasms (Bishop 1987; Mariani-Costanini et al. 1989). Much interest has been focused on the c-*erb*B-2 poto-oncogene product, a transmembrane cellular protein similar in structure to the epidermal growth factor (EGF) receptor with protein tyrosine kinase activity. A 30 kDa factor (gp30) secreted from MDA-MB-231 human breast cancer cells has been shown to be a possible ligand for the c-*erb*B-2 molecule (Lupu et al. 1990). Amplification of the gene has been observed in a variety of human neoplasms, including adenocarcinomas of the breast, the stomach, the colon, the lung and the salivary glands (Cohen et al. 1989; Falck and Gullick 1989; Gullick 1990; Oda et al. 1990; Park et al. 1989; Semba et al. 1985). In human breast adenocarcinoma amplification of the c-*erb*-2 gene is closely related to the levels of expression of the proto-oncogene product (Borg et al. 1990; Corbett et al. 1990;

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Table 1. Immunohistochemical expression of the c-*erb*B-2 proto-oncogene product in various histopathological samples of the mammary gland ($n=474$)

	Total	c-*erb*B-2 immunoreactivity	
		Present	Absent
Normal parenchyma			
Normal breast parenchyma	3	0	3
Benign epithelial tumours			
Fibroadenoma	11	0	11
Papilloma	1	0	1
Adenoma of nipple	1	0	1
Other non-malignant lesions			
Fibrocystic disease	9	0	9
Sclerosing adenosis	2	0	2
Mastitis	3	0	3
Radial scar	2	0	2
Epithelial hyperplasias			
Intraductal hyperplasia without atypia	15	0	15
Intraductal hyperplasia with atypia	9	0	9
Lobular hyperplasia with atypia	2	0	2
Carcinomas in situ			
Ductal of comedo type	16	11	5
Ductal, micropapillary	8	4	4
Ductal, cribriform	3	2	1
Ductal, solid	3	1	2
Ductal, mixed	2	1	1
Invasive carcinomas <10 mm			
Ductal, well differentiated	23	3	20
Ductal, moderately differentiated	36	6	30
Ductal, poorly differentiated	33	5	28
Ductal of comedo type	4	3	1
Lobular	6	0	6
Papillary	2	0	2
Mucinous	3	0	3
Invasive carcinomas >10 mm			
Ductal, well differentiated	16	1	15
Ductal, moderately differentiated	93	21	72
Ductal, poorly differentiated	114	33	81
Ductal of comedo type	17	11	6
Lobular	26	5	21
Medullary	5	0	5
Mucinous	6	2	4

Venter et al. 1987). Elevated expression of the c-*erb*B-2 protein in human breast adenocarcinoma has been reported to be related to early recurrence and poor survival (Slamon et al. 1987, 1989).

In the present study we investigated the immunohistochemical expression of the c-*erb*B-2 proto-oncogene product at different stages of tumour development in a comprehensive series comprising 474 human breast specimens, including genetically stable and genetically unstable conditions, as reflected by their crude nuclear DNA content.

Materials and methods

A total of 474 routinely formalin-fixed and paraffin-embedded surgical specimens were investigated. The series included the following samples: 32 benign and 26 hyperplastic lesions, 32 carcinomas in situ and 384 invasive breast neoplasms (Table 1). There were 107 mammographically detected cases in the latter group of less than 10 mm in largest diameter. This subgroup of invasive carcinomas was investigated as a separate entity, because malignant breast tumours of less than 10 mm represent an early stage in development and differ from invasive breast neoplasms of larger diameter in that they are characterized by a generally excellent prognosis. Among the group of invasive carcinomas of more than 10 mm, in 71 cases specimens from involved regional lymph node metastases at the time of initial operation were available. All samples were classified according to the World Health Organization (WHO) histological typing of breast tumours (World Health Organization 1981). The criteria for typical and atypical hyperplasias were those described by Page and Rogers (1987).

Immunohistochemical staining was performed using a avidin-biotin immunperoxidase complex technique. Tissue sections, 4 μm thick, were prepared from formalin-fixed, paraffin-embedded spec-

imens, dewaxed, and dehydrated. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in distilled water for 20 min. Non-specific staining was blocked with 1% bovine serum albumin in TRIS-buffered saline (TBS) for 1 h. The sections were then incubated with a polyclonal antibody against the cytoplasmic domain of the human c-*erb*B-2 protein raised in sheep (OA-11-854, Cambridge Research Biochemicals Cambridge, UK) at a dilution 1:1200 in TBS at 4° C overnight. After washing in TBS, the sections were processed according to the routine avidin-biotin-peroxidase complex technique. Biotinylated rabbit anti-sheep affinity-purified antibody (BA-6000; Vector, Burlingame, Calif.) was used at a dilution of 1:200 together with 1% inactivated human serum in TBS for 45 min and, subsequently, a Vectastain "Elite" staining kit with diaminobenzidine as chromogen. After light counterstaining with haematoxylin the slides were dehydrated and mounted.

Fresh frozen sections were processed in the same way, except that they were fixed in chloroform and acetone (1:1) for 10 min, and endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol.

A known case of carcinoma in situ of comedo type served as a positive control. Sections of the same case incubated with inactivated sheep serum instead of the primary antibody were used as negative controls.

Different techniques for nuclear DNA measurements were used on Feulgen-stained specimens. Image cytometry was performed on tissue sections of 4 μm thickness in those cases representing normal parenchyma, benign and hyperplastic lesions, as well as in carcinomas in situ and in small primary breast cancers less than 10 mm in diameter. In a minor subgroup of the invasive breast carcinomas greater than 10 mm in diameter, cytometrical DNA measurements were performed using tumour imprints on glass slides. In the majority of the invasive neoplasms of more than 10 mm, the nuclear DNA pattern was assessed on archival, May-Giemsa-Grünwald (MGG) stained smears, upon which the primary diagnosis of breast carcinoma had been based. MGG stained smears were destained, and restained according to the Feulgen technique as described previously (Fallenius et al. 1986). The DNA assessment techniques, including staining, internal standardization and tumour cell selection were those previously described (Fallenius et al. 1988a; Schimmelpennig et al. 1990).

The cytometrical DNA histograms were classified into four different types according to the criteria described by Auer et al. (1980) (Fig. 1). Briefly, the type I histogram has a single distinct peak in the diploid or near diploid region. Type II histograms have a well-circumscribed peak in the G_2M region of the normal diploid

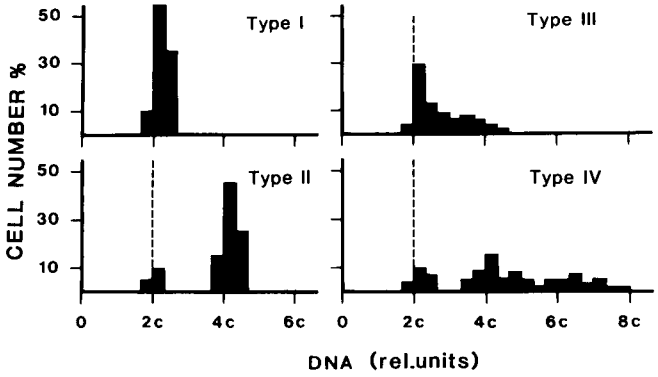

Fig. 1. Nuclear DNA histogram types according to Auer et al. (1980) that were found to be associated with the clinical outcome in human breast cancer. Type I (diploid) and type II (tetraploid) had a favourable prognosis; types III and IV (aneuploid) had a worse clinical outcome in a comprehensive investigation comprising 409 primary mammary adenocarcinomas with long-term clinical follow-up (Fallenius et al. 1988a, b)

population or two distinct peaks within the G_0/G_1 and the G_2/M region, the latter containing at least 20% of all cell counts. Only a negligible number of cells scatter between those two peaks or exceed them. Histograms of type III have either one peak in the G_0/G_1 region of the normal cell population and a considerable number of scattered cells in the S-phase region of that diploid peak or they have two peaks in the G_0/G_1 and the G_2/M region with scattered DNA cell counts between them. Type IV histograms are characterized by highly aneuploid DNA distribution patterns and increased DNA values exceeding the normal G_2/M region.

Results

Samples were classified as immunoreactive when distinct cell membrane staining was observed, although even a faint cytoplasmic staining was noted in most of these cases (Fig. 2). Staining results were recorded as absent, weak, moderate and strong. Cell membrane staining was with slight variations uniformly distributed throughout

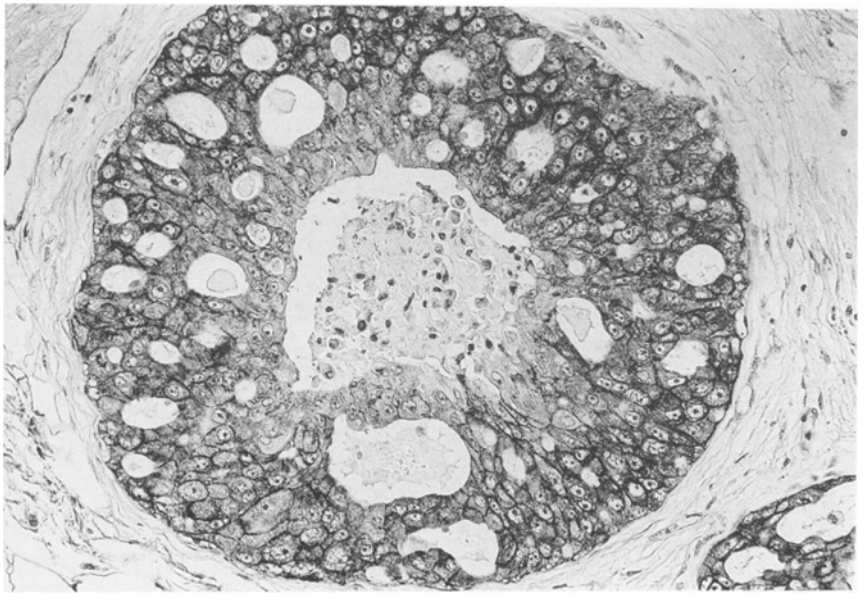

Fig. 2. Immunohistochemical staining of the c-*erb*B-2 proto-oncogene product in a formalin-fixed, paraffin-embedded tissue section of intraductal mammary carcinoma of comedo type. Note the distinct outlining of the neoplastic cell membranes and the uniform staining pattern throughout the lesion. Counterstained with haematoxylin, ×400

the neoplastic cell population. When present, intraductal carcinomas had the same staining pattern as adjacent invasive carcinomas. In a pilot series of 50 cases of breast adenocarcinomas both sections of formalin-fixed, paraffin-embedded specimens and fresh frozen material of the same cases were stained with the polyclonal antibody OA-11-854. Immunohistochemical staining results in the former type of preparation exhibited a stronger and more distinct staining pattern of cell membranes as compared to the frozen sections. There were no frozen specimens that were immunoreactive where the corresponding paraffin-embedded specimen of the same case did not immunoreact. Consequently, the findings reported in this study are based on immunohistochemical staining results obtained from paraffin-embedded specimens.

The results of immunohistochemical staining in the various histopathological subtypes of breast samples are summarized in Table 1. C-*erb*B-2 immunohistochemical membrane staining was not seen in normal breast parenchyma, benign lesions or epithelial proliferations, represented by ductal and lobular hyperplasias with and without atypia. Occasionally, metaplastic apocrine epithelium demonstrated a diffuse intracytoplasmatic staining pattern, but without co-existent specific outlining of the cell membranes. Consequently, this was not recorded as c-*erb*B-2 immunoreactivity by the criteria described above.

In contrast, carcinomas in situ and invasive neoplasms of the breast exhibited varying degrees of c-*erB*-2 immunoreactivity (Table 1). The highest levels of c-*erb*B-2 protein expression were observed in carcinomas in situ, particularly those of comedo type. Intraductal carcinomas of non-comedo type showed immunoreactivity in 8 of 16 cases, while 11 of 16 intraductal carcinomas of comedo type were immunoreactive.

Small primary breast adenocarcinomas less than 10 mm in diameter exhibited c-*erb*B-2 immunoreactivity in 17 of 107 cases (16%), whereas primary breast neoplasms more than 10 mm in diameter showed cell membrane immunostaining in 73 of 277 cases (26%). Among both groups of invasive neoplasms (those of <10 mm and those of >10 mm in diameter) ductal adenocarcinomas of comedo-type immunoreacted most often, followed by ductal adenocarcinomas of non-comedo type. In the latter histopathological subgroup, immunohistochemical expression of the c-*erb*B-2 protein was frequently found to be associated histopathologically with poor differentiation of the tumour cells.

Specimens from regional lymph node metastases were available in 71 of the 277 invsive breast neoplasms of more than 10 mm. Here, lymph node positive primary tumours immunoreacted in 23 of 71 cases (32%), while node negative primary tumours exhibited immunoreactivity in 50 of 206 cases (24%). All specimens from lymph nodes of the 23 node positive, c-*erb*B-2 immunoreactive primary tumours immunoreacted as well. No regional lymph node metastasis was immunoreactive when the corresponding primary tumour did not stain. The periglandular tissue of lymph node metastases from three different cases had intralymphatic tumour thrombi which showed strong c-*erb*B-2 immunostaining.

The samples representing normal breast parenchyma and benign mammary conditions were all characterized by DNA histograms of diploid type [type I according to Auer et al. (1980)]. In contrast, epithelial proliferations – ductal hyperplasias with and without atypia – occasionally showed varying amounts of scattered DNA values in the S-phase region of the normal G_0/G_1 peak and exceeding the G_2/M region. Of the 15 ductal hyperplasias without atypia, 9 exhibited diploid DNA profiles of type I, 5 demonstrated DNA histograms of type III, and 1 sample had an aneuploid histogram of type IV. The corresponding figures in the 9 ductal hyperplasias with atypica were 3 cases with diploid histograms of type I, 2 specimens with histograms of type III and 3 lesions showed aneuploid histograms of type IV. The 2 atypical lobular hyperplasias both had cell nuclei exhibiting diploid DNA profiles.

The immunohistochemical staining pattern of the c-*erb*B-2 proto-oncogene product in the malignant mammary tumours in relation to cytometrical DNA histogram types is summarized in Table 2 and, in schematic form, in Fig. 3. Carcinomas in situ were frequently c-*erb*B-2 immunoreactive, even when the neoplastic cell nuclei of the tumours exhibited diploid DNA distribution patterns. However, intraductal carcinomas were

Table 2. Immunohistochemical expression of the c-*erb*B-2 oncogene product and cytometrical DNA histogram type (according to Auer et al. 1980) in mammary carcinomas in situ ($n=32$), invasive primary carcinomas <10 mm ($n=107$) and invasive primary carcinomas >10 mm in diameter ($n=277$)

Cytometrical DNA histogram type	c-*erb*B-2 immunoreactivity	Carcinomas in situ	Invasive primary carcinomas <10 mm	Invasive primary carcinomas >10 mm
I	Present	4	4	8
	Absent	6	57	69
II	Present	1	0	14
	Absent	1	6	28
III	Present	1	1	3
	Absent	1	2	23
IV	Present	13	12	48
	Absent	5	25	84
Total	Present	19 (59%)	17 (16%)	73 (26%)
	Absent	13 (41%)	90 (84%)	204 (74%)

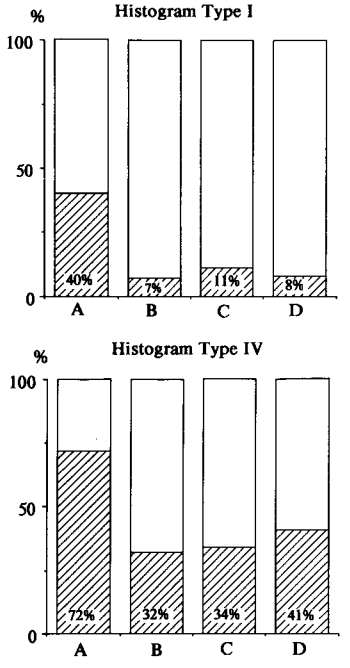

Fig. 3. Immunohistochemical expression of the c-*erb*B-2 proto-oncogene product and frequency of DNA histogram types I (diploid) and IV (aneuploid) according to Auer et al. (1980) in 32 carcinomas in situ (**A**), 107 invasive mammary carcinomas of less than 10 mm (**B**), and in 206 lymph node negative (**C**), as well as in 71 lymph node positive invasive neoplasms more than 10 mm in diameter (**D**)

Table 3. Immunohistochemical expression of the c-*erb*B-2 oncogene product and cytometrical DNA histogram type (according to Auer et al. 1980 in specimens from 71 invasive primary mammary neoplasms with, and 206 without regional lymph node metastases at the time of initial operation; in the 23 lymph node positive, immunoreactive cases shown below, the corresponding lymph node metastases were also equipped with c-*erb*B-2 immunoreactive tumour cells

c-*erb*B-2 Immunoreactivity	Cytometrical DNA histogram type				Total
	I	II	III	IV	
Lymph node positive invasive carcinomas >10 mm ($n=71$)					
Present	1	4	1	17	23 (32%)
Absent	11	7	6	24	48 (68%)
Lymph node negative invasive carcinomas >10 mm ($n=206$)					
Present	7	10	2	31	50 (24%)
Absent	58	21	17	60	156 (76%)
Total	77	42	26	132	277 (100%)

particularly immunoreactive when they were of DNA aneuploid type.

Invasive carcinomas of DNA diploid type rarely had c-*erb*B-2 immunoreactive tumour cells, irrespective of tumour size and lymph node status. In contrast, small and large invasive neoplasms that were characterized by aneuploid histograms of type IV frequently showed immunohistochemical expression of the c-*erb*B-2 protein. Here, the highest degree of immunoreactivity was seen in lymph node positive invasive carcinomas (Table 2, Fig. 3).

A detailed analysis of the inter-relationship of cytometrical DNA histogram type and c-*erb* B-2 immunoreactivity in 71 lymph node positive and in 206 lymph node negative mammary neoplasms more than 10 mm in diameter is given in Table 3. No differences in the frequency of histogram types were observed between lymph node positive and lymph node negative carcinomas. However, lymph node positive tumours were more often equipped with c-*erb*B-2 immunoreactive neoplastic cells than lymph node negative neoplasms. This relationship was even more evident in DNA aneuploid samples of histogram type IV.

Discussion

Convincing evidence has accrued showing that complex nuclear DNA alterations, reflected by DNA aneuploidy, are of decisive importance in the growth of various human malignant tumours (Osborne 1989). Cytometric DNA assessment of the nuclei of tumour cells is gaining an important role as a tool for malignancy grading and even differential diagnosis of neoplastic diseases (McGuire et al. 1990).

Increasing evidence suggests that proto-oncogene products with various functions are largely involved in the genesis and progression of human malignant tumours (Callahan 1989; Varley et al. 1989). One such proto-oncogene, c-*erb*B-2, encodes for a growth-receptor-like transmembrane phosphoglycoprotein which probably functions in an, as yet unknown, growth signal pathway (Maguire and Greene 1989). Amplification and over-expression of the oncogene have been noted in adenocarcinomas of different origins and several studies have reported a correlation between c-*erb*B-2 amplification and/or over-expression and clinical outcome, particularly in mammary adenocarcinomas (Borg et al. 1990; Gullick 1989; Slamon et al. 1987, 1989, Wright et al. 1989). However, studies on human breast cancers have reported controversial results on the clinical significance of c-*erb*B-2 oncoprotein amplification (Ali et al. 1988; Zhou et al. 1989).

In order to form a better understanding of the expression of the c-*erb*B-2 proto-oncogene product in mammary tumorigenesis, this study was conducted in a comprehensive series of human breast samples, including normal parenchyma, benign conditions, hyperplastic lesions and malignant neoplasms, that might reflect different stages of breast cancer development and different degrees of karyotype stability.

In the present study normal breast parenchyma and benign mammary lesions showed diploid DNA profiles and lacked c-*erb*B-2 proto-oncogene expression. In contrast, samples representing proliferative disease of the breast (ductal and lobular hyperplasias with and without atypia) occasionally exhibited scattered DNA values above the normal G_0/G_1 peak and partially exceeding the G_2/M region, as reflected by DNA aneuploid histogram types. The cytometric findings seem to agree with the morphological features of these histopathological conditions. Ductal hyperplasias with atypica are histopathologically often characterized by hyperchromatic cell nuclei, along with other criteria that are also seen in carcinoma in situ (Page and Rogers 1987), No c-*erb*B-2 immunoreactivity was noted in these samples of epithe-

lial proliferative breast disease. Tsutsumi et al. (1990) occasionally observed heterogeneous c-*erb*B-2 expression in frozen sections of benign breast tissue. Antigen demonstration was less sensitive in paraformaldehyde-prefixed sections as in acetone-postfixed specimens. However, those results were obtained by the use of an monoclonal antibody against the extracellular domain of the c-*erb*B-2 protein. The lack of c-*erb*B-2 expression in benign mammary parenchyma and hyperplastic lesions reported herein is in close agreement with the findings of Gustersson et al. (1988a) and Nesland et al. (1991). As we reported, they also occasionally noted a faint, diffuse cytoplasic immunostaining, using polyclonal antibodies, but no specific cell-membrane-related reactivity, as demanded for classification as "c-*erb*B-2 immunoreactive". In conclusion, the cytometrical findings in epithelial proliferative breast disease suggest that already at this very early stage complex nuclear DNA damages may have occurred, and they might serve as an additional indicator for the possible development of these lesions towards malignancy. However, the DNA irregularities seen in epithelial hyperplasias do not seem to engage the c-*erb*B-2 proto-oncogene, indicating that genomic changes as reflected by DNA ploidy alterations precede c-*erb*B-2 oncogene amplification.

Of the rather limited number of intraductal carcinomas included in this study, there was a high rate of over-expression of the c-*erb*B-2 oncoprotein. In an elaborate study of 74 cases of ductal carcinomas in situ, Ramachandra et al. (1990) found 44 (59%) c-*erb*B-2 immunoreactive specimens. Among these, carcinomas in situ of comedo type were the most frequent. This closely agrees with the findings of this study, and similar figures of c-*erb*B-2 immunoreactivity in intraductal mammary carcinomas were reported by others (Bartkova et al. 1990; Gustersson et al. 1988b; Van de Vijver et al. 1988a). In addition to what has been reported earlier, the results reported herein show that both DNA euploid and aneuploid samples exhibited high levels of c-*erb*B-2 immunoreactivity. However, there was a significant difference in immunoreactivity even between these two subtypes, in that the level of expression was substantially higher in DNA aneuploid intraductal carcinomas. The same kind of interrelationship was found in an immunohistochemical study on the c-*erb*B-2 expression of 107 ductal carcinomas in situ conducted at our laboratory (Schimmelpenning et al. 1992). The reasons for the high level of c-*erb*B-2 expression in carcinomas in situ remain unclear. Apparently a larger fraction of ductal carcinomas in situ expresses the c-*erb*B-2 protein than of infiltrating tumours. It has thus been suggested that mammary in situ carcinomas might represent a special subtype that fail to progress to clinically detectable invasive carcinomas in the lifetime of the patient (Ramachandra et al. 1990). It must be the task of a larger study with long-term clinical follow-up data to elucidate the question whether c-*erb*B-2 expression is related to the malignant potential of mammary carcinomas in situ.

In the current investigation only minor differences were noted between the immunohistochemical staining behaviour of small primary invasive adenocarcinomas of less than 10 mm, and those of larger diameter. Genetically stable invasive tumours, (where the neoplastic cell nuclei exhibit diploid DNA profiles), rarely exhibited c-erbB-2 immunoreactivity, irrespective of tumour size and nodal status. In contrast, genetically unstable neoplasms (characterized by aneuploid DNA distribution patterns), frequently showed elevated levels of c-*erb*B-2 expression. These findings are in general agreement with what has been reported previously (Bacus et al. 1990; Ro et al. 1989). In addition, the results of this study seem to indicate that this inter-relationship is also true of lesions represented by DNA histogram of types II and III. Invasive breast cancer specimens exhibiting DNA histograms of type II (tetraploid DNA profile) have a relatively high grade of genetic instability and were frequently equipped with c-*erb*B-2 immunoreactive cells. DNA histograms of type III probably reflect diploid tumor cell populations (high karyotype stability) with a high proliferation rate. As was the case in diploid lesions, the degree of immunoreactivity in the neoplastic cells of samples with type III DNA histograms was low. The data seem to indicate that the immunohistochemical expression of the c-*erb*B-2 oncoprotein in diploid lesions occurs only occasionally, and without significant increase during tumour progression. In contrast, the findings in small DNA aneuploid invasive carcinomas and even in carcinomas in situ support the suggestion that complex DNA alterations, engaging the c-*erb*B-2 proto-oncogene, are already present at an early stage of tumour development.

Expression of the c-*erb*B-2 proto-oncogene product was noted more frequently in lymph node positive, than in lymph node negative adenocarcinomas. This finding agrees with data reported by other groups, and was interpreted as an indicator for the perhaps clinically more aggressive behaviour of c-*erb*B-2 immunoreactive breast adenocarcinomas (Guerin et al. 1989; Seshadri et al. 1989). Samples with immunoreactive lymph node metastases had a concomitant expression of the c-*erb*B-2 protein in the primary tumour. Van de Vijver et al. (1988b) found the same type of condomitant immunohistochemical staining pattern between primary tumour and metastases in 8 mammary adenocarcinomas. Similar data was reported by others (Kraus et al. 1989; Lacroix et al. 1989; Varley et al. 1987). These observations may indicate an increased ability of a breast adenocarcinoma subpopulation, bearing a c-*erb*B-2 oncogene with elevated levels of immunohistochemical expression, to metastatize. Previous studies have shown that local and distant metastases from mammary carcinomas in general exhibit the same nuclear DNA profile as the primary tumour (Auer et al. 1984b; Erhardt and Auer 1985). It has therefore been suggested that mammary adenocarcinomas generally show a high degree of karyotype stability, and that the progress of the malignant disease is more likely to be due to a net increase and or dissemination of tumour cells exhibiting similar genetic properties and malignant potential than to a progressive dedifferentiation and increase of malignancy of the tumour cells. In order to further elucidate this question, it would be interesting to investigate the expression of the c-*erb*B-2

proto-oncogene product in local and distant metastases arising during the course of the neoplastic disease.

Further findings of the current investigation were c-*erb*B-2 immunoreactive intralymphatic tumour thrombi. Though convincing evidence is difficult to obtain, one may speculate that these tumour masses could possibly be engaged in a growth stimulating signal transduction pathway, and thus, yield growth advantage compared to non-immunoreactive neoplastic cell clusters.

In conclusion, the results of the current investigations in a comprehensive series of human mammary specimens seem to support the concept that crude DNA measurements indicate the absence of significant changes in the relative genetic stability during tumour progression. However, the present data demonstrate that alterations at the gene level (such as gene amplification) may occur during tumour progression and could relate to the malignant protential of tumour cell populations. This is supported by the observation that genetically stable tumours show low numbers of c-*erb*B-2 immunoreactive lesions, which remain virtually unchanged during progression. In contrast, genetically unstable tumour variants frequently exhibit both a high degree of c-erbB-2 expression and a further increase of the latter during the course of the neoplastic disease. It will be the subject of a future study to see whether immunohistochemical c-*erb*B-2 protein expression can contribute prognostic information in addition to other known variables, including nuclear DNA content.

Acknowledgements. This study was supported by the Swedish Medical Research Council, the Swedish Cancer Society, the Cancer Society of Stockholm, the King Gustav V Jubilee Fund, and the Research Funds of the Faculty of Medicine at the Karolinska Institute, Stockholm.

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