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20q13.2 Amplification in intraductal hyperplasia adjacent to in situ and invasive ductal carcinoma of the breast

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Abstract The 20q13 region harboring recently described putative oncogenes is frequently amplified in invasive ductal carcinoma (IDC). The aim of this study was to examine the 20q13 copy number in intraduct hyperplasia (IH), atypical duct hyperplasia (ADH), and ductal carcinoma in situ (DCIS) adjacent to IDC. In 5 patients, comparative genomic hybridization (CGH) after laser microdissection revealed 20q13 amplification in four of five cases of IH, in all of three cases of IH with atypia, all five of DCIS, and all five of IDC. Fluorescence in situ hybridization (FISH) confirmed the amplification at 20q13.2 in IH in the two specimens analyzed. The amplification rate, however, was higher in DCIS and IDC. In phenotypically normal ductal epithelium normal values were found for 20q13 copy number by FISH (n=2) and CGH (n=5). Although the number of cases presented here is small, our results suggest that mutations in the 20q13.2 region in IH may be associated with accelerated proliferation and hyperplasia of the ductal epithelium. Progression to DCIS and ICD is accompanied by a further increase in the 20q13.2 copy number.

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Introduction

The evolution of ductal carcinoma of the breast is considered to be a multistep process which is supposed to develop by way of the histopathologically defined stages of intraduct hyperplasia (IH), atypical duct hyperplasia (ADH), ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). IH without atypia seems to be a potential initial step in this process [11].

At the molecular level, it is suggested that amplification of different oncogenes (e.g. c-*erb*B2) and mutation or loss of tumor suppressor genes (e.g. *p53*) occur together with a cumulative effect during breast cancer carcinogenesis, especially at the stage of DCIS [2, 11]. However, the exact timing of most molecular genetic events during tumorigenesis and how they correlate with defined histopathological stages are largely unknown.

Amplification of the long arm of chromosome 20 has been described as a common finding in IDC and in lymph node metastases examined by means of comparative genomic hybridization (CGH) [1, 5, 9]. More recently, CGH studies have shown that a DNA gain of 20q is also present phatase PTPN1/PTP1B, zinc finger protein 217 (ZNF217) and NABC1 (novel amplified in breast cancer-1) genes are being considered as candidate oncogenes [3, 7, 14].

The aim of this present study was to investigate whether a DNA gain on 20q, and especially an amplification at 20q13.2, can be detected even in the potential precursor lesions of IDC. To this end, lumpectomy specimens containing IH, ADH, and DCIS lesions adjacent to the invasive cancer were examined by FISH and CGH.

Materials and methods

Formalin-fixed and paraffin-embedded tissue from lumpectomy specimens of five patients with IDC were investigated. These

Table 1 Comparative genomic hybridization (CGH) results of 20q13 in various histopathological lesions from the lumpectomy specimens of five patients with ductal breast carcinoma. Presence (+) or absence (–) of 20q13 amplification (*n.a.* not available, *ER* estrogen receptor, *PR* progesterone receptor, + positive, – negative)

Case	UICC, grading, ER/PR[a]	Normal epithelium	Intraductal hyperplasia	Atypical duct hyperplasia	Ductal carcinoma in situ	Invasive ductal carcinoma
1	pT1b, G2, ER–/PR+	–	+	+	+[b]	+
2	pT1c, G2, ER+/PR–	–	+	+	+[c]	+
3	pT1c, G3, ER+/PR+	–	+	n.a.	+[c]	+
4	pT2, G2, ER–/PR+	–	+	+	+[b]	+
5	pT2, G3, ER+/PR+	–	–	n.a.	+[c]	+

[a] Classification according to the International Union Against Cancer) [13]

[b] Cribriform, low nuclear grade (well differentiated)

[c] Comedo-type, high nuclear grade (poorly differentiated)

cases were highly selected on the basis that IH, and/or ADH, and DCIS were present adjacent to IDC. The histological classification of H&E-stained sections was performed independently by two investigators (M.C., M.W.). DCIS was classified according to Rosen and Oberman [12], and nuclear grading was performed following the criteria of Holland et al. [8]. IDC was graded according to Elston and Ellis [6]. For tumor classification the criteria of the UICC [13] were used. Estrogen receptor (ER) and progesterone receptor (PR) was determined by immunohistochemistry as described [15]. Serial 5-µm sections of the tissue blocks were used for FISH analysis and laser microdissection, followed by comparative genomic hybridization (CGH). With both methods, corresponding areas on sequential sections were investigated. For FISH analysis, DNA probes for the centromeric region of chromosome 20 (D20Z1, biotin labeled, Oncor Appligene, Heidelberg, Germany) and for the subchromosomal region 20q13.2 (Spectrum Orange labeled, Vysis, Stuttgart, Germany) were used. Signals in at least 200 nuclei per histological lesion were counted after confocal laser scanning microscopy (Zeiss LSM 410). Controls consisted of phenotypically normal ductal epithelium *from the same cases* for CGH (n=5) and FISH (n=2). For a detailed description of the CGH and FISH methods used and for the evaluation by confocal laser scanning microscopy the reader is referred to the work of Aubele et al. [1] and Wagner et al. [16]. Statistical analysis was performed using the Chi-square test.

Results

CGH from microdissected tissue showed the amplification of 20q13 in IDC and DCIS of all five patients as well as in IH of four of the five (Table 1). In three patients small foci of ADH were present, all displaying a gain of 20q13. The CGH profiles in DCIS and IDC suggested an additional increase of the whole chromosome 20 (see Fig. 1B), comparable to the FISH results with the centromeric probe (see Fig. 1D). All five control samples revealed normal profiles on CGH.

FISH analysis in paraffin sections revealed 0.8–1.1 signals for both 20q13.2 and the centromeric probe D20Z1 in phenotypically normal ductal epithelium (Table 2). As shown in Fig. 1, because of the sectioning most normal epithelial cells exhibited one signal per nucleus. In both cases of IH (without atypia), increased copy numbers of 20q13.2 were evident (P<0.1). There was a further increase of the mean number of 20q13.2 signals per nucleus in DCIS and IDC (Table 2, Fig. 1, P<0.1). The percentage of nuclei with three and more signals for 20q13.2 increased from IH (24%) to DCIS (60%) and IDC (70%), as shown for patient 1 in Table 1, and was also high in one lymph node metastasis (74%; patient 1). In IH no amplification of the centromeric region of chromosome 20 was detected, with the mean number of signals per nucleus being similar to that in normal ductal eptithelium.

Discussion

The present study describes for the first time an amplification of a chromosomal region, i.e. 20q13, harboring putative oncogenes, which was present at the stage of IH without atypia, a probable precursor of IDC. These results suggest that 20q13 amplification is one of the earliest genetic changes in the development of ductal carcinoma of the breast. The fact that 20q13 gains were also detected in ADH, DCIS and IDC of the same specimens suggests a clonal link between IH and IDC in those cases. The amount of 20q13 amplification increased from the earliest (IH) to the later (DCIS, IDC) stages of carcinogenesis.

Table 2 Mean number and error of the mean of fluorescent in situ hybridization (FISH) signals for 20q13.2 and the centromere of chromosome 20 (D20Z1) in lumpectomy specimens of two breast cancer patients (*n.d.* not done, *n.a.* not available)

Case	Normal epithelium		Intraductal hyperplasia		Ductal carcinoma in situ		Invasive ductal carcinoma		Lymph node metastases	
	D20Z1	20q13	D20Z1	20q13	D20Z1	20q13	D20Z1	20q13	D20Z1	20q13
1	0.9±0.06	0.8±0.06	1.1±0.04	1.9±0.06	1.3±0.05	2.8±0.11	1.8±0.08	3.0±0.14	1.5±0.07	3.3±0.11
2	1.0±0.11	1.1±0.10	1.0±0.05	1.6±0.06	n.d.	n.d.	1.7±0.08	3.1±0.11	n.a.	n.a.

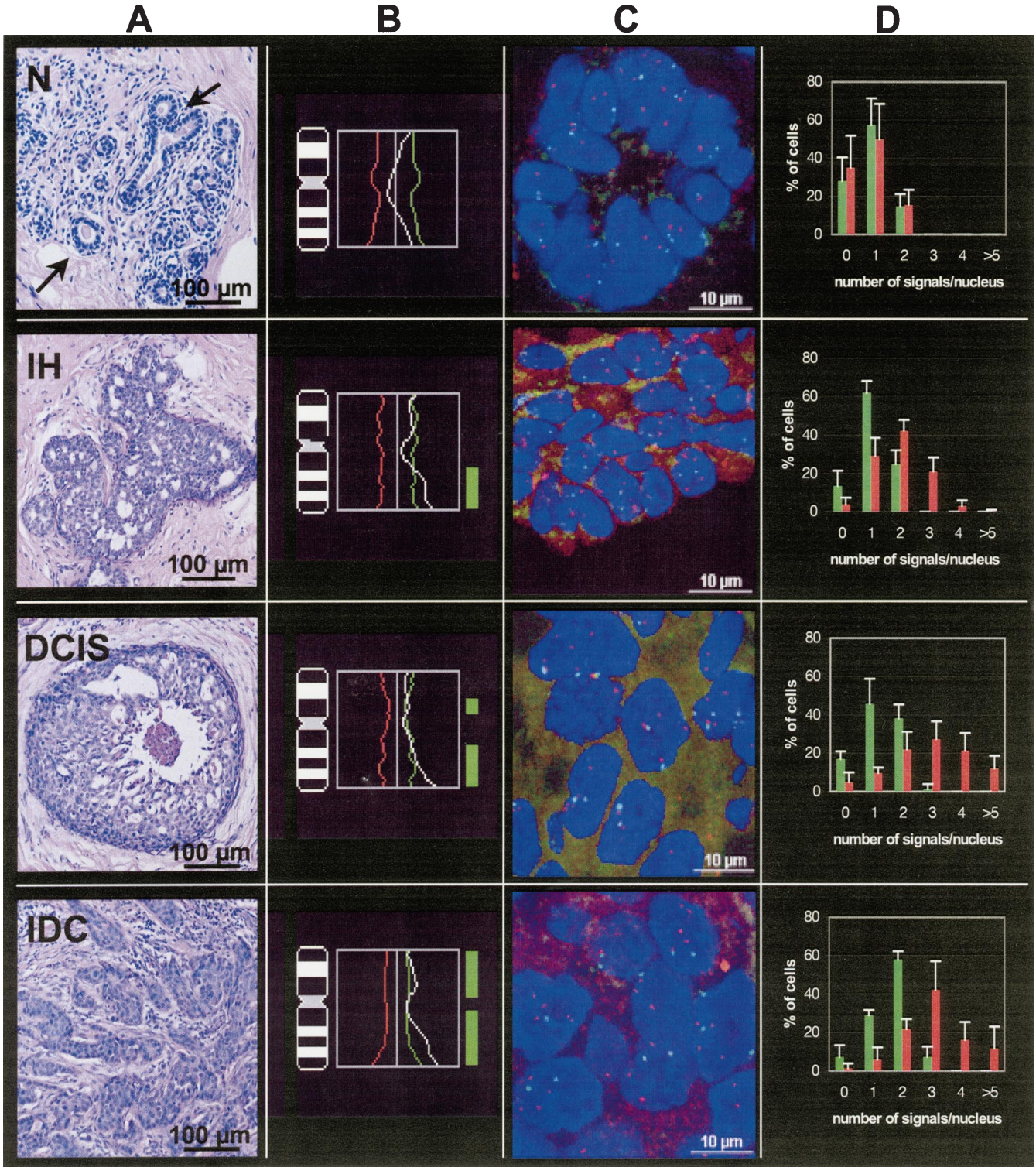

Fig. 1A–D Fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) results in case 1. **A** Phenotypically normal terminal duct epithelium (*N*, *arrows*), intraductal hyperplasia (IH), ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) are depicted from H&E-stained paraffin sections. **B** CGH profiles of chromosome 20, demonstrating gain of the 20q13 region in IH, DCIS and IDC. DNA from normal duct epithelium, IH, DCIS and IDC was labeled with a green fluorescent dye and cohybridized with a red-labeled reference DNA to normal human metaphase spreads. The red-to-green ratios (*white line*, with statistical confidence limits) along chromosome 20 have been measured by image analysis. DNA amplifications are marked by *green vertical bars* on the *right*. **C** FISH analysis of the sequential section. Probes for centromere 20 (Cy5, *green*) and 20q13 (Spectrum Orange, *red*) have been hybridized simultaneously. Nuclei are counterstained with DAPI (*blue*). The section thickness was determined in each region by laser scanning microscopy (5 µm). **D** Frequency distribution of hybridization signals per nucleus for centromere 20 and the 20q13 probe with at least 200 nuclei counted per histopathological lesion

Only a few DNA alterations have been detected at the early stage of IH in breast tissue. Loss of heterozygosity (LOH) at various loci has been shown in 0–15% of IH cases without atypia in benign breast biopsies [11]. IH adjacent to IDC shared LOH with the invasive cancer at one or more loci in 37% of cases [4], suggesting a role for mutations of tumor suppressor genes in the development of IH. However, so far, oncogene amplification has not been considered a very early step in breast cancer development [11]. 17p Amplification and c-*erb*B2 overexpression, for instance, occurred at the stage of DCIS but was not present in IH [2].

To date, the search for possible candidate genes at 20q13.2, and also along the 20q arm, is not complete. Three independently amplified, but also frequently co-amplified, chromosome regions involved in breast cancer have been described on 20q. These regions harbor the highly amplified RMC20C001 sequences at 20q13.2, the lesser amplified PTPN1 region encoding for a nonreceptor tyrosine phosphatase 3 megabases further proximal, and the AIB3/AIB4 (amplified in breast cancer) region at 20q11 [4]. More recently, five genes and one pseudogene have been found after molecular cloning of a 1-megabase region at 20q13.2 [3]. Two previously unknown genes, zinc finger protein 217 (ZNF217) and NABC1 (novel amplified in breast cancer-1), were amplified and overexpressed in several cell lines and breast cancer specimens. NABC1 seems to encode a 858-aminoacid protein of unknown function, and ZNF217 encodes alternatively spliced, Kruppel-like DNA transcription factors, each consisting of a DNA-binding domain and a proline-rich transcription activation domain. Both NABC1 and ZNF217 are considered to be candidate oncogenes involved in breast cancer tumorigenesis [3].

Although the number of cases investigated here is low, an important role for amplified oncogenes at 20q13.2 during the complex process of breast cancer development can be suggested. Amplifications at the 20q13.2 region may give a stimulus to normal epithelial cells for accelerated proliferation and formation of hyperplastic ducts. Additional molecular genetic events, including the shift from low- to high-level amplification of 20q13.2, could contribute to the transformation to neoplasia, i.e. DCIS and IDC.

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