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Chromosome alterations and E-cadherin gene mutations in human lobular breast cancer

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Summary We have studied a set of 40 human lobular breast cancers for loss of heterozygosity (LOH) at various chromosome locations and for mutations in the coding region plus flanking intron sequences of the E-cadherin gene. We found a high frequency of LOH (100%, 31/31) at 16q21–q22.1. A significantly higher level of LOH was detected in ductal breast tumours at chromosome arms 1p, 3p, 9p, 11q, 13q and 18q compared to lobular breast tumours. Furthermore, we found a significant association between LOH at 16 q containing the E-cadherin locus and lobular histological type. Six different somatic mutations were detected in the E-cadherin gene, of which three were insertions, two deletions and one splice site mutation. Mutations were found in combination with LOH of the wild type E-cadherin locus and loss of or reduced E-cadherin expression detected by immunohistochemistry. The mutations described here have not previously been reported. We compared LOH at different chromosome regions with E-cadherin gene mutations and found a significant association between LOH at 13 q and E-cadherin gene mutations. A significant association was also detected between LOH at 13q and LOH at 7q and 11q. Moreover, we found a significant association between LOH at 3 p and high S phase, LOH at 9p and low ER and PgR content, LOH at 17p and aneuploidy. We conclude that LOH at 16q is the most frequent chromosome alteration and E-cadherin is a typical tumour suppressor gene in lobular breast cancer. © 1999 Cancer Research Campaign

Keywords: E-cadherin; lobular breast cancer; mutations; LOH; tumour suppressor gene

Loss of heterozygosity (LOH) has been studied at many chromosome regions in sporadic breast cancers and at least 15 different chromosome arms have shown frequent LOH (Ingvarsson, 1999). LOH at 16q is one of the most frequently occurring genetic events in sporadic breast cancer (~67% of the informative cases), indicating the presence of one or more tumour suppressor genes at 16q (Tsuda et al, 1994; Skirnisdottir et al, 1995).

The E-cadherin gene is one of the candidate tumour suppressor genes at 16q22.1, which is one of the smallest deletion regions at 16q (Cleton-Jansen et al, 1994). E-cadherin is expressed on the cell surface in most epithelial tissues (Takeichi, 1990). The transmembrane molecule E-cadherin is considered to be one of the key molecules for the formation of the intercellular junctional complex and for the establishment of cell polarization (Gumbiner et al, 1988). The cytoplasmic tail of E-cadherin is linked via catenins to the actin cytoskeleton (Cowin, 1994), whereas the extracellular domain is involved in a molecular zipper mediating cell–cell adhesion (Shapiro et al, 1995). E-cadherin showed an important invasion suppressor activity in vitro (Frixen et al, 1991). Reduced E-cadherin expression is associated with invasiveness in breast cancer (Siitonen et al, 1996). Activation of E-cadherin can cause growth retardation of tumour cells (Navarro et al, 1991; St Croix et al, 1998). Mutations of the gene E-cadherin have been reported so far in gastric carcinomas (Becker et al, 1993, 1994), gastric carcinoma cell lines (Oda et al, 1994), cancers of the endometrium and ovarium (Risinger et al, 1994), breast cancer cell lines (Hiraguri et al, 1998) and lobular breast cancers (Kanai et al, 1994; Berx et al, 1995*a*, 1996). Furthermore, germline mutations in the E-cadherin gene have been described in familial gastric cancer (Gayther et al, 1998; Guilford et al, 1998). These results strongly suggest that the E-cadherin gene is a tumour suppressor gene.

By screening four exons (5, 6, 7 and 8) of the E-cadherin gene, Kanai et al (1994) found that two (10%) of 20 lobular breast carcinomas showed point mutations. Berx et al (1996) reported 27 E-cadherin mutations detected from a majority of 16 exons of E-cadherin gene in a series of 48 lobular breast carcinomas. These mutations were obviously scattered over the whole E-cadherin gene, particularly in the exons encoding the extracellular domain (Kanai et al, 1994; Berx et al, 1996). Interestingly, no mutations were identified in 50 breast cancers of other histological subtypes by Berx et al (1996).

In order to study the difference of genetic alterations in lobular and ductal types of breast tumours we analysed LOH at 1p, 3p, 6q, 7q, 9p, 11q, 13q, 16q, 17q, 18q and 20q. We also investigated the association between LOH at 16q and lobular histological type in order to understand the involvement of 16q in the aetiology of lobular breast carcinomas. Furthermore, we screened a set of lobular breast tumours in an attempt to find new E-cadherin mutations.

MATERIALS AND METHODS

Patients and tumour material

All 40 patients were diagnosed histologically as lobular breast cancers at Department of Pathology of the University Hospital of Iceland. Primary breast carcinoma tissue was obtained on the day of surgery. Blood samples from the patients were collected in EDTA and if not processed immediately the tumour tissue and

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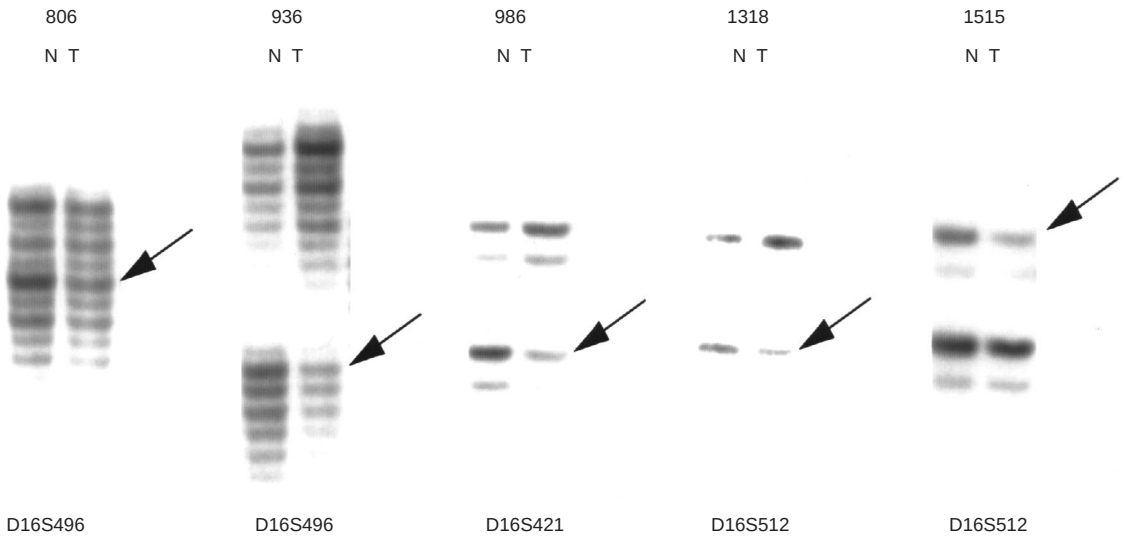

Figure 1 LOH in matched normal (N) and tumour (T) tissues from five lobular breast cancer patients with E-cadherin gene mutations. Case numbers are shown at the top. Symbols at the bottom indicate the markers used. LOH in tumours is indicated by arrows

blood were quick frozen at –80°C. All relevant information about the tumours, e.g. size, node status, receptor status (oestrogen receptor (ER) and progesterone receptor (PR)), ploidy and S phase fraction, was recorded by the same department. In this study 204 invasive ductal breast tumours were used as controls for LOH analysis (Huiping et al, 1998).

DNA isolation

Salting out procedure (Miller et al, 1988) and phenol extraction methods were used to obtain DNA from whole blood and tumour samples respectively.

LOH determination

Microsatellite markers used for LOH analysis of chromosome 1p were: D1S233, D1S496, D1S209, D1S488, and D1S435. The microsatellite markers used for chromosome 3p were: D3S1211, D3S1029, D3S1217, D3S1210 and D3S1101. The markers used for 6q were: D6S262, D6S292, D6S409, D6S290 and D6S305. The markers used for 7q were: D7S518, D7S515, D7S523, D7S471 and D7S500. The markers used for 9p were: D9S156, D9S157, INFA, D9S171 and D9S104. The markers used for 11q were: D11S907, INT-2, D11S35, D11S4206, D11S925 and D11S921. The markers used for 13q were: D13S260, D13S171, D13S267, D13S219 and D13S263. The markers used for 16q were: D16S503, D16S496, D16S421, D16S545 and D16S512 for the 16q21–22.1 region containing the E-cadherin gene (Genome Database). The markers used for 17p were: D17S945, D17S921, D17S953, D17S925, D17S798 and D17S933. The markers used for 17q were: D17S800, D17S855, D17S1322, D17S579 and D17S784. The markers used for 18q were: D18S67, D18S474, D18S51, D18S70 and D18S61. The markers used for 20q were: D20S199, D20S118, D20S191, D20S119 and D20S196. The polymerase chain reaction (PCR) products were separated in an acrylamide sequencing gel and transferred to a positively charged nylon membrane, Hybond-N+ (Amersham, Aylesbury, UK) and baked for at least 2 h at 80°C. The non-radioactive detection method used to visualize the PCR products has been described previously (Vignal et al, 1993). Autoradiograms were inspected visually by at least two reviewers, comparing the intensity of alleles from normal and tumour DNA. The absence or a significant decrease of one allele in the tumour compared to the normal reference sample was considered as LOH.

PCR–SSCP analysis

All 16 exons of E-cadherin gene were screened for inactivation mutations with a PCR–SSCP (single-strand conformation polymorphism) analysis on genomic DNA templates. The primers used in the SSCP analysis were described in Berx et al (1995*a*) and ordered from Pharmacia Biotech. Genomic DNA was used at 30 ng per 25 µl reaction mixture containing 5 pmol of the forward and reverse primers, 2.5 nmol of each dNTP, 0.5 units of DynaZyme polymerase. The samples were amplified in 35 cycles composed of 30 s of denaturation at 95°C, 60 s of annealing at 55–70°C, and finally 60 s of extension at 72°C. A hot start was used by adding the enzyme during the first cycle at about 72°C, after a preincubation time of 5 min at 94°C. A 4 µl aliquot of PCR products was mixed with 7 µl of formamide dye (95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol), denatured at 94°C for 10 min and snapcooled on ice. Aliquots of 2 µl were analysed simultaneously on two non-denaturing polyacrylamide gels (5% acrylamide with 2% cross-linking), either containing 5% glycerol or lacking glycerol. Electrophoresis was performed in 1 × TBE on vertical gels (390 × 330 × 0.4 mm) at 6w overnight or for 6 h at room temperature. The PCR products were visualized as the microsatellite markers.

Sequencing of PCR products

Samples with abnormal mobility bands were amplified again for 35 cycles as described above. A 5 µl aliquot of the PCR product was then incubated with 10 U exonuclease I and 2 U shrimp alkaline phosphatase to remove excess of primers and dNTPs (US70995, Amersham). Sequences of both strands were determined by thermo

Table 1 LOH at different chromosome regions and immunohistochemical staining (IHCS) of E-cadherin in lobular breast tumours

Tumour sample	1 p	3 p	6 q	7 q	9 p	11 q	13 q	16 q	17 p	17 q	18 q	20 q	E-cad IHCS
402	ND	+	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
676	+	–	–	–	–	–	–	+	ND	ND	ND	–	–
743	–	–	–	–	+	+	–	+	ND	+	–	+	ND
745	–	–	–	–	–	–	–	+	ND	ND	ND	–	ND
806*	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	–
811	–	–	–	+	–	+	+	+	–	ND	–	–	ND
856	–	–	–	–	–	–	–	+	ND	+	ND	–	+
897	+	+	–	–	–	+	–	+	–	+	+	+	ND
908	+	+	+	–	–	–	–	+	ND	ND	ND	–	ND
936*	+	–	–	+	+	+	+	+	+	–	+	+	(+)
986*	–	–	–	–	–	+	+	+	ND	–	ND	–	(+)
1032	–	–	ND	–	ND	–	–	+	ND	ND	ND	ND	(+)
1036	–	–	–	+	–	–	–	+	–	–	–	–	ND
1061	–	–	+	–	–	+	–	+	ND	–	ND	+	–
1118	–	–	+	–	–	–	+	+	ND	ND	ND	+	–
1225	–	ND	–	–	–	–	–	+	–	–	+	+	(+)
1258	–	–	–	–	–	–	+	+	–	–	–	+	–
1301	–	–	+	–	–	+	+	+	–	+	–	–	–
1318*	–	–	–	+	–	+	+	+	–	–	+	–	–
1327	–	–	–	+	–	–	+	+	–	–	–	–	–
1338	+	–	–	+	–	–	+	+	+	+	–	+	–
1341	–	–	ND	+	ND	+	ND	+	ND	ND	ND	ND	–
1367	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
1371	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	(+)
1475	–	–	–	+	–	+	–	ND	+	–	+	–	(+)
1478	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	–
1483	+	–	–	–	–	–	–	+	+	–	–	–	–
1499	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–
1514	–	–	–	–	–	–	–	+	–	–	–	–	+
1515*	+	+	–	–	–	–	–	+	–	–	–	–	–
1518	–	+	+	+	–	+	+	+	+	–	–	+	–
1520*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–
1524	+	+	–	+	+	–	–	ND	–	ND	–	–	–
1534	+	–	+	–	–	–	–	ND	+	+	ND	+	–
1545	–	–	–	–	+	–	–	ND	–	–	–	–	–
1546	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–
1548	+	–	–	–	+	–	–	+	–	+	–	–	–
1549	+	–	+	+	–	+	–	ND	–	+	–	–	–
1575	–	–	+	+	–	–	–	ND	–	+	–	+	ND
1583	–	+	–	–	–	–	–	+	–	+	+	–	–
Useful	11	7	8	12	5	12	10	31	6	10	6	11	29
Total	32	32	30	32	30	32	31	31	22	24	22	30	31
%	34	22	27	38	17	38	32	100	27	42	27	37	94

+, positive LOH or staining; –, negative LOH or staining; (+), weak staining; *, somatic mutation in the E-cadherin gene; ND, not determined

sequenase DNA polymerase (Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit, Amersham) using either one of the original PCR primers.

Immunohistochemical staining

Immunohistochemistry was performed on 5-μm sections from paraffin-embedded tumour tissue blocks with monoclonal antibody HECD-1 (Zymed Laboratories, South San Francisco, CA, USA) using the antigen retrieval protocol described by Hazelbag et al (1995).

Statistical analysis

A χ^2 test was used to assess the relationship between LOH in lobular tumours and LOH in ductal tumours at 1p, 3p, 6q, 7q, 9p, 11q, 13q, 16q, 17q, 18q and 20q. We also analysed the association of LOH at different chromosome regions with the categorized prognostic variables, LOH at other chromosome regions and E-cadherin gene mutations by χ^2 test and Fisher's exact test.

RESULTS

LOH analysis

LOH by the use of polymorphic microsatellite markers at chromosomes 1p, 3p, 6q, 7q, 9p, 11q, 13q, 16q, 17p, 17q, 18q and 20q in 40 lobular tumours was analysed (Figure 1). LOH for these chromosome regions ranged from 17% to 100% (Tables 1 and 2). The highest percentage of LOH was detected at the 16q21–q22.1 region where all examined cases (31/31) were positive. Table 2 shows the χ^2 analysis comparing LOH in lobular tumours with LOH in ductal tumours investigated in our laboratory at the different chromosome regions. There was a significant association

Table 2 Chi-square analysis comparing loss of heterozygosity (LOH) at different chromosome regions of tumour DNA to histological types

Chromosome region	Histological type	LOH/total	%	*P*
1 p	Lobular	11/32	34%	
	Ductal	111/194	57%	0.02[c]
3 p	Lobular	7/32	22%	
	Ductal	48/96	50%	0.0054[b]
6 q	Lobular	8/30	27%	
	Ductal	50/112	45%	0.08
7 q	Lobular	12/32	38%	
	Ductal	54/180	30%	0.40
9 p	Lobular	5/30	17%	
	Ductal	60/134	45%	0.0044[b]
11 q	Lobular	12/32	38%	
	Ductal	62/107	58%	0.04[c]
13 q	Lobular	10/31	32%	
	Ductal	88/146	60%	0.0044[b]
16 q	Lobular	31/31	100%	
	Ductal	57/78	73%	<0.001[a]
17 q	Lobular	10/24	42%	
	Ductal	28/46	61%	0.13
18 q	Lobular	6/22	27%	
	Ductal	123/192	64%	<0.001[a]
20 q	Lobular	11/30	37%	
	Ductal	39/117	33%	0.73

[a]99.9% confidence interval. [b]99% confidence interval. [c]95% confidence interval.

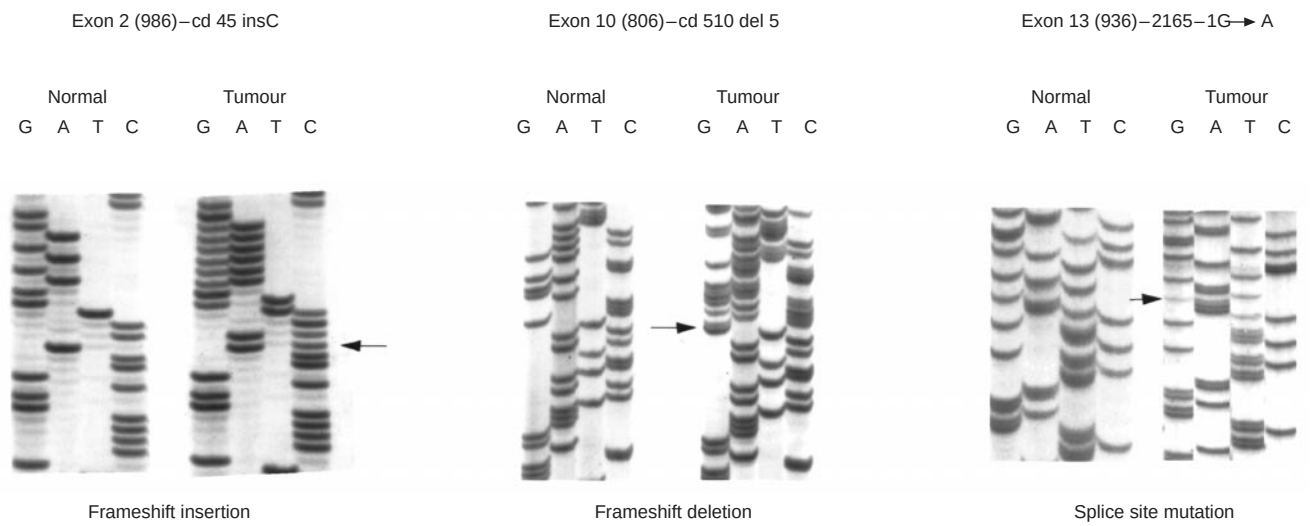

Figure 2 Three examples of sequence analysis of abnormally shifted PCR–SSCP bands, yielding three different mutation types as indicated at the bottom. The arrows indicate the following sequence changes: (**A**) one basepair insertion cd 45 ins C; (**B**) five basepair deletion cd 510 del cccag; (**C**) splice site mutation 2165–1 G→A. The sample numbers are in parentheses

between lobular tumours and LOH at 16q (99.9% confidence interval). There was also a significant association between ductal tumours and LOH at 1p, 3p, 9p, 11q, 13q and 18q (Table 2). By comparing all investigated chromosome regions in lobular breast tumours, a significant association was detected between LOH at 13q and LOH at 7q ($P = 0.049$) and between LOH at 13q and LOH at 11q ($P = 0.049$).

Mutational analysis of 40 lobular breast cancers by PCR–SSCP

We studied a set of breast cancers from 40 patients for occurrence of E-cadherin mutations. Mobility shifts were found in the amplicons 1, 2, 3, 4–5, 10, 13, 14 and 16 (Table 3). Tumours with mutation in the E-cadherin gene, 936, 986, 1318 and 1515, show LOH at several chromosomes in addition to chromosome 16q: chromosome 1p, 7q, 9p, 11q, 13q, 17p, 18q and 20q; chromosome 11q and 13q; chromosome 7q, 11q, 13q and 18q; and chromosome 1p and 3p respectively (Table 1).

DNA sequence analysis

By direct sequence analysis of the PCR products, five truncation mutations were identified among the 40 lobular tumours (Figure 2 and Table 3). Three frameshift insertions and two frameshift deletions were found, all resulting in premature downstream stop

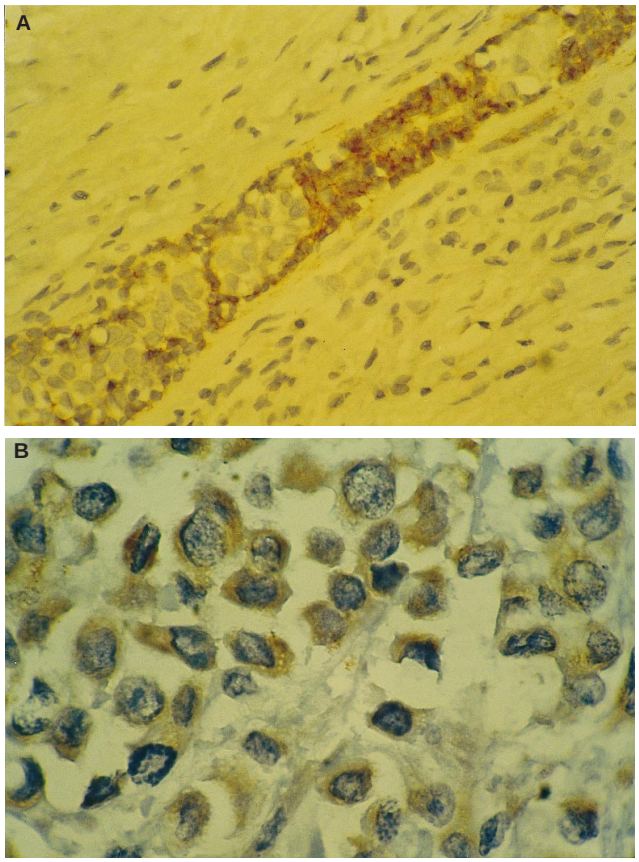

Figure 3 (**A**) Case no. 1338. Normal duct with positive staining for E-cadherin in non-neoplastic ductal cells and lobular carcinoma in situ within the duct negative for E-cadherin. In the surrounding tissue infiltrating lobular carcinoma cells are negative for E-cadherin. (**B**) Case no. 936. Infiltrating lobular carcinoma cells showing mild diffuse cytoplasmic staining for E-cadherin

codons (Table 3). Most of the insertions and deletions were rather small, causing 1-bp to 5-bp changes. Only one larger deletion of 22 bp was identified. We also identified one putative splice site mutation for exon 13 (Figure 2 and Table 3). In the mutated splice acceptor sequence of exon 13, GT was converted by a single base substitution to AT (Figure 2 and Table 3). All of the six mutations were found only once and have not been previously reported. Sequencing of DNA for blood from all six patients with mutations in breast tumours indicates that the mutations are not germline, but tumour-specific.

Besides the six mutations, we also identified nine different polymorphisms of which four have been described before (Risinger et al, 1994; Berx et al, 1995*a*, 1996). Besides the frequent polymorphism in the intron 4, a previously unreported but frequent polymorphism (four out of 38) was identified in the intron 15 sequence from amplicon 16. Polymorphisms are shown in Table 3.

Immunohistochemistry

Of the 31 lobular tumours analysed by immunohistochemistry, 29 (94%) showed no or reduced membrane-associated E-cadherin staining with monoclonal antibody HECD-1 and only two showed a clear membrane-associated expression of E-cadherin (Figure 3 and Table 1). In particular, four samples 806, 986, 1318 and 1515 with truncation mutations plus LOH at 16q22.1 were negative or showed reduced E-cadherin protein expression (Table 3 and Figure 3). All lobular breast tumours lack membrane staining, while two of them show weak cytoplasmic staining.

Association analysis of the lobular breast tumours

We found an association between E-cadherin gene mutations and LOH at 13q and 16q ($P = 0.05$). A significant association was also found between LOH at 3p and high S phase, LOH at 9 p and low ER and PR content, LOH at 17p and aneuploidy ($P = 0.033$, 0.011, 0.001, 0.014 respectively). A trend was detected between LOH at 1p and tumours larger than 2 cm ($P = 0.052$).

DISCUSSION

In this study, LOH at 16q22.1 was detected in all lobular breast tumours examined. This is a higher rate than those reported in previous studies (Tsuda et al, 1994; Skirnisdottir et al, 1995; Berx et al, 1996) presumably due to difference in the set of markers used. LOH at 16 q in lobular tumours was compared with LOH at 16 q in ductal tumours using the same microsatellite markers. The detected association suggests that the chromosome 16q22.1 region contains one or more suppressor genes particularly relevant to lobular breast carcinogenesis. LOH for the 16q22.1 region has been detected in several carcinoma types besides breast cancer, also suggesting the presence of tumour suppressor genes at this region (Tsuda et al, 1990; Bergerheim et al, 1991; Sato et al, 1991). We also compared LOH at other chromosome regions in lobular and ductal breast tumours and found a higher LOH at 1 p, 3 p, 9 p, 11 q, 13 q and 18 q in ductal tumours, indicating a difference in genetic alterations in the two histological types of breast cancers.

The human E-cadherin gene has been mapped to chromosome 16q22.1 (Mansouri et al, 1988; Natt et al, 1989). Its expression was reduced in several types of human carcinomas (Shimoyama et al, 1991; Bussemakers et al, 1992; Inoue et al, 1992; Umbas et al, 1992). Mutations of the E-cadherin gene have been identified in human tumours and tumour cell lines (Becker et al, 1993, 1994; Kanai et al, 1994; Oda et al, 1994; Risinger et al, 1994; Berx et al, 1995*a*, 1996). These observations in combination with LOH at 16q22.1 indicate that the E-cadherin gene is a tumour suppressor gene. Our data reported here consolidate the evidence for E-cadherin playing an important role as a typical tumour suppressor in lobular breast carcinomas. Since no mutations in the E-cadherin gene have been detected in tumours in the breast of the ductal histological type (Berx et al, 1996), this provides the clearest evidence of molecular difference in the two main histological types of breast carcinomas. Nonetheless, reduced expression of E-cadherin has been found in both lobular and ductal breast cancers (Oka et al, 1993; Gamallo et al, 1996) and detection of chromosome 16q22.1 is the highest documental loss of a chromosome region in sporadic breast cancers of both histological types (Skirnisdottir et al, 1995; this study). Two explanations of this difference are possible, either: (1) a gene other than the E-cadherin gene is the target of the 16q22.1 deletion in ductal compared to lobular carcinomas of breast cancer; or (2) the progression of ductal carcinomas is more sensitive to loss of one copy of the E-cadherin gene, and corresponding reduction of expression, than lobular breast carcinomas, where both copies need to be eliminated for further progression to malignant invasive growth. The LOH detected in this study is probably due to loss of genetic material for chromosome 16q22.1 since

Table 3 Summary of E-cadherin gene mutations and polymorphisms detected in a series of 40 lobular breast tumours

Amplicon No.[a]	Tumour sample	Mutation site[b]	Mutation	Nucleotide change	LOH[c]	E-cad IHC[d]
1	1318	cd 8	frameshift	del 22 (stop at cd 48)[e]	yes	–
2	986	cd 45	frameshift	ins C (stop at cd 58)	yes	(+)
2	1520	cd 51	frameshift	ins G (stop at cd 58)	NT	–
10	806	cd 510	frameshift	del 5 (stop at cd 534)[f]	yes	–
13	936	intron 13	splice site	2165–1 G→A	yes	(+)
14	1515	cd 726	frameshift	ins T (stop at cd 747)	yes	–

Amplicon	Site	Polymorphism	Observed frequency
3	intron 2	tttctccc→tttctgccc	2/38
3	cd 115	ACG(Thr)→ACA(Thr)	1/38[g]
4–5	intron 4	gaaac→gaaag	6/38[h]
13	cd 692	GCC(Ala)→GCT(Ala)	3/38[i]
14	cd 751	AAC(Asn)→AAT(Asn)	1/38[j]
14	cd 764	GAC(Asp)→GAT(Asp)	1/38
16	cd 878	GGC(Gly)→GGT(Gly)	1/38
16	intron 15	ttgag→tttag	4/38
16	intron 15	ttctt→ttgtt	1/38

[a]Amplicons contain besides exons also flanking intronic sequences and in the case of amplicon 4–5 the whole intron 4 is present, too. [b]cd, codon. [c]LOH, loss of heterozygosity at 16q22.1. [d]E-cad IHC: E-cadherin specific immunohistochemistry. NT, no tumour material available for LOH analysis. –, no cell surface associated staining. (+), weak or cytoplasmic staining, +, normal staining. [e]22 bp deletion at codon 8 of exon 1: 5′-ctctcggcgctgctgctgctgc-3′. [f]5 bp deletion at codon 510 of exon 10: 5′-cccag-3′. [g–j]These polymorphisms were also reported by Berx et al. (1995a). [i]This polymorphism was also reported by Risinger et al (1994). [j]This polymorphism was reported previously by Risinger et al (1994) and by Becker et al (1994).

reduced expression of the E-cadherin is detected as well, but our method in detecting LOH does not exclude a duplication of a locus due to mitotic recombination.

Altogether, we examined 40 lobular breast tumours on the genomic level for E-cadherin mutations. Besides polymorphisms, six mutations were identified by our E-cadherin PCR/SSCP analysis. None of the mutations described here have been reported before. In agreement with the mutations reported by Berx et al (1996), most of the mutations (5/6) were caused by frameshifts, usually resulting in premature stop codons (Table 3). Although a large series of lobular tumours was analysed, we could not identify any non-sense mutation, in contrast with a previous report on five non-sense mutations in a series of 48 lobular tumours (Berx et al, 1995*a*, 1996). Besides truncation mutations we also found a splice site mutation, affecting the acceptor splice site and probably causing skipping of exon 14 (Table 3). This particular tumour shows a weak cytoplasmic E-cadherin staining. Becker et al (1993, 1994) have found frequent skipping of exon 8 or 9 in diffuse gastric carcinomas. It is noteworthy that exon 14 is encoding the part of the transmembrane domain necessary for binding membrane and the part of the cytoplasmic domain necessary for binding β-catenin and plakoglobin (γ-catenin) (Aberle et al, 1994). E-cadherins are linked to the actin cytoskeleton by the introduction of catenins, resulting in tight cell–cell adhesion (Cowin, 1994). The aberrant transmembrane domain and cytoplasmic domain can affect the cell–cell adhesion. Interestingly, the six novel mutations were scattered over all five E-cadherin domains, including the signal and precursor sequences (Table 3).

The various truncated E-cadherin peptides are likely to be secreted and to have some residual function which could interfere with proper cell–cell adhesion in the peritumoral tissues (Berx et al, 1996). Furthermore, these fragments and even small decapeptides containing the HAV sequence, known to be involved in homophilic recognition, can disturb proper cell–cell adhesion (Blaschuk et al, 1990). Therefore, it may be interesting to investigate the treatment of lobular tumours with related antibodies that can be synthesized in vitro according to the genes with truncation or splice site mutations.

For all informative lobular breast tumours, which we found to lack membrane E-cadherin expression, we identified both LOH of 16q22.1 harbouring the E-cadherin locus and mutations in the remaining allele, in accordance with the classical two-hit theory of Knudson for tumour suppressor genes (Knudson, 1985). This supports the results reported by Berx et al (1995*a*, 1996). This is also supported by an association between E-cadherin gene mutations and LOH at 16q. Presumably, LOH at 13q could enhance the lobular tumour growth triggered by E-cadherin gene mutation and LOH at 16q because we also found an association between E-cadherin gene mutations and LOH at 13q. Transfection of MET/6 mouse mammary carcinoma cells, which lack E-cadherin, with an exogenous E-cadherin expression vector, resulted in tighter adhesion of multicellular spheroids and a reduced proliferative fraction in three-dimensional culture (St Croix et al, 1998). Exposure to E-cadherin-neutralizing antibodies in three-dimensional culture simultaneously prevented adhesion and stimulated proliferation of E-cadherin transfectants as well as a panel of human colon, breast and lung carcinoma cell lines that express functional E-cadherin (St Croix et al, 1998). From these, it can be concluded that E-cadherin, classically described as an invasion suppressor, is also a major growth suppressor.

We identified loss of or reduced E-cadherin expression in 29 out of 31 lobular breast tumours, in accordance with earlier studies (Gamallo et al, 1993; Rasbridge et al, 1993). However, only six out of 29 lobular tumours lacking E-cadherin expression showed detectable E-cadherin mutations, suggesting that loss of one copy of the E-cadherin gene may affect the expression of the remaining allele. This is lower frequency of mutations than reported by Berx et al (1995*a*, 1996). The number of mutations reported here is

probably underestimated, either due to low efficiency of SSCP or that some tumours were lacking data from all exons. In addition to this the reduced expression may be due to transcriptional defects (Ji et al, 1997). Synthesis of mRNA could be affected by mutations in the promoter region, or in intron located regulatory sequences, whereas stability of mRNA could be affected by mutations in the untranslated regions (Berx et al, 1996). CpG-island overspanning intron 1 of the E-cadherin gene (Berx et al, 1995*b*) was found to be densely methylated in breast cancer cell lines, and this correlated with loss of E-cadherin expression (Graff et al, 1995). Moreover, p53 protein accumulation and c-erbB-2 protein overexpression may play a role in regulation of E-cadherin expression (Bukholm et al, 1997).

Catenins link E-cadherin molecules to the actin cytoskeleton and lay a solid foundation for the tight cell–cell adhesion (Cowin, 1994). Therefore, loss of or reduced catenin expression also interferes with cell–cell adhesion. Simultaneous loss of E-cadherins and catenins have been detected in invasive lobular breast cancer and lobular carcinoma in situ (De Leeuw et al, 1997). Thus, it may be important to detect mutations in the catenin genes in lobular breast tumours or other epithelial carcinomas.

A number of studies have revealed that chromosome deletions in breast cancers may occur in preferred combinations with respect to growth advantage, e.g. 1p and 3p (Ragnarsson et al, 1996), 7q and 1p (Kristjansson et al, 1997), 3p and 6q (Bragadottir et al, 1995), 9p and 6q (Eiriksdottir et al, 1995), 13q and 17p (Anderson et al, 1992), 11p and 17p (Takita et al, 1992), 18q and 1p, 7q, 9p, 13q and 17q (Huiping et al, 1998). In order to determine whether there are specific combinations of chromosome deletions in lobular breast tumours, we compared LOH at different chromosome regions and found a weak association between LOH at 13q and LOH at 7q and 11q.

LOH at different chromosome regions was compared with various clinicopathological variables of the lobular breast carcinomas. A significant association was found between LOH at 9p and low ER and low PR. This suggests that LOH at 9p could be involved in the loss of ER and PR content. A weak association was found between LOH at 3p and high S phase fractions and a trend between LOH at 1p and tumour larger than 2 cm. This suggests that genes at 3p and 1p could possibly have a restraining effect on the rate of cell proliferation, and the loss of them would lead to a rapid growth. We also found a weak association between LOH at 17p and aneuploidy, indicating that LOH at 17p could be associated with an unstable genome.

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