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Four human breast cancer cell lines with biallelic inactivating α -catenin gene mutations

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Abstract Mutations of *E-cadherin* have been identified in half of lobular breast cancers and diffuse-type gastric cancers, two tumor subtypes with remarkably similar pathological appearances including small rounded cells with scant cytoplasm and a diffuse growth pattern. A causal role for *E-cadherin* gene mutations in the lobular breast cancer phenotype was recently demonstrated in *E-cadherin* knock-out mice. These observations suggested that another gene in the E-cadherin tumor suppressor pathway might be mutated in lobular breast cancers with wild-type *E-cadherin* genes. Here, we identified *E-cadherin* gene mutations exclusively in human breast cancer cell lines that grow with a rounded cell morphology. Using expression analyses and gene mutation analyses, we have identified four biallelic inactivating α -catenin mutations among 55 human

breast cancer cell lines. All four α -catenin mutations predicted premature termination of the encoded proteins, and concordantly, none of the four mutant cell lines expressed α -catenin proteins. Importantly, three of the α -catenin mutant cell lines had the rounded cell morphology and all 14 cell lines with the rounded cell morphology had mutations of either *E-cadherin* or α -catenin. As anticipated, loss of α -catenin protein expression was associated with the lobular subtype in primary breast cancers. Together, our observations suggest that α -catenin may be a new tumor suppressor gene that operates in the E-cadherin tumor suppressor pathway.

Keywords Cell adhesion · Cell morphology · E-cadherin · Lobular breast cancer · Tumor suppressor gene

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Introduction

The E-cadherin/catenin protein complex consists of the cytoplasmic proteins α -catenin, β -catenin, γ -catenin, and p120-catenin (p120ctn) and the transmembrane protein E-cadherin. The armadillo proteins β -catenin and γ -catenin bind directly to the intracellular carboxy-terminal tail of E-cadherin and p120ctn binds more membrane-proximal to E-cadherin. The vinculin-related protein α -catenin acts as a molecular switch and binds either to β -catenin or γ -catenin as a monomer or to the actin cytoskeleton as a homodimer. Extracellular, E-cadherin forms homodimers with E-cadherin proteins on adjacent epithelial cells in a calcium-dependent fashion. The E-cadherin/catenin protein complex thus maintains the integrity of epithelial tissues through cell–cell adhesion (reviewed in [1, 2]).

Inactivation of the *E-cadherin* tumor suppressor gene in human cancer may involve mutational inactivation or transcriptional silencing by transcriptional repressors and/or promoter hypermethylation (reviewed in [3–5]). Mutations in the *E-cadherin* gene had predominantly been found in breast cancers of the lobular subtype, but also in diffuse-type gastric cancers, two tumor subtypes that have remarkable similarities in their morphological appearance [6–9]. The association of *E-cadherin* gene mutations with this typical morphology suggested causality for *E-cadherin* mutations. Indeed, mutational inactivation of *E-cadherin* has been shown to be causal for a lobular cancer phenotype in conditional knock-out mice [10]. However, not all human lobular breast cancers harbor *E-cadherin* mutations. We therefore hypothesized that another gene in the E-cadherin pathway might be mutated in these tumors.

Here, we have used a model of 55 human breast cancer cell lines to study *E-cadherin* inactivation. We found an exclusive association of *E-cadherin* gene mutations with a characteristic rounded cell morphology of the mutant cell lines. We then identified biallelic inactivating α -catenin gene mutations in *E-cadherin* wild-type cell lines with the rounded cell morphology.

Materials and methods

Breast cancer cell lines

The 55 human breast cancer cell lines used in this study are listed in Table 1. Cell lines EVSA-T, MPE600, and SK-BR-5/7 were kind gifts of Dr. N. de Vleeschouwer (Institut Jules Bordet, Brussels, Belgium), Dr. H.S. Smith (California Pacific Medical Center, San Francisco, CA, USA) and Dr. E. Stockert (Sloan-Kettering Institute for Cancer Research, New York, NY, USA), respectively. The SUM cell lines were generated in the Ethier laboratory (available at <http://www.asterand.com>). Cell lines OCUB-F and -M had been generated from the same tumor, and were obtained from Riken Gene Bank (Tsukuba, Japan). The other cell lines were obtained from ATCC (Manassas, VA, USA). All cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, in standard culture flasks.

Clinical breast cancers

Formalin-fixed paraffin-embedded primary breast cancers included 33 lobular, 48 ductal, 22 medullary, 22 mucinous, and 14 tubular breast cancers. All 139 breast cancers were obtained from the archive of the Pathology department at Erasmus MC, randomly selected from diagnosis years 1990

through 2005 based on their histology. Tissue microarrays (TMAs) were constructed by punching three 0.6-mm cores from representative areas of each tumor and transferring them into a recipient paraffin block using an ATA-27 automated tissue microarrayer (Beecher Instruments, Sun Prairie, WI, USA). The Medical Ethical Committee at Erasmus MC has approved the study (MEC 02-953), which was carried out according to the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands (<http://www.fmwv.nl>).

Gene mutation analysis

E-cadherin and α -catenin (ENSG00000039068 and ENSG0000044115) coding sequences were analyzed by direct sequencing of PCR-amplified fragments from genomic DNA, using an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and intronic primers (Supplementary Table S1). All identified mutations were confirmed by sequencing of an independently amplified DNA template and all deletions were confirmed by performing a duplex PCR including primers for the *ZEB2* gene (ENSG00000169554) that served as positive control.

Promoter methylation analysis

Methylation specific PCR (MSP) for *E-cadherin* CpG1 and CpG3 was done using the EZ Methylation Kit (Zymed, Orange, CA) with reported primers [11] under our standard PCR conditions [12]. For azacytidine assays, exponentially growing cells were treated for 3 days with 10 μ M 5-aza-2'-deoxycytidine (Sigma, Steinheim, Germany) and total RNA was isolated on day 4 using the RNeasy kit (Qiagen, Hilden, Germany).

Expression analysis

Reverse transcriptase (RT)-PCR for *E-cadherin*, α -catenin, β -catenin, γ -catenin, and *p120ctn* was done using the Qiagen one-step RT-PCR kit and gene-specific exonic primers (Supplementary Table S2).

Immunohistochemistry was performed using the EnVision+ kit (DAKO, Glostrup, Denmark). To unmask antigens, slides were boiled for 20 min in Citrate buffer at pH 6 (Klinipath, Duiven, The Netherlands) for α -catenin or Tris-EDTA buffer at pH 9 (Klinipath) for the other antigens. Primary monoclonal antibodies and their dilutions were: E-cadherin 1:25 (DAKO, clone NCH-38), α -catenin 1:40 (Santa Cruz Biotechnology (Santa Cruz, CA, USA), clone G-11), β -catenin 1:100 (BD Transduction Laboratories (Franklin Lakes, NJ, USA), clone 14), γ -catenin 1:120 (BD Transduction Laboratories, clone 15),

Table 1 *E-cadherin* and α -*catenin* gene status and *E-cadherin*/catenin expression patterns of 55 breast cancer cell lines

Breast cancer cell line	Cell morphology	<i>E-cadherin</i> gene status	<i>E-cadherin</i> methylation	<i>E-cadherin</i> transcript	<i>E-cadherin</i> protein	α - <i>Catenin</i> gene status	α - <i>Catenin</i> transcript	α - <i>Catenin</i> protein	β - <i>Catenin</i> transcript	β - <i>Catenin</i> protein	γ - <i>Catenin</i> transcript	γ - <i>Catenin</i> protein	<i>p120ctn</i> transcript	<i>p120ctn</i> protein
HCC202	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1008	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1419	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1500	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1569	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1599	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1806	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1954	Epithelial	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
BT474	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
BT483	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
HCC1937	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
MCF-7	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
MDA-MB-175 VII	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
MDA-MB-361	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
MDA-MB-415	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
SUM52PE	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
SUM185PE	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
SUM190PT	Epithelial	Wild-type	nd	+	+	Wild-type	+	+	+	+	+	+	+	+
SUM225CWN	Epithelial	Wild-type	nd	+	+	Wild-type	+	+	+	+	+	+	+	+
T47D	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
UACC812	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
UACC893	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
ZR75-1	Epithelial	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
CAMA-1	Rounded	IF mutant	-	+	+	Wild-type	+	+	+	+	+	+	+	+
EVSA-T	Rounded	IF mutant	-	+	+	Wild-type	+	+	+	+	+	+	+	+
MPE600	Rounded	IF mutant	-	+	+	Wild-type	+	+	+	+	+	+	+	+
MDA-MB-134 VI	Rounded	PT mutant	-	+	-	Wild-type	+	+/-	+	-	+	+/-	+	+
SUM44PE	Rounded	PT mutant	nd	+	-	nd	+	+/-	+	-	+	+/-	+	+
OCUB-F, -M	Rounded	PT mutant	-	+	+/-	Wild-type	+	+	+	+/-	+	+/-	+	+
MDA-MB-453	Rounded	PT mutant	-	+/-	-	Wild-type	+	+/-	+	-	+	+/-	+	+
SK-BR-5	Rounded	PT mutant	-	+/-	-	Wild-type	+	+	+	-	+	+/-	+	+
ZR75-30	Rounded	PT mutant	-	+/-	-	Wild-type	+	+	+	+/-	+	+/-	+	+
SK-BR-3	Rounded	PT mutant	-	-	-	Wild-type	+	+	+	-	+	+/-	+	+
HCC2218	Rounded	PT mutant	nd	nd	-	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1187	Rounded	Wild-type	nd	nd	nd	PT mutant	nd	-	nd	nd	nd	nd	nd	nd

Table 1 continued

Breast cancer cell line	Cell morphology	<i>E-cadherin</i> gene status	<i>E-cadherin</i> methylation	<i>E-cadherin</i> transcript	<i>E-cadherin</i> protein	α -Catenin gene status	α -Catenin transcript	α -Catenin protein	β -Catenin transcript	β -Catenin protein	γ -Catenin transcript	γ -Catenin protein	<i>p120ctn</i> transcript	<i>p120ctn</i> protein
MDA-MB-330	Rounded	Wild-type	-	+	+	PT mutant	+	-	+	+	+	+	+	+
MDA-MB-468	Rounded	Wild-type	-	+	+	PT mutant ^a	+	-	+	+	+	+	+	+
MDA-MB-157	Spindle	Methylated	CpG1/CpG3	+/-	-	PT mutant ^b	+	-	+	+	+	+/-	+	+
BT549	Spindle	Methylated	CpG1/CpG3	-	-	Wild-type	+	+	+	+	+/-	+/-	+	+
Hs578T	Spindle	Methylated	CpG1/CpG3	-	-	Wild-type	+	+	+	+	+	+/-	+	+
MDA-MB-435s	Spindle	Methylated	CpG1/CpG3	-	-	Wild-type	+	+	+	+	+	-	+	+
MDA-MB-436	Spindle	Methylated	CpG1/CpG3	-	-	Wild-type	+	+	+	+	+	+/-	+	+
SUM159PT	Spindle	Methylated	CpG1/CpG3	-	-	Wild-type	+	+	+	+	+	+	+	+
SUM1315MO2	Spindle	Methylated	CpG1/CpG3	-	-	Wild-type	+	+/-	+	+	+/-	-	+	+
MDA-MB-231	Spindle	Methylated	CpG1/CpG3	+/-	-	Wild-type	+	+	+	+	+	-	+	+
SK-BR-7	Spindle	Methylated	CpG1/CpG3?	+	+	Wild-type	+	+	+	+	+	+	+	+
SUM102PT	Spindle	Methylated	CpG1	+	nd	Wild-type	+	nd	+	nd	+	nd	+	nd
SUM149PT	Spindle	Methylated	CpG1	+	+	Wild-type	+	+	+	+	+	+	+	+
SUM229PE	Spindle	Methylated	CpG1	+	+	Wild-type	+	+	+	+	+	+	+	+
BT20	Spindle	Wild-type	-	+	+	Wild-type	+	+	+	+	+	+	+	+
HCC38	Spindle	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC70	Spindle	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1143	Spindle	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
HCC1395	Spindle	Wild-type	nd	nd	nd	Wild-type	nd	nd	nd	nd	nd	nd	nd	nd
DU4475	Other	Wild-type	-	+	+/-	Wild-type	+	+	+	++	+	+/-	+	+/-

Breast cancer cell lines are organized by their morphology and by their *E-cadherin* and α -catenin gene status as determined by mutation analysis of all coding exons and for *E-cadherin* also by methylation specific PCR and azacytidine assays. *E-cadherin* and catenin transcript and protein expression was determined by RT-PCR and immunohistochemistry

IF in-frame, *PT* protein truncation, - not detectable, +/- low or barely detectable expression, + clearly detectable expression, *nd* not determined

^a Two shortened α -catenin transcripts

^b Transcript is only detectable up to exon 7

and p120ctn 1:150 (BD Transduction Laboratories, clone 98).

Results

E-cadherin gene mutations are found exclusively among breast cancer cell lines with rounded morphology

We have used a model of 55 human breast cancer cell lines to study E-cadherin inactivation. The *E-cadherin* gene status was analyzed for gene mutations by sequencing and for promoter methylation by MSP and azacytidine assays. Together, 11 *E-cadherin* mutant cell lines and 12 cell lines with methylation at the *E-cadherin* gene promoter were identified (Table 1, mutations detailed in [13] and c.1_1565del1565 in HCC2218). Three distinct morphology groups were apparent among the breast cancer cell lines: those that grow with epithelial cells, with rounded cells, and with spindle cells (Fig. 1, Table 1). Importantly, all 11 *E-cadherin* mutant cell lines had the rounded cell morphology, whereas all 12 *E-cadherin* methylated cell lines had the spindle cell morphology. All cell lines with the epithelial cell morphology had a normal *E-cadherin* gene status. The two different inactivation mechanisms of E-cadherin thus appear to have a profound impact on cell morphology.

E-cadherin gene mutations are identified in half of lobular cancers, but essentially not in other subtypes of breast cancer [6, 7, 14]. The presence of non-cohesive small rounded cells with scant cytoplasm is a cytological characteristic of the lobular pathological subtype of human breast cancer [15]. Concordantly, mutations in *E-cadherin* were shown to be causal for the lobular subtype of breast cancer [10]. Indeed, the rounded cell morphology of the breast cancer cell lines is characterized by rounded cells with scant cytoplasm. The rounded cells may grow in clusters, grape-like bunches or so-called Indian files of cells, or as single cells (Fig. 1). The cell cultures typically also contain adherent cells that grow in epithelial sheets but these display diminished cell–cell adhesion. The proportion of rounded cells versus adherent cells varies per cell line (ranging from 10 to 90%), and the rounded cells may be attached to the adherent cells or float freely in the culture medium. The 14 breast cancer cell lines with the rounded cell morphology thus appear to resemble lobular breast cancers. Consistent with this notion, four *E-cadherin* mutant cell lines from our collection were known to be derived from breast cancers with lobular characteristics (EVSA-T, MDA-MB-134VI, MDA-MB-330, and SUM44PE; [16–18] and personal communication Dr. S.P. Ethier).

Truncating α -catenin mutations in four human breast cancer cell lines

Although *E-cadherin* mutation was shown to be causal for the lobular subtype of breast cancer, not all lobular breast cancers harbor *E-cadherin* mutations [6, 7]. Similarly, three rounded breast cancer cell lines from our collection had wild-type *E-cadherin* genes, suggesting that mutations in another gene of the E-cadherin tumor suppressor pathway might be involved. To seek evidence that another gene in the E-cadherin pathway might be mutated, we performed expression analysis of α -catenin, β -catenin, γ -catenin, and p120ctn by RT–PCR and immunohistochemistry on cell line TMAs (Table 1). Interestingly, four wild-type *E-cadherin* cell lines had lost α -catenin protein expression and two of these cell lines also had a shortened α -catenin transcript. Subsequent analysis of the complete coding sequence of α -catenin revealed four biallelic α -catenin mutations among 55 human breast cancer cell lines. In cell line MDA-MB-468, α -catenin exons 4 and 5 were homozygously deleted from the genome, resulting in two aberrant transcript lengths (Fig. 2a; c.302_588del287). One transcript lacked exons 4 and 5, predicting a premature termination of the encoded α -catenin protein (r.302_588del287; p.D102IfsX8). The other transcript lacked exons 3 through 5, predicting an in frame deletion of 161 amino acids (r.106_588del1483; p.V36_Q196del161). As we did not detect α -catenin protein expression in MDA-MB-468 with a C-terminal antibody, it appears that the second transcript is not being translated. In cell line MDA-MB-157, we were not able to amplify α -catenin exons 8 and 9 from the genome (Fig. 2b). Using various combinations of intronic and exonic primers for both exons 8 and 9, we never observed PCR amplification products for exon 8 and, depending on the primer combination, shorter or no amplification products for exon 9. Transcript analysis by RT–PCR allowed amplification of exons 1 through 7, but none of the more 3' located exons. Together, these results suggested a complex genomic rearrangement or translocation in MDA-MB-157, involving deletion of α -catenin exon 8 and partial deletion of exon 9. Finally, cell lines HCC1187 and MDA-MB-330 both harbored a nonsense mutation (Fig. 2c and d; c.2032C>T/p.Q678X and c.1322C>G/p.S441X, respectively). Additionally, we identified two SNPs and two silent mutations in 22 cell lines (Supplementary Table S3). Regrettably, original (uncultured) tumor material was not available for any of the four α -catenin mutant breast cancer cell lines, precluding confirmation that the mutations had been present in the original tumors. Together, we identified four biallelic α -catenin mutations among 55 breast cancer cell lines.

Importantly, three of the four α -catenin mutant breast cancer cell lines have the rounded cell morphology. All

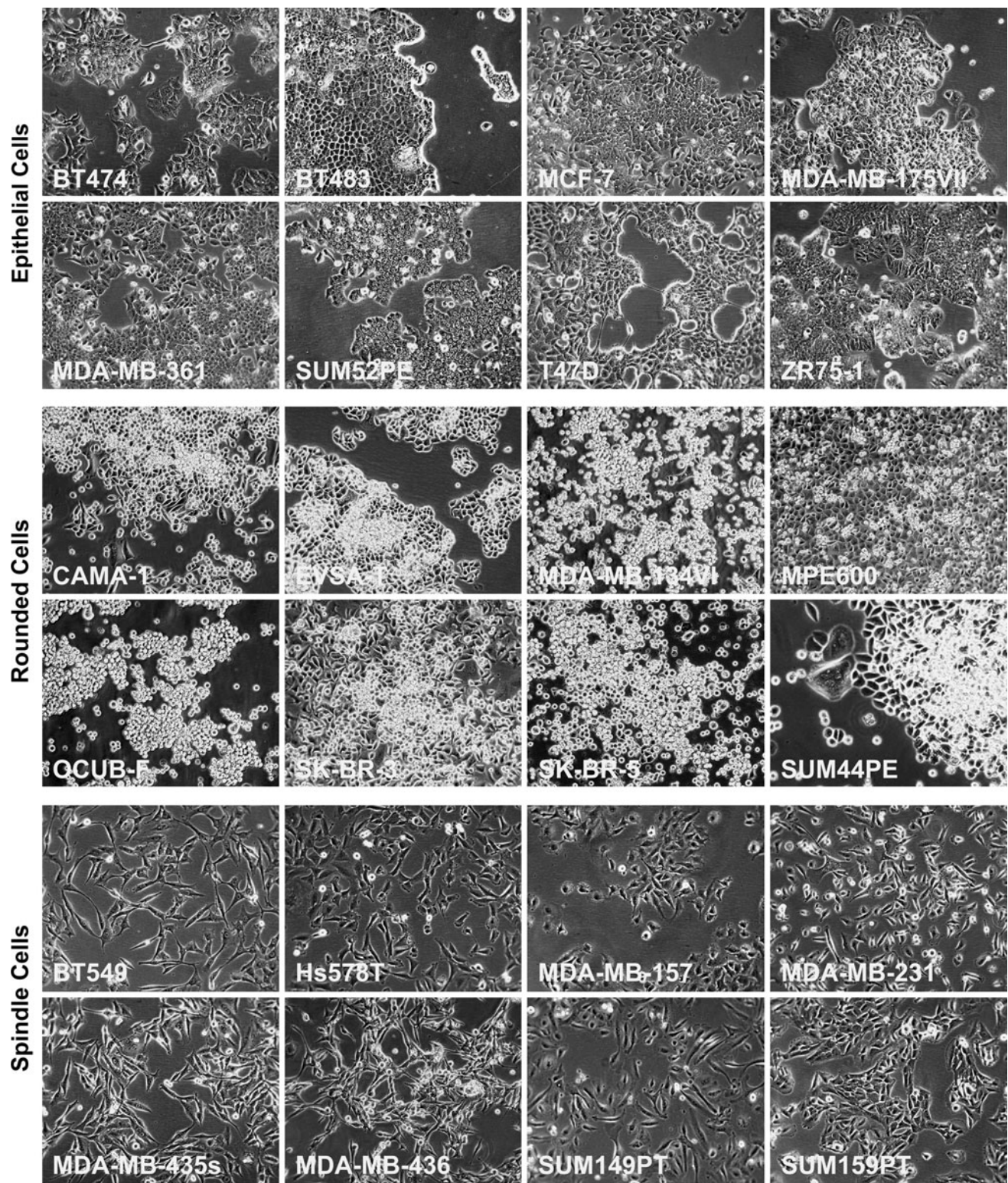


Fig. 1 Morphology of human breast cancer cell lines. Eight examples are given for each of three morphology groups: epithelial cells that all have wild-type *E-cadherin* genes and normal E-cadherin protein

expression; rounded cells that all have mutant *E-cadherin* genes; and spindle cells that all have methylation at the *E-cadherin* promoter region

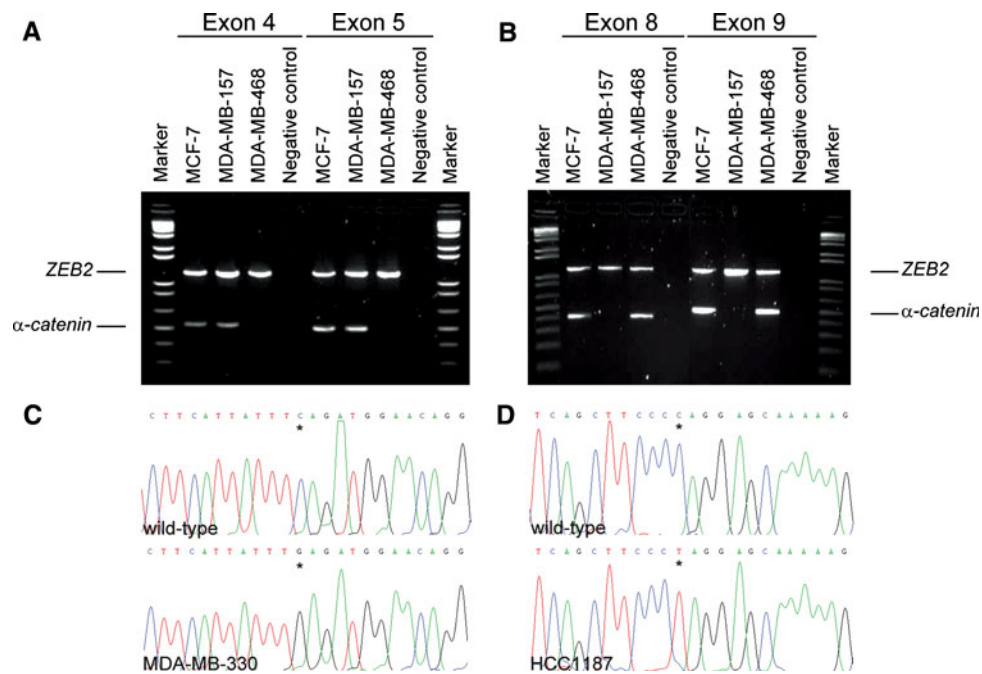


Fig. 2 Identification of α -catenin gene mutations among 55 breast cancer cell lines. **a** Homozygous deletion of exons 4 and 5 of α -catenin in cell line MDA-MB-468 and **b** Homozygous deletion of exons 8 and 9 of α -catenin in cell line MDA-MB-157. Duplex PCR amplification products are shown for three breast cancer cell lines, using primers specific for *ZEB2* and α -catenin (upper and lower fragments, respectively). Negative control, template negative control;

Marker, size marker 1 kb+ DNA ladder (Invitrogen). **c** α -catenin nonsense mutation identified in MDA-MB-330 and **d** α -catenin nonsense mutation identified in HCC1187. Mutations were identified by PCR amplification and sequencing of genomic DNA (lower electropherograms). The wild-type α -catenin gene sequence is shown for comparison (top electropherograms)

together, 14 out of the 55 breast cancer cell lines from our collection had the rounded cell morphology, with three of these carrying biallelic inactivating mutations in α -catenin and the other 11 carrying biallelic inactivating mutations in *E-cadherin*. Our results thus support α -catenin as a putative new tumor suppressor gene in the E-cadherin tumor suppressor pathway.

Loss of α -catenin protein expression associates with clinical lobular breast cancers

To substantiate our premise that α -catenin operates in the E-cadherin tumor suppressor pathway, we have evaluated α -catenin protein expression in clinical breast cancers.

Therefore, we have constructed a tissue microarray of 139 formalin-fixed paraffin-embedded (FFPE) primary breast cancers that included five pathological subtypes of breast carcinoma. Protein expression analysis by immunohistochemistry revealed absence of α -catenin protein expression in 14 out of 139 tumors (10%) and absence of E-cadherin protein expression in 37 of 139 tumors (27%), with 13 tumors that had lost expression of both proteins (Table 2). As expected, loss of E-cadherin protein expression was particularly pronounced among lobular breast cancers (79% of 33 tumors) and did not exceed 20% of tumors from other pathological subtypes. Most important, loss of α -catenin protein expression was also particularly pronounced among lobular breast cancers (33% of 33 tumors) and did not

Table 2 E-cadherin and α -catenin protein expression among 139 clinical breast cancers

Pathological subtype	No. of tumors	Loss of α -catenin protein expression	Loss of E-cadherin protein expression	Loss of α -catenin and E-cadherin protein expression
Lobular	33	11/33 (33%)	26/33 (79%)	11/33 (33%)
Ductal	48	2/48 (4%)	4/48 (8%)	1/48 (2%)
Mucinous	22	1/22 (4%)	4/22 (18%)	1/22 (5%)
Medullary	22	0/22 (0%)	3/22 (14%)	0/22 (0%)
Tubular	14	0/14 (0%)	0/14 (0%)	0/14 (0%)
Total	139	14/139 (10%)	37/139 (27%)	13/139 (9%)

exceed 5% in the other subtypes (Table 2). Loss of α -catenin protein expression thus was significantly associated with breast cancers of the lobular subtype (χ^2 , $P < 0.001$), supporting our observation that α -catenin may be a tumor suppressor that operates in the E-cadherin pathway.

Discussion

We have identified four human breast cancer cell lines with biallelic inactivating α -catenin mutations among 55 human breast cancer cell lines, suggesting that α -catenin is a new tumor suppressor gene. Similar to the *E-cadherin* mutant breast cancer cell lines, three of the α -catenin mutant cell lines had the rounded cell morphology. All four α -catenin mutant breast cancer cell lines had lost α -catenin protein expression and, concordantly, loss of α -catenin protein expression was most pronounced among clinical breast cancers of lobular pathology. It was therefore quite surprising that we detected only two α -catenin gene mutations among 107 fresh-frozen lobular breast cancers and 12 α -catenin-negative paraffin-embedded breast cancers, both of which were of unclear oncogenic significance (data not shown). The question arises whether we have failed to detect α -catenin mutations in the clinical cancer samples or that we have not analyzed the appropriate sample cohort. In this respect, diffuse-type gastric cancer is a likely candidate, particularly because both somatic and germline *E-cadherin* mutations have been associated specifically with this tumor type [8, 9, 19, 20].

Pending identification of α -catenin mutations in clinical tumor samples, the argument for α -catenin as a new tumor suppressor gene is compelling. The four α -catenin mutant breast cancer cell lines that we identified here all had biallelic mutations that predicted premature termination of the encoded proteins—a classical hallmark of a tumor suppressor gene. But even more convincing was the mutual exclusive occurrence of α -catenin and *E-cadherin* gene mutations in breast cancer cell lines with the rounded cell morphology. Rounded cells with scant cytoplasm is a defining feature of lobular breast cancers as well as diffuse-type gastric cancers. In concordance, α -catenin mutations were shown to be causative for the diminished cell adhesion in α -catenin mutant cell lines [21–24]. Together, these results strongly suggest that α -catenin and *E-cadherin* operate in the same tumor suppressor pathway. Our observation that α -catenin and E-cadherin protein expression was lost predominantly among clinical lobular breast cancers further substantiates the functional relationship between the two proteins. Whatever the mutation detection method, the tumor type or subtype, it seems a matter of time and effort that α -catenin mutations are found in clinical tumors.

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