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Cadherin-Catenin Complex Dissociation in Lobular Neoplasia of the Breast

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Abstract

Background—E-cadherin (E-CD) inactivation with loss of E-CD-mediated cell adhesion is the hallmark of lesions of the lobular phenotype. E-CD is typically absent by immunohistochemistry in both lobular carcinoma in situ (LCIS) and invasive lobular lesions, suggesting it occurs early in the neoplastic process. In laboratory models, downstream post-transcriptional modifiers such as TWIST and SNAIL contribute to the dissociation of the intracellular component of the cadherin-

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Disclosure/Conflict of Interest

The authors report no conflicts of interest. The authors have control of all primary data and agree to allow the journal to review the data if requested.

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catenin complex (CCC), resulting in tumor progression and invasion. We hypothesized that complete CCC dissociation may play a role in lobular neoplasia progression. Here we explore the relationship between loss of E-CD and dissociation of the CCC in pure LCIS and LCIS associated with invasive cancer.

Methods—Fresh-frozen tissues were obtained from 36 patients undergoing mastectomy for pure LCIS (n=11), LCIS with ILC (n=18) or LCIS with IDC (n=7). Individual lesions were subject to laser-capture microdissection and gene-expression analysis (Affymetrix HG-U133A 2.0). Immunohistochemistry for ER,PR,HER2, E-CD,N-CD, α - β -, and phospho β -catenin, TWIST, and SNAIL were evaluated in normal, in situ, and invasive components from matched formalin-fixed paraffin-embedded samples(n=36). CCC-dissociation was defined as negative membranous E-CD, α - and β -catenin expression.

Results—E-CD was negative in all LCIS and ILC lesions, and positive in all normal and IDC lesions. Membranous α and β -catenin expression decreased with the transition from LCIS to ILC (pure LCIS 82%;LCIS w/ILC 28%;ILC 0%), while TWIST expression increased (pure LCIS low;LCIS w/ILC moderate;ILC high). Gene expression paralleled IHC staining patterns with a stepwise downregulation of E-CD, α and β -catenin from normal to LCIS to invasive lesions, and increasing expression of TWIST from normal to LCIS to ILC.

Conclusions—Loss of E-CD expression is an early event in lobular neoplasia. Decreasing membranous catenin expression in tandem with increasing levels of TWIST across the spectrum of lobular lesions suggests CCC dissociation is a progressive process.

Keywords

Lobular carcinoma; E-cadherin; catenin; dissociation; gene expression

Introduction

Since the first description of lobular carcinoma in situ (LCIS) by Foote and Stewart in 1941, confusion has existed regarding its true biologic potential. Epidemiological data supporting its role as a marker of increased risk for the future development of breast cancer rely largely on the finding that patients with LCIS are equally likely to develop both ductal and lobular cancers, and that cancers in the contralateral breast occur at similar rates. However, emerging evidence of genotypic similarities suggesting clonality between coexisting LCIS and invasive lobular breast cancers suggests that a subset of LCIS exhibits true precursor potential [1-4]. Data on the mechanisms that may underlie the development or progression of LCIS to invasive cancer remain limited.

Loss of E-cadherin (E-CD)-mediated cell adhesion is a characteristic finding in lobular breast cancer, reflected in the typical discohesive appearance of the individual neoplastic cells [5]. It is well documented that epithelial tumors lose E-CD partially or completely as they progress toward malignancy, and seminal studies have demonstrated both strong anti-invasive and anti-metastatic roles for E-CD [6, 7]. E-CD mediates cell adhesion by binding cytoplasmic catenins to the membrane to form an adherens junction known as the cadherin-catenin complex (CCC). α -catenin connects E-CD to the actin cytoskeleton to maintain the structure and polarity of the cell, and regulates the interaction between E-CD and β -catenin.

β -catenin binds the C-terminal cytoplasmic domain of E-CD [8, 9]. In normal cells, cytoplasmic levels of β -catenin are typically low due to rapid targeting of excess β -catenin to the proteasome. Recent studies have suggested that phosphorylation of β -catenin results in its translocation to the nucleus where it binds the transcription factor Tcf/Lef, thereby promoting cell proliferation and activation of the Wnt pathway [10]. E-CD also inhibits the ligand activation of other tyrosine kinase receptors, namely EGFR, HER2-neu, IGF-1R, and the HGF receptor c-Met, and also inhibits expression of the pro-migratory/pro-metastatic cadherin molecule N-cadherin (N-CD) [11].

Loss of E-CD-mediated cell adhesion is a complex process which can be partial or complete, occur at any stage of neoplasia, and have varied downstream effects on cell signaling [9]. Mutational inactivation of the E-CD gene leads to loss of function of the *extracellular* component of the CCC in lobular breast cancer and occurs early in the neoplastic process, as it is evident in LCIS [12-16]. Because LCIS is considered to be only a marker of risk for invasive cancer, this suggests that additional alterations are required to potentiate any subsequent invasive process. In laboratory models, post-transcriptional modifiers such as TWIST, SNAIL, and SLUG contribute to the subsequent dissociation of the *intracellular* component of the CCC leading to invasion and metastasis [17-19]. Complete (extracellular and intracellular) dissociation of the CCC facilitates tumor progression, invasion, and migration. Whether disruption of the CCC plays a role in the progression of select LCIS lesions to invasive lobular carcinoma (ILC) remains unclear.

We hypothesized that complete dissociation of the CCC may play a role in the transition of LCIS to invasive cancer, indicating precursor potential of select LCIS lesions. The aim of this study was to explore the relationship between loss of E-CD and dissociation of the CCC in pure LCIS as compared to LCIS associated with concurrent invasive cancer.

Materials and Methods

This study was approved by the Institutional Review Board (IRB) of Memorial Sloan-Kettering Cancer Center (MSKCC), New York, NY. Patients with LCIS with or without concurrent invasive disease undergoing mastectomy were prospectively enrolled in an IRB-approved protocol and fresh frozen tissue blocks were obtained (IRB No. 01-135). Sections from the frozen tissue blocks and patient-matched formalin fixed paraffin embedded blocks were prepared for hematoxylin and eosin (H&E) staining and reviewed to confirm the presence of areas of interest (normal epithelium, LCIS +/- concurrent invasive cancer) and also to determine the quality of morphologic preservation.

Gene Expression Analysis

Using a tissue preparation protocol previously published by our group [20], selective sampling of patient-matched normal epithelial cells, LCIS, and invasive cancer was performed by laser capture micro-dissection of fresh frozen tissue specimens (PALM laser catapult system, Zeiss, Bernried, Germany). High-quality RNA was extracted from microdissected cells, isolated, and purified using standard methodologies (mirVana miRNA Isolation Kit, Ambion Applied Biosystems, Foster City, CA), and subject to gene expression analysis. Samples were labelled and hybridized to Affymetrix HG-U133A 2.0

oligonucleotide arrays and scanned with GeneChip System confocal scanner (Agilent Technologies, Santa Clara, CA). Images were visually inspected and analyzed using Affymetrix's Microarray Suite version 5.0 (MAS 5.0, Affymetrix, Santa Clara, CA) algorithm for quality control.

In this study, we analyzed the gene expression of e-cadherin (CDH1) α -catenin (CTNNA1), β -catenin (CTNNB1), Snail (SNAIL), and Twist (TWIST1). For genes represented by more than 1 probe set, the probe set closest to the 3' end of the mRNA sequence was chosen for analysis. Raw data were imported, converted, and normalized using the RMA method, and the mean expression values among patient-matched samples of normal epithelium, LCIS, and invasive cancer were analyzed using the LIMMA method (R software) [21]. A separate unpaired analysis was carried out between LCIS with and without invasive cancer. A p-value of < 0.05 was considered significant.

Immunohistochemistry (IHC)

Several consecutive sections, 5 μ m thick, were prepared from the paraffin blocks and placed on silanized glass slides. The antibodies, clones, dilutions, pre-treatment conditions, and sources for immunohistochemical studies are listed in Table 1. All cases underwent IHC analysis for estrogen receptor (ER), progesterone receptor (PR), HER2neu, E-CD, α -catenin, and β -catenin. Additional immunohistochemical analysis for N-CD, TWIST, and SNAIL was undertaken for cases that showed evidence of loss of E-CD expression. Positive and negative controls for each antibody were stained in parallel.

Three investigators reviewed the slides (MM, VPA, DG). ER and PR were considered positive if any nuclear staining was present. HER2/neu was scored on a 0 to 3+ scale using ASCO/CAP 2007 recommendations [22]. E-CD, N-CD, and the catenins (α and β) were recorded as positive or negative for membranous staining; myoepithelial cells intermixed with LCIS cells were excluded from this analysis. Complete CCC-dissociation was defined as loss of membranous staining for E-CD, α -catenin, and β -catenin. Phospho- β -catenin immunostaining was recorded based on cellular localization (cytoplasmic/nuclear) and intensity (low/moderate/high). TWIST and SNAIL were individually assessed based on percent of neoplastic cells showing nuclear staining (low: $< 25\%$; moderate: $25\text{--}50\%$; high: $> 50\%$), and collectively by median expression (% positive).

Comparisons were made between pure LCIS cases, LCIS associated with invasive cancer (both ductal and lobular phenotype), and the invasive cancers themselves. Fisher's exact test was used to evaluate the differences in protein expression between groups (significance level=0.05).

Results

Fifty-six patients were accrued for this study. Twelve patients underwent mastectomy in the setting of pure LCIS, 32 patients had LCIS with concurrent ILC, and 9 patients had LCIS with concurrent infiltrating ductal carcinoma (IDC)/ductal carcinoma in situ (DCIS). Fresh frozen tissue samples were suitable for gene expression analysis for 5 (42%) patients with pure LCIS, 23 (72%) patients with LCIS and concurrent ILC, and 7 (78%) patients with

LCIS and concurrent IDC/DCIS. Archival FFPE tissue blocks were available for all IHC analyses in 11 (92%) patients with pure LCIS, 18 (56%) patients with LCIS and concurrent ILC, and 7 (78%) patients with LCIS and concurrent IDC. All LCIS lesions were of the “classic” histological phenotype. The median patient age at the time of surgery was 50 years (range,37-79); there was no statistically significant difference in median age between patients with pure LCIS (45.5yrs) compared to LCIS with invasive (52 years) or between cases with frozen samples (49.5yrs) or FFPE samples (50yrs).

Gene expression analysis

Among 23 patient-matched samples of normal epithelium, LCIS and ILC, E-CD ($p<0.001$), α -catenin ($p=0.027$) and β -catenin ($p=0.013$) were significantly downregulated in ILC compared to normal epithelium, while LCIS showed intermediate levels of expression for those same genes. E-CD expression was downregulated from normal to LCIS ($p=0.005$) and further downregulated from LCIS to ILC ($p<0.001$). Significant differences in β -catenin gene expression were observed between ILC and normal ($p=0.044$), but not between LCIS and normal epithelium or LCIS and ILC. α -catenin expression demonstrated the same pattern as β -catenin (normal vs ILC, $p=0.045$). Twist was overexpressed in ILC compared to normal and LCIS ($p=0.027$). Snail showed no difference in expression among different cell types in ILC triplicates (Table 2A).

Among 7 patient-matched normal-LCIS-IDC/DCIS triplicates, we did not observe any significant differences in expression of this gene set. However, mean expression levels of E-CD, α -catenin and β -catenin were higher in IDC than ILC, and mean expression levels of SNAIL and TWIST were lower in IDC than ILC. These differences observed between ILC and IDC lesions demonstrate that the disruption of the CCC does not play a major role in the progression of lesions with ductal phenotype. The mean transcript abundances for IDC triplicates are reported in Table 2B.

Box plots for CDH1, α -catenin, and β -catenin, and TWIST and SNAIL mRNA expression levels across the spectrum of lobular and ductal neoplasias are shown in Figure 1.

We did not observe any significant difference in gene expression between samples from patients with pure LCIS as compared to samples from patients with LCIS and concurrent invasive cancer (Table 2C).

Immunohistochemistry

All 11 pure LCIS lesions, 18 LCIS with ILC lesions, and 18 ILC lesions were ER-positive and PR-positive compared to 4/7 (57%) IDC/DCIS lesions. HER2 staining intensity varied across all lesions, ranging from 0 to 2+. FISH was not performed (Table 3).

Cadherin-Catenin Complex

Membranous E-CD expression was absent in all in situ and invasive lobular lesions, confirming dissociation of the extracellular component of the CCC, whereas all normal breast epithelium and all IDC lesions were E-CD positive. Complete loss of membranous α -catenin staining occurred in only 2/11 (18%) cases of pure LCIS, in 13/18 (72%) cases of

LCIS associated with ILC, and in 4/7 (57%) cases of LCIS associated with IDC. In contrast, complete loss of membranous α -catenin was observed in all 18 ILCs (100%) and in none of the IDCs (0%) ($p<0.001$; Figure 2A and Figures 3A, 3C, 3E).

Patterns of β -catenin staining were similar to those observed for α -catenin. Complete loss of β -catenin was observed in 5/11 (46%) cases of pure LCIS, in 15/18 (89%) cases of LCIS associated with concurrent ILC, and in 6/7 (86%) cases of LCIS with IDC. With respect to the invasive lesions, complete loss of β -catenin was observed in 16/18 ILCs (89%) and in none of the IDCs (0%) ($p<0.001$; Figure 2B and Figures 3B, 3D, 3F).

Collectively, these observations demonstrate that all normal epithelial cells and IDCs in this series have an intact CCC as measured by the presence of strong E-CD, α -catenin, and β -catenin protein expression, whereas the degree of CCC dissociation varied across the different sets of lobular neoplasia. Specifically, in the absence of concurrent invasive disease, only 2/11 (18%) LCIS lesions demonstrated complete dissociation of the CCC compared to 11/18 (61%) cases of LCIS with ILC and 16/18 (89%) ILCs ($p<0.001$; Figure 2C).

Changes associated with CCC dissociation

To measure downstream effects of either extracellular or complete intracellular and extracellular dissociation of the CCC, we performed IHC for phospho- β -catenin, N-CD, and the transcription regulators TWIST and SNAIL. None of the normal epithelial cells, or neoplastic lobular or ductal cells in this series, showed positive immunoreactivity for N-CD.

TWIST and SNAIL nuclear staining were both frequently observed across the spectrum of lobular lesions. Cytoplasmic staining was also present, but our analysis focused on nuclear staining as this is the accepted cellular location of the transcriptional modulators. We observed an inverse correlation between TWIST and E-CD/catenin expression. Median TWIST expression increased with the transition from LCIS (low) to ILC (high), and high nuclear expression of TWIST was associated with complete dissociation of the CCC ($p=0.031$) (Figure 4A-B and Figure 6A-C). SNAIL expression was diffuse and intense in the majority of the LCIS and ILC lesions, and not significantly different between the 3 groups ($p=0.206$; Figure 5 and Figure 6D). There was no observed pattern of differential SNAIL expression between lesions with respect to CCC dissociation.

Cytoplasmic phospho- β -catenin expression was also seen in all lobular lesions independent of CCC status. The median cytoplasmic phospho- β -catenin expression was greater in ILC (high) compared to LCIS with invasive disease (moderate) or pure LCIS (low) (Figures 6E, 6F). Nuclear phospho- β -catenin expression was seen in the majority of lobular lesions. Median expression of nuclear phospho- β -catenin was greater in ILC (moderate) compared to LCIS with invasive disease (low) or pure LCIS (low).

Discussion

Despite sharing many morphologic and molecular features with ILC, LCIS has been historically considered a risk marker, but not a true precursor, of invasive breast cancer [4].

In this study, we investigated the expression of E-CD and other molecules involved in the maintenance of the CCC at the level of both mRNA and protein expression in pure LCIS and LCIS associated with invasive cancer. To accomplish this, we prospectively harvested fresh frozen samples from patients with LCIS undergoing mastectomy and optimized a protocol for preservation of high-quality RNA during laser-capture microdissection (LCM) of these very small lesions [20]. To our knowledge, this is the first report combining IHC and gene expression profiles obtained from fresh frozen lesions across the spectrum of lobular neoplasia. Our data demonstrate that complete dissociation of the CCC at the cell membrane is limited to lesions of the lobular phenotype and is a progressive process from LCIS to ILC.

The importance of understanding the molecular underpinnings in the progression of lobular neoplasia is highlighted by emerging evidence that some LCIS may in fact share clonality with associated ILC lesions, suggesting a true precursor role [1-4]. Some authors further advocate a low-grade pathway of breast carcinogenesis where low-grade in situ lesions, including LCIS, serve as a precursor of low-grade invasive cancer of either the lobular or ductal phenotype [23-26]. Comparing standard molecular markers such as ER, PR, and HER2 of associated lesions provides a simple way to evaluate their potential relatedness, yet these similarities do not provide sufficient evidence to support or refute a role of LCIS as a precursor lesion.

At the mRNA level, we observed a significant and monotonically downregulated mean expression of E-CD from normal epithelium to LCIS to ILC. It is interesting to note that although the mean levels of expression are different, the wide range of E-CD expression in LCIS—and notably ILC—overlaps with the range of expression in the normal epithelium. This phenomenon is not appreciated on IHC, where all LCIS and ILC lesions demonstrated complete loss of E-CD staining, implying that post-translational mechanisms play an important role in the regulation of E-CD protein expression. In addition to E-CD loss, we observed a stepwise increase in the frequency of complete loss of membranous α and β -catenin protein expression across cases from pure LCIS to LCIS with concurrent invasive cancer to ILC, demonstrating that complete dissociation of the CCC is a progressive process in lobular neoplasia. This observation is supported at the mRNA level, where the greatest difference in expression of E-CD, α -catenin, and β -catenin was observed in the transition between LCIS and ILC. Although we did not investigate mechanisms of E-CD regulation in this study, our observations demonstrate that E-CD is transcribed in lobular lesions, both in situ and invasive, and then subject to further regulation at different stages of lobular neoplasia. Comparisons between ILC and IDC demonstrated differences in Wnt pathway activation at both gene and protein expression levels, not only in E-CD and catenin levels, but also downstream as observed in TWIST expression.

According to genomic models of breast carcinogenesis, LOH at 16q is a common finding among low-grade lesions of both the lobular and ductal phenotype, and further CDH1 inactivation in the retained allele is unique to lobular carcinomas.[24, 27] Mutations, deletions, promoter hypermethylation, transcriptional repression and, more recently, miRNA modulation, have all been proposed as potential mechanisms of E-CD inactivation [9, 13, 14, 28-33]. Sarrio et al [34] described complete loss of E-CD staining in 39/51 (76%) ILC

and matched LCIS cases, abnormal or reduced staining in 7/51 (14%) cases, and retained E-CD membranous staining in 5/51 (10%) of samples. In the same series, a CDH1 mutation was identified in 10 (22%) of ILCs; loss of heterozygosity (LOH) was observed in 30/41 (73%) ILCs, and CDH1 promoter hypermethylation was identified in 19/46 (41%) cases. CDH1 promoter hypermethylation was also identified in 9 of 30 cases with LOH. LOH was strongly associated with negative staining of E-CD, but this relationship was not present in all cases.

Vesuna et al demonstrated that breast cancer cell lines and human breast tumors with lower expression levels of E-CD showed higher levels of TWIST. The inverse correlation was significant in cell lines and showed a trend in frozen samples from breast tumors of different histologic grades, where grade III tumors exhibited a 2.9-fold increase in TWIST expression and a 1.2-fold decrease in E-CD expression compared to grade I tumors [32]. Although all invasive lesions in our series were classified as grade I, TWIST was measurable by IHC and gene expression. Median nuclear TWIST expression increased with the transition from LCIS (low) to ILC (high). Furthermore, high nuclear expression of TWIST was associated with complete extracellular and intracellular dissociation of the CCC. At the mRNA level, we also observed an inverse correlation between E-CD and TWIST in ILCs (lowest E-CD level and highest TWIST level among all groups).

In contrast, although laboratory models support the role of SNAIL in dissociation of the intracellular component of the CCC, in our study, SNAIL expression was diffusely high in all groups, with no statistical difference observed either by IHC or gene expression and no correlation with dissociation of the CCC. SNAIL is a zinc finger transcription factor that triggers the epithelial-mesenchymal transition (EMT) by directly repressing E-CD expression and inducing an invasive and migratory phenotype. Increased SNAIL expression has been associated with poorly differentiated tumors, lymph node metastases, distant metastases, and decreased disease-free and overall survival [18, 35]. Further work is needed to determine whether SNAIL contributes to CCC dissociation in lobular neoplasia.

We acknowledge that not having a perfect match between patients with frozen and paraffin samples is a limitation of our study, that despite optimization of protocols, some cases displayed either small lesions, poor-quality or low RNA yield, and that in some cases, archival blocks were not available for study. As LCIS is classically an incidental microscopic finding in breast tissue, all prior reports have utilized FFPE tissues for analysis and, as such, our study using both fresh frozen samples and matched paraffin lesions when possible is a unique contribution to the literature on this topic. Our study also differs from others in that we used strict criteria, defined as complete absence of staining, for loss of E-CD expression by IHC. Others have considered any reduction in expression as sufficient for E-CD inactivation or have combined cases with absent or reduced staining in the same group [28, 34, 36-40]. Such differences in criteria may complicate comparisons across reported series.

Strict morphologic review of all IHC staining also demonstrated that in some cases, LCIS cells were intermixed with myoepithelial cells in varied proportions. Myoepithelial cells can be selectively ignored when scoring cases by IHC; however, complete elimination of

myoepithelial cells from LCM samples is not always feasible. Thus the gene expression presented here is representative of the lobular microenvironment, and we estimate that the samples are enriched by at least 80% of neoplastic cells, with many having higher levels of purity. Other cells located outside the lumen, such as normal fibroblasts, fat cells, and inflammatory cells, are selectively excluded by the LCM process. A previous study reported no statistical differences in E-CD, α -catenin, and β -catenin mRNA levels as evaluated by qPCR in frozen samples of normal epithelium, ILC, and IDC [41], but the investigators did not use a purified cell population. Our study is the first to investigate the genetic profile of a reasonable number of fresh frozen LCIS lesions, and we demonstrated that the gene and protein expression of E-CD, β -catenin, α -catenin, and other selected downstream elements of the Wnt pathway are concordant overall. At the level of gene expression, we were not able to distinguish significant differences between LCIS with and without concurrent invasive cancer. However, this may also be an effect of small sample size in the group of LCIS without invasive disease.

In summary, we have shown that complete disruption of the CCC at the cell membrane occurs as a stepwise process across lesions with the lobular phenotype. Disruption of the CCC is fully developed in most cases of ILC and is not present in IDCs. This observation is supported at the mRNA level, where the greatest difference in expression of E-CD, α -catenin, and β -catenin was observed in the transition between LCIS and ILC. Additional efforts, such as use of whole genome arrays to examine clonality of LCIS and associated invasive lesions are needed to improve our understanding of lobular carcinogenesis and the precursor potential of LCIS.

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Figure 1. mRNA expression (Log2 scale) for normal epithelium and individual lesions in the spectrum of lobular and ductal neoplasia: (A, CDH1 [E-cadherin]); (B, CTNNB1 [β -catenin]); (C: CTNNA1 [α -catenin]), (D, Twist1 [Twist]); (E: Snai1 [Snail]).

Figure 2.

(A) Number of cases showing loss of membranous α -catenin staining according to type of lobular lesion and IDCs ($p < 0.001$). (B) Number of cases showing loss of membranous β -catenin staining according to type of lobular lesion subtype and IDCs ($p < 0.001$). (C) Number of cases showing complete dissociation of the cadherin-catenin complex at the cell membrane ($p < 0.001$).

Figure 3.

Lack of membranous α -catenin (A, 100x) and β -catenin (B, 100x) immunoreactivity on LCIS cells. Residual non-neoplastic cells in lobules and ducts provide positive internal control. Positive membranous immunoreactivity for α -catenin (diffuse) (C, 200x) and β -catenin (focal) (D, 200x) in a case of pure LCIS. ILC cells showing similar patterns of immunoreactivity for α -catenin (diffuse) (E, 200x) and β -catenin (focal) (F, 200x).

Figure 4.

(A) Percentage of neoplastic cells with nuclear staining for TWIST across lobular neoplasia subtypes ($p < 0.001$). (B) Relationship between the percentage of neoplastic cells with nuclear staining for TWIST and Cadherin-Catenin Complex dissociation across lobular neoplasia subtypes ($p = 0.031$).

Figure 5.
SNAIL staining across the lobular neoplasia subtypes (p=0.206).

Figure 6.

TWIST nuclear staining is low in pure LCIS (A, 400x), moderate in LCIS associated with invasive (B, 100x) and high in ILC (C, 200x). High nuclear and cytoplasmic staining for SNAIL is present in ILC (D, 200x). Nuclear Phospho- β -catenin staining is present in LCIS, both in the nucleus and cytoplasm. (E, 200x; F, 400x).

Table 1

Antibodies, clones, dilutions, pre-treatment conditions, and sources for immunohistochemical studies.

Antibody	Origin	Clone	Dilution	Incubation	Retrieval	Detection	Staining location	Scoring
ER	Dako	1D5	1:100	30mn	EDTA pH9	Horse radish peroxidase polymer	Nuclear	Any positivity
PR	Dako	PgR636	1:100	30mn	Citrate pH6.0	Horse radish peroxidase polymer	Nuclear	Any positivity
HER2	Dako	Rabbit	as per kit	30mn	EDTA pH9	Horse radish peroxidase polymer	Membranous	Herceptest
ECAD	Dako	NCH-38	1:50	30mn	Citrate pH6.0	Horse radish peroxidase polymer	Membranous	Any positivity
NCAD	Dako	6G11	1:25	30mn	Citrate pH6.0	Horse radish peroxidase polymer	Membranous	Any positivity
αCatenin	BD	5/a-catenin	1:25	45mn	Citrate pH6.0	Biotin-Streptavidin-HRP	Membranous	Any positivity
βCatenin	Cell Marque	14	1:2	45mn	EDTA pH8	Horse radish peroxidase polymer	Membranous	Any positivity
PhosphoβCatenin	Abcam	Rabbit poly for S33 / S37	1:40	120mn	Citrate pH6.0	Horse radish peroxidase polymer	Nuclear and Cytoplasmatic	Any positivity
Twist	Abcam	Rabbit poly	1:100	60mn	Citrate pH6.0	Horse radish peroxidase polymer	Nuclear	Low = <25% Mod = 26-50% High = 50%+
Snail	Abcam	Rabbit poly	1:100	60mn	Citrate pH6.0	Horse radish peroxidase polymer	Nuclear and Cytoplasmatic	Low = <25% Mod = 26-50% High = 50%+

Table 2A

Comparison of mean mRNA expression levels in patient-matched normal, LCIS and ILC samples for the cadherin-catenin complex molecules and related transcription regulators.

Probe #	Gene Symbol	Gene Name	NL	LCIS	ILC	Overall p-value	LCIS-NL p-value	ILC-NL p-value	ILC-LCIS p-value
201131_s_at	CDH1	cadherin 1, type 1, E-cadherin (epithelial)	11.37	10.91	9.54	<0.001	0.005	<0.001	<0.001
210844_x_at	CTNNA1	catenin (cadherin-associated protein), alpha 1, 102kDa	10.26	10.16	10.05	0.037	0.275	0.045	0.192
201533_at	CTNNA1	catenin (cadherin-associated protein), beta 1, 88kDa	7.92	7.75	7.72	0.013	0.087	0.044	0.816
219480_at	SNAI1	snail homolog 1 (Drosophila)	4.34	4.34	4.40	0.870	0.967	0.552	0.475
213943_at	TWIST1	twist homolog 1 (Drosophila)	7.58	7.46	8.15	0.027	0.503	0.030	0.002

Table 2B

Comparison of mean mRNA expression levels in patient-matched normal, LCIS and IDC samples for the cadherin-catenin complex molecules and related transcription regulators.

Probe #	Gene Symbol	Gene Name	NL	LCIS	IDC	Overall p-value	LCIS-NL p-value	IDC-NL p-value	IDC-LCIS p-value
201131_s_at	CDH1	cadherin 1, type 1, E-cadherin (epithelial)	10.88	11.21	10.35	0.135	0.497	0.617	0.387
210844_x_at	CTNNA1	catenin (cadherin-associated protein), alpha 1, 102kDa	10.32	10.28	10.15	0.334	0.892	0.532	0.360
201533_at	CTNNA1	catenin (cadherin-associated protein), beta 1, 88kDa	8.31	8.09	8.18	0.413	0.136	0.389	0.555
219480_at	SNAI1	snail homolog 1 (Drosophila)	4.22	4.21	4.24	0.091	0.925	0.927	0.826
213943_at	TWIST1	twist homolog 1 (Drosophila)	7.50	7.02	7.58	0.359	0.077	0.790	0.162

Table 2C

Comparison of mean mRNA expression levels between LCIS with and without concurrent invasive disease for the cadherin-catenin complex molecules and related transcription regulators.

Probe #	Gene Symbol	Gene Name	LCIS only	LCIS with invasive	p-value
201131_s_at	CDH1	cadherin 1, type 1, E-cadherin (epithelial)	11.34	11.01	0.466
210844_x_at	CTNNA1	catenin (cadherin-associated protein), alpha 1, 102kDa	10.07	10.08	0.972
201533_at	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	8.13	8.15	0.896
219480_at	SNAI1	snail homolog 1 (Drosophila)	4.22	4.33	0.369
213943_at	TWIST1	twist homolog 1 (Drosophila)	7.27	7.66	0.356

Table 3

Estrogen receptor (ER), progesterone receptor (PR), and HER2 immunohistochemical status for individual lesions.

	Pure LCIS N=11	LCIS w/ ILC N=18	ILC N=18	LCIS w/IDC N=7	IDC N=7
ER					
Pos	11 (100%)	18 (100%)	18 (100%)	5 (71%)	4 (57%)
Neg	0	0	0	2 (29%)	3 (43%)
PR					
Pos	11 (100%)	18 (100%)	18 (100%)	6 (86%)	5 (71%)
Neg	0	0	0	1 (14%)	2 (29%)
HER2neu					
Neg (0/1+)	11 (100%)	15 (83%)	17 (94%)	7 (100%)	7 (100%)
++	0	3 (17%)	1 (6%)	0	0
+++	0	0	0	0	0
E-Cadherin					
Neg	11 (100%)	18 (100%)	18 (100%)	7 (100%)	0
Pos	0	0	0	0	7 (100%)

LCIS, lobular carcinoma in situ; ILC, invasive lobular carcinoma; IDC, invasive ductal carcinoma. FISH not performed.