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Simultaneous bilateral breast carcinoma: Histopathological characteristics and CD44/catenin-cadherin profile

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Summary. Aims: Family history of breast carcinoma, multicentric tumor foci in one breast, and in situ lobular carcinoma increase the risk of bilateral breast cancer (BBC), synchronous or metachronous. Synchronous tumors are designated as simultaneous breast carcinoma if they appear at the same time. The CD44 family and cadherin/catenin immunophenotype of this group of BBCs has not yet been evaluated. The aim of this study was to compare clinicopathological characteristics and immunohistochemical profiles of simultaneous BBC and corresponding lymph node metastases in eight patients. Methods and results: In toto 15 primary and 9 metastatic tumors were evaluated. The expression of CD44 variant isoforms, ß-catenin, E, P and N-cadherin were evaluated by immunohistochemistry. Rare types of breast carcinoma were frequent in this group of patients. There were 6 pleomorphic lobular, 5 invasive ductal of usual type, 3 atypical medullary carcinomas, 2 mucinous and one invasive micropapillary carcinoma. The expression CD44v6 was most frequent, followed by CD44v3-10, CD44v5, and CD44v3. CD44v4 was generally not expressed. E-cadherin was expressed in 80% primary tumors, 40% expressed N-cadherin, and 66% expressed P-cadherin. Conclusions: Generally, simultaneous carcinomas had different morphology and different immunophenotype. Each primary tumor was more similar to its corresponding metastatic tumor than to the contralateral primary tumor.

Key words: Simultaneous bilateral breast cancer, Immunohistochemical profile, CD44, Cadherin, Catenin

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Introduction

Women with a strong family history of breast cancer, with multicentric tumor foci in one breast, and with in situ lobular carcinoma have a high incidence of bilateral breast cancer, synchronous or metachronous (Heron et al., 2000). Synchronous are tumors detected in each breast simultaneously (i.e. in the same period) or within 6 months (Heron et al., 2000; Jobsen et al., 2003; Intra et al., 2004), but the time interval ranges from 1 to 12 months (Kelmendi de Ustaran and Meiss, 1988; Coradini et al., 1998). Metachronous tumors occur at different times. No previous studies evaluated simultaneous breast carcinoma separate from other synchronous carcinoma. Bilateral breast carcinomas are much more frequent in *BRCA1/2*, *Pten* and *ATm* families than in the general population (Olsson, 2001).

One challenge in the field of bilateral breast cancer is whether the second tumor is a new primary or a metastasis from the first carcinoma. Morphological criteria, such as histological type, grading, presence of an in situ component, and steroid receptor status are the most commonly explored factors (Dawson et al., 1991; Coradini et al., 1998; Intra et al., 2004). The histological type of breast cancer is the same on both sides in 57% to 68% (Sterns and Fletcher, 1991; Gogas et al., 1993; Heron et al., 2000). In some reports a prevalence of lobular infiltrating breast carcinomas among bilateral cases is observed (Kiang et al., 1980).

Deranged expression and/or function of adhesion molecules have been implicated in malignant tumor development and progression. Changes in E-cadherin, ß-catenin and CD44 expression occur early in breast carcinogenesis and these adhesion molecules are involved in tumor differentiation (Bankfalvi et al., 1999). CD44 is one of the main cell surface receptors for extracellular matrix components and is increased in many human cancers. In a recent report Al-Hajj et al. suggested that CD44+CD24-/low lineage cells exhibit properties of cancer stem cells (Al-Hajj et al., 2003).

Contrarily, loss of intercellular adhesion molecules, such as cadherins and catenins were reported in conjunction with increased tumor growth and metastatic potential (Jiang and Mansel, 2000).

The aim of the present study was to explore the phenotypic characteristic of simultaneous bilateral breast cancer. We show that this group of patients has very high incidence of pleomorphic lobular carcinomas as well as other rare types of breast carcinoma and that the primary tumors have different morphology and CD44 family and cadherin/catenin family immunophenotype.

Materials and methods

Patients

Eight patients with simultaneous bilateral breast cancer were included in the study. The cases were selected from the archives of the Department of Pathology, Alexander University Hospital, Sofia, Bulgaria and Department of Pathology, The Norwegian Radium Hospital, Oslo, Norway for a 30-year period (between 1970 and 2000). Clinical and pathological reports and slides were available for all cases. Paraffin tissue blocks were also available for all cases with an exception of the left-sided breast tumor in case 8.

Histological examination

The tumors were classified in accordance with World Health Organization (WHO) recommendation (WHO, 1982). All tumors were graded using the method of Elston and Ellis (Elston and Ellis, 1993).

Immunohistochemistry

Immunostaining for anti-panCD44 and variant isoforms, E-cadherin, P-cadherin, N-cadherin and ß-catenin were performed on primary tumors and corresponding lymph node metastases. The antibodies and conditions of immunostaining are listed in Table 1. Four-micron, formalin-fixed, paraffin-embedded tissue sections were cut and mounted on super frost/plus slides and air-dried for 24 hours at 37°C. Sections were dewaxed in xylene and rehydrated in graded series of alcohol to distilled water. Subsequently, antigen retrieval by heating in microwave oven in either 10 mM citrate buffer (pH 6,0) or EDTA buffer (pH 8,0) at highest power (800 W) was performed. B-SA Super Sensitive Multilink Immunodetection System was used for detection (BioGenex Laboratories, San Ramon, CA). The antigen-antibody-enzyme complex was visualized by diaminobenzidin (DAB). Sections were counterstained with haematoxylin. Appropriate negative and positive controls were used in each staining run.

The immunohistochemical reaction was evaluated semi-quantitatively. Only membranous reactivity was recorded for CD44 and cadherin/catenin family. Less than 5% positive cells were recorded as negative. An intense brown staining in 5-30% tumor cells was considered weakly positive (1+), in 31-50% tumor cells was considered moderately positive (2+), and in more than 50% of tumor cells as strongly positive (3+).

Statistical analysis

Wilcoxon Signed Ranks Test, crosstables and chi square test or linear-by-linear association tests were used for statistical analyses of the differences between left and right tumor and each primary tumor and corresponding lymph node metastasis.

Results

The mean age at presentation was 47 years (range 32-71 years). Half of the patients were younger than 40. Four of the patients developed generalized metastatic disease after only 12 months (mean interval 14 months) and after an average of 24 months these patients died of widespread disease. Only patient 7 had long-term survival being alive and without disease 31 years after diagnosis of simultaneous breast carcinoma.

Histopathology

Six patients had pleomorphic (G2 or G3) invasive

Table 1. Antibody list.

ANTIBODY (CLONE)	SOURCE	DILUTION	HIER
Pan-CD44 (DF 1485)	Dako, Glostrup, Denmark	1:100	A
CD44v3 (VFF-327v3)	Bender Medsystems	1:400	A
CD44v4 (VFF-11)	Novocastra, Newcastle upon Tyne, UK	1:100	A
CD44v5 (VFF-8)	Novocastra, Newcastle upon Tyne, UK	1:50	A
CD44v6 (VFF-18)	Novocastra, Newcastle upon Tyne, UK	1:50	B
CD44v3-v10 (polyclonal)	Bender Medsystems	1:400	B
E-cadherin (HECD-1)	Zymed, South San Francisco, CA	1:1000	D
P-cadherin (56)	Transduction Laboratories, San Diego, CA	1:200	C
N-cadherin (3B9)	Zymed, South San Francisco, CA	1:1	C
ß-catenin (14)	Transduction Laboratories, San Diego, CA	1:4000	D

HIER: Heat-induced epitope retrieval; A: 4 x 5 min citric buffer pH 6; B: 2 x 5 min citric buffer pH 6; C: 4 x 5 min EDTA pH 8; D: 2 x 5 min EDTA pH 8.

lobular carcinoma in one breast (Table 2, Fig. 1). However, in these six patients the contralateral carcinoma was never of the same type; one had atypical medullary carcinoma and the remaining 5 had invasive ductal carcinoma. Six ductal carcinomas were of the usual type, one was micropapillary invasive ductal carcinoma, and one was mucinous. Patient 5 had invasive ductal carcinoma in both breasts and patient 7 had atypical medullary carcinoma in both breasts. An in situ component was detected in 7 of the 16 tumors, but only in 4 carcinomas extensive in situ component (more that 50% of tumor volume) was observed. In three cases it was found only unilateraly (case 1 – left breast – LCIS, case 4 and 8 – left and right breast – DCIS). In case 3 in both breasts DCIS and LCIS were diagnosed and in case 5 both ductal carcinomas had extensive in situ component.

Only patients 3 and 6 did not have axillary lymph node metastases from the right primary tumor, while the contralateral tumor had local metastatic spread.

Immunophenotype

The results of immunohistochemical analysis are shown in Table 3. Similar to their differences in histopathology, primary bilateral tumors were not similar for CD44 family or cadherin/catenin family expression. However, similarities were found between primary and their metastatic tumors as described below. In addition, when all tumors were analyzed separately, a number of positive associations was found for the expression of CD44 family, as well as cadherin/catenin family.

CD44 family

CD44 was expressed in 10/15 (66%) of the primary tumors. There was a positive linear association between primary and metastatic tumors (p=0.01). CD44v6 exon product expression was found in all primary tumors. Similar to CD44 expression, there was a positive linear association between primary and metastatic tumors (p=0.01). The tumors with strong expression of CD44v3 exon product did not have metastases (p=0.03, Chi-square test). CD44v4 exon product was negative in all except one primary tumor and one lymph node metastasis, where the level of expression was weak.

As expected, strong linear association was observed between the levels of expression of CD44 and CD44v6 ($p<0.0001$), CD44v3 (p=0.001), and CD44v3-v10 (p=0.002), as well as between CD44v3-10 and CD44v3 (p=0.003), CD44v6 (p=0.001) exon products. A weak trend for positive linear association was found for CD44v3-10 and CD44v5 (p=0.09) exon products.

Cadherin-catenin family

Results are summarized in Table 4 and Fig. 1. Of primary tumors, 80% expressed E-cadherin, 67% expressed P-cadherin, 40% expressed N-cadherin, and 87% expressed b-catenin. All of the invasive ductal carcinomas showed strong expression of E-cadherin, while those pleomorphic lobular and atypical medullary carcinomas, which were positive, generally had weak expression of E-cadherin.

A positive linear association was found between primary and metastatic tumors for expression of P-cadherin (p=0.01) and ß-catenin (p=0.02) respectively. A strong linear association was also found for expression of E-cadherin and ß-catenin (p=0.008), and P-cadherin and N-cadherin (p=0.02).

In addition, a positive linear association was found for expression of ß-catenin and CD44 (p=0.01) and CD44v3 (p=0.001) exon product.

Discussion

Synchronous breast carcinomas were previously studied by several authors (Dawson et al, 1991; Sterns

Table 2. Clinicopathological data.

PATIENT	AGE	LEFT BREAST	AXILLARY LNs (positive/total)	pTNM	RIGHT BREAST	AXILLARY LNs (positive/total)	pTNM	FOLLOW UP
1	56	IDC, G2	26/27	PT3N3	PLC, G3	18/22	PT2N1	43 months, alive
2	70	MUC, G2	9/14	PT2N2	PLC, G3	24/25	PT1bN2	1 yr: DOD
3	45	IDC, G3	5/17	PT2N1	PLC, G2	0/14	PT2N0	32 months, alive
4	71	PLC, G2	1/7	PT1N1biii	IDC, G3	inadequate	PT1aNx	25 months, alive
5	32	IDC, G3	5/9	PT4N1	IDC, G3	2/10	PT1bN2	1 yr: Bone metastases 3 yr: DOD
6	36	PLC, G2	3/14	T1bN1	AMC	0/17	PT3N0	19 mo: Bone metastases 2 yr: DOD
7	34	AMC	1/16	PT1N1	AMC	inadequate	PT2Nx	31 years, alive
8	33	PLC, G2	not performed	PTxNx	IDC, G3	not performed	PTxNx	1 yr: DOD

LN: Lymph node; IDC: invasive ductal carcinoma; PLC: pleomorphic lobular carcinoma; MUC: Mucinous carcinoma; AMC: atypical medullary carcinoma; G2: grade 2; G3: grade 3; DOD: dead of disease.

and Fletcher, 1991; Hungness et al., 2000).

However, no previous studies investigate phenotype of the simultaneous breast carcinomas as a separate group. A great predominance of pleomorphic lobular carcinomas was found in our patients with simultaneous breast carcinoma. Interestingly, in all of these patients, the contralateral tumor was never of the same histologic type. Similarly, the immunophenotype in relation to CD44 family and cadherin/catenin family expression was also dissimilar. In recently published studies the

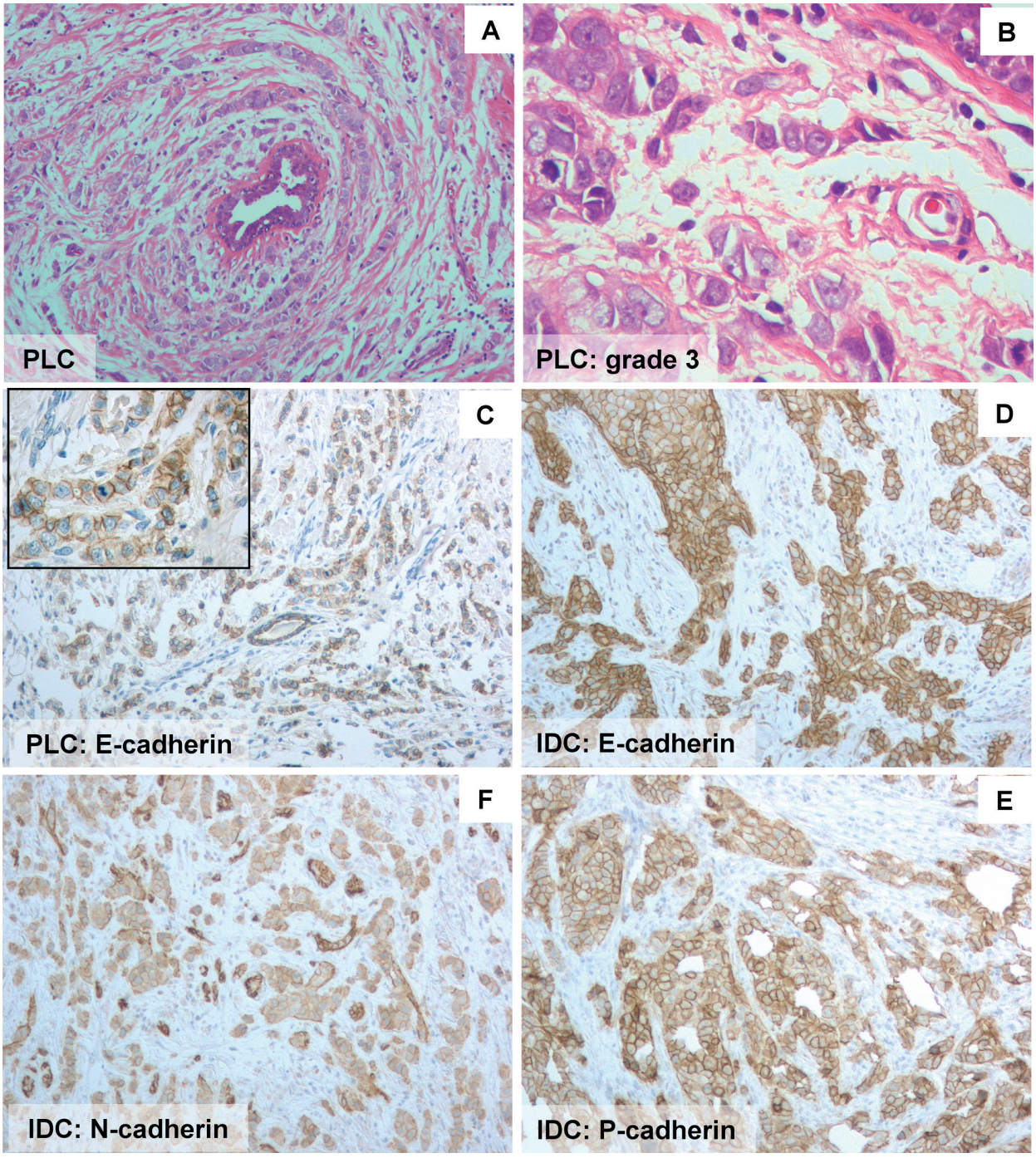

Fig. 1. A typical morphology of pleomorphic invasive lobular carcinoma (**A**, x 10 and **B**, x 40) with weak expression of E-cadherin (**C**, x 10, inset, x 40) in contrast to invasive ductal carcinoma strongly expressing E-cadherin (**D**, x 10), N-cadherin (**E**, x 10) and P-cadherin (F, x 10) (B-SA).

genetic profile of contralateral tumor was also different, which ruled out clonal relationship, i.e. metastasis to a contralateral breast (Regitnig et al., 2004; Teixeira et al., 2004).

More similarities were found between the primary and their corresponding metastatic tumors than between the two primary tumors. Specifically, CD44, CD44v6, P-cadherin, and ß-catenin were similarly expressed in primary and their corresponding metastatic tumors. Even in the two patients with the same bilateral histology (patient 5 with grade 3 invasive ductal carcinoma and patient 7 with atypical medullary carcinoma) the immunophenotype favored two independent primary tumors, rather than metastasis to a contralateral breast.

Only one patient was a long-term survivor (patient 7). This patient was 34-years old at the time of diagnosis and had bilateral atypical medullary breast carcinoma. Even though the family history was negative to the best of our knowledge, the age of the patient and the morphology of the tumors suggest BRCA-1 related

Table 3. Immunohistochemical profile of the primary tumors and corresponding metastases.

PATIENT	LOCATION	CD44	CD44v3	CD44v4	CD44v5	CD44v6	CD44v3+10	m Ecad	m Pcad	m Ncad	m ,cat
1	Left breast	2+	1+	negative	3+	3+	1+	1+	1+	negative	negative
	Lymph node metastasis	2+	1+	negative	2+	3+	2+	negative	negative	negative	1+
	Right breast	negative	negative	negative	2+	1+	negative	3+	2+	negative	3+
	Lymph node metastasis	negative	negative	negative	negative	1+	negative	2+	1+	negative	3+
2	Left breast	negative	negative	negative	1+	2+	negative	3+	1+	negative	3+
	Lymph node metastasis	1+	negative	negative	negative	3+	1+	3+	1+	negative	3+
	Right breast	1+	negative	negative	negative	2+	negative	1+	negative	1+	2+
	Lymph node metastasis	2+	1+	negative	negative	3+	1+	3+	1+	negative	3+
3	Left breast	2+	1+	negative	1+	3+	2+	3+	3+	3+	3+
	Lymph node metastasis	3+	negative	negative	negative	3+	1+	3+	3+	2+	3+
	Right breast	3+	3+	negative	negative	3+	2+	1+	1+	negative	1+
4	Left breast	1+	1+	negative	negative	3+	1+	3+	negative	negative	3+
	Lymph node metastasis										
	Right breast	3+	2+	negative	1+	3+	1+	1+	1+LCIS, negative ILC	negative	negative
5	Left breast	negative	negative	negative	negative	1+	negative	3+	negative	1+	3+
	Lymph node metastasis	negative	negative	negative	negative	1+	negative	2+	negative	1+	3+
	Right breast	negative	negative	negative	negative	1+	1+	2+	negative	negative	3+
	Lymph node metastasis	negative	negative	negative	negative	negative	negative	2+	negative	negative	2+
6	Left breast	1+	negative	negative	negative	1+	negative	2+	3+	negative	3+
	Lymph node metastasis	1+	negative	1+	1+	2+	negative	3+	3+	1+	3+
	Right breast	1+	negative	negative	negative	1+	negative	1+	3+	3+	3+
	Recidive	negative	negative	negative	1+	1+	negative	3+	3+	negative	3+
7	Left breast	2+	negative	negative	1+	2+	1+	3+	2+	1+	3+
	Lymph node metastasis	negative	negative	negative	negative	1+	negative	2+	2+	1+	3+
	Right breast	negative	negative	negative	1+	1+	negative	1+	3+	negative	3+
8	Right breast	1+	negative	1+	3+	3+	2+	3+	3+	1+	3+

Table 4. Immunohistochemical profile of the primary tumors and corresponding metastases.

	PRIMARY TUMOR (%)			CORRESPONDING LYMPH NODE METASTASES (%)		
	Negative	1+	2+/3+	Negative	1+	2+/3+
CD44	5/15 (33)	5/15 (33)	5/15 (33)	4/9 (44)	2/9 (22)	3/9 (33)
CD44v3	10/15 (67)	3/15 (20)	2/15 (13)	7/9 (78)	2/9 (22)	0/9 (0)
CD44v4	14/15 (93)	1/15 (7)	0/15 (0)	8/9 (89)	1/9 (11)	0/9 (0)
CD44v5	7/15 (47)	5/15 (33)	3/15 (20)	7/9 (78)	1/9 (11)	1/9 (11)
CD44v6	0/15 (0)	6/15 (40)	9/15 (60)	1/9 (11)	3/9 (33)	5/9 (56)
CD44v3-v10	7/15 (47)	5/15 (33)	3/15 (20)	5/9 (56)	3/9 (33)	1/9 (11)
m E-cadherin	3/15 (20)	3/15 (20)	9/15 (60)	1/9 (11)	0/9 (0)	8/9 (89)
m P-cadherin	5/15 (33)	3/15 (20)	7/15 (47)	3/9 (33)	3/9 (33)	3/9 (33)
m N-cadherin	9/15 (60)	4/15 (27)	2/15 (13)	5/9 (56)	3/9 (33)	1/9 (11)
m ß-catenin	2/15 (13)	1/15 (7)	12/15 (80)	0/9 (0)	1/9 (11)	8/9 (89)

tumors, which are known to have relatively good prognosis (Lakhani et al., 1998, 2002).

CD44 family

The review of the literature is shown in Table 5. The significance of CD44v6 exon product in breast carcinogenesis is still putative, but in some reports the expression of this variant isoform in breast carcinoma ranges from 13% to 100% (Kaufmann et al., 1995; Tempfer et al., 1996; Berner et al., 2003). In general, most studies report a high percentage of primary and metastatic breast carcinoma to express CD44v6 exon product, which is also shown in our study. CD44v5 exon product was detected mainly in invasive ductal carcinomas. Overall, 53% of primary tumors expressed CD44v5 exon product, which is similar to previously reported 56% (Tempfer et al. 1996). However, we have found CD44v5 exon product expression in only 22% of metastatic tumors in contrast to previously reported 94% (Tempfer et al. 1996). Generally, CD44 is highly expressed in both ductal and lobular carcinoma of the breast, but lower expression was reported for node positive patients and for intraductal papillary carcinoma (Saddik and Lai, 1999). Even though in some studies anti-pan-CD44 antibodies were reported as specific for CD44s, including clone DF1485 (Saddik and Lai, 1999), we believe that reactivities with constant regions of the CD44 cannot justify claim of specificity for CD44s because those constant regions are also present in all variant forms (Naot et al., 1997). Our results show expected associations in immunoreactivity for all but CD44v3-10 and CD44v5 exon products expression. Incoherent immunoreactivity, an inability to demonstrate immunoreactivity for CD44v5 in Namalwa transfectants with v3-10 was previously shown by Martegani et al. (Martegani et al., 1999). The failure to immunodetect specific products on cell lines and possibly human tissues is probably due to the different exon assortment in each CD44v molecule.

Cadherin/catenin family

The review of the literature is shown in Table 6. E-cadherin could be expressed in up to 17% of lobular carcinoma (Acs et al., 2001). However, none of the previous studies examined pleomorphic lobular carcinomas. We have found expression of E-cadherin in 2 of the 5 pleomorphic lobular carcinomas. Since the number of cases is small, it is difficult to know whether pleomorphic lobular carcinomas express E-cadherin more frequently than the usual type of lobular

Table 5. CD44 family – Literature review.

STUDY	SPECIMEN TYPE (N)	METHODS	COMMENTS
CD44s			
Saddik and Lai, 1999	Paraffin sections(21)	IHC	CD44s is expressed in normal breast epithelium and intraductal papilomas; less then 10% of cells in papillary carcinomas show CD44s positivity.
Roca et al., 1998	Frozen tissue samples (43)	RT-PCR, IHC	No correlation between CD44v expression and disease outcome. 81% have complex patterns. Infiltrating carcinomas express CD44s, v3 and v6.
Joensuu et al., 1993	Paraffin sections (311)	IHC	Strong CD44s expression in node positive IDC is associated with poor outcome. CD44s is expressed in 62% of poorly differentiated ER- tumors.
CD44 v			
Berner and Nesland, 2001	Paraffin sections (39)	IHC	Membranous CD44v6 expression is associated with alveolar and classical/alveolar variant of ILC. Membranous CD44v5 staining is higher in LN+ cases. 82% of ILC are CD44s positive, 59% are CD44v3, 90% are v5 and v3-10, 23% are v6 and 74% are v7 positive.
Foekens et al., 1999	Frozen sections (467)	IHC	CD44v6 expression in LN- patients is associated with favorable prognosis. CD44v6 is positive in 65% of tumors and CD44v10 in 33%.
Bankfalvi et al., 1999	Paraffin sections (142)	IHC	Increased CD44v4 and CD44v7, and decreased E-cadherin expression associated with LN+ status. Lack of CD44v6 and reduced E-cadherin correlate with poor survival. All CD44s and variant isoforms are expressed in IDC. In G2 and G3 vs. low G1 tumors there is progressive loss of v3 and v6, slight increase of standard and v4, and significant expression of v5. ILC gain CD44s, v5 and v7 and lost v4 and v6 compared with LCIS.
Tempfer et al., 1996	Paraffin sections (115)	IHC	CD44v5 and v6 expression correlated with poor survival. CD44v6 expression correlated with histologic grade and lymph node metastases. CD44v5 is expressed in 56%, v6 in 24% and v7-8 in 15% of primary breast carcinomas. CD44v5 is expressed in 94%, v6 in 92% and v7-8 in 89% of the LN metastases.
Kaufmann et al., 1995	Frozen sections (130)	IHC	CD44v3, v5 and v6 correlate with poor overall survival. CD44v3 is positive in 52%, v7/8 in 37% and v10 in 45% of primary tumors. CD44v5 is positive in 68% and CD44v6 in 84% of primary tumors. CD44v6 is positive in 100% of lymph node metastases.

Table 6. Cadherin-catenin family – Literature review.

STUDY	SPECIMEN TYPE (N)	METHODS	COMMENTS
E-cadherin			
Lei et al., 2002	Fresh human tumor samples (108)	DHPLC (WAVE) method	No significant difference in the allelic frequency between patients with breast cancer (28%) and controls (27%). Somatic mutations in E-cadherin gene (CDH1) in 5% of ductal and 12% of lobular carcinomas. CDH1 mutations are present in both IDC and ILC; germline mutations in CDH1 are not characteristic for familial breast cancer.
Rieger-Christ et al., 2001	Paraffin sections (33)	PCR, SSCP, DNA sequencing, IHC	LCIS were E-cadherin and ß-catenin negative. LCIS was negative in 13/16 cases and 10/16 ILC were also E-cadherin negative.
Gillett et al., 2001	Paraffin sections (470)	IHC	E-cadherin and α-catenin were associated with positive ER. γ-catenin was associated with negative ER. E-cadherin was associated with poor prognosis and increased number of positive lymph nodes. E-cadherin was negative or weakly positive in 38% of the cases, whereas ß-catenin was negative or weakly positive in 22%.
Madhavan et al., 2001	Paraffin sections (51)	IHC	E- and P-cadherin were down regulated in node-positive tumors. E-cadherin was down regulated in high-grade tumors. E-cadherin was negative or weakly positive in 30% of LN- vs. 79% of LN+ cases. E-cadherin strong expression in 13% LN- and 0% LN+ patients; P cadherin was negative in 35% LN- vs. 80%LN+ cases and was strongly expressed in 30% LN- vs. 12% LN+ cases.
Salahshor et al., 2001	Fresh human tumor samples (31)	SSCP, DHPLC	Correlation between Ala592Thr alteration and ductal-comedo type tumors. No pathogenic germline mutations in CDH1 gene. One somatic mutation and one germline mutation (Ala592Thr) was found – the frequency of that mutation was 0.56% in non-BRCA patients and 0.83% in sporadic group.
Parker et al., 2001	Paraffin sections (174)	IHC	E-cadherin correlates with tumor type, ER status and histologic grade. E-cadherin was strongly positive in 29% in G1 tumors and 38% in G3 tumors (p=0.031).
Bukholm et al., 2000	Paraffin sections (34 primary and 34 metastatic)	IHC	Metastatic tumors are characterized by re-expression of E-cadherin, α-, and ß-catenin, and down-regulation of γ-catenin in.
N-cadherin			
Nieman et al., 1999	Cell cultures	IHC, In vitro invasion and motility analysis	N-cadherin-positive cells have fibroblastic phenotype. N-cadherin promotes an invasive phenotype. In cells coexpressing E- and N-cadherin, N-cadherin dominates over E-cadherin function and promotes motility and invasion. P-cadherin-positive cells also have fibroblastic morphology, but not invasive properties.
P-cadherin			
Rasbridge et al., 1993	Frozen and paraffin sections (123)	IHC	No association of the different distributions of E-cadherin with a particular subtype of lobular carcinoma was detected. E-cadherin was absent in LCIS and in 83% of ILC. P-cadherin was not expressed in benign breast and only rarely in carcinomas.
Soler et al., 1999	Paraffin sections (183)	IHC	P-cadherin expression had strong correlation with poor prognosis, inverse correlation with steroid receptor expression, and in IDC positive correlation with high histologic grade. P-cadherin was positive in 52% of cases. N-cadherin expression was found in 48% cases.
ß-catenin			
Vaziri et al., 2001	31 BRCA1 carriers and 81 BRCA1 negative tumors.	Allele specific amplification	BRCA1 positive tumors were more frequently Ki-67 and b-catenin positive. ß-catenin was expressed in 58% of BRCA1 mutation carriers and 34% in control group.
Lin et al., 2000	Cell lines, 123 patient samples	CSGE, IHC Western blot, IHC	Strong correlation between b-catenin and cyclin D1 expression. High ß-catenin correlated with poor prognosis.
Gonzalez et al., 1999	Frozen sections (55)	IHC	E-cadherin expression correlated with expression of α- and ß/γ catenin. Loss of E-cadherin was associated with higher tumor grade and lobular morphology.
Hashizume et al., 1996	Paraffin sections (66)	IHC	No correlation between expression of E-cadherin, α- and ß-catenin, and lymph node metastases.70% of IDC expressed α- and ß-catenin, and E-cadherin. 80% of ILC were negative. Complete loss of α- and ß-catenin, and E-cadherin was more frequent in diffuse than in the solid type of IDC.

carcinoma. On the other hand medullary and mucinous carcinomas are frequently E-cadherin positive. Accordingly, we found E-cadherin expression in 2 of the 3 atypical medullary carcinomas and 100% invasive ductal carcinomas.

Little is known about N-cadherin and P-cadherin expression in breast carcinomas. In breast cancer cell line model, inappropriate expression of non-epithelial N-cadherin downregulated E-cadherin expression and promoted cellular motility (Nieman et al., 1999; Hazan et al., 2000; Gamallo et al., 2001). In a study by Soler et al. (Soler et al., 1999), cytoplasmic N-cadherin expression was found in a small population of cells in 48% of the tumors, but no association was found with clinical outcome. We show expression of N-cadherin in 40% of simultaneous breast carcinomas. However, we found no association between N-cadherin and E-cadherin expression and found that they both could be expressed at high levels in the same tumor. P-cadherin is expressed in highly aggressive tumors and is strongly associated with poor prognosis (Gamallo et al., 2001). Soler at al. found also a complete absence of this marker in lobular carcinoma (Soler et al., 1999). Four of ten primary tumors, which expressed P-cadherin, were pleomorphic lobular carcinomas. Even though our series is small, our results also suggest that P-cadherin is expressed in highly aggressive tumors.

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