Research Article

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Cytoplasmic Localization of β-Catenin is a Marker of Poor Outcome in Breast Cancer Patients

Elena López-Knowles[1], Sarah J. Zardawi[1], Catriona M. McNeil[1,3,4,5], Ewan K.A. Millar[1,6,7,8], Paul Crea[10], Elizabeth A. Musgrove[1,9], Robert L. Sutherland[1,9], and Sandra A. O'Toole[1,2,9,11]

Abstract

β-catenin is involved in cell adhesion through catenin-cadherin complexes and as a transcriptional regulator in the Wnt signaling pathway. Its deregulation is important in the genesis of a number of human malignancies, particularly colorectal cancer. A range of studies has been undertaken in breast cancer, with contradictory associations reported among β-catenin expression, clinicopathologic variables, and disease outcome. We undertook an immunohistochemical study measuring the levels and subcellular localization of β-catenin in 292 invasive ductal breast cancers with known treatment and outcome. No association with breast cancer–specific death was observed for cytoplasmic or membrane expression alone; however, a continuous score representing both locations (membrane minus cytoplasmic expression: MTC score) was associated with a worse outcome in univariate analysis ($P = 0.004$), and approached significance in a multivariate analysis model that included lymph node, progesterone receptor (PR), and HER2 status ($P = 0.054$). Therefore, the MTC score was used for further statistical analyses due to the importance of both the subcellular location and the levels of expression of β-catenin. An association was identified between high cytoplasmic expression (low MTC score), and high tumor grade ($P = 0.004$), positive Ki67 ($P = 0.005$), negative estrogen receptor (ER) ($P = 0.005$), positive HER2 ($P = 0.04$) status, and an active phosphoinositide 3-kinase pathway ($P = 0.005$), measured as *PIK3CA* mutations ($P = 0.05$) or PTEN loss ($P = 0.05$). Low cytoplasmic expression (high MTC score) was associated with the luminal A subtype ($P = 0.004$). In conclusion, a low β-catenin MTC score is associated with an adverse outcome in breast cancer, which may be of mechanistic significance in the disease process. *Cancer Epidemiol Biomakers Prev; 19(1); 301–9. ©2010 AACR.*

Introduction

Breast cancer is the most common invasive cancer in women in industrialized nations and is second only to lung cancer as the leading cause of cancer-related death in women. Current treatments, which are largely determined by the estrogen receptor, HER2 status, or clinicopathologic variables, such as tumor size and grade, and lymph node status, are useful in guiding therapeutic decision making, but there is a pressing need to develop new biomarkers and therapeutic strategies to combat the disease.

Numerous prognostic factors have been evaluated in breast cancer patients to predict clinical outcome, and there are multiple lines of evidence that suggest an important role for β-catenin in breast cancer. β-catenin is an oncogene, and its dysregulation or mutational activation can lead to cancer (1). In breast cancer, mutations are rare (2), unlike in colon or hepatocellular cancers, but the level of expression or activation of β-catenin may be associated with breast cancer progression. Previous data, however, have presented mixed results about the subcellular localization of β-catenin, and its relationship with clinicopathologic variables and disease outcome.

β-catenin is a critical component of cadherin-based cell-cell adhesion, has a central role in transcriptional regulation in the Wnt signaling pathway, and is an important intermediate in many other signal transduction pathways, such as the phosphoinositide 3-kinase(PI3K)/AKT pathway. β-catenin is located at the cell membrane, and the cytoplasm and/or nucleus. At the cell membrane, it is bound to the cytoplasmic domain of type I cadherins, and is essential for the structural organization and function of cadherins by linking through α-catenin to the actin

Authors' Affiliations: [1]Cancer Research Program, Garvan Institute of Medical Research; [2]Discipline of Pathology, Faculty of Medicine, University of Sydney; [3]Department of Medical Oncology, Westmead Hospital, Sydney, New South Wales, Australia; [4]Breast Cancer Institute of New South Wales and [5]Western Clinical School, University of Sydney, Westmead Hospital, Westmead, New South Wales, Australia; [6]Department of Anatomical Pathology, South Eastern Area Laboratory Service, St George Hospital, Kogarah, New South Wales, Australia; [7]Department of Pathology, School of Medicine, University of Western Sydney, Campbelltown, Australia; [8]School of Medical Sciences, Faculty of Medicine and [9]St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Kensington, New South Wales, Australia; [10]Department of Surgical Oncology, St Vincent's Hospital, Darlinghurst, New South Wales, Australia; and [11]Department of Anatomical Pathology, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia

Corresponding Author: Sandra A. O'Toole, Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, New South Wales 2010, Australia. Phone: 612-9295-8338; Fax: 612-9295-8321. E-mail: s.otoole@garvan.org.au

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cytoskeleton (3). This complex is stabilized by serine/threonine phosphorylation of β-catenin (4). Other phosphorylation events, however, such as tyrosine phosphorylation of β-catenin by epidermal growth factor receptors or Src, among others, lead to its dissociation from the adherens complex and transfer to the cytoplasm (5). Cytosolic β-catenin is subsequently degraded or translocated to the nucleus. In the absence of Wnt ligands, β-catenin binds to a complex formed by glycogen synthase kinase 3β (GSK3β), adenomatous polyposis coli, and axin (6). This complex facilitates the phosphorylation of the NH2 terminus of β-catenin by GSK3β or other kinases, targeting it for degradation by the ubiquitin-proteasome pathway (7). Activation of Wnt signaling is initiated by Wnt ligands binding to two receptor molecules, frizzled proteins and lipoprotein receptor–related proteins 5 and 6 (LRP-5/6). This leads to GSK3β inactivation and β-catenin accumulation in the cytoplasm with translocation to the nucleus. There it interacts with TCF/LEF to control transcription of downstream target genes, such as cyclin D1 and c-Myc.

β-catenin stability is also regulated by numerous Wnt-independent mechanisms, and can be modulated by ErbB2, p53, and Pin1 (8), among others.

The aim of this study was to evaluate β-catenin expression in a cohort of breast cancer patients, and investigate the link between subcellular localization and levels of β-catenin expression with known clinicopathologic variables and outcome, as well as the PI3K/AKT signal transduction pathway and Notch1 as a Wnt pathway downstream marker.

Materials and Methods

Patients and Tumors

Cases were drawn from the St Vincent's Campus Outcome Cohort, which comprised 292 invasive ductal carcinomas of the breast from patients treated by a single surgeon (P.C.) between February 1992 and August 2002 at St Vincent's Hospital, Sydney, Australia. Ethics approval was granted for the use of pathology specimens and cognate clinicopathologic data (Human Research Ethics Committee of St Vincent's Hospital, Sydney, Australia). A more detailed description of the clinicopathologic characteristics of the cohort is published elsewhere (9). Forty percent of tumors were >20 mm, 45% were grade >2, 43% were lymph node positive, 68% were ER positive, 57% were PR positive, and 18% were HER2 positive by fluorescence *in situ* hybridization (>2.2 ratio of HER2:chromosome 17 centrosome; ref. 10). Median age was 54 y, and patients were treated with endocrine therapy (49%), chemotherapy (38%), or both (24%). Cases were prospectively followed-up for a median of 64 mo, and the outcome events measured were recurrence (local or distant; 25%) and metastasis (23%); all deaths were recorded but only breast cancer–related deaths (18%) were considered for survival analyses.

Tissue Microarray Construction

A total of 18 tissue microarrays containing two cores of each tumor sample were constructed from the formalin-fixed, paraffin-embedded tumor material from each patient in the cohort, as previously reported. The tissue microarrays were produced with the use of the MTA-1 manual tissue arrayer (Beecher Instruments, Woodland, CA).

Immunohistochemistry

Three micron sections of each tissue microarray were cut, deparaffinized, and used for immunostaining. The β-catenin mouse monoclonal antibody (BD Transduction Laboratories, Franklin Lakes, NJ) was used at a 1:200 dilution for 60 min at room temperature. Antigen retrieval was done with the use of Dako solution (pH 6.0; S1699, Dako, Carpentaria, CA) for 30 min in a water bath. A Dako autostainer was used for immunostaining (Dako). Reactions were developed with the use of diaminobenzidine, and sections were hematoxylin counterstained. Colon cancer tissues were used as a positive control and invasive lobular breast carcinoma as a negative control. Immunohistochemistry protocols, and scoring and analysis for $p27^{Kip1}$, $p21^{Cip1/Waf1}$, cyclin E1, cyclin D1, and Ki67 are described elsewhere (9). Protocols, scoring, and analysis of the PI3K/AKT pathway are described in Lopez-Knowles et al. (11), and of Notch 1 in Zardawi et al. (12).

Immunohistochemical Scoring

Scoring was completed by a specialist breast pathologist (S.A.O'T.) and a scientist (S.J.Z.) blinded to the clinical and pathologic information; in cases of discrepancy a consensus was reached by conferencing. All tumors were assessed for both intensity (1+ to 3+), and the proportion of cells staining positive in the nucleus, cytoplasm, and cell membrane. A histoscore (H) was calculated by multiplying the percentage of positively stained cells with each category of staining intensity. β-catenin expression was found predominantly in the membrane and cytoplasm of cells, with only three cases showing detectable nuclear expression; hence, we described a histoscore value for the membrane expression (H1) and the cytoplasmic expression (H2) independently, and then derived a third histoscore (H3), which represents both categories of expression as one continuous variable (membrane minus cytoplasmic histoscore) as a measure of subcellular localization, the "membrane to cytoplasmic score" (MTC). When this continuous value is positive, the tissue shows predominantly membranous expression, and when the value is negative, the tissue shows mainly cytoplasmic expression (examples shown in Fig. 1).

Statistical Analyses

To assess the independence of two categorical variables, the χ^2 test was applied when the variables

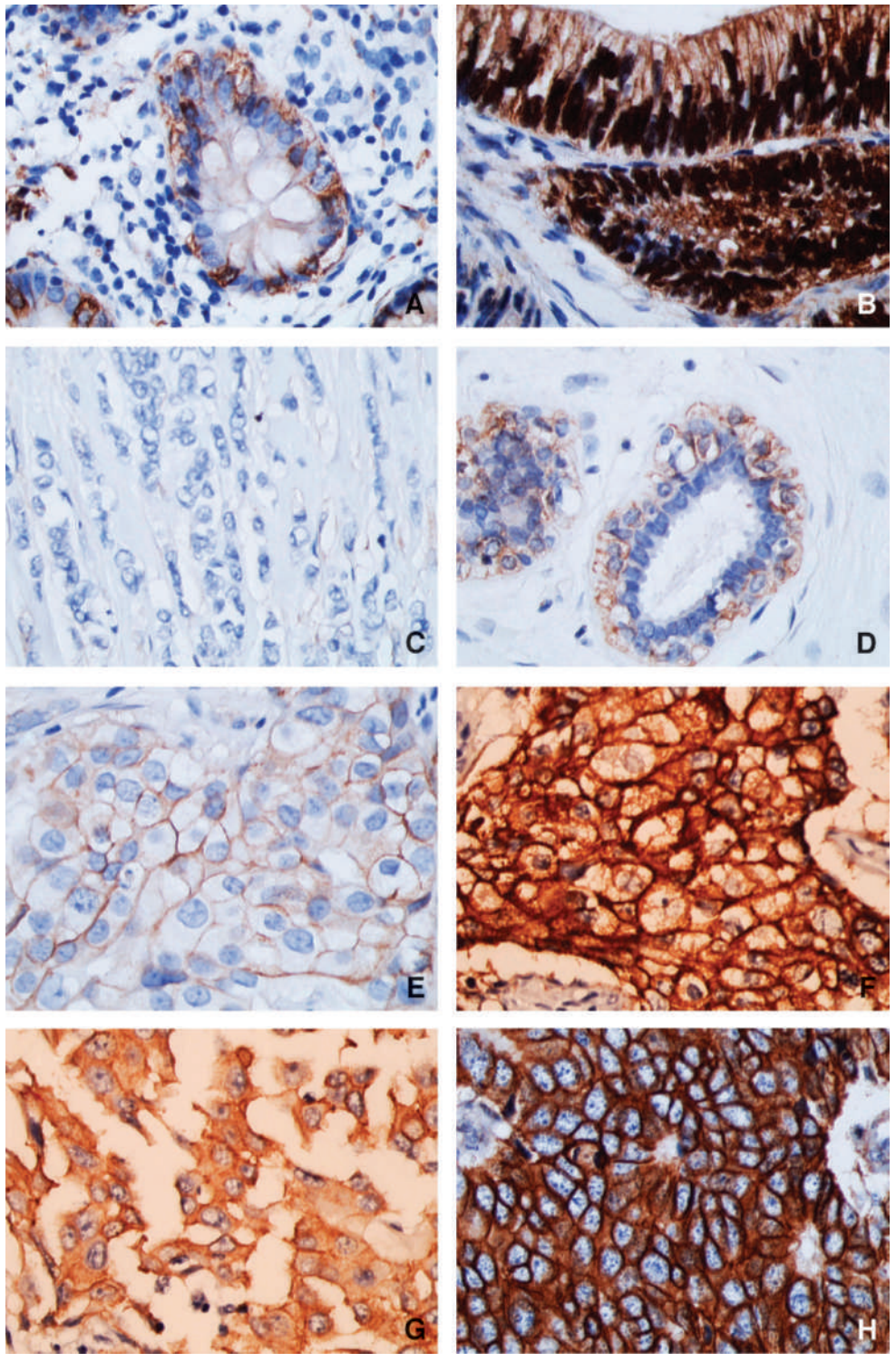

Figure 1. Patterns of expression of β-catenin expression in human tissues. **A.** Normal colonic crypts show a membranous pattern of expression. **B.** Invasive colon adenocarcinoma showing predominantly nuclear expression. **C.** Invasive lobular breast carcinoma negative control lacks expression of β-catenin. **D.** Normal breast duct with membranous expression in the basal cell layer. **E.** Weak predominantly membranous expression in invasive ductal carcinoma. **F.** Strong predominantly membranous expression in invasive ductal carcinoma. **G.** Predominantly cytoplasmic expression in invasive ductal carcinoma. **H.** Strong membranous and cytoplasmic expression in invasive ductal carcinoma. All images, ×400 magnification; hematoxylin counterstained.

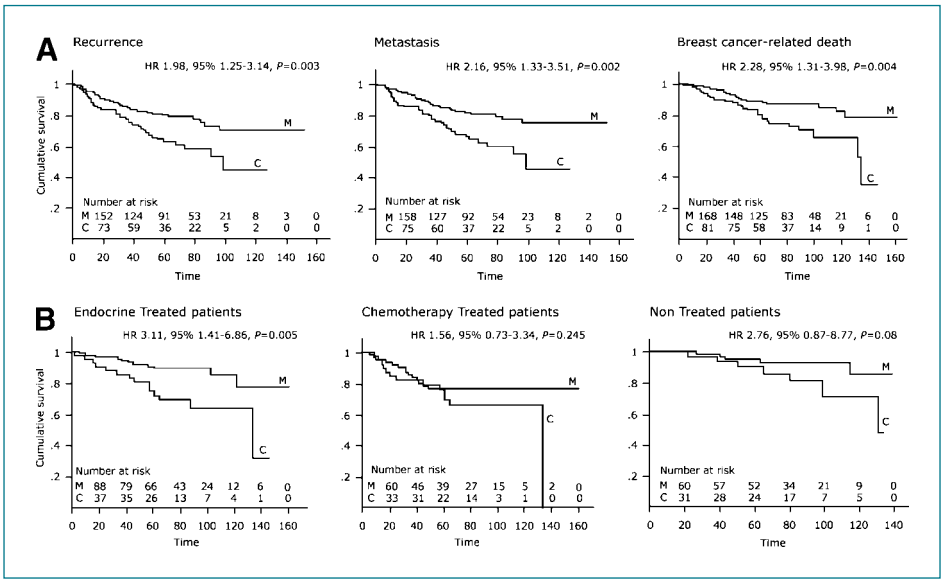

Figure 2. Kaplan-Meier curves illustrating the relationship between β-catenin expression, and disease progression and treatment. **A.** Recurrence, metastasis, and cancer-specific death stratified by a cytoplasmic (*C*; β-catenin MTC score expression <0) or membrane (*M*; β-catenin MTC score expression >0) expression. The number of patients at risk, and the HRs, 95% CIs, and *P*-value of the association by log rank testing are shown. **B.** Breast cancer–specific death in patients treated with adjuvant endocrine (tamoxifen) therapy or chemotherapy, and in untreated patients, stratified by cytoplasmic or membrane staining.

were dichotomous, the Mann-Whitney test was applied when one variable was dichotomous and the other continuous, and the Spearman rank correlation was applied when both variables were continuous. Kaplan-Meier survival curves and Cox proportional hazard ratios (HR) were estimated to obtain risks of recurrence, metastasis, and death from breast cancer after adjusting for other confounding variables. Results were considered significant at the two-sided $P < 0.05$ level. Statview version 5.0 was used for the analysis (Abacus Systems, Berkeley, CA).

Results

β-Catenin Expression

Normal colon and adenocarcinoma of the colon were used as controls for β-catenin expression. Normal colonic mucosa showed membranous expression of β-catenin (Fig. 1A), whereas adenocarcinoma showed strong nuclear and cytoplasmic expression (Fig. 1B). Invasive lobular carcinoma of the breast was used as a negative control, showing complete absence of expression (Fig. 1C). Of the original 292 invasive ductal carcinomas, 276 tumors were able to be evaluated once uninformative cores (e.g. folded or missing) were excluded. β-catenin showed moderate membranous expression in the myoepithelial/basal cell layer of normal ducts (Fig. 1D). In contrast, β-catenin expression was observed in both the cytoplasm and the membrane compartments of carcinoma cells. Four percent of cases (12 of 276) showed only membranous expression (Fig. 1E), 5% of cases (15 of 276) showed only cytoplasmic expression, and the remainder showed expression in both subcellular compartments (Fig. 1F-H). Nuclear expression was seen in only three cases. The average membrane H1 score was 125 and the median was 120. Because membranous expression did not show a normal distribution and no optimal cut point was observed, the median was used as a threshold for survival analysis. The average cytoplasmic H2 score was 104 and the median was 80. Again, cytoplasmic expression did not show a normal distribution and no optimal cut point was identified; therefore, the median was used as a threshold for survival analysis. When one continuous H3 score was calculated for β-catenin expression by subtracting the cytoplasmic H2 score from the membranous H1 score (the MTC score), the average expression of β-catenin was 21 and the median was 10. This variable had a normal distribution across the whole cohort of 276 breast cancers. To study the association with outcome, we dichotomized the data with a value <0 of the continuous β-catenin expression; a score <0 would be mainly cytoplasmic expression, and one >0 would represent predominantly membranous expression. To test other means of obtaining one continuous variable that represented both the cytoplasmic and membrane staining observed, two other variables were calculated. First, the ratio dividing the membrane and the cytoplasm scores was calculated, and the scores dichotomized at the median. Second, the membrane plus the cytoplasmic scores were added, dichotomizing the data again at the median score. Both these scores showed no significant association with outcome (data not shown).

β-Catenin Expression, Disease Outcome, and Treatment

Membranous and cytoplasmic expression of β-catenin alone did not show an association with breast cancer–specific death [HR, 0.88; 95% confidence interval (95% CI), 0.507-1.554; $P = 0.67$ and HR, 1.493; 95% CI, 0.853-2.613; $P = 0.16$, respectively], and only cytoplasmic expression showed a weak association with recurrence

(HR, 1.79; 95% CI, 1.122-2.854; $P = 0.01$) and metastasis (HR, 1.79; 95% CI, 1.097-2.932; $P = 0.02$). However, in a univariate analysis a shift to high cytoplasmic expression, measured as a negative MTC score, was associated with recurrence (HR, 1.98; 95% CI, 1.25-3.14; $P = 0.003$), metastasis (HR, 2.16; 95% CI, 1.33-3.51; $P = 0.002$), and breast cancer–specific death (HR, 2.28; 95% CI, 1.31-3.98; $P = 0.004$; Fig. 2A). In a multivariate analysis incorporating all clinicopathologic variables reported in Table 1, β-catenin approached independent predictor status for breast cancer–related death in a model that included lymph node status, PR, and HER2 status (HR, 1.77; 95% CI, 0.98-3.18; $P = 0.054$).

Next, patients were distributed into three treatment subgroups (i.e., endocrine therapy, chemotherapy, and no adjuvant treatment), and associations between β-catenin and breast cancer–specific death were assessed. In patients treated with endocrine therapy ($n = 140$), β-catenin shift to cytoplasmic expression was associated with breast cancer–specific death (HR, 3.11; 95% CI, 1.41-6.86; $P = 0.005$). There was no such association, however, in the chemotherapy-treated patients ($n = 107$; (HR, 1.56; 95% CI, 0.73-3.34; $P = 0.245$) or in the patients who did not receive adjuvant treatment ($n = 99$; HR, 2.76; 95% CI, 0.87-8.77; $P = 0.08$; Fig. 2B).

β-Catenin Expression and Clinicopathologic Variables

The association between β-catenin expression in the cytoplasm, membrane, or both, and the clinicopathologic variables was evaluated (Table 2). High membranous expression was associated with a large tumor size >20 mm ($P = 0.005$), a high tumor grade >2 ($P = 0.01$), and positive lymph node status ($P = 0.02$). It was also associated with ER-negative ($P = 0.04$) and PR-negative ($P = 0.01$) status. High cytoplasmic expression was also associated with a large tumor size >20 mm ($P = 0.0009$), a high tumor grade >2 ($P < 0.0001$), and positive lymph node status ($P = 0.03$). It was also associated with ER-negative ($P < 0.0001$) and PR-negative ($P = 0.0005$) status, and a high proliferation rate, as assessed by Ki67 ($P < 0.0001$). The MTC score showed an association between cytoplasmic expression, and higher grade ($P = 0.004$), ER-negative ($P = 0.005$) and HER2-positive ($P = 0.04$) status, and positive Ki67 ($P = 0.005$).

Table 2. Clinicopathologic association of β-catenin expression

		Membrane H1	Cytoplasmic H2	MTC H3
		P	P	*P*
Tumor size				
<20 mm	164			
>20 mm*	112	**0.0054***	**0.0009***	0.7355
Tumor grade				
≤2	150			
>2*	126	**0.0153***	**<0.0001***	**0.0042***
LN status				
0	152			
>0*	121	**0.0235***	**0.0348***	0.9403
Age, y				
<50	102			
>50	174	0.1166	0.9882	0.1783
ER				
Negative*	86	**0.0456***	**<0.0001***	**0.0052***
Positive	188			
PR				
Negative*	117	**0.0116***	**0.0005***	0.5411
Positive	158			
HER2				
Negative	217			
Positive*	51	0.5167	0.1511	**0.0412***
Ki67				
Negative	128			
Positive*	120	0.4825	**<0.0001***	**0.0054***

*Significantly associated with β-catenin expression.

Table 1. Univariate and multivariate outcome analysis for clinicopathologic variables, hormone receptor status, and β-catenin

	Variable	HR	95% CI	*P*
Univariate	Grade >2	3.520	1.930-6.418	**<0.0001**
	Size >20 mm	2.468	1.415-4.304	**0.0015**
	Lymph node status >0	3.691	2.025-6.729	**<0.0001**
	Age >50 y	1.427	0.799-2.551	0.2297
	ER+	0.300	0.172-0.524	**<0.0001**
	PR+	0.170	0.087-0.333	**<0.0001**
	HER2 FISH+	3.491	1.956-6.229	**<0.0001**
	MTC H3 <0	2.28	1.309-3.980	**0.0036**
Multivariate	Lymph node status >0	3.295	1.781-6.098	**0.0001**
	PR+	0.213	0.107-0.422	**<0.0001**
	HER2 FISH+	2.429	1.293-4.564	**0.0058**
	MTC H3 <0	1.775	0.989-3.187	**0.0545**

Abbreviation: FISH, fluorescence *in situ* hybridization.

β-Catenin Expression and Cell Cycle Markers

β-catenin expression increases with cell cycle progression, and is located in the cytoplasm and nucleus during the cell cycle. Because a role for β-catenin in cell proliferation and apoptosis has been previously identified (13), and expression was associated with Ki67 in our cohort, we investigated potential relationships between β-catenin expression and several cell cycle proteins of importance in breast cancer (Table 3).

Table 3. Association of β-catenin expression and molecular markers

	N.	Membrane H1 *P*	Cytoplasmic H2 *P*	MTC H3 *P*
Cell cycle markers				
Cyclin D1		Rho −0.113	Rho −0.154	Rho 0.019
		0.0625	**0.0108***	0.7568
Cyclin E1		Rho 0.344	Rho 0.364	Rho 0.217
		<0.0001*	**<0.0001***	**0.0003***
p21		Rho 0.041	Rho −0.018	Rho 0.086
		0.4971	0.7604	0.1515
p27		Rho −0.231	Rho −0.363	Rho 0.124
		0.0001*	**<0.0001***	**0.0407***
Notch1				
<67%	133			
>67%*	93	**0.0077***	**<0.0001***	0.1731
PI3K/AKT pathway				
PIK3CA				
Wt	146			
Mut*	12	0.1806	0.4805	**0.0528***
PIK3CA				
No change	174			
Amp	28	0.9375	0.1885	0.1342
PTEN				
H >0	181			
H = 0*	70	**0.0232***	**<0.0001***	**0.0599***
pAKT				
H <22	191			
H >22	60	0.5274	0.8769	0.7144
PI3K/AKT				
Wt	53			
Altered*	134	0.2798	0.0989	**0.0057***

*Significantly associated with β-catenin expression.

An association was identified between membranous expression of β-catenin and high cyclin E1 ($P < 0.0001$) and low $p27^{Kip1}$ ($P = 0.0001$). An association was also identified between cytoplasmic expression of β-catenin, and low cyclin D1 ($P = 0.01$), high cyclin E1 ($P < 0.0001$), and low $p27^{Kip1}$ ($P < 0.0001$) expression levels. When the MTC score was analyzed, an association was confirmed between predominantly membranous expression and high cyclin E1 ($P = 0.0003$), and predominantly cytoplasmic expression and loss of $p27^{Kip1}$ ($P = 0.04$).

β-Catenin Expression and Signal Transduction Pathways

β-catenin is involved in Wnt signaling and is downstream of GSK3β, which is also involved in the PI3K/AKT pathway. We therefore next assessed the association of Notch, as an interactor of the Wnt signaling pathway, and pAKT, PTEN, and *PIK3CA*, as components of the PI3K/AKT pathway, with β-catenin expression (Table 3).

Cross-talk between the Wnt and Notch pathways can occur through a number of mechanisms: through the physical binding of Notch1 to β-catenin (14); through their association with common cofactors (15); through GSK3β phosphorylation of Notch, modulating its transcriptional activity (16); and through β-catenin–mediated transcriptional activation of the Notch ligand Jagged1 (17). We identified an association between high cytoplasmic ($P < 0.0001$) and high membrane ($P = 0.007$) β-catenin expression and high Notch1 expression.

PIK3CA mutations and copy number, PTEN expression, a negative regulator of the PI3K pathway, and pAKT expression were previously characterized in this cohort of patients (11). A positive association was identified between *PIK3CA* mutations and cytoplasmic location of β-catenin, measured by the MTC score ($P = 0.05$). A positive association was also identified between loss of PTEN expression, and high cytoplasmic expression ($P < 0.0001$) and high membranous expression ($P = 0.02$) of β-catenin. The MTC score confirmed this association

between PTEN loss and cytoplasmic location of β-catenin ($P = 0.05$). Finally, a surrogate variable for an active PI3K/AKT pathway, measured as an alteration of at least one member of the PI3K/AKT pathway studied, was associated with cytoplasmic location of β-catenin, as measured by the MTC score ($P = 0.005$).

Therefore, an active PI3K/AKT pathway, measured by *PIK3CA* mutations, PTEN loss, or an alteration in any of the three variables studied in the pathway, is associated with β-catenin protein expression located predominantly in the cytoplasmic compartment of the tissue.

β-Catenin Expression and Breast Cancer Subtypes

Breast cancer patients were distributed into five molecular phenotypes or subtypes with the use of criteria similar to those recently described by Cheang (18), but with the use of fluorescence *in situ* hybridization to determine HER2 status (9, 10). The five phenotypes were luminal A (ER+ and/or PR+, HER2−), luminal B (ER+ and/or PR+, HER2+), HER2 (ER− and PR−, HER2+), "basal-like" (ER−, PR−, HER2−, CK5/6+ and/or EGFR+), and unclassified (negative for all markers).

When cases were distributed into these five phenotypes, a high membranous β-catenin H1 score was associated with the basal-like ($P = 0.008$) subtype. A high cytoplasmic β-catenin H2 score was also associated with the basal-like ($P < 0.0001$) and HER2 ($P = 0.01$) subtypes, and a low cytoplasmic expression ($P < 0.0001$) was associated with the luminal A subtype. A high MTC score was also associated with the luminal A subtype ($P = 0.004$; Table 4).

Discussion

β-catenin has previously been shown to be a potential prognostic marker in breast cancer; however, the associations with clinicopathologic variables and outcome measures reported in different studies are discrepant. We therefore evaluated β-catenin expression in a cohort of invasive ductal breast cancers with known patient clinicopathologic variables, outcome, and treatment. A shift to high cytoplasmic β-catenin expression was associated with high-grade tumors, high proliferation rate, ER-negative and HER2-positive status, and worse outcome. The latter was significant in univariate analysis, and approached independence as a prognostic marker of breast cancer–specific death ($P = 0.054$) in a model including lymph node, PR, and HER2 status.

Table 4. β-catenin expression and breast cancer subtypes

		Membrane H1	Cytoplasmic H2	MTC H3
		P	*P*	*P*
Basal-like	31	**0.0079 (+)**	**<0.0001 (+)**	0.1796
HER2	24	0.3973	**0.0135 (+)**	0.1155
Luminal A	169	0.1037	**<0.0001 (–)**	**0.0045 (–)**
Luminal B	27	0.1085	0.6455	0.0974
Unclassified	16	0.4595	0.7109	0.9654

NOTE: (+) indicates a positive correlation and (–) a negative correlation.

β-catenin expression is localized in the nucleus in tissues like colorectal cancer (19); however, in the breast only two studies have shown nuclear localization (20, 21), whereas most publications report a cytoplasmic or membranous pattern of expression in breast cancer cells. Previously, β-catenin cytoplasmic expression was reported to be associated with worse outcome in three studies (20, 22, 23), whereas others have identified no such relationship (24-26). Two studies have reported an association between cytoplasmic expression and ER-positive status (23, 27), whereas others have found no association (25, 28). Contradictory results are also reported for its associations with tumor size and grade, patient age, and lymph node status.

Confirming the findings of most previous studies, β-catenin expression was identified only in the membrane and cytoplasm of the breast cancer tissues analyzed. The molecular mechanisms that allow the transfer of membrane bound to cytoplasmic localization is largely unknown, but could be due to changes in adenomatous polyposis coli (APC) or axin, which maintains high β-catenin levels in the cytoplasm (29), APC shuttling (30), or galectin-3 recruiting β-catenin from cell adhesion to signaling pathways (31). Low APC expression is associated with β-catenin overexpression and localization in the cytoplasm in breast cancer cells (32). In breast cancer, *APC* (33) and *Axin1* mutations are rare (32), but other events, such as methylation, as described in the APC promoter of invasive breast cancer patients (34), could regulate APC levels and thus β-catenin accumulation. Also, APC protein truncation leading to an increase in cytosolic levels of β-catenin have been identified in breast cancer cell lines (35).

When analyzing downstream markers, such as c-Myc and cyclin D1, no association was identified. Two studies have shown an association between cyclin D1 and β-catenin expression (20, 32), although two further studies have not identified this association (25, 36). This lack of consensus could be due to cytoplasmic rather than nuclear localization of the protein.

One critical issue related to previous studies is the selection of either membrane or cytoplasmic expression for scoring and statistical analysis. A review of previous literature shows marked discrepancies in the selection of a threshold, and the scoring of either of the two locations to measure β-catenin activation or aberrant expression. We have calculated a new score, which evaluates the expression in both locations without defining arbitrary thresholds. Both locations are then taken into consideration by calculating a MTC histoscore. This serves as a surrogate marker for β-catenin localization, with low

MTC expression measuring a decrease in membrane expression and an increase in cytoplasmic expression.

PIK3CA mutations, PTEN loss, and the overall PI3K active pathway lead to a decrease in GSK3β and thus an increase in β-catenin cytoplasmic accumulation. The association of *PIK3CA* mutations, PTEN loss, and overall PI3K pathway activation with a shift of expression to the cytoplasm, and the lack of association between the activated pathway and either membrane or cytoplasmic expression alone, highlight the strength of this combined variable to characterize the localization of β-catenin expression. Recently, an important role for PI3K/AKT/β catenin pathway has been identified in the regulation of malignant stem/progenitor cell populations, suggesting that inhibiting this pathway could be an effective method to target tumorigenic breast cancer cells (37).

No association with breast cancer–specific death was observed with either membrane or cytoplasmic expression alone; however, a shift to cytoplasmic expression, as defined by a MTC score <0, is significantly associated with recurrence, metastasis, and breast cancer–specific death, highlighting the importance of β-catenin localization and the pathways that converge on this protein in breast cancer. A shift to high cytoplasmic expression also shows a strong trend toward being an independent prognostic marker of breast cancer–specific death ($P = 0.054$).

These observations point to the importance of the membrane to the cytoplasm shift score. This measures a decrease in β-catenin at the adherens junctions involved in cell adhesion, which could have an effect on cell invasion, tumor metastasis, and the increase in β-catenin cytoplasmic accumulation due to the activation of pathways converging on this protein (such as the PI3K/AKT pathway). This accumulation can lead to activation of numerous target genes that play essential roles in normal development and in human cancers (38).

Interestingly, we observed an association between β-catenin cytoplasmic expression and poor outcome in patients treated with endocrine therapy in the cohort as a whole. This trend supports preliminary data by Hiscox et al., which showed that tamoxifen-resistant breast cancer cells had increased cytoplasmic accumulation of β-catenin (39). Taken together, these data suggest a potential role for β-catenin in endocrine resistance, but this requires further investigation, particularly in an appropriate clinical trial cohort.

In conclusion, our study shows that a shift in subcellular localization of β-catenin is associated with alterations in cell cycle regulatory proteins and signaling transduction pathways in clinical breast cancer samples. Furthermore, cytoplasmic accumulation of β-catenin is associated with poor prognosis in invasive ductal carcinoma. It is possible that in a larger cohort, true independence may be established, and further efforts are needed to validate these findings in independent breast cancer cohorts.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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