

Prognostic Significance of Abnormal β -catenin Expression in Breast Carcinoma

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Background : The subcellular localization and activity of β -catenin are tightly regulated within the cell. The aim of this study was to analyze the aberrant β -catenin expression in breast carcinomas and to determine its clinical significance. **Methods :** Fifty five cases of breast carcinoma were immunostained with monoclonal antibodies against β -catenin. Normal expression of β -catenin was defined as exclusive membranous staining. Abnormal expression of β -catenin was reclassified into 3 categories: complete or partial loss of membranous staining (LOM) without cytoplasmic staining and nuclear staining, LOM with cytoplasmic staining and without nuclear staining, and LOM with nuclear staining and with/without cytoplasmic staining. **Results :** Normal membranous β -catenin expression was detected in 25 (45.5%) of 55 cases of breast carcinoma. Thirty cases with abnormal β -catenin expression comprised 9 cases (16.1%) showing LOM without cytoplasmic and/or nuclear staining, 20 cases (36.4%) showing LOM with cytoplasmic staining and without nuclear staining, and one case (1.8%) showing LOM with nuclear and cytoplasmic staining. Abnormal β -catenin expression was significantly correlated with lymph node metastasis ($p=0.03$). LOM with cytoplasmic and/or nuclear expression was significantly correlated with poor disease free survival by univariate ($p=0.03$) and multivariate analyses ($p=0.03$). In addition, it was correlated with poor overall survival with a borderline significance ($p=0.059$). **Conclusions :** This study suggests that the cytoplasmic and/or nuclear expression of β -catenin can be used as a biologic marker for predicting disease recurrence and poor patients' survival in breast carcinomas.

Key Words : Beta catenin; Immunohistochemistry; Survival; Carcinoma, ductal, breast

Breast cancer is one of the most common carcinomas in women. The prognosis of breast cancer patients is profoundly influenced by the classical prognostic variables such as histological grade, tumor size, lymph node status and vascular invasion as well as by the predictors of therapeutic responses such as the expression of estrogen receptor and c-erbB-2. A large number of genetic alterations have been identified in invasive breast carcinomas, and many of them are of potential prognostic or predictive value.¹

β -catenin is a ubiquitous protein. It was primarily found to be a cell-cell adhesion molecule. It binds directly to the cytoplasmic domain of E-cadherin, a cell surface protein that maintains intercellular adhesiveness and plays a critical role in cancer invasion and metastasis.^{2,3} Free β -catenin can act independently of the cadherins, and function as a regulator of the nuclear transcription factors in the Wnt signaling pathway.^{4,5} The Wnt signaling pathway has a major role in controlling a cell's fate, its adhesion and the cellular polarity during embryonic development.⁴⁻⁶ Wnt signals through a family of cell-surface receptors stimulate sev-

eral pathways, the central one of which involves β -catenin and adenomatous polyposis coli (APC). In resting cells (not exposed to Wnt), β -catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β -catenin, and the intracellular levels of β -catenin become low. When cells are stimulated by secreted Wnt molecules or when APC is mutated, the destruction complex is deactivated, β -catenin degradation does not occur and its cytoplasmic level increases. β -catenin translocates to the nucleus where it forms a complex with a T cell factor (Tcf) that upregulates cellular proliferation by increasing the transcription of several genes involved in the cell cycle.⁷⁻⁹

Aberrant activation of the Wnt/ β -catenin pathway is one of the most frequent signaling abnormalities known in human cancer.¹⁰⁻¹³ Several mechanisms have been reported to cause this deregulation, including deletion of the APC gene, mutation of β -catenin and activation of the Wnt pathway.⁷⁻⁹ Although the deletion of APC and mutations of β -catenin have been found

in many types of cancers, evidences for comparable mutations in breast cancers are lacking.⁴⁻⁶ However, Wnt signals are strongly implicated in the initial development of the mammary rudiments, and in the ductal branching and alveolar morphogenesis that occurs during pregnancy.^{4,5} Transgenic expression of Wnt1 or Wnt10b in the mouse mammary gland leads to lobuloalveolar hyperplasia with a major risk of progression onward to carcinoma.^{4,5}

The aim of this study was to analyze the aberrant β -catenin expression in invasive ductal carcinomas of the breast, and to elucidate its clinical significance as an independent prognostic marker.

MATERIALS AND METHODS

Samples

Formalin fixed and paraffin embedded tissues from 55 cases of invasive ductal carcinoma of the breast were obtained from the files of the Department of Pathology, Dankook University Hospital. The tissues were taken from breast cancer patients who had been treated at the hospital from 1997 to 1999. For each case, the haematoxylin-eosin stained sections were examined and the histologic grade was assessed according to the Elston and Ellis¹³ method. The pathologic reports and clinical records were reviewed, and the clinical follow-up data, including patients' survival and cancer recurrence or metastasis, were collected retrospectively. The mean follow-up period of the patients was 46.5 months (range: 1-86 months).

Immunohistochemistry

The sections were deparaffinized in xylene and a graded series of ethanol solutions. The endogenous peroxidase activity was blocked by 10 min incubation in 3% hydrogen peroxide/methanol buffer, and then the sections were rinsed in phosphate-buffered saline (PBS). The slides were incubated with normal bovine serum for 30 min at room temperature to reduce the nonspecific background staining. To enhance the immunoreactivity, microwave antigen retrieval was performed for 10 min in a citrate buffer (pH 6.0). The sections were subsequently incubated with primary antibodies at a dilution of 1:200 for 1 h at room temperature. The primary antibodies used were monoclonal antibodies for β -catenin (HMF2, Novocastra, Newcastle, UK). The LSAB kit (DAKO, Carpinteria, CA, USA) was used as a detection system. After washing the sections, they were incu-

bated with biotinylated secondary antibody for 30 min at room temperature and were rinsed with PBS. Antigen-antibody complexes were visualized using a peroxidase-conjugated streptavidin with 3,3'-diaminobenzidine as a chromogen. The slides were counterstained with Mayer's haematoxylin, rinsed in tap water, and then mounted.

Evaluation of immunohistochemical staining

The adjacent normal breast tissue was used as an internal positive control for β -catenin, and the staining of β -catenin was normally seen at the cell-to-cell borders. β -catenin staining was recorded as normal and abnormal. The normal expression of β -catenin was defined as exclusively complete membranous staining of a similar intensity to that in the adjacent normal epithelia. Abnormal expression of β -catenin was reclassified into 3 categories: complete or partial loss of membranous staining (LOM) without cytoplasmic and nuclear staining, LOM with cytoplasmic staining and without nuclear staining, and LOM with nuclear staining and with/without cytoplasmic staining. The cases with strong membranous staining with faint granular cytoplasmic staining were considered normal.

Statistical analysis

Statistical analysis for the relationship between the aberrant β -catenin expression and the clinicopathologic parameters was performed by using the χ^2 test or Fisher's exact test. The disease free and overall survival curves were plotted using the Kaplan-Meier method, and differences between the survival curves were tested by using the log-rank test. Multivariate analysis for covariates showing statistical significance in univariate analysis was performed using the Cox proportional hazards model. The results were considered to be statistically significant when the p-values were less than 0.05. All statistical analyses were conducted using the SPSS 11.0 statistical software program (SPSS Inc., Chicago, IL, USA).

RESULTS

The normal breast tissue showed the strong membranous expression of β -catenin in the epithelium. Faint cytoplasmic staining was infrequently detected, but no nuclear staining was demonstrated in the normal breast tissue.

Normal membranous β -catenin expression was detected in

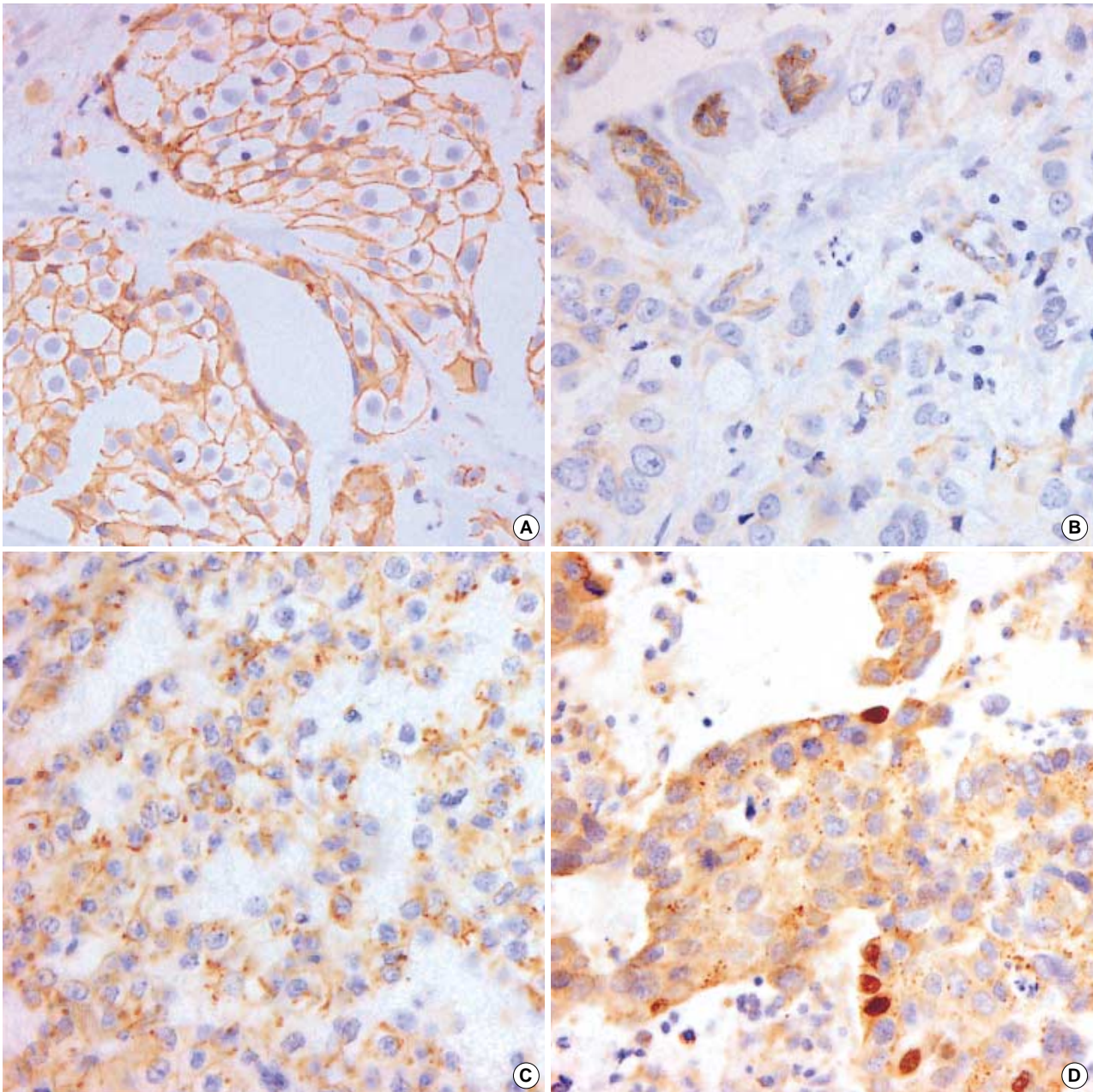


Fig. 1. Immunohistochemical expression of β -catenin in invasive ductal carcinomas of the breast. Normal expression shows strong complete membranous staining (A) similar to the entrapped normal ducts (left upper portion of B). Abnormal expression comprises loss of membranous expression (LOM) without cytoplasmic and nuclear expression (B), LOM with cytoplasmic expression and without nuclear expression (C), LOM with focal nuclear and diffuse cytoplasmic expression (D). Cytoplasmic expression is diffuse and coarsely granular or dot-like (C, D).

25 (45.5%) of 55 cases of invasive ductal carcinomas, and abnormal β -catenin expression in 30 cases (54.5%). Thirty cases with abnormal β -catenin expression comprised 9 cases (16.4%) showing LOM without cytoplasmic and/or nuclear staining, 20 cases (36.4%) showing LOM with cytoplasmic staining, and one case (1.8%) showing LOM with nuclear and cytoplasmic staining

(Fig. 1 and Table 1). Cytoplasmic staining of β -catenin was diffuse and coarsely granular or dot-like. Cytoplasmic β -catenin was frequently accumulated in the perinuclear areas. In one case, nuclear staining was present in about 5% of tumor cells, and was accompanied by LOM and abundant perinuclear cytoplasmic staining.

Table 1. Incidence of normal and abnormal expression of β -catenin in the cases of breast carcinomas

	No. of case (%)
Normal membranous expression	25 (45.5)
LOM without cytoplasmic and nuclear expression	9 (16.4)
LOM with cytoplasmic expression and without nuclear expression	20 (36.4)
LOM with nuclear expression and with/without cytoplasmic expression	1 (1.8)
Total	55

LOM, complete or partial loss of membranous expression.

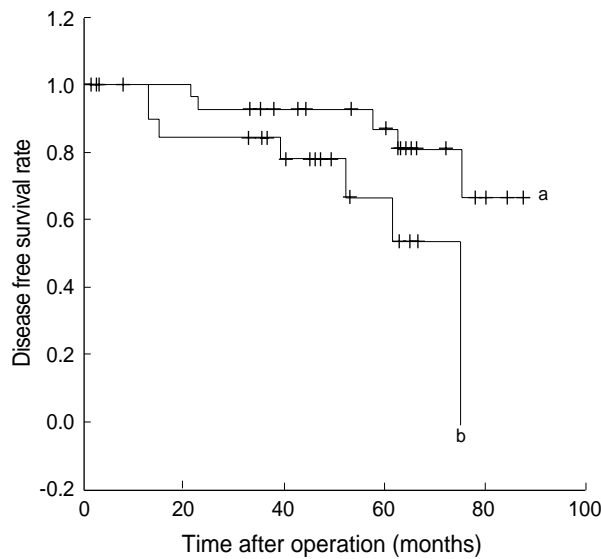


Fig. 2. Kaplan-Meier analysis for disease free survival shows significantly decreased disease free survival rate in patients who had breast cancers with loss of membranous expression with cytoplasmic and with/without nuclear expression of β -catenin (b), than patients who had breast cancers with normal expression of β -catenin (a) ($p=0.03$).

The relationship between the abnormal β -catenin expression and the clinicopathologic parameters are shown in Table 2. Abnormal β -catenin expression was significantly correlated with lymph node metastasis ($p=0.03$), and was associated with tumor stage with borderline significance ($p=0.06$). LOM with cytoplasmic and with/without nuclear expression was not correlated with the histologic grade, lymph node metastasis or tumor stage (Table 3).

During follow-up period, 12 cases (21.8%) metastasized to various organs such as the lung, the liver and the brain, and 4 cases (7.3%) died of the breast cancer. Abnormal β -catenin expression was not correlated with the disease free survival or overall survival. However, LOM with cytoplasmic and with/without nuclear expression was inversely correlated with the disease free survival by univariate ($p=0.03$) and multivariate ($p=0.03$) analyses (Fig. 2). LOM with cytoplasmic and/or nuclear expression

Table 2. Relationship between cases of abnormal expression of β -catenin and clinicopathologic parameters in breast carcinomas

	Total	Abnormal expression of β -catenin (%)	p value
Histologic grade			NS
I	3	0 (0)	
II	32	18 (56.3)	
III	20	12 (60.0)	
Lymph node metastasis			0.03
Absence	31	13 (41.9)	
Presence	24	17 (70.8)	
Stage			0.06
I-II	47	23 (48.9)	
III-IV	8	7 (87.5)	

NS, not significant.

Table 3. Relationship between loss of membranous expression with cytoplasmic expression and with/without nuclear expression of β -catenin and clinicopathologic parameters in breast carcinomas

	Total	LOM with cytoplasmic and with/without nuclear expression (%)	p value
Histologic grade			NS
I	3	0 (0)	
II	32	13 (40.6)	
III	20	8 (40.0)	
Lymph node metastasis			NS
Absence	31	9 (29.0)	
Presence	24	12 (50.0)	
Stage			NS
I-II	47	16 (34.0)	
III-IV	8	5 (62.5)	

LOM, complete or partial loss of membranous expression; NS, not significant.

was correlated with poor overall survival with a borderline significance ($p=0.059$). The tumor stage was also associated with poor overall survival ($p=0.042$).

DISCUSSION

The Wnt/ β -catenin pathway has been studied in many cancers.¹⁰⁻¹³ Almost 100% of colon cancers have either mutated β -catenin or deleted APC, which is expected to activate the β -catenin pathway.⁷⁻⁹ In the present study, the abnormal β -catenin expression was noted in more than 50% of breast cancer specimens. This incidence is in accord with those of the previous reports.^{15,16} This fact implies that the Wnt/ β -catenin pathway is activated in breast cancers.^{17,18} However, the specific mutations that might account for the β -catenin deregulation have not been

well documented yet in breast cancers.^{4,5} Several reports^{4-6,19-23} suggested that the stabilization of β -catenin could result from abnormalities in other signaling pathways besides the Wnt signals. Such pathways include those regulated by tumor suppressor PTEN, by the kinases of the epidermal growth factor receptor (EGFR) family, and by the function of p53.⁴⁻⁶ Recent studies show that deregulated β -catenin not only promotes tumors but also can lead to p53 induction in parallel. A second arm of this feedback loop was proposed when it was shown that high levels of the wild-type p53 promoted β -catenin degradation through other serial interactions that were independent of the Wnt signaling pathway.¹⁹⁻²³

The β -catenin activity is determined by the subcellular localization. The subcellular localization and activity of β -catenin are tightly regulated within the cell. The free cytosolic pool of β -catenin is unstable and it is subject to rapid proteolytic degradation. It has been well documented that accumulated β -catenin in the cytoplasm and/or the nucleus increased when the cells stabilized β -catenin and, consequently, the activated β -catenin/Tcf4 activity. β -catenin was localized solely at the plasma membrane of the cell when its transactivation activity was low.⁴⁻⁶ Expression of nuclear β -catenin has been rarely demonstrated in breast cancers,¹⁵ compared to other cancers such as colon cancer,⁶⁻⁸ stomach cancer,¹¹ gallbladder cancer,¹² ovarian cancer.¹³ In the present study, focal nuclear expression of β -catenin was observed only in one case. Frequent cytoplasmic expression and rare nuclear expression in breast cancers reflect that the different signaling pathways may affect β -catenin in breast cancers.

Previous reports indicated that the absent or decreased membranous expression of β -catenin was associated with tumor invasiveness and a poor prognosis in many cancers.^{7,8,10-12} In the present study, LOM with/without the cytoplasmic and/or nuclear expression of β -catenin was correlated with the presence of lymph node metastasis, but it was not correlated with the disease free survival or the overall survival. However, LOM with the cytoplasmic and/or nuclear expression was significantly correlated with disease free survival by the univariate and multivariate analyses. These results suggest that LOM with the cytoplasmic and/or nuclear expression of β -catenin is a stronger biologic indicator for poor prognosis than LOM alone in breast cancers.

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