Int J Clin Exp Pathol 2015;8(11):14989-14994
www.ijcep.com /ISSN:1936-2625/IJCEP0016903

Original Article

Clinical implications of β-catenin protein expression in breast cancer

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Received September 25, 2015; Accepted October 27, 2015; Epub November 1, 2015; Published November 15, 2015

Abstract: The objective of this study was to examine the expression and significance of β-catenin in the diagnosis and prognosis of breast cancer. Overall, 241 patients with histologically confirmed breast cancer who had undergone radical surgery were enrolled in this study. β-catenin protein expression in breast cancer samples was evaluated by immunohistochemistry. β-catenin was expressed in Nuclei/Plasma of the samples from 41 patients. β-catenin protein expression correlated with the histological grade of the tumor ($P<0.05$) and Ki-67 labeling ($P<0.01$). Survival analysis showed that β-catenin expression negatively correlated with breast cancer-specific survival. Our results showed prominent expression of β-catenin in breast cancer and strongly implicate the β-catenin in tumor promotion.

Keywords: Breast cancer, β-catenin, tumor marker, survival, Ki-67

Introduction

Breast cancer is the most common type of cancer in women worldwide [1]. In 2008, 1,380,000 women were diagnosed with breast cancer with more than 458,400 deaths in the same year [2]. Tumor invasion and metastasis account primarily for poor prognosis and result from a cascade of events [3]. Current treatments are largely determined by estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status or by clinicopathologic variables such as tumor size, tumor grade, and lymph node status. Although these parameters guide therapeutic decision making, an invasion and metastasis related marker is still eagerly needed to develop new biomarkers and therapeutic strategies to combat breast cancer [4, 5]. Identification of novel biomarkers and an understanding of their clinical significance would benefit both current therapies and prognosis.

β-catenin is an important intermediate in several signal transduction pathways including the Wnt pathway. It interacts with E-cadherin, a critical regulator of cell-cell adhesion, in plasma membrane and is responsible for the maintenance of cell polarity [6]. Nuclear accumulation of β-catenin resulting from aberrant Wnt signaling or mutation of the β-catenin gene leads to breast cancer [7]. In combination with Tcf/lymphoid enhancer factor (Lef), nuclear β-catenin transactivates the gene encoding cyclin D1, which induces mammary hyperplasia [7]. Nuclear β-catenin also upregulates the expression of pro-invasive proteins [8]. Therefore, the subcellular distribution of β-catenin remarkably affects the phenotype and behavior of tumor cells. Overaccumulated cytoplasmic β-catenin might also be a malignant marker for breast cancer, because of a certain relationship between cytoplasmic and nuclear β-catenin in adenocarcinomas [9].

In the present study, we examined the expression and location of β-catenin in 241 breast cancer samples, and its relationship to the clinicopathologic characteristics and prognoses of breast cancer patients.

Materials and methods

Patients and tissue specimens

This study enrolled 241 patients with histologically confirmed breast cancer who had undergone mastectomy in the Surgical Oncology

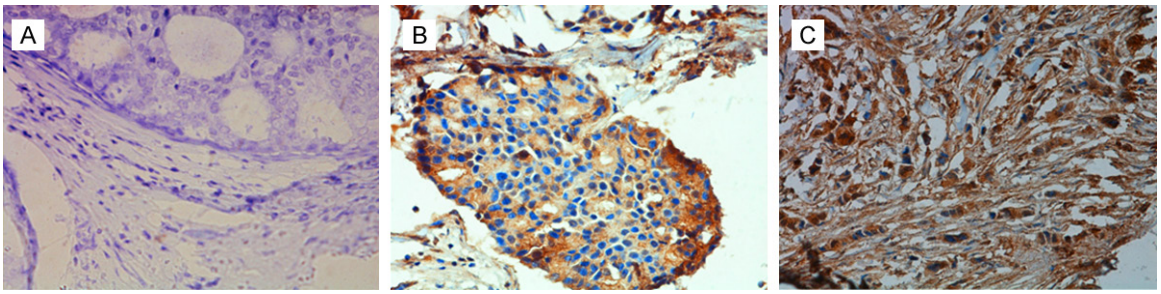

Figure 1. A. Representative β-catenin immunohistochemical staining results in a breast tumor at 400× magnification. Tissues had no non-specific stain. B. Representative β-catenin immunohistochemical staining results in a breast tumor at 400× magnification. β-catenin is expressed in breast cancer mesenchyme. C. Representative β-catenin immunohistochemical staining results in a breast tumor at ×400 magnification. β-catenin is present in the cytoplasm of breast cancer cells.

Department of the First Affiliated Hospital of China Medical University from January 2006 to September 2007. All patients had received curative surgery, and resected specimens and more than 10 lymph nodes were histopathologically examined. Complete clinical records and expression of ERs, PRs, HER2, and Ki-67 were also examined. The study protocol was approved by the Ethics Committee of China Medical University, and informed consents were obtained from all participants in the study.

Antibodies

Rabbit monoclonal antihuman β-catenin antibodies (Santa Cruz Biotechnology, CA, USA) were diluted and applied as recommended by the manufacturer. Horseradish peroxidase conjugated secondary antibody was used in this study. Mesenchymal were regarded as positive control, while the primary antibody was substituted by phosphate-buffered saline in negative control.

Immunohistochemical analysis

Tissue samples were fixed in a 4% formaldehyde solution (pH 7.0), embedded in paraffin, sliced into 4-μm-thick sections, and placed on glass slides pretreated with 3-aminopropyltriethoxysilane. Tissue samples were stained with hematoxylin and eosin to determine β-catenin protein expression. Immunohistochemical staining of β-catenin was performed as previously described [10]. Samples were observed via an inversion fluorescence microscope (Olympus, Tokyo, Japan) at high magnification (400×). β-catenin expression was scored semiquantitatively according to the following criteria: a score of 0 if <1% of morphologically unequivocal neoplastic cells discretely expressed cytoplasmic β-catenin; a score of 1+ if ≥1 and <10% of morphologically unequivocal neoplastic cells discretely expressed cytoplasmic β-catenin; and a score of 2+ if ≥10% of morphologically unequivocal neoplastic cells discretely expressed cytoplasmic β-catenin. Grades 1+ and 2+ were considered β-catenin positive.

Statistical analysis

All data were analyzed using SPSS software (version17.0; SPSS Inc., IL, USA). Relationships between β-catenin expression and other parameters were evaluated using the chi-square test and Fisher's exact test. Regression values indicated no correlation (0.0-0.2), a low degree of correlation (0.2-0.4), a moderate degree of correlation (0.4-0.6), a marked degree of correlation (0.6-0.8), or a high degree of correlation (0.8-1.0). Disease-specific survival was analyzed according to the Kaplan-Meier method. Multivariate analysis was performed using the Cox proportional hazards model with forward stepwise selection. *P* values less than 0.05 were considered statistically significant.

Results

Patient characteristics

The overall mean age of the patients in our study was 51 years (range 33-72 years). Among the 241 patients, 139 had no lymph node metastases. Based on postoperative clinical stage, adjuvant treatments were administered at the time of diagnosis in accordance with

Table 1. Correlations between growth patterns and clinicopathological features (n=241)

Variables	N	β-catenin (-) (n=154)	Mesenchyme (+) (n=46)	Nuclei/Plasma (+) (n=41)	*P* value
Age					0.686
≤ 45	85	57	16	12	
>45	156	97	30	29	
Tumor size					0.906
T1	49	34	8	7	
T2	184	115	36	33	
T3	8	5	2	1	
Histological grade					0.030
I	30	23	4	3	
II	138	91	30	17	
III	73	40	12	21	
Metastatic nodes					0.541
Positive	102	68	20	14	
Negative	139	86	26	27	
TNM stage					0.574
I	11	7	2	2	
II	177	116	35	26	
III	53	31	9	13	
ER status					0.719
Positive	90	59	18	13	
Negative	151	95	28	28	
PR status					0.753
Positive	95	62	19	14	
Negative	146	92	27	27	
Her-2 status					0.490
Positive	91	61	18	12	
Negative	150	93	28	29	
Ki-67 status					0.006
≤ 14%	81	63	10	8	
>14%	160	91	36	33	

established national comprehensive cancer network guidelines. β-catenin, ER, PR, and HER2 were expressed in the nucleus and/or cytoplasm in 41 (17.0%), 90 (37.3%), 95 (39.4%), and 91 (37.8%) patients, respectively. The numbers of patients with clinical stage I, II, and III cancer were 11 (4.6%), 177 (73.4%), and 53 (22.0%), respectively. Histological grades were I, II, and III for 30 (12.4%), 138 (57.3%), and 73 (30.3%) patients, respectively.

Correlations between β-catenin expression and clinicopathological features

Immunohistochemical examination showed that β-catenin was expressed in the nucleus and/or cytoplasm of breast cancer cells (**Figure 1C**) and mesenchymal (positive control) (**Figure 1B**). β-catenin was not detected when the primary antibody was omitted (negative control) (**Figure 1A**). β-catenin expression was observed in 41 (17.0%) patients and was not associated with age, histological type, tumor stage, or lymph node metastasis (**Table 1**). Overall, breast cancers patients with a Ki-67 labeling index ≥14% expressed more β-catenin than patients with a Ki-67 labeling index <14% (*P*=0.006). β-catenin expression also significantly correlated with histological tumor grade (*P*<0.05): grade I tumors displayed low β-catenin expression, whereas grade III tumors displayed strong β-catenin expression (*P*<0.05). β-catenin expression did not significantly differ in patients older than 45 years of age compared with those 45 years of age or younger.

Correlations between β-catenin expression and ER, PR, HER2, and Ki-67 expression

Univariate analysis showed β-catenin expression significantly correlated with Ki-67 expression (*P*=0.006) but not ER, PR, or HER2 expression (**Table 1**).

Survival outcome

Survival analysis showed that total β-catenin expression (including mesenchyme and nuclei/cytoplasm) did not significantly correlated with breast cancer-specific survival in the 241 patients in our study (*P*>0.05, log-rank test; **Figure 2**). While, subgroup survival analysis showed that there is statistical significance between different expression statuses of β-catenin. β-catenin-negative patients clearly

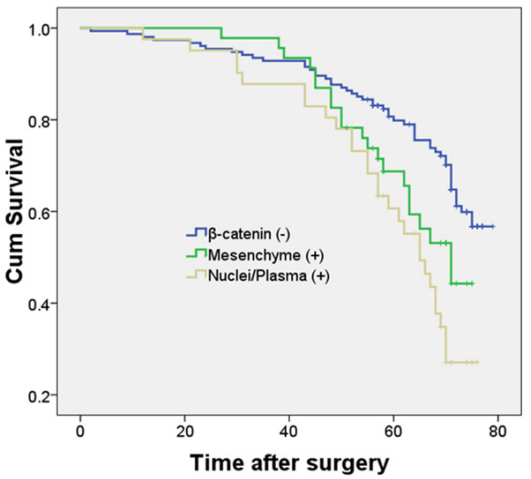

Figure 2. The 5-year survival rate in patients with different expression status of β-catenin (*P*=0.753).

Table 2. Subgroup survival analysis of different expression status of β-catenin

Characteristic	*P* value
β-catenin (-) vs Mesenchyme (+)	0.142
β-catenin (-) vs Nuclei/Plasma (+)	<0.01
Mesenchyme (+) vs Nuclei/Plasma (+)	0.039

had a longer survival time. Expression of β-catenin in the nucleus and/or cytoplasm was associated with shorter breast cancer-specific survival compared with the absence of β-catenin expression (*P*<0.01, log-rank test; **Table 2**). Furthermore, there was association between β-catenin expressed in the nucleus/cytoplasm and that of mesenchyme (*P*<0.05, log-rank test; **Table 2**). However, expression of β-catenin in the mesenchyme was not association with shorter breast cancer-specific survival compared with the absence of β-catenin expression (*P*>0.05, log-rank test; **Table 2**).

Discussion

Breast cancer is a clinically heterogeneous disease [11]. Many factors influence the prognosis and probability of responding to systemic therapies (e.g., tumor size, grade, and histological type, lymph node involvement, and ER, PR, and HER2 status), but they do not fully describe the diverse clinical courses of breast cancer [5-7]. Consequently, much effort has been focused on the clinical significance, interrelationships, and discovery of biomarkers-all aimed at optimal utilization of available therapies and the development of novel therapies based on improved cancer models.

β-catenin has many functions including the regulation of cell-cell adhesion and transcription. The canonical Wnt signaling pathway stabilizes cytoplasmic β-catenin; in the absence of Wnt signals, a destruction complex phosphorylates serine/threonine sites in the N-terminal domain of β-catenin, which leads to its ubiquitination and proteosomal destruction. Wnt ligands prevent β-catenin degradation via Frizzled receptor-mediated signaling through the low-density lipoprotein-related proteins (LRPs). Proteins downstream of the LRPs (Disheveled and axin) inhibit the destruction complex, resulting in cytoplasmic accumulation of β-catenin and its consequent translocation to the nucleus. In the nucleus, β-catenin associates with the Lef DNA-binding proteins and upregulates cyclin D1 and c-Myc, which promote the infiltration and metastasis of tumor cells [12]. Because its stability and abundance is regulated by the Wnt pathway, β-catenin is an ideal molecule for determining the molecular basis of malignancy. β-catenin accumulates in many malignant cancers in breast, ovarian, glioma, and prostate, and a related abnormal activation of the Wnt pathway occurs frequently in these cancer [13-18].

A previous study of β-catenin alterations in colorectal cancer found that β-catenin activation correlated with better cancer-specific and overall survival among obese patients and that the status of β-catenin influenced tumor cell behavior [19]. This study has considerable clinical implications. Therefore, we thought that β-catenin status might serve as a predictive biomarker in breast cancer.

In our study, β-catenin was expressed in the nucleus, cytoplasm, and/or mesenchyme of 36.1% of breast cancer patients. Besides, its expression appeared to increase as tumors progressed: histological stage I tumors had the lowest β-catenin protein expression levels, while stage III tumors had the highest. These findings suggest that β-catenin may be a marker of advanced breast cancer and thus may represent a new diagnostic or therapeutic target for detection of advanced cancers and timely intervention. Of the four pathological indicators of breast cancer (ER, PR, HER2, and Ki-67) that we examined, only Ki-67 expression correlated

with β-catenin expression. Therefore, β-catenin may promote breast cancer by stimulating cell proliferation, but further experiments are required to elucidate the exact mechanism. As a result, we suggest that examination of β-catenin expression and subcellular location may benefit our understanding of biological behavior and growth patterns.

In a survival analysis, β-catenin expressed in the nucleus and/or cytoplasm significantly correlated with poor postoperative disease-specific survival. In addition, there was a difference between β-catenin expression in the nucleus/cytoplasm and that of mesenchyme, but no difference between β-catenin expression in the mesenchyme and the β-catenin negative group in breast cancer-specific survival.

Brabletz et al. [9] found abnormal β-catenin expression in the 89% of the high clinical grade adenocarcinomas examined; specifically, increases in β-catenin expression in the nucleus were accompanied by diffuse cytoplasmic expression of β-catenin. It is possible that abnormal accumulation of β-catenin in the nucleus resulted in the loss of E-cadherin and consequent cell polarity and cell adhesion. This in turn may have simultaneously activated cell shedding and induced the expression of invasion-related genes, causing the release of tumor cells from the tumor into blood and lymph vessels. In addition, numerous studies have shown that β-catenin participates in the epithelial-mesenchymal transition (EMT) by regulating the expression of vimentin. The release of β-catenin from dismantled E-cadherin-containing cell-cell junctions during EMT and the consequent β-catenin-mediated gene transactivation are important events in EMT regulation [20]. Therefore, β-catenin may play a key role in the infiltration or metastasis of tumor cells.

There are some differences between our results and previously reported results. López-Knowles et al. [21] found that cytoplasmic β-catenin expression was not only associated with high-grade tumors and high proliferation rates, but also with ER and HER2 expression and lymph node metastasis. Reasons for differences between their study and ours include discrepancies between basic and clinical research.

In conclusion, we believe that our findings provide important information about a potential prognostic breast cancer marker. However, owing to the small sample size and other conditions of this study, the specific role of β-catenin in breast carcinoma remains unclear. More studies are needed to elucidate the underlying mechanisms of breast cancer and the impact of β-catenin on breast cancer.

Acknowledgements

This study was funded by China National Natural Science Foundation (No. 81441052 and 81402384).

Disclosure of conflict of interest

None.

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