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Asia-Pacific Journal of Blood Types and Genes

2018, 2(2):111-116

APJBG

β-catenin is up-expressed and predicts poor overall survival of breast cancer

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ABSTRACT

Breast cancer (BC) is one of the most common malignant tumors in women.The majority of BC cells contain at least one or more up-expressed oncogene. β-catenin is found overexpressed in various epithelial cell cancers and has the function of inducing cancer cell proliferation, invasion and metastasis. However, the expression of β-catenin and its prognostic value in BC is not yet clear. In this study, mRNA and β-catenin proteins expressed in BC tissues have been explored. Quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC) on tissue microarrays (TMA) were performed to examine the level of β-catenin mRNA and protein in BC tissues. The association between β-catenin and clinical characteristics and prognostic value were also explored. β-catenin mRNA and protein were found over-expressed in BC tissues when compared with matched tumor neighbor tissues. A high degree of β-catenin staining in BC tissues was significantly associated with tumor size, Ki67 expression, lymph node status and TNM stage. β-catenin up-expression was also able to predict poor overall survival (OS) rates. These results indicated that β-catenin may be a useful prognostic molecular biomarker for BC patients.

Keywords: β-catenin, breast cancer, prognosis, biomarker

INTRODUCTION

Breast cancer (BC) is one of the most common malignant tumors in women, and the incidence is increasing, with more than 1.2 million new cases diagnosed each year worldwide[1–2]. β-catenin is the ultimate signaling molecule in theWnt/β-catenin signaling pathway[3–5]. In the absence of Wnt ligands, most β-catenin protein in the cytoplasm are able to combine with E-cadherin in the cell membrane, as well as with APC, Axin and GSK3 and CK Ⅰ, causing the degradation of composites, and possibly the phosphorylation of different residues and the degradation of β-catenin. The β-catenin signal has been confirmed as playing an important role in the development and transformation of tumors, such as colon cancer, pancreatic cancer, breast cancerand gastric cancer[3,6–7]. It has been reported that the Wnt/β-catenin signaling pathway is involved in the proliferation, invasion, metastasis and resistance of gastric cancer cells[8]. Meanwhile, interference with the Wnt/β-catenin signaling pathway can inhibit the proliferation, invasion and metastasis of gastric cancer and increase the sensitivity of cancer cells to chemotherapy drugs[9].

At present, little is known about the level of β-catenin expression, and its prognostic value in BC is not yet clear. Therefore, the relationship between β-catenin expression, clinical characteristics, and overall survival rates for BC have been explored in this study.

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Conflict of interests: The authors have declared no conflict of interests.

MATERIALS AND METHODS

Patient specimens

Two hundred and fifty-five cases of BC were obtained from the department of pathology, Nanjing First Hospital, from 2008 to 2012. These samples include 255 cases of cancer tissue with 43 cases of matched tumor neighbor tissues. Clinical information for tissue donor patients included age, location, tumor size, TNM stage, ER/PR/HER2, Ki-67, histologic grade, lymph node status, metastasis and overall survival. The study protocol was approved by the hospital´s Human Research Ethics Committee.

Quantitative real-time polymerase chain reaction (qRT-PCR)

β-catenin expression levels in 32 pairs of human BC tissues were compared with matched tumor tissues. Cell RNA was extracted by TRIzol reagent (Invitrogen, USA) and then reverse transcribed into cDNA using a PrimeScript™ RT reagent kit (Takara, Japan), according to the manufacturer´s protocol. Human GAPDH served as the internal control. The primers used in the study were as follows; GAPDH F: 5´ -GAAGGTGAAGGTCGGAGTC-3´, and R: 5´ -GAAGATGGTGATGGGATTTC-3´. β-catenin F, 5´- GAAACGGCTTTCAGTTGAGC -3´, and R, 5´-CTGGCCATATCCACCAGAGT -3´ (Genescript. China). qRT-PCR was performed on an ABIPRISM 7500HT Sequence Detection System (Applied Biosystems, USA) in 96-well plates. Relative expression levels were calculated as ratios normalized against those of GAPDH. Results were normalized to respective internal controls. The Ct-value for each sample was calculated using the ΔΔCt method, and results were expressed as $2^{-\Delta\Delta Ct}$.

Tissue microarrays construction (TMA) and immunohistochemistry

The tissues from 255 BC and 43 matched tumor-adjacent tissues were formalin-fixed and paraffin-embedded for the study. TMA was produced in the department of pathology, Nanjing first hospital, Nanjing, Jiangsu, China, using the Tissue system Quick-Ray (Unitma, Korea) manual. Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded BC and produced in the recipient paraffin blocks. The methods and IHC score can be seen in our previous articles. β-catenin was detected using amonoclonal antibody mouse anti-human β-catenin (dilution 1:100) (Santa Cruz, USA).

Statistical analysis

The statistical analysis was performed using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL). The Student´s *t*-test and Pearson χ^2 test were used to determine the statistical significance between the different groups. Both the Kaplan-Meier method and a LogRrank test were used to evaluate the significant difference between overall survival(OS) in patients. The univariate and multivariate hazard ratios for the variables were analyzed by a cox proportional hazards model. A two-tailed *P*-value of less than 0.05 was considered as statistically significant.

RESULTS

The level of β-catenin mRNA expression of BC tissues

The expression level of β-catenin mRNA through qRT-PCR in 32 pairs of breast cancer tissues were detected. The results showed that β-catenin mRNA was over-expressed in BC tissues compared to matched tumor neighbor tissues, with an average upregulation of 2.176±1.773 (*P* <0.001, ***Fig.1***).

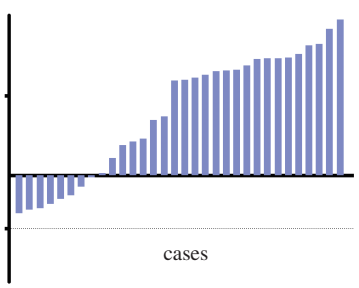

Fig. 1 **β -catenin mRNA expression in 32 pairs breast cancer tissues.** β-catenin mRNA expression was detected by qRT–PCR and normalized to GAPDH.

β –catenin protein expression of BC tissues compared with tumor–adjacent tissues by IHC

β-catenin protein was localized to the cell membrane and cytoplasm (***Fig. 2***). Using the x-tile software for TMAs data analysis(http://www.tissuearray.org/rimmlab) [10], the cutoff point was defined according to OS in BC patients. A cutoff value of 110 was selected, 0–110 were considered low expression, and 111–300 were considered high expression.

Highly expressed β-catenin expression in BC tissues (159/255, 62.35%) were higher than tumor-adjacent tissues (8/43, 18.61%), χ^2=33.98, *P*<0.001 (***Table 1***).

Association of β–catenin protein expression with clinicopathologic characteristics in BC patients

As shown in ***Table 2***, high β-catenin staining in BC

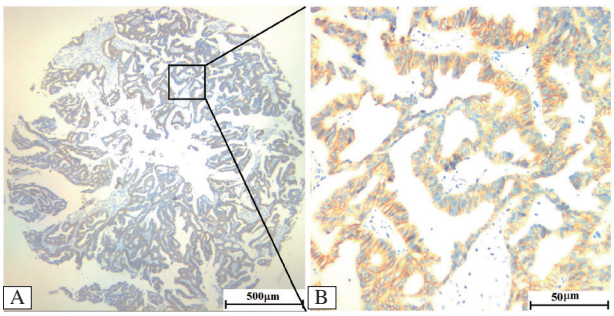

***Fig. 2* Representative pattern of β–catenin protein expression in BC tissues on TMA sections.** High expression of β–catenin in breast cancer tissues (IHC score, 270), β–catenin staining with ×4 (bar=500 μm) (A), with ×20 (bar=50 μm) (B).

tissues was significantly associated with tumor size (χ^2= 5.066, P = 0.012), Ki-67 expression (χ^2= 4.384, P = 0.029), lymph node status (χ^2= 4.304, P = 0.032 and TNM stage (χ^2= 4.904, P = 0.026). However, the present study did not find a significant association between β-catenin protein expression and tumor location, age, pathology stage, ER, PR, HER2, metastasis,etc (***Table 2***).

Table 1 **β –catenin expression in BC tissues compared with tumor–adjacent tissues**

Group	*n*	β–catenin expression [*n*(%)]	
		Low or no	High
Breast tissues cancer	255	96 (37.65)	159 (62.35)
Tumor–adjacent tissues	43	35 (81.39)	8 (18.61)

χ^2=33.98,P<0.001.

Up-expression of β-catenin protein correlates with poor OS of BC patients

To evaluate the relationship between β-catenin and prognostic factors in BC patients, both univariate and

Table 2 **Associations between high β -catenin expression and clinicopathologic characteristics in BC patients**

Characteristic	*n*	β-catenin expression [*n*(%)]		χ^2	*P* valae
		Low or no	High		
Tumor location				0.550	0.734
Left	154	70 (49.35)	84 (54.55)		
Right	101	43 (42.57)	58 (57.43)		
Age				2.198	0.087
Premenopausal	110	55 (50.00)	55 (50.00)		
Postmenopausal	145	62 (42.76)	83 (57.24)		
Pathology stage				0.137	0.341
1	47	20 (42.55)	27 (57.45)		
2	159	70(45.28)	89 (55.97)		
3	49	20 (40.81)	29 (59.18)		
Tumor size				5.066	0.012
⩽ 2cm	59	37 (62.72)	22 (37.29)		
> 2cm	195	82 (42.05)	114 (58.46)		
ER				1.473	0.178
Positive	98	38 (38.78)	60 (61.22)		
Negative	157	74 (47.13)	83 (52.87)		
PR				0.944	0.379
Positive	125	51 (43.20)	74 (59.20)		
Negative	130	62 (48.50)	68 (51.50)		
HER2				0.042	0.817
Positive	93	43 (46.20)	50(53.80)		
Negative	162	73 (45.06)	89(54.94)		
Ki-67				4.384	0.029
Positive	136	56 (41.18)	80 (58.82)		
Negative	119	61 (51.26)	58 (48.74)		
Lymph node status				4.304	0.032
N0	111	66 (59.46)	45 (40.54)		
N1+2+3	144	61 (42.36)	83 (57.64)		
Metastasis					
M0	64	27 (42.19)	37 (57.81)	0.023	0.487
M1	191	83 (43.46)	108 (56.54)		
TNM stage				4.904	0.026
1	90	46 (51.11)	44 (48.89)		
2	97	43 (44.33)	54 (55.67)		
3	68	20 (29.41)	48 (70.59)		

multivariate analyses have been used(***Table 3***). High β-catenin protein expression (HR: 3.416, 95% CI: 1.432–3.243; *P*<0.001) was associated with a shorter survival in univariate analysis, along with tumor size (HR: 2.962, 95% CI: 1.189–3.604; *P*= 0.028), Ki-67 expression (HR: 3.483, 95% CI: 1.388–3.432; *P*=0.021), lymph node status (HR: 2.988, 95% CI: 1.521–2.097; *P*=0.042), metastasis (HR: 2.342, 95% CI: 1.126–2.963; *P*=0.034) and TNM stage (HR: 4.012, 95% CI: 1.623–3.624; *P*<0.001). In multivariate analysis, up-expressed β-catenin (HR: 2.634, 95% CI: 1.012–3.023; *P*=0.011), Ki-67 expression (HR: 2.415, 95% CI: 1.023–2.362; *P*=0.022) and TNM stage (HR: 2.823, 95% CI: 1.402–3.243; *P*=0.021) was associated with poor OS. Furthermore, data from the Kaplan-Meier survival curves showed that high β-catenin expression was significantly associated with poor OS in BC patients (***Fig.3***). These results indicated that β-catenin is a prognostic marker for BC patients.

DISCUSSION

BC is the most common malignancy in women worldwide[11–12]. According to statistics, the incidence of BC in China is 169 000, which ranks it as the second most common in malignant tumor[13]. The occurrence of BC is closely related to environment and lifestyle, including nutritional status andweight loss, which has been confirmed as an effective primary prevention measure. Furthermore, a reduction in mortality and improvements in the survival of BC patients could be improved through the effective screening of higher risk groups[14].

β-cateninmainly exists in the cell membrane and cytoplasm of various types of cells and has many functions[15]. The main function of β-catenin is to regulate adhesion between cells causing the expression of certain genes. The expression level of β-catenin

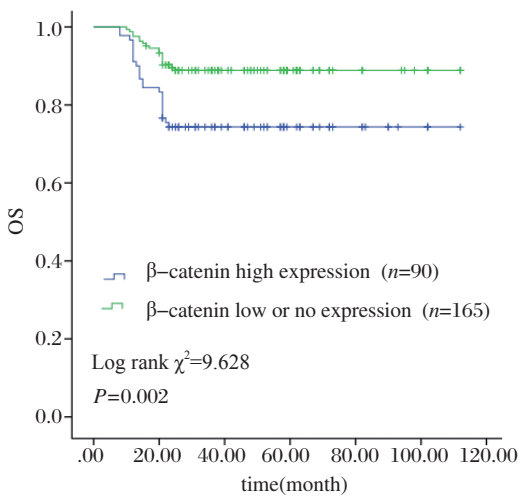

***Fig. 3* Survival curves for breast cancer using the Kaplan–Meier method and log–rank test.** Overall survival curves for patients with β-catenin high expression (blue line, 1), and low or no expression (green line, 2).

***Table 3* Associations between high β–catenin expression and clinicopathologic characteristics in BC patients**

prognostic indicator	Univariate analysis			Multivariate analysis		
	HR	P>|z|	95% CI	HR	P>|z|	95% CI
β–cateninex pression						
High vs. Low or no	3.416	<0.001	1.432–3.243	2.634	0.011	1.012–3.023
Location						
left vs.right	0.93	0.572	0.534–1.285			
Age						
premenopausal vs.postmenopausal	0.834	0.926	0.501–1.934			
Pathology stage						
1 *vs*.2 *vs*.3	0.623	0.623	0.531–1.383			
Tumor size						
≤2CM *vs*.>2CM	2.962	0.028	1.189–3.604			
ER						
Positive *vs*. negative	1.834	1.013	0.623–2.433			
PR						
Positive *vs*. negative	0.823	0.523	0.412–1.653			
HER2						
Positive *vs*. negative	1.153	1.823	0.595–2.623			
Ki–67						
Positive *vs*. negative	3.483	0.021	1.388–3.432	2.415	0.022	1.023–2.362
Lymph node status						
N0 *vs*. N1+2+3	2.988	0.042	1.521–2.097			
Metastasis						
M0 *vs*. M1	2.342	0.034	1.126–2.963			
TNM stage						
1 *vs*. 2 *vs*. 3	4.012	<0.001	1.623–3.624	2.823	0.021	1.402–2.234

can also directly regulate the Wnt signaling pathway. When β-catenin degradation is blocked, this can lead to an accumulationand enters the nucleus. Thereby leads to the occurrence of cancer by activating the Wnt signaling pathway[9,16].

This study, explored β-catenin mRNA and protein levels in BC tissues through qRT-PCR and IHC. β-catenin mRNA levels were much high than in matched neighboring matched tissues. The results were similar toNadanaka´s research[17], which studied the increased cytoplasmic and nuclear β-catenin levels observed in basal-like breast cancers *in vitro*. The study further examined the protein levels in BC patients through microarray examination, which included 255 samples of BC tissue, and associated clinical and follow-up data. As with mRNA expression, high β-catenin protein expression was detected in a larger proportion of BC tissues (159/255, 62.35%) than in matched tumor tissues (8/43, 18.61%). Furthermore, the authors also found that high β-catenin staining in BC tissues was significantly associated with tumor size, Ki-67 expression, lymph node status, TNM stage. β-catenin up-expression was also a predictor of poor OS.

The mutation and abnormal expression of β-catenin is known to be found in many tumors, so β-catenin is highly likely tobe related to the occurrence and development of these tumors. Ma *et al*[18]. reported that β-catenin may play a critical role in BC immunity, particularly in HER2-enriched and triple negative BC (TNBC), and may serve as a potential target for regulating immune infiltrates in breast cancer. Others reported that the abnormal expression of β-catenin was associated with poorer prognoses in cancer patients[19–20]. In the present study, the relationship between β-catenin and HER2 expression could not be found. This may be due to the differences in the molecular characteristics and epidemiology of BC. These reports nonetheless supported our hypothesis, that β-catenin may be a useful prognostic molecular biomarker for BC patients.

This study had several limitations: it is the lacking of an appropriate assay to explore the role of β-catenin *in vivo* and *in vitro*, and a further examination is needed to explore the molecular mechanism of β-catenin in BC.

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(Received 07 Feburary 2018, Revised 04 April 2018, Accepted 17 April 2018)