RESEARCH ARTICLE

Predictive Value of the Pattern of β-Catenin Expression for Pathological Response to Neoadjuvant Chemotherapy in Breast Cancer Patients

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Abstract

Purpose: This study aimed to explore the association of β-catenin expression pattern with pathological response after neoadjuvant chemotherapy in breast cancer (BC) patients. Materials and Methods: In this retrospective exploratory study, data for 50 BC patients who received neoadjuvant chemotherapy were recorded. β-catenin expression in tumours was assessed using immunohistochemistry and classified as either membranous or cytoplasmic according to the pattern of staining. Distributions of different clinico-pathological parameters according to β-catenin expression were assessed using the Chi-square test. Logistic regression analysis was used to assess any relation of the pattern of β-catenin expression with the pathological response. Results: Cytoplasmic β-catenin expression was detected in 34% of BCs. Among our cases, 52% were hormonal receptor (HR)-positive, 24% were HER2-positive, 74% were clinical stage III and 74% received both anthracycline and taxane-based chemotherapy. Patients with cytoplasmic expression were more commonly younger than 40 years at diagnosis (cytoplasmic, 41.2% vs. no cytoplasmic expression, 12.1%, p=0.03). By doing t-test, cytoplasmic β-catenin expression was linked with a higher body mass index compared to membranous-only expression (mean± SD 33.0 ± 4.47 *vs.* 29.6 ±6.01, respectively, p=0.046). No significant associations were found between β-catenin expression and other parameters such as HR and HER2 status, or clinical stage. Complete pathological response (pCR) rate was twice as great in patients with membranous expression but without statistical significance (membranous-only, 33.3% *vs.* cytoplasmic, 17.6%, OR= 2.3, 95% CI= 0.55-9.87, p=0.24). Conclusions: This study suggests that cytoplasmic β-catenin expression may be linked with lower probability of achieving pCR after neoadjuvant chemotherapy. These data need to be validated in a larger cohort of patients.

Keywords: β-catenin - breast cancer - neoadjuvant chemotherapy

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Introduction

Neoadjuvant chemotherapy (NAC) has been the standard of care for patients with inflammatory and locally advanced breast cancer (BC). It is increasingly used in operable breast tumours to allow for breast conservation and improving cosmetic results. Nowadays, NAC is increasingly utilized to assess the activity of new therapeutic agents to accelerate their clinical development (Fisher et al., 1998; Bear et al., 2006; Van der Hage et al., 2001). However, NAC carries the risk of disease progression in a proportion of patients. Biomarkers that predict response to therapy may eliminate unnecessary treatments and could predict pathological complete response (pCR) which may be linked with improved long term survival (Cortazar et al., 2014). Several clinical, pathological and molecular predictors of response to NAC have been studied (Rouzier et al., 2005; Hess et al., 2006; Nolen et al., 2008).

β-catenin is a multifunctional protein located at the intracellular side of the cytoplasmic membrane. It has a critical role in cell-to-cell adhesion by linking cadherins to the actin cytoskeleton and has a central role in transcriptional regulation in the Wnt signaling pathway (Geyer et al., 2011). Indeed, upon Wnt activation, β-catenin is translocated from the membrane to the cytoplasm and then to the nucleus, where it interacts with transcriptional activators to modulate a number of target genes associated with increased growth, invasion and cellular transformation, such as c-MYC 2 and cyclin D1 (Geyer et al., 2011).

Noteworthy, high level of cytoplasmic and nuclear localization of β-catenin is characteristic of cancer stem cell populations that are resistant to chemotherapeutics and capable of initiating new tumours (Chau et al., 2012; Sinnberg et al., 2011). Several studies have reported

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increased cytoplasmic and nuclear β-catenin expression in primary BC, especially basal-like cancers (Lin et al., 2000; Prasad et al., 2007; Khramtsov et al., 2010).

However, the association of β-catenin with survival outcome in BC has been a matter of controversy. Although some have reported that aberrant β-catenin expression is linked with poor outcome (Lopez-Knowles et al., 2010), others have failed to demonstrate such correlation (Pedersen et al., 2002; Wong et al., 2002). This is not surprising, given the difficulties in assessing β-catenin/Wnt pathway activation in BC due to its complexity, challenging interpretation of β-catenin subcellular localization, specificity of different antibodies and other technical issues (Chung et al., 2004).

The present study aimed to address the distribution of β-catenin expression (membranous vs. Cytoplasmic) in invasive BC and its correlations with clinico-pathological features in addition to exploring its value in predicting pathological response to neoadjuvant chemotherapy in BC patients.

Materials and Methods

Study population

The study group involved female patients with histologically confirmed BC who received neoadjuvant chemotherapy from September 2012 to September 2013 at King Abdullah Medical City in Saudi Arabia. Paraffin blocks of diagnostic biopsies must be available for β-catenin testing.

Study design and procedures:

In this retrospective exploratory study, clinico-pathological data was collected including age at diagnosis, menopausal status, tumour multicentricity, type and number of chemotherapy cycles (<6 *vs*. 6-8), pre-chemotherapy tumour size and lymph node (LN) status as assessed at baseline mammograms. Clinical stage before starting chemotherapy was recorded according to the TNM staging system of the American Joint Committee on Cancer (AJCC), 7th edition. Tumour phenotype (ER, PR, HER2 status) and Ki67 expression were recorded from reports of diagnostic biopsies taken before starting chemotherapy. Using immune-histochemistry (IHC), β-catenin expression was assessed and was classified as either membranous or cytoplasmic according to the pattern of staining.

Pathological tumour size and nodal status were recorded from pathological reports of definitive breast surgery. We assessed the relation of β-catenin expression (cytoplasmic vs. no cytoplasmic expression) with pCR and pathological stage. We defined pCR as the absence of any invasive carcinoma or ductal carcinoma in situ at the breast or axillary LNs at the time of definitive breast surgery. Approval from the institutional review board was obtained before starting the study procedures.

Statistical analysis

The data was analyzed using STATA version 11.0. Numeric data was presented as mean values +/- standard deviation (SD). Categorical variables were presented as percentages. Distributions of different clinico-pathological parameters and tumour phenotype according to β-catenin expression pattern (cytoplasmic vs. no cytoplasmic expression) were assessed using Chi-square test. Logistic and ordinal regression analyses were used to assess the relation of the pattern of β-catenin expression with pCR and pathological stage respectively. The relations of β-catenin expression with numerical variables such as age and body mass index (BMI) were assessed using t-test. An alpha level of <0.05 was considered significant for all two tailed comparisons.

Results

Cytoplasmic β-catenin expression was detected in 34% of cases. Simultaneous membranous and cytoplasmic expression was detected in 16% of cases. Among our patients, 52% were hormonal receptors (HR)-positive, 24% were HER2-positive, 74% had clinical stage III, 78% had clinical T3/T4 tumours and 74% received both anthracycline and taxane-based chemotherapy

Table 1. Patient Characteristics

	Number	percentage
Age		
40 or less	11	22%
More than 40	39	78%
Multicentricity		
No	34	68%
yes	16	32%
DCIS		
No	36	72%
Yes	14	28%
Lymphovascular invasion		
No	34	68%
yes	16	32%
ER		
Negative	26	52%
positive	24	48%
PR		
Negative	28	56%
Positive	22	44%
Pathologic response		
No	36	72%
yes	14	28%
Pathological stage		
0-2	35	70%
3	15	30%
Her 2neu		
Negative	38	76%
Positive	12	24%
Tumor size		
T1 &T2	11	22%
T3&T4	39	78%
Clinical Stage		
Stage I & II	13	26%
Stage III	37	74%
Pathology		
IDC	47	94%
Other	3	6%
Grade		
I	2	4%
II	27	54%
III	21	42%

Table 2. Distribution of Different Parameters According to B-Catenin Expression Pattern

	Cytoplasmic β- catenin	Membranous β- catenin	P-value
Age			
40 or less	7(41.2)	4(12.1)	0.03
More than 40	10(58.8)	29(87.9)	
Multicentricity			
No	13(76.5)	21(63.6)	0.52
yes	4(23.5)	12(36.4)	
DCIS			
No	11(64.7)	25(75.8)	0.51
Yes	6(35.3)	8(24.2)	
Lymphovascular invasion			
No	10(58.8)	24(72.7)	0.35
yes	7(41.2)	9(27.3)	
ER			
Negative	8(47.1)	18(54.5)	0.77
positive	9(52.9)	15(45.5)	
PR			
Negative	9(52.9)	19(57.6)	0.77
Positive	8(47.1)	14(42.4)	
Her 2neu			
Negative	14(82.4)	24(72.7)	0.51
Positive	3(17.6)	9(27.3)	
Tumour size			
T1 &T2	6(35.3)	5(15.2)	0.15
T3&T4	11(64.7)	28(84.7)	
Clinical stage			
Stage I &Stage II	5(29.4)	8(24.2)	0.74
Stage III	12(70.6)	25(75.7)	
Pathology			
IDC	15(88.2)	32(97.0)	0.26
Other	2(11.8)	1(03.0)	
Menopausal status			
Premenopausal	13(76.5)	17(51.5)	0.09
Postmenopausal	4(23.5)	16(48.5)	
Grade			
I	0	2(06.1)	0.56
II	10(58.8)	17(51.5)	
III	7(41.2)	14(42.4)	

Table 3. Pathological Response According to B-Catenin Expression Pattern

	Cytoplasmic β-catenin	Membranous β-catenin	P-value
Pathological response			
No	14(82.4)	22(66.7)	0.33
Yes	3(17.6)	11(33.3)	
Pathological stage			
0-II	10(58.8)	25(75.8)	0.22
III	7(41.2)	8(24.2)	
Pathological stage			
0	3(17.6)	11(33.3)	0.65
I	2(11.8)	6(18.2)	
II	5(29.4)	8(24.2)	
III	7(41.2)	8(24.2)	

(Table 1). Patients with cytoplasmic expression were more commonly younger than 40 years at diagnosis (cytoplasmic; 41.2% vs. no cytoplasmic expression; 12.1%, p=0.03) (Table 2). Similarly, by doing t-test, cytoplasmic β-catenin expression was linked with younger age at diagnosis (mean± SD; 43.71± 8.89 vs. 51.55 ±8.97, p=0.005). In addition, cytoplasmic β-catenin expression was linked with higher body mass index compared to no cytoplasmic expression (mean± SD, 32.95 ± 4.47 vs. 29.55 ±6.01, respectively, p=0.046).

Patients with cytoplasmic expression were more commonly premenopausal although not statistically significant (cytoplasmic; 76.5% vs. no cytoplasmic expression; 51.5%, p=0.09). No significant associations were found between other parameters and β-catenin expression including HR and HER2 status, clinical stage, tumour grade, multicentricity and lymphovascular invasion (Table 2). Noteworthy, no significant correlation between the pattern of β-catenin expression and Ki67 level (mean± SD, cytoplasmic; 59.06 ±36.98 vs. no cytoplasmic expression; 54.91 ±32.43, p=0.28).

pCR was found in 28% of patients, while partial response and stable disease were found in 60% and 12% of patients respectively. pCR rate was almost doubled in patients without cytoplasmic expression but without reaching statistical significance (no cytoplasmic expression, 33.3% vs. Cytoplasmic, 17.6%, OR= 2.3, 95% CI= 0.55-9.87, p=0.33). Similarly, patients with cytoplasmic expression had more frequently advanced pathological stage (III) compared to no cytoplasmic expression, although not statistically significant (41.2% *vs*. 24.3% respectively, p=0.22) (Table 3).

Discussion

The Wnt/β-catenin signalling pathway plays a critical role in many biological processes, such as embryonic development, stem cell growth and tumour cell survival (Clevers 2006). Activation of the Wnt pathway by Wnt ligands through binding to frizzled proteins and lipoprotein receptor-related proteins 5 and 6 (LRP-5/6), prevents phosphorylation and degradation of β-catenin by the GSK3β/APC/axin destruction complex. β-catenin then accumulates in the cytoplasm and is translocated to the nucleus where it interacts with target genes associated with increased growth, invasion, and cellular transformation (Nelson, Nusse, 2004). Wnt/β-catenin signaling has been implicated in different stages of mammary gland development and is important for mammary oncogenesis (Prosperi, Goss, 2010).

In normal mammary ducts and glands, membranous β-catenin staining is uniformly localized at the intercellular borders (Jiang et al., 2009). In a study that assessed β-catenin expression in tissue samples from 20 benign breast diseases, β-catenin was expressed in the nuclei of myoepithelial and luminal epithelial cells, in addition to moderate membrane staining in the non-tumorous ducts of breast tissues (Jiang et al., 2009). In invasive breast cancer, β-catenin expression displayed different patterns from that of normal tissues with significantly increased cytoplasmic and nuclear staining (Bertolo et al., 2008).

Several studies have evaluated β-catenin expression in BC and its association with outcome (Dolled-Filhart et al., 2006; Lin et al., 2000; Bukholm et al., 1998). In the study of Li et al., 2014, aberrant β-catenin expression was observed in 64.5 % of patients and there was no difference in β-catenin expression in the four molecular subtypes

of BC. Furthermore, the same authors reported that aberrant β-catenin expression was significantly associated with adverse outcome in the entire cohort as well as in each of the different molecular subtypes. Similarly, in another study involving triple negative BC patients, elevated expression of cytoplasmic/nuclear β-catenin was associated with poor outcome (Xu et al., 2012). In a third study involving 215 breast cancer patients, disease-free survival and overall survival were significantly shorter in patients expressing cytoplasmic β-catenin (Fanelli et al., 2008)

In our study, cytoplasmic β-catenin expression was associated with younger age at diagnosis and higher BMI; features usually linked to worse survival outcome. Higher BMI was associated with worse outcome after neoadjuvant chemotherapy. Dawood et al. (Dawood et al., 2008) displayed worse survival outcome in obese and over-weight patients with locally advanced BC compared to normal/underweight counterparts. Similarly, in a recent meta-analysis including 213,075 patients from 82 studies, obesity was associated with poor overall and BC survival in pre- and post-menopausal BC patients (Chan et al., 2014).

In our study, cytoplasmic expression was linked with reduced probability of achieving pCR and higher chance of having advanced pathological stage after NAC, although not reaching statistical significance given the small number of patients included in the study. This may point to the potential role of cytoplasmic β-catenin expression in predicting resistance to NAC and underscores the need for further exploration of this potential biomarker in a larger cohort of patients to set a more definitive conclusion. Noteworthy, the lack of association of β-catenin expression with Ki67 expression may point to its potential value as predictive/prognostic marker independent of the level of tumour proliferation.

In conclusion, this study suggests that cytoplasmic β-catenin expression may be linked with lower pathological response after neoadjuvant chemotherapy. These data need to be validated in a larger cohort of patients.

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