RESEARCH ARTICLE

Prognostic Value of β-catenin Expression in Breast Cancer Patients: a Meta-analysis

De-Pu Zhang[1,2&], Xiao-Wei Li[3&], Jing-He Lang[1*]

Abstract

Background: β-catenin plays a crucial role in the progression of breast cancer (BC) and a prognostic role of in BC patients has been widely reported. However, controversy still remains. Materials and Methods: Identical search strategies were used to search relevant literature in electronic databases updated to July 1, 2014. Individual hazard ratios (HRs) and 95% confidence intervals (CIs) were extracted and pooled HRs with 95%CIs were used to evaluate the strength of association between positive β-catenin expression in different subcellular locations and survival results of BC patients. Subgroup and meta-regression analyses were performed to explore heterogeneity. Funnel plots of Begg's and Egger's linear regression test were used to investigate publication bias. Heterogeneity and sensitivity were also assessed. All the work was completed using STATA. Results: A total of 2,204 patients from 12 evaluative studies were finally included. Pooled HRs and 95%CIs suggested that β-catenin expression in cytoplasm/nucleus had an unfavorable impact on both overall survival (OS) (HR: 1.93, 95%CI: 1.40-2.65) and disease free survival (DFS)/ recurrent free survival (RFS) (HR: 1.60, 95%CI: 1.20-2.13) in BC patients. However, here was no significant association between β-catenin expression in the membranes with OS (HR: 0.65, 95%CI: 0.42-1.02) or DFS/RFS (HR: 0.66, 95%CI: 0.38-1.13). Publication bias was absent in all of the four outcomes. Sensitivity analysis revealed that the results of this meta-analysis were robust. Conclusions: Positive β-catenin expression in cytoplasm/nucleus rather than in membrane is a significant prognostic factor in patients with BC who have been surgically treated.

Keywords: β-catenin - breast cancer - prognosis - meta-analysis

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Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer death in females, and affects 1.38 million individuals worldwide every year (Lee et al., 2012). Prognostic factors are frequently helpful in the clinical management of BC patients and have the potential to improve the quality of individual care for these patients (Saurel et al., 2010). Several independent prognostic factors for patient survival have been identified, including TNM (primary tumor, lymph nodes, metastasis) stage, tumor grade, hormone receptor status and patient age (Colozza et al., 2005; Hayes, 2005).

However, BC is a malignant disease with multiple factors involved in, and it has been reported that molecular mechanisms may affect tumor growth and progression, thereby potentially limit the prognostic value of most potential prognostic biological markers (Coradini and Daidone, 2004). As such, it is indispensable to find new biomarkers which are well regarded as suitable predictors and seem to be more accurate predictions of clinical outcome of patients with BC.

Tumor cells escaping from the primary tumor is the initial step of metastasis, in which cell-cell and cell-matrix interactions play a role. Cell adhesion molecules (CAMs) enable these interactions (Gillett et al., 2001). β-cateninis one of the essential components of CAMs and plays a crucial role in cell-cell adhesion and tissue remodeling. It participates in cell-cell adhesion by binding to the intracellular domain of E-cadherin to form an adherens junction known as the cadherin-catenin complex. β-catenin is also important in Wnt/β-catenin signaling pathway which is known to play a role different stages of mammary gland development and is important for mammary oncogenesis (Incassati et al., 2010; Prosperi and Goss, 2010). Indeed, aberrant β-catenin expression has been widely reported to be related to poor differentiation, lymph node spread, and metastasis in BC (Cheng et al., 2012). However, reports of β-catenin expression in BC and its association with outcome are limited and controversial. Some authors have reported that β-catenin expression is associated with poor prognosis (Lin et al., 2000; Khramtsov et al., 2010; Geyer et al., 2011; Mukherjee et al., 2012; Weissenbacher et al., 2013; Li et al., 2014), but others have failed to demonstrate a correlation between β-catenin expression and outcome (Gillett et al., 2001;

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Chung et al., 2004; Park et al., 2007; Fanelli et al., 2008; Kim et al., 2010; Logullo et al., 2010; Lopez-Knowles et al., 2010; Xu et al., 2012; Ho et al., 2013; Pang et al., 2013).

The discrepancies in conclusions may attribute to some of the defects in individual study such as small sample size or low statistical power. The current study aims to gain a better insight into the correlation between β-catenin expression and survival of BC patients using a meta-analysis. The results showed that overexpression of β-catenin in the cytoplasm and nucleus, rather than in the membrane, was associated with a worse outcome. The meta-analysis suggested that postoperative detection of β-catenin expression in BC would help us develop better therapy strategies, distinguish high risk populations from the patients undergoing surgery and make better follow-up plans.

Materials and Methods

Search strategy and study selection

Relevant articles looking at β-catenin expression and survival results in BC patients were retrieved in PubMed, Ovid, EMBASE, Web of Science and China National Knowledge Infrastructure (CNKI) database update to July 1, 2014. Articles were identified using the following combined search terms: ("breast cancer" or "breast neoplasm" or "breast carcinoma") and ("Beta-catenin" or "β-catenin" or "CTNNB1"). No lower date or language limits were applied initially, but for full-text review and data analysis, only papers in English or Chinese were included finally. In order to minimize the deviation caused during the search process, references in all retrieved articles were scanned to identify other potentially applicable reports. The following inclusion criteria must be met in order to ensure the high quality of this article: *i)* the patients with primary BC who underwent surgical resection were investigated; *ii)* β-catenin protein expression (not mRNA) was measured in tumor tissue (not serum or plasma); *iii)* the method to evaluate β-catenin expression was immunohistochemistry (IHC); *iv)* it was a full paper that assessed the association between β-catenin expression and clinical outcome (overall survival (OS), recurrent free survival (RFS), disease free survival (DFS)) in BC; *v)* hazard ratio (HR) and 95% confidence intervals (CIs) could be obtained from the article or calculated them based on the information in the paper with methods developed by Parmar (Parmar et al., 1998), Williamson (Williamson et al., 2002)and Tierney (Tierney et al., 2007).

Study was excluded based on any of the following criteria: *i)* it was review, letter or experiment on animal models; *ii)* it lacked key information for HR estimation analysis; *iii)* it mentioned the β-catenin mutation instead of the β-catenin expression. When an individual author published several articles with data obtained from the same patient population, only the newest or most informative article was selected.

In selecting literature, we first screened the title and abstract to see whether they met the including criteria. Then, based on the initial screening, we scrutinized the full manuscript of studies that needed further examination. Two reviewers (Depu Zhang and Xiaowei Li) independently determined study eligibility. Disagreements were resolved by consensus.

Clinical outcomes used in the meta-analysis

OS and data relevant to the relative recurrence risk of BC were recognized as the clinical out comes for our meta-analysis of the association between β-catenin expression and prognosis in BC patients. Many studies have assessed the OS and RFS outcomes or alternatively the DFS. The definition of each outcome was as follows: OS, time from the date of diagnosis to death; RFS, time from the date of diagnosis to a recurrent BC; and DFS, time from the date of diagnosis to the first distant metastasis or death from BC without a recorded relapse.

Data extraction

Data extracted from the literature included: name of first author, publication year, country, median age of patients, median follow-up time, study sample size, the percentage of β-catenin positive, subcellular location of β-catenin, definition of β-catenin positive (cut-off), outcomes, method of HR estimation, method of survival analysis, HR and 95%CI. If the above information were not mentioned in the original study, the item was treated as "not reported (NR)". Inconsistencies in the research process were resolved through debate and consultations.

Quality assessment

Study quality was assessed in duplicate independently by two investigators (Depu Zhang and Xiaowei Li) according to the Newcastle-Ottawa quality assessment scale (NOS) as recommended by the Cochrane Non-Randomized Studies Methods Working Group (Wells G; Maxwell et al., 2006; Millett et al., 2008). This scale was developed to assess the quality of nonrandomized studies, specifically cohort and case-control studies. According to the NOS, studies were assessed based on three broad perspectives: patient population and selection, study comparability and outcome of interest. We considered studies that met six or more of the NOS criteria as high quality (Wells, 2010).

Statistical analysis

HR and 95%CI were used as the effective value to measure the impact of β-catenin expression on survival of BC patients in this meta-analysis result for its distinctive advantages: accounting for censoring, including all data and describing all of patients' experience. In the individual study, some of them provided HR and 95%CI directly. For some other studies not given these data clearly, we calculated from available data by using the methods illustrated by Tierney et al. (2007) and Parmar et al. (1998), which has been widely applied in meta-analysis about prognostic factors. The available data refer to the total number of events, the number of patients in each group, the log-rank statistic and its P-value or the O-E statistic (difference between numbers of observed and expected events). If the only existing survival data were in the form of figures, the Kaplan-Meier survive cure was read by Engauge Digitizer version 4.1 (free software down-

loaded from http:// sourceforge.net) to reconstruct the HR. Additional information and data needed for meta-analytic calculations were obtained by emailing the authors. Furthermore, when univariate and multivariate analysis were both obtainable, the latter was chosen to be pooled because survival result in BC is affected by multiple factors. By convention, an observed HR>1 implied a worse survival for the group with increased β-catenin expression. This influence of β-catenin expression on survival was considered as statistically significant if the corresponding 95%CI for the summary HR did not overlap 1.

Heterogeneity across studies was evaluated by Chi-square-based Q statistical test. The I^2 statistic (I^2=0-40%, no or moderate heterogeneity; I^2> 40%, significant heterogeneity) was also calculated to quantify the proportion of the total variation due to study heterogeneity (Ioannidis et al., 2007). A P>0.10 for the Q-test indicated a lack of heterogeneity among the studies. Fixed-effect model was used if there was no significant heterogeneity. Otherwise, the random-effect model was used. If there existed heterogeneity, subgroup analysis and meta-regression analysis were performed to find the main studies that may contribute to the heterogeneity. Funnel plots of Begger's and Egger's linear regression test were used to investigate any possible publication bias (Egger et al., 1997), which was indicated when the P value from Egger's test was <0.05. Sensitivity analysis was performed to examine the stability of the pooled results.

Statistical calculations were all performed using STATA version 12.0.

Results

Study characteristics

A total of 1006 papers potentially eligible for inclusion were confirmed with the initial search strategy mentioned above. Through the first review by reading the title and

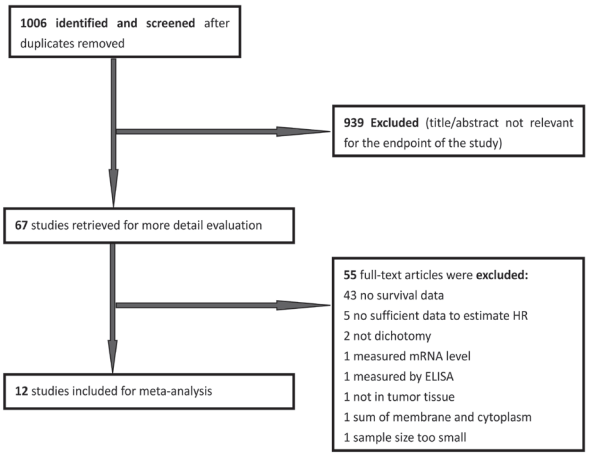

Figure 1. Study Flow Chart for the Process of Selecting the Final 12 Publications

Table 1. Characteristics of Eligible Studies in Meta-analysis

Author	Country	Age Median (range)	Median Follow-up (months)	N	Pos. (%)	Location	Cut-off	Outcomes measured	HR estimate	Survival analysis	HR (95%CI)	Quality stars (NOS)
Shiaw-Yih Lin, 2000	USA	48(26-87)	48	123	60.2	C/N	≥1%	OS	A	U	2.81(1.67-4.74)	7
					39.8	M	≥1%	OS	A	U	0.36(0.21-0.60)	
C.E.Gillett, 2001	UK	53(21-85)	>120	466	77.7	M	≥2/3	OS	A	U	1.30(0.94-1.78)	8
				417	78.4	M	≥2/3	RFS	A	U	1.32(0.98-1.77)	
				480	46.3	C	≥2/3	OS	A	U	1.15(0.88-1.51)	
				466	76.0	C	≥2/3	RFS	A	U	1.18(0.92-1.52)	
Fanelli, M. A., 2008	Argentina	55(27-81)	120	195	86.2	C/N	≥1%	OS	HR	M	0.96(0.53-1.76)	7
					86.2	C/N	≥1%	DFS	A	U	2.21(1.34-3.66)	
					13.8	M	≥1%	OS	HR	M	1.04(0.57-1.88)	
					13.8	M	≥1%	DFS	A	U	0.45(0.27-0.75)	
Hyun-Soo Kim, 2009	Korea	49(23-74)	71	99	82.8	M	≥70%	OS	HR	U	0.57(0.21-1.58)	8
					86.9	C	≥20%	OS	HR	U	1.33(0.31-5.75)	
Ângela Flávia Logull, 2009	Brazil	56(33-80)	NR	54	70.4	M	≥10%	OS	A	U	0.56(0.20-1.60)	7
Elena López-Knowles, 2010	Australia	54(NR)	64	292	NR	M	CS	OS	HR	U	0.88(0.51-1.55)	8
					NR	C	CS	OS	HR	U	1.49(0.85-2.61)	
					NR	M	CS	RFS	HR	U	0.51(0.32-0.80)	
					NR	C	CS	RFS	HR	U	1.79(1.12-2.85)	
Khramtsov, A. I., 2010	USA	NR	100	131	NR	C	≥70%	OS	HR	M	2.91(1.48-5.73)	8
					NR	N	≥30%	OS	HR	M	2.24(1.12-4.93)	
Felipe C Geyer, 2011	UK	NR	67	221	11.3	N	CS	OS	A	U	2.39(1.12-5.10)	9
					11.3	N	CS	DFS	A	U	1.82(0.92-3.59)	
Wen-Huan Xu, 2012	China	NR	80	85	64.7	C/N	≥10%	OS	HR	M	3.39(0.71-16.25)	7
					64.7	C/N	≥10%	RFS	HR	M	1.75(0.66-4.62)	
Hui Pang2013	China	46(25-72)	75	170	34.1	M	CS	OS	HR	M	0.63(0.29-1.38)	9
					34.1	M	CS	DFS	HR	M	0.62(0.28-1.37)	
Ho, S. K.2013	Singapore	45(15-82)	42	185	38.9	C	CS	OS	A	U	2.81 (0.38-20.96)	8
Shuguang Li2014	China	NR	48	169	64.5	C/N	≥1%	OS	A	U	3.19(1.78-5.74)	7
					35.5	M	≥1%	OS	A	U	0.31(0.17-0.56)	

NR not reported, C/N defining either nucleus or cytoplasm staining or both as positive, N defining nucleus staining as positive, C defining cytoplasm staining as positive, M defining membrane staining as positive, CS complex score combining intensity and percentage of β-catenin expression, HR HR reported in text, A HR available data or Kaplan–Meier curves, U Univariate, M multivariate

abstract of the articles, 939 articles were excluded which were not relevant to our aim. The remaining 67 papers were approved through scrutinizing the entire paper by two independent authors (Zhang and Li), and 55 papers were excluded. Among the 55 excluded papers, 43 papers were not researches on survival analysis, which didn't contain follow-up data. Five literatures had no sufficient data to analyze and one analyzed the relationship between β-catenin mRNA level and the outcome in BC. One study measured β-catenin protein expression in carcinoma-associated fibroblasts rather than tumor tissue and β-catenin protein expression was measured by ELISA in another. Statistical methods in two studies measuring β-catenin expression was not dichotomy. Furthermore, one study provided information about the sum of β-catenin expression in membrane and cytoplasm, which is not suitable for our category. The number of samples was too small in one paper. Eventually, 12 studies met the inclusion criteria were included (Figure 1).

The major characteristics of retained studies were listed in Table 1. The meta-analysis was performed assessing the association of β-catenin expression in the cytoplasm/nucleus with OS on 11 studies and DFS or RFS on 5 studies. There were 8 studies utilized OS to assess the prognostic value of β-catenin expression in the membrane in BC patients and 5 studies used DFS or RFS as the indicator. The total number of patients in the included studies was 2204 ranged from 54 to 480 per study. The reported median age of patients ranged from 45 to 55 years across the eligible studies. Some of the studies defined the cut off value by complex score combining intensity and percentage of β-catenin expression, while other studies only used the percentage of β-catenin expression to define positive expression with the cut off value varied from 1%

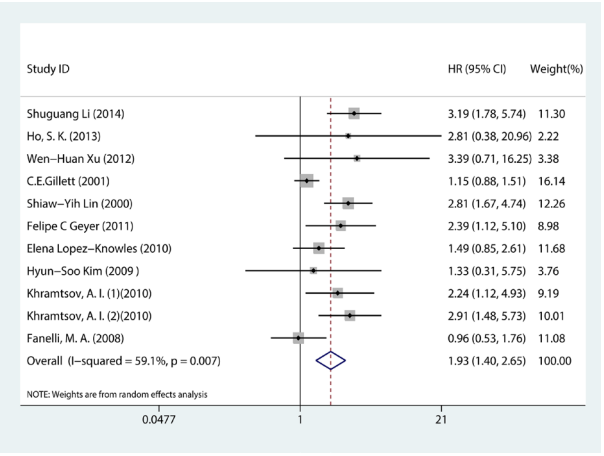

Figure 2. Forest Plot of β-catenin Expression in Cytoplasm/Nucleus and Overall Survival in Breast Cancer Patients

Table 2. Subgroup-analysis of the Association betweenβ-catenin Expression in the Cytoplasm/Nucleus or Membrane and Overall Survival

Subcellular location	Subgroup	No. of studies	No. of patients	HR(95%CI)	Meta-regression	Heterogeneity		
						χ^2	*P*	I^2 (%)
C/N	Overall effect	11	2111	1.93 (1.40-2.65)		24.43	0.007	59.10
	County				0.27			
	Asia	4	538	2.88 (1.75-4.74)		1.23	0.745	66.00
	Non-Asia	7	1573	1.76 (1.24-2.52)		17.65	0.007	59.10
	Subcellular location				0.927			
	C/N	4	572	2.19(1.16-4.13)		10.01	0.018	70.00
	C	5	1187	1.56(1.05-2.34)		6.94	0.139	42.40
	N	2	352	2.31(1.36-3.93)		0.01	0.905	0.00
	Evaluation standards				0.975			
	percentage	8	1413	1.96(1.30-2.95)		23.07	0.002	69.70
	CS	3	698	1.80(1.16-2.80)		1.16	0.561	0.00
	HR estimate				0.578			
	Reported in text	6	933	1.73(1.17-2.56)		7.49	0.187	33.30
	By estimated	5	1178	2.18(1.25-3.79)		16.92	0.002	76.40
	Analysis model				0.968			
	Univariate	7	1569	1.95(1.29-2.93)		17.06	0.009	64.80
	Multivariate	4	542	1.94(1.05-3.58)		7.11	0.068	57.80
M	Overall effect	8	1568	0.65(0.42-1.02)		29.94	0	76.60
	County				0.24			
	Asia	3	438	0.44(0.27-0.70)		2.32	0.314	13.70
	Non-Asia	5	1130	0.78(0.46-1.32)		18.28	0.001	78.10
	Evaluation standards				0.71			
	percentage	6	1106	0.62(0.34-1.11)		29.47	0	83.00
	CS	2	462	0.79(0.50-1.24)		0.46	0.498	0.00
	HR estimate				0.446			
	Reported in text	4	756	0.83(0.59-1.17)		1.58	0.663	0.00
	By estimated	4	812	0.54(0.34-1.25)		28.08	0	89.30
	Analysis model				0.371			
	Univariate	5	911	0.55(0.27-1.13)		28.32	0	85.90
	Multivariate	3	657	0.87(0.61-1.25)		0.99	0.608	0.00

C/N defining either nucleus or cytoplasm staining or both as positive, N defining nucleus staining as positive, C defining cytoplasm staining as positive, CS complex score combining intensity and percentage of β-catenin expression

to 70%. β-catenin positive expression rate was observed ranging from 11.3% to 86.9%. Among all of the included studies, HR and 95%CI were obtained from the original articles directly in five studies, and data were calculated based on the available information in six individual studies. The remaining one paper, HR and 95%CI for OS was provided directly and that for DFS had to be calculated based on the available information. Two articles got 9 NOS scale, five got 8 and five got 7 (see Table, Supplemental Digital Content 1, which demonstrates details of scale).

Impact of β-catenin Expression in Cytoplasm/Nucleus on OS and DFS/RFS of BC in the meta-analysis

The meta-analysis was performed on 11 studies (one article provided information about association of β-catenin expression in cytoplasm and nucleus with OS respectively, which we processed independently as two studies in the meta-analysis) assessing the association of β-catenin expression in cytoplasm/nucleus with OS. The pooled HR was 1.93 (95%CI: 1.40-2.65; Z=4.04; P=0.000) (Figure 2) with heterogeneity (I^2=59.1% P=0.007). Five studies assessed the association of β-catenin expression in cytoplasm/nucleus with DFS/RFS; the pooled HR was 1.60 (95%CI: 1.20-2.13; Z=3.22; P=0.001) (Figure 3A) without significant heterogeneity (I^2=39.8% P=0.156). These results suggested that β-catenin overexpression in cytoplasm/nucleus was significantly correlated with a worse prognosis of BC and that β-catenin overexpression in cytoplasm/nucleus was an independent prognostic factor in BC.

To explain the heterogeneity in OS, subgroup and meta-regression analysis were performed by the study location, subcellular location of β-catenin expression, evaluation standards of positive, methods of HR estimate and analysis model. The results indicated that a significant relationship between β-catenin expression in the cytoplasm/nucleus and OS was exhibited in articles defining positive by complex score combining intensity and percentage of β-catenin expression (HR 1.80, 95%CI: 1.16-2.80, Z=2.63, P=0.009) without heterogeneity (I^2=0% P=0.561) (Table 2). When we just focus on the β-catenin expression in the nucleus, no heterogeneity (I^2=0% P=0.905) (Table 2) was observed, too. Additionally, no significant heterogeneity was detected (I^2=33.3% P=0.187) when the HRs were reported in text (HR 1.73, 95%CI: 1.17-2.56, Z=2.72, P=0.006) (Table 2) .When the analysis of OS was limited to studies with same study location or with same model of statistical analysis (multivariate or univariate), heterogeneity still existed (Table 2). However, the results of meta-regression analysis showed that none of the five factors had significant association with the heterogeneity (all P>0.05). Moreover, subgroup analysis on the five factors did not alter the significant prognostic impact ofβ-catenin expression.

Impact of β-catenin Expression in Membrane on OS and DFS/RFS of BC in the meta-analysis.

Eight eligible studies were assessed to found that there was no significant association between β-catenin overexpression in the membrane with OS; the combined HR was 0.65 (95%CI: 0.42-1.02, Z=1.89, P=0.059) with heterogeneity (I^2=76.6% P=0.000) (Figure 4). Five studies assessed the association of β-catenin expression in the membrane with DFS/RFS; the pooled HR was 0.66 (95%CI: 0.38-1.13; Z=1.52; P=0.130) (Figure 3) with heterogeneity (I^2=80.6% P=0.000). These studies indicated that β-catenin overexpression in the membrane had no relationship with prognosis of BC.

To explain the heterogeneity, meta-regression and subgroup analysis were performed by the study location, evaluation standards of positive, methods of HR estimate and analysis model (Table 2). The results of meta-regression analysis showed that none of the factors had significant association with the heterogeneity (all P>0.05). Subgroup analysis indicated that a significant relationship between β-catenin expression in the membrane and OS was exhibited in studies from Asia (HR 0.44, 95%CI: 0.27-0.7, Z=3.47, P=0.001) with no significant heterogeneity (I^2=13.7% P=0.314). No significant relationship between β-catenin expression in the membrane and OS were observed in other three subgroups.

Publication bias analysis

Both Egger's test and Begger's funnel plot were used to check the publication bias in this meta-analysis. The

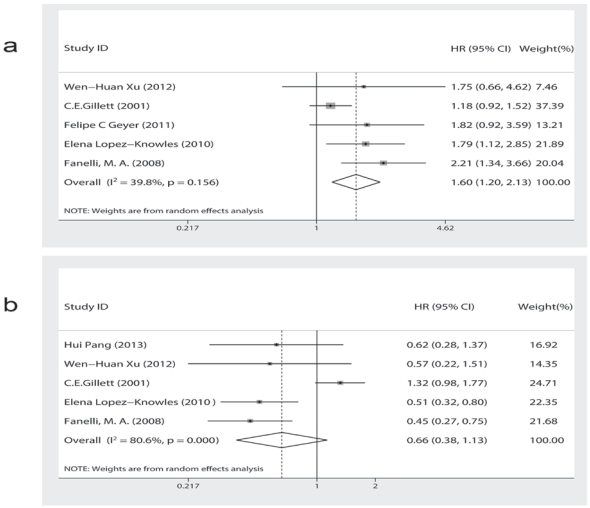

Figure 3. Forest plot of β-catenin expression in (a) cytoplasm/nucleus or (b) membrane and DFS/RFS in breast cancer patients

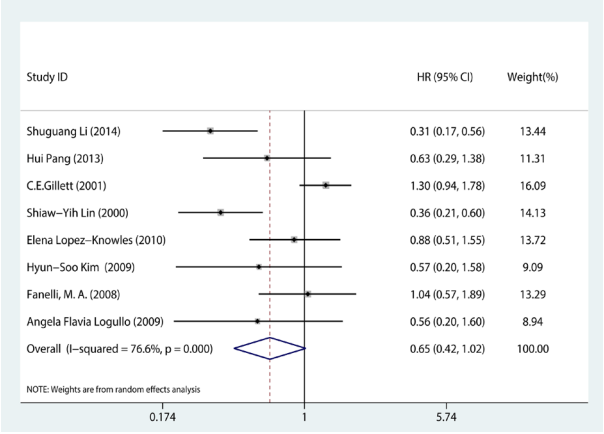

Figure 4. Forest plot of β-catenin expression in membrane and overall survival in breast cancer patients

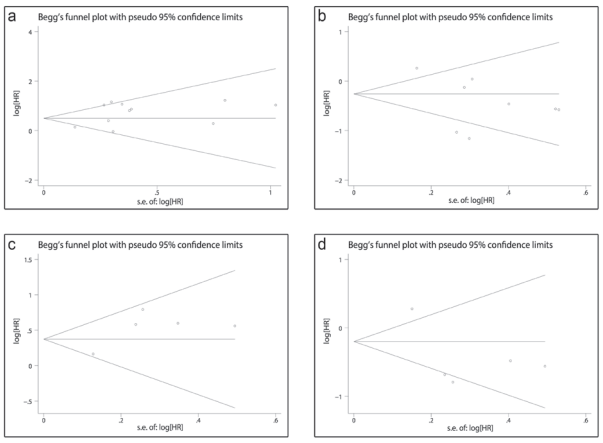

Figure 5. Begger's Funnel Plot of the Meta-analysis Assessing. A) β-catenin expression in cytoplasm/nucleus and overall survival. **B)** β-catenin expression in membrane and overall survival. **C)** β-catenin expression in cytoplasm/nucleus and DFS/RFS. **D)** β-catenin expression in membrane and DFS/RFS in breast cancer patients. No publication bias was observed in the four outcomes

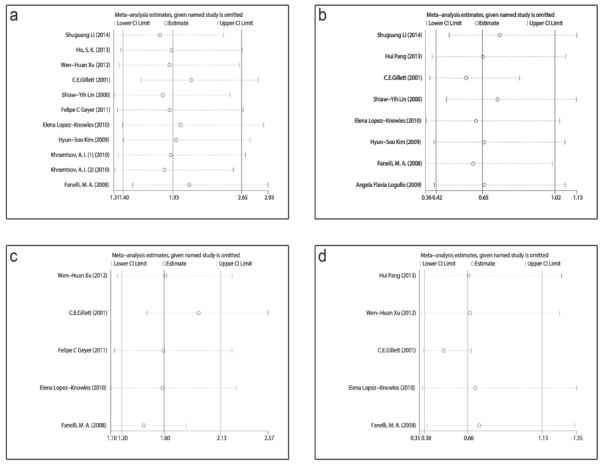

Figure 6. Sensitivity Analyses of the Meta-analysis Assessing. A) β-catenin expression in cytoplasm/nucleus and overall survival. **B)** β-catenin expression in membrane and overall survival. **C)** β-catenin expression in cytoplasm/nucleus and DFS/RFS. **D)** β-catenin expression in membrane and DFS/RFS in breast cancer patients. Omission of any study did not affect the whole estimate results significantly in the four outcomes

funnel plots presented no proof of obvious publication bias for studies in all of the four outcomes. Further estimation using Egger's linear regression test also failed to reveal any support for significant publication bias: Cytoplasm/Nucleus with OS (t=1.85, P=0.097); Cytoplasm/Nucleus with DFS/RFS (t=2.10, P=0.126); Membrane with OS (t=-1.53, P=0.176); Membrane with DFS/RFS (t=-1.56, P=0.217). The funnel plots were shown in Figure 5.

Sensitivity analysis

In order to gauge results stability, a sensitivity analysis, in which one study was deleted at a time, was performed. No individual study significantly influenced the combined HR of the four outcomes by sensitivity analysis, suggesting the robust result of this meta-analysis. The detailed results were shown in Figure 6.

Discussion

BC is the most common cancer in woman with high mortality and its prognosis is still poor despite remarkable advances in treatment (Jemal et al., 2011; ZZ et al., 2012). Alteration of molecular biological markers in tumor tissues has become an important part in predicting the prognosis of patients with BC and seemed more specific than markers currently used in clinical such as TNM stage, weight loss and lymph node metastasis. There are many reports about the prognostic significance of β-catenin in BC (Lin et al., 2000; Gillett et al., 2001; Lim and Lee, 2002; Chung et al., 2004; Dolled-Filhart et al., 2006; Nakopoulou et al., 2006; Park et al., 2007; Fanelli et al., 2008; Paredes et al., 2008; Khramtsov et al., 2010; Kim et al., 2010; Logullo et al., 2010; Lopez-Knowles et al., 2010; Geyer et al., 2011; Cheng et al., 2012; Mukherjee et al., 2012; Xu et al., 2012; Ho et al., 2013; Pang et al., 2013; Weissenbacher et al., 2013; Li et al., 2014). However, results were inconsistent. Thus, a quantitative meta-analysis was warranted. The pooled results indicated that β-catenin overexpression in the cytoplasm/nucleus rather than membrane was significantly associated with poor prognosis in patients with BC.

β-catenin, a multifunctional intracellular protein, is at the hub of multiple signaling pathways, especially the Wnt signaling pathway. It is expressed in three main forms: membrane, cytoplasm and nucleus localization. It plays a critical role in stabilizing cell -cell adhesion through forming cadherin-catenin complexes with E-cadherin and actin filaments when it is located in the membrane. The other two forms are mainly involved in regulation of the Wnt signaling pathway. Cytoplasmic β-catenin, maintaining at a low level in the absence of a Wnt ligand, is bound by a destruction complex within which Axin and adenomatous polyposis coli (APC) serve as scaff olds. Bound β-catenin is phosphorylated by glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1), then ubiquitinated by beta-transducing repeat-containing protein (β-TrCP) and targeted for proteasomal destruction. Binding of Wnt ligand inhibits the destruction complex, allowing β-catenin to accumulate in the cytoplasm and undergoes translocation to the nucleus, where it binds Lef/T-cell factor (TCF) transcription factors and activates specific Wnt target genes.

The role of Wnt/β-catenin signaling in human tumors has been supported by many studies, but to our knowledge, the exact impact on BC remains unclear. The possible explanations are these studies did not categorize the β-catenin subcellular localization.

In normal BC tissues, β-catenin expression is located mainly in the membrane, and cytoplasmic or nuclear localization was not observed in previous studies (Sadot et al., 2002; Wang et al., 2011). β-catenin expression and/or localization are often abnormal in human BC (Jonsson et al., 2000; Lin et al., 2000; Ryo et al., 2001; Ozaki et al., 2005; Dolled-Filhart et al., 2006; Hayes et al., 2008). Increased cytoplasmic and nuclear β-catenin levels which is considered to be indicative of Wnt pathway activation (Fanelli et al., 2008; Hayes et al., 2008; Khramtsov et al., 2010; Lopez-Knowles et al., 2010; Geyer et al., 2011; Ye et

al., 2014) have been documented in 40% of primary BCs.

In this meta-analysis, we categorize the β- catenin by subcellular localization. The association of β-catenin expressions in membrane or cytoplasm/nucleus with prognosis in BC is analyzed respectively. It is proved that β-catenin overexpression in cytoplasm/nucleus was significantly associated with an unfavorable prognosis in patients with BC. Nevertheless, we did not find a significant impact of β-catenin expression in membrane as a poor prognostic factor for BC.

In the present study, sources of heterogeneity were also explored. The study location, subcellular location of β-catenin expression, evaluation standards of positive, methods of HR estimate and analysis model were quite different across studies, creating significant heterogeneity. This heterogeneity could potentially affect the meta-analysis results. Accordingly, we used random effects models to analyze the data, but the models did not identify the source of heterogeneity. To clarify the source of heterogeneity in this study, subgroup and meta-regression analysis were performed.

As for β-catenin overexpression in cytoplasm/nucleus with OS, when the analysis was performed without consideration of these factors, heterogeneity was detected (I^2=59.1% P=0.007). Although no significant heterogeneity was detected in some subgroups, the results of meta-regression analysis showed that none of the five factors had significant association with the heterogeneity (all P>0.05).

What is noteworthy is that, subgroup analysis by subcellular location showed a statistically significant impact of β-catenin expression as a prognostic factor in either nucleus or cytoplasm. However, we still noted that some of the included studies (Lin et al., 2000; Fanelli et al., 2008; Xu et al., 2012; Li et al., 2014) defined either cytoplasm or nucleus as positive β-catenin expression, which made it difficult to explain which part of accumulation of β-catenin played the most important influence on prognosis in BC patients, only to find that positive β-catenin expression by this definition has significant association with OS. This result confirms our conclusion that β-catenin expression in cytoplasm/nucleus, which is considered to be indicative of Wnt pathway activation, was significantly associated with an unfavorable prognosis in patients with BC.

As for β-catenin overexpression in membrane with OS, the results of meta-regression analysis showed that none of the factors had significant association with the heterogeneity (all P>0.05). It is worth noting that a significant relationship between β-catenin expression in the membrane and OS was exhibited in studies from Asia with no significant heterogeneity by subgroup analysis. In consideration of only a total of 438 patients from 3 studies in this subgroup, more well-designed prospective studies with larger sample sizes are needed to support this conclusion.

Publication bias is a major concern that may cause bias. In this research, neither Egger's test nor Begger's funnel plot showed evidence of publication bias in all of the four outcomes. Nevertheless, we still need to consider the fact that studies with positive result tend to be accepted by journals, whereas the studies with negative results are often rejected or not even submitted. The study included in our meta-analysis was restricted only to articles published in English or Chinese, which probably provided additional bias. Additionally, among the excluded studies, five studies were excluded because of insufficient data. None of the five studies reported significant association between reduced β-catenin expression and survival in BC. All of the above factors could lead to possible bias and should not be neglected. Sensitivity analysis also showed that omission of any single study did not have significant impact on the combined risk estimates in all of the four outcomes. This made the results of this meta-study more reliable to some extent.

Despite our efforts to conduct a comprehensive analysis, some limitations remain to be addressed. In this meta-analysis, to minimize the heterogeneity, the included studies were required to measure β-catenin expression by IHC, which was the most applied method. However, the heterogeneity still existed. On one hand, Methodological differences of IHC may contribute to heterogeneity. The treatments to the patients among the studies were not exactly the same, which may influence the results of survival time between the studies. However, no sufficient information was provided to explain it. On the other hand, the primary antibody used had a significant influence on the sensitivity of IHC. However, the used of antibody in studies was also varied widely. Other factors such as storage time and revelation time may also cause potential bias. We could not perform subgroup analysis to explore this influence because few studies offered the concrete data.

There was also large difference in the definition of positive expression of β-catenin among the studies. Some of the studies defined positive by complex score combining intensity and percentage of β-catenin expression, while other studies only used the percentage with the cut off value varied from 1% to 70%. Up to date, no uniform standard to define the positive expression of β-catenin was recognized, which may cause potential bias.

The method of HR estimate requires stated. For the studies that HR and 95%CI were not reported directly, they were calculated from the available data mentioned in the publish articles. If even no available data was provided, we had to extrapolate the value from the survive curve based on the published method (Parmar et al., 1998; Tierney et al., 2007). This approach may have caused errors due to the inaccuracies in reading survive curve, so we try our best to minimize errors by reading the curve by two reviewers independently. It seems that the HR estimated from the curve may be less trustworthy than obtained directly. Consequently, we compared the HR and 95%CI with the published results to make sure of the accuracy of the estimated HR

Most of studies included in the pooled analyses of BC outcomes were analyzed by univariate analysis. Multivariate analysis is more credible than univariate analysis because confounding factors are taken into account. Nevertheless, different covariates were adjusted in each study, thus leading bias to the pooled results. Besides, other clinical factors such as age, TNM, hormone

receptor status and different postoperative treatment in each study might lead to bias.

Considering these limitations existing in this meta-analysis, our results should be rigorous exposition and the conclusions of this meta-analysis should also be drawn carefully.

In conclusion, positive β-catenin expression in cytoplasm/nucleus rather than in membrane is a significant prognostic factor in patients with BC who have been surgically treated. It is potentially a useful biomarker for predicting prognosis in BC. As a prognostic factor for BC patients, β-catenin expression in cytoplasm/nucleus can help to make decisions for therapeutic of the BC patients. Larger prospective studies with long-term follow-up are needed by multivariate analysis, which takes into account the well-known prognostic factors in BC.

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