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Nuclear expression and/or reduced membranous expression of β-catenin correlate with poor prognosis in colorectal carcinoma

A meta-analysis

Shizhen Zhang, MS[a], Zhen Wang, PhD[a], Jinlan Shan, MS[a], Xiuyan Yu, PhD[a], Ling Li, PhD[b], Rui Lei, PhD[c], Daozhe Lin, PhD[d], Siqi Guan, MS[e], Xiaochen Wang, PhD[a,*]

Abstract

Background: The differential subcellular localizations of β-catenin (including membrane, cytoplasm, and nucleus) play different roles in the progression of colorectal cancer (CRC). However, the correlation between each subcellular localization of β-catenin and the prognosis of CRC patients remains undetermined.

Methods: Systematic strategies were applied to search for eligible published studies in the PubMed, Embase, and Web of Science databases. The correlation between each subcellular localizations of β-catenin expression and patients' clinicopathological features or prognosis was analyzed.

Results: Finally, this meta-analysis, including 6238 cases from 34 studies, revealed that β-catenin overexpression in the nucleus (HR: 1.50[95% CI: 1.08–2.10]) or reduced expression of β-catenin in the membrane (HR: 1.33[95% CI: 1.15–1.54]) significantly correlated with lower 5-year overall survival (OS). Conversely, overexpression of β-catenin in the cytoplasm (HR: 1.00[95% CI: 0.85–1.18]) did not show significant association with 5-year OS.

Conclusion: This study suggested that β-catenin overexpression in the nucleus or reduced expression in the membrane, but not its overexpression in cytoplasm, could serve as a valuable prognostic predictor for CRC. However, additional large and well-designed prospective studies are required to verify our results.

Abbreviations: CRC = colorectal cancer, CI = confidence interval, HR = hazard ratio, OR = odds ratio, OS = overall survival, DFS = disease-free survival.

Keywords: β-catenin, colorectal cancer, meta-analysis, prognosis

1. Introduction

Colorectal cancer (CRC) is the fourth most frequent human malignancies worldwide.[1] Although the mortality of colorectal cancer has been decreased by almost 35%through earlier screening and better treatment modalities,[2] CRC remains the second highest cause of cancer-related deaths. The 5-year survival rate for CRC exceeds 50%, but it is highly variable depending on the stage of the disease.[3] The molecular pathways involved in the tumorigenesis of CRC are complicated and heterogeneous. Fearon and Vogelstein[4] have reported a series of genetic alterations, including the activation of certain oncogenes and the

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All analyses were based on previous published studies; thus, no ethical approval and patient consent were required

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[a] Department of Oncology and Cancer Institute (Key Laboratory of Cancer Prevention & Intervention, National Ministry of Education, Provincial Key Laboratory of Molecular Biology in Medical Sciences), Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [b] Division of Hematopoietic Stem Cell and Leukemia Research, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA, [c] Department of Plastic Surgery, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, [d] Department of surgical oncology, Third Affiliate Hospital of Wenzhou Medical University, Wenzhou, [e] Department of Reproductive, Integrated Chinese and Western Medicine Hospital of Zhejiang Province, Hangzhou, China.

[*] Correspondence: Xiaochen Wang, Department of Surgical Oncology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China (e-mail: wangxiaochen@zju.edu.cn).

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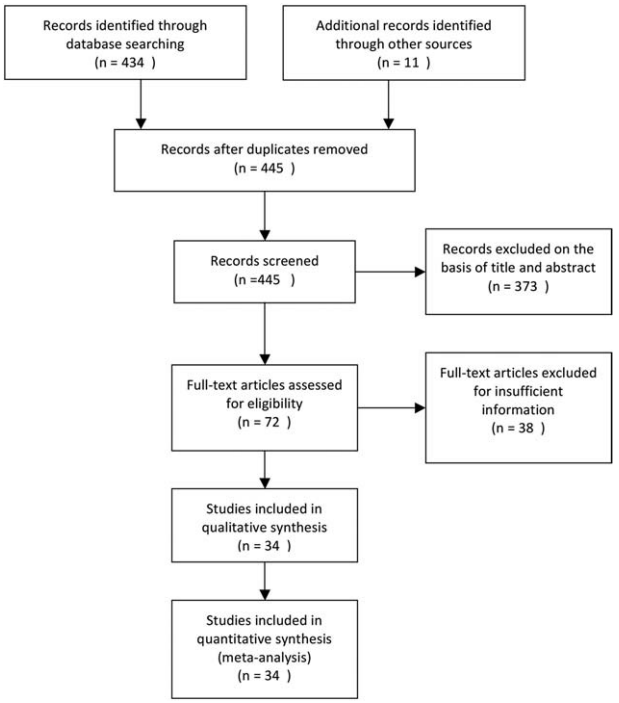

Figure 1. Flow diagram of study selection procedure.

inactivation of particular tumor suppressor genes, which are responsible for colorectal tumorigenesis.

β-catenin localizes in the membrane, cytoplasm, or nucleus and exerts different functions related to cell differentiation and proliferation. Membranous β-catenin was identified as a protein associated with E-cadherin in maintaining cell-to-cell interactions. Interestingly, the membranous expression of β-catenin exerts a restrictive effect on tumor cell movement and growth. Loss of β-catenin expression on the cell surface increases cell motility, growth, and transformation and thus promotes tumorigenesis.[5] Cytoplasmic β-catenin, which can translocate to the nucleus and activate the downstream target genes relevant to cell proliferation, migration, invasion, cell cycle progression and metastasis, serves as a downstream transcriptional transactivator through int/Wingless family (Wnt) transduction signaling.[6] Pre-existing intracellularβ-catenin directly connects scaffolding proteins Axin, adenomatous polyposis coli(APC), serine/threonine kinases, Casein kinase 1 alpha (CK1α), and glycogen synthase kinase 3β (GSK3β) to form a destruction complex without Wnt ligands.[7] However, if the APC is inactivated, Axinor β-catenin mutates; therefore, free β-catenin cannot be degraded and accumulates in the cytosol. Subsequently, β-catenin translocates to nucleus as a co-factor for T-cell factor (TCF) family of transcription factors to activate the downstream Wnttarget genes.[6] This aberrant Wnt/β-catenin-TCF signaling plays a key role in the development and progression of colorectal cancer. The Wnt/β-catenin pathway has been recognized to play a critical role in maintaining the stem cell features by targeting genes,

Table 1

Characteristics of the studies of the nuclear β-catenin expression.

Author, year	Type	Country	No. of patients	Mean ages	Gender (M/F)	N (%)	DOP	Antibody source	Dilution	Score
Youssef, 2015	CRC	Egypt	72	NA	35/37	23 (31.94%)	>10%	DAKO	1:100	6
Rania, 2015	CRC	Tunisia	124	62.9 (25–85)	72/52	72 (58.1%)	>5%	Santa Cruz	1:100	6
Balzi, 2015	CRC	Italy	321	NA	171/150	156 (48.6%)	>5%	DAKO	1:200	5
Wangefjord, 2013	CRC	Sweden	527	NA	250/277	368 (69.8%)	Moderate	BD Pharmingen	1:5000	5
Jung, 2013	CRC	Korea	349	63 (27–88)	208/141	246 (70%)	>0%	DAKO	1:500	7
Toth, 2012	RC	Hungary	79	68.8 (35–85)	40/39	36 (45.6%)	>10%	TL	1:100	4
Andras, 2012	CRC	Hungary	90	66.9 (35–91)	52/49	39 (43%)	>10%	TL	1:100	8
Ozguven, 2011	CRC	Turkey	60	62.3 (16–82)	38/22	31 (52%)	>0%	Immunovision	NA	5
Morikawa, 2011	CRC	USA	955	67.1	381/574	439/(46%)	Moderate	TL	1:200	6
Mstsuoka, 2011	CRC	Japan	156	65.5 (22–86)	99/57	43 (28%)	>20%	Zymed	1:400	8
Sun, 2011	CRC	China	67	NA	43/24	30 (44.8%)	>10%	Santa	1:100	8
Magnusson, 2010	CRC	Sweden	312	NA	149/96	81 (26%)	Moderate	TL	1:1000	6
Filiz, 2010	CRC	Turkey	138	65 (21–89)	83/55	8 (5%)	Weak	Labvision Corporation	NA	5
Fang, 2010	CRC	China	142	55 (15–78)	80/62	100 (70.4%)	Moderate	DAKO	1:200	4
Pancione, 2009	CRC	Italy	72	70.5 (35–89)	44/28	13 (18.1%)	Moderate	TL	1:200	5
Togo, 2008	CRC	Japan	183	66 (27–95)	115/68	154 (84.1%)	Moderate	NA	1:1600	5
Chen, 2008	CRC	China	60	54.8 (28–83)	24/36	13 (22%)	>10%	BZGBB	1:100	6
Martensson, 2007	CRC	Germany	67	(37–88)	39/28	45 (67%)	>5%	Sigma	1:750	6
Bravou, 2006	CRC	Greece	125	119 (95.2)	NA	119 (95.2%)	>10%	BD Bioscience	1:1000	5
Kim, 2005	CRC	Korea	124	NA	NA	50 (40.3%)	>5%	TL	NA	6
Bondi, 2004	CC	Norway	162	71.1 (45–94)	74/88	36 (22.2%)	>1%	TL	1:2000	4
Fernebro, 2004	RC	Sweden	257	68 (32–92)	173/96	146 (56.8%)	Weak	TL	1:5000	5
Ougolkow, 2002	CC	Japan	202	60 (24–75)	110/92	107 (53%)	>10%	TL	1:100	5
Chung, 2001	CRC	USA	543	NA	NA	114 (21%)	Moderate	TL	1:2500	4
Maruyama, 2000	CRC	Japan	96	NA	NA	63 (65.5%)	>10%	TL	1:250	8
Wong, 2003	CRC	China	60	63.8 (32–91)	39/21	25 (42%)	>300	TL	1:200	4
Lee, 2013	CRC	Korea	305	63.6 (25–86)	189/144	38 (12.5%)	>30%	DAKO	1:400	6
Jang, 2012	CRC	Korea	218	NA	134/84	158 (72.5%)	>30%	BD Bioscience	1:50	5
Khiari, 2012	CRC	Tunisia	150	61.5 (17–98)	76/71	23 (15.3%)	Moderate	Vision Biosystems	1:100	6

BZGBB = Beijing Zhongshan Golden Bridge Biotechnology, CC = colon cancer, CRC = colorectal cancer, DOP = definition of positive, N(%) = the number and percentage of tissue samples with β-catenin overexpression in nucleus(N), NA = not available, NO = number, RC = rectal cancer, Score = quality score of the literatures, TL = transduction laboratories.

Table 2

Characteristics of the studies of the β-catenin expression in cytoplasm.

Author, year	Type	Country	No. of patients	Mean ages	Gender (M/F)	C (%)	DOP	Antibody source	Dilution	Score
Youssef, 2015	CRC	Egypt	72	NA	35/37	38 (52.8%)	>10%	DAKO	1:100	6
Balzi, 2015	CRC	Italy	321	NA	171/150	222 (69.2%)	>5%	DAKO	1:200	5
Gao, 2014	CRC	China	181	NA	105/76	42 (23.3%)	>50%	BZGBB	1:500	6
Morikawa, 2011	CRC	USA	955	67.1	381/574	455 (47.6%)	Moderate	TL	1:200	6
Filiz, 2010	CRC	Turkey	138	65 (21–89)	83/55	107 (77.5)	Moderate	Labvision	NA	5
Magnusson, 2010	CRC	Sweden	312	NA	149/96	86 (27.6%)	Moderate	TL	1:1000	6
Chen, 2008	CRC	China	60	54.8 (28–83)	24/36	25 (41.7%)	>10%	BZGBB	1:100	6
Fernebro, 2004	RC	Sweden	252	68 (32–92)	173/96	238 (94.4%)	Weak	TL	1:5000	5
Maruyama, 2000	CRC	Japan	96	NA	NA	65 (67.7%)	>10%	TL	1:250	8

BZGBB = Beijing Zhongshan Golden Bridge Biotechnology, C(%) = the number and percentage of tissue samples with β-catenin overexpression in cytoplasm(C), CC = colon cancer, CRC = colorectal cancer, CST = cell signaling technology, DOP = definition of positive, NA = not available, NO = number, RC = rectal cancer, Score = quality score of the literatures, TL = transduction laboratories.

such as Lgr5, Ascl2, and Sox9.[8–10] The hyperactivation of Wnt/β-catenin signaling enhances the invasive and metastatic potential of CRC cells.[11] Knockdown of β-catenin in CRC cells dampens cell proliferation and invasion.[12,13] Nuclear β-catenin expression detected by immunohistochemistry has been reported to be associated with high tumor burden and worse survival outcomes of CRC.[14–17] However, other studies did not present this association.[18,19] The variable and contradictory results were also observed regarding the correlation between the reduced membranous β-catenin expression and the prognosis in patients with CRC.[17,19–21] A previous analysis suggested that β-catenin overexpression in nucleus, rather than in cytoplasm, was associated with poor prognosis of CRC.[22] In this paper, we collected and added updated articles regarding β-catenin expression in CRC to reanalyze the prognostic value of β-cateninin cytoplasm and/or nucleus in CRC patients. More importantly, we also extracted relevant data to analyze the prognostic significance of reduced membranous β-catenin expression in patients with CRC. The pooled results suggested that β-catenin overexpression in nucleus or reduced β-catenin expression in the membrane was associated with worse prognosis of CRC.

2. Methods

2.1. Literature selection

We systematically searched the PubMed, Embase, and Web of Science databases to identifying pertinent articles published prior to November 2015. The terms used in the search were as follows with all possible combinations: "β-catenin, Beta-catenin, or CTNNB1," "WNT/β-catenin signal pathway," "prognostic, prognosis, or survival," and "colorectal neoplasms, colorectal cancer, colorectal carcinoma, colorectal tumor." The reference lists associated with all the studies were also inspected for additional available studies.

2.2. Inclusion and exclusion criteria

To obtain high-quality literatures to meet the high standards for this meta-analysis, studies must fulfill the following criteria: (1) patients included have definite pathological diagnosis of colorectal carcinoma; (2) β-catenin expression was evaluated by immunohistochemistry in the CRC tissue; (3) evaluation of the correlation between β-catenin expression and CRC pathological features and overall survival (OS) or disease-free survival (DFS) were done; and (4) studies included were published in English. In addition, the following articles were excluded: (1) articles published in a non-English language; (2) articles where the relevant data provided could not be extracted; (3) articles where no relevant data was provided for necessary analysis; (4) duplicated articles; (5) cut-off scoring of positive immunoreactivity of β-catenin expression in nucleus was higher than 30%; and (6) the quality of included study was too low (score < 4). The studies were evaluated and selected by 2 reviewers (XY and JS), and the disagreements were settled by a third reviewer (DL). Then, the eligible articles were included for further data processing.

Table 3

Characteristics of the studies of the reduced β-catenin expression in the membrane.

Author, year	Type	Country	No. of patients	Mean ages	Gender (M/F)	Reduced M (%)	DOP	Antibody source	Dilution	Score
Rania, 2015	CRC	Tunisia	124	62.9 (25–85)	72/52	72 (58.1%)	<95%	Santa Cruz	1:100	6
Balzi, 2015	CRC	Italy	321	NA	171/150	29 (9%)	<95%	DAKO	1:200	5
Gao, 2014	CRC	China	181	NA	105/76	107 (59.1%)	<80%	BZGBB	1:500	6
Salim, 2013	CC	Sweden	85	NA	NA	60 (60%)	<50%	T L	1:1000	4
Kamposioras, 2013	CRC	Greece	106	71 (31–88)	65/41	NA	Moderate	Novocastra	1:350	7
Mstsuoka, 2011	CRC	Japan	156	65.5 (22–86)	99/57	99 (63.5%)	<67%	Zymed	1:400	8
Morikawa, 2011	CRC	USA	955	67.1	381/574	485 (50.8%)	Weak	T L	1:200	6
Fang, 2010	CRC	China	142	55 (15–78)	80/62	101 (71.1%)	<70%	DAKO	1:200	4
Filiz, 2010	CRC	Turkey	138	65 (21–89)	83/55	128 (92.8%)	Moderate	Labvision	NA	5
Boo, 2007	CRC	Korea	138	57.9 (18–82)	79/59	47 (34.1%)	<80%	DAKO	NA	5
Bravou, 2006	CRC	Greece	125	NA	NA	99 (79.2%)	<80%	BD	1:1000	6
Fernebro, 2004	RC	Sweden	252	68 (32–92)	173/96	117 (46%)	Weak	T L	1:5000	4
Maruyama, 2000	CRC	Japan	96	NA	NA	67 (69.8%)	<70%	T L	1:250	8

BZGBB = Beijing Zhongshan Golden Bridge Biotechnology, CC = colon cancer, CRC = colorectal cancer, DOP = definition of positive, NA = not available, R C = rectal cancer, reduced M(%) = the number and percentage of tissue samples with reduced β-catenin expression in membrane(M), Score = quality score of the literatures, TL = transduction laboratories.

Table 4

Characteristics of the studies of the nuclear β-catenin expression in the invasive front of cancer.

Author, year	Type	Country	No. of patients	Mean ages	Gender (M/F)	N (%)	DOP	Antibody source	Dilution	Score
Ouglkow, 2001	CC	Japan	202	60 (24–75)	110/92	18 (9.0%)	>10%	TL	1:100	5
Gao, 2014	CRC	China	181	NA	105/76	30 (16.6%)	>50%	BZGBB	1:500	6
Wang, 2014	RC	China	178	64 (35–78)	126/52	93 (52.2%)	>50%	CST	1:50	4

BZGBB = Beijing Zhongshan Golden Bridge Biotechnology, CC = colon cancer, CRC = colorectal cancer, CST = cell signaling technology, DOP = definition of positive, N(%) = the number and percentage of tissue samples with β-catenin overexpression in nucleus (N), NA = not available, NO = number, RC = rectal cancer, Score = quality score of the literatures, TL = transduction laboratories.

2.3. Data extraction and quality evaluation

For each eligible study, the following information were extracted by 2 reviewers (SZ and XW): (1) first author's name and country and the publication year; (2) number of patients; (3) age and gender of patients; (4) characteristics of the disease; (5) accumulated percentage of the different subcellular locations of β-catenin expression; (6) antibody source and dilution rate; and (7) definition of β-catenin positive. The quality of the eligible studies was evaluated using the Newcastle–Ottawa scale (NOS), which was described previously.[22,23]

2.4. Statistical analysis

The STATA (version 12.0, Stata Corp. College Station, TX) was utilized for this meta-analysis. Odds ratios (ORs) with

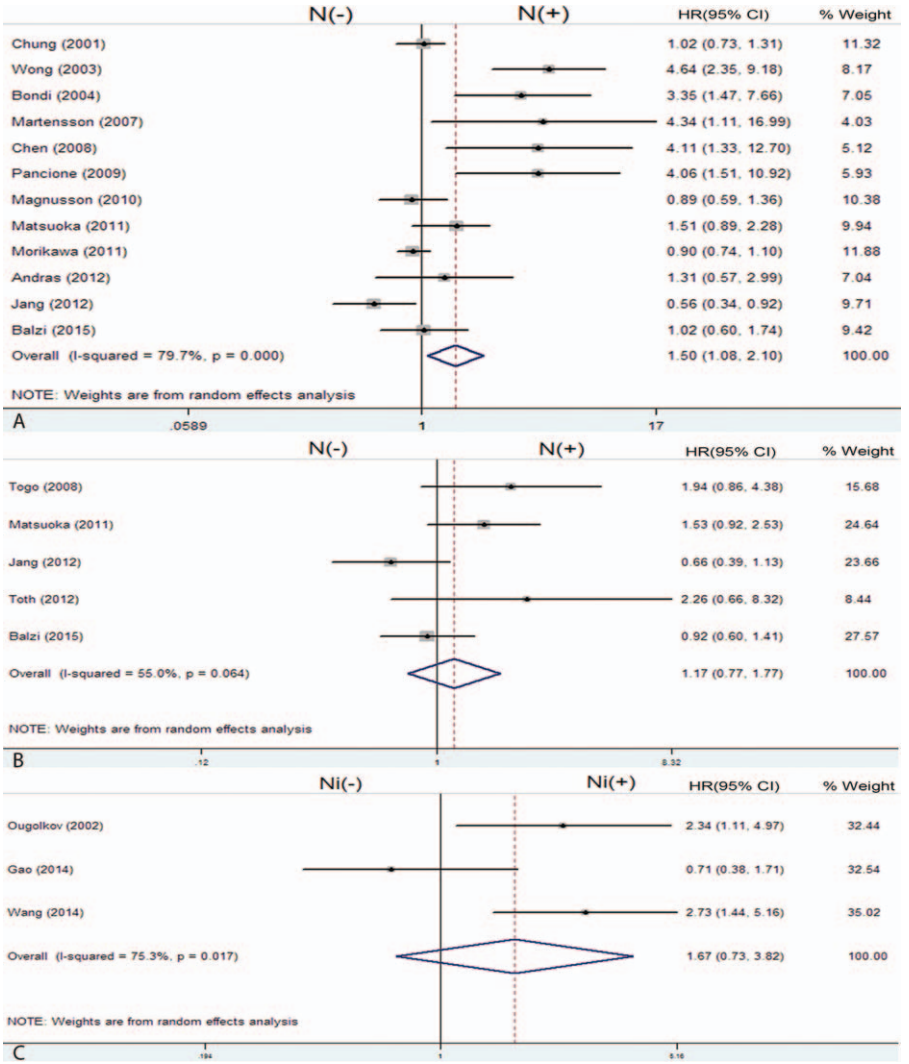

Figure 2. Forest plot of hazard ratio for the association of β-catenin expression with survival. (N(−):β-catenin negative expression in the nucleus; N(+):β-catenin overexpression in the nucleus). (A) HRs with corresponding 95% CIs of the β-catenin expression in nucleus with 5-year OS; (B) HRs with corresponding 95% CIs of the β-catenin expression in nucleus with DFS; (C) HRs with corresponding 95% CIs of the nuclear β-catenin expression in the invasive front of tumor with 5-year OS. CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, OS = overall survival.

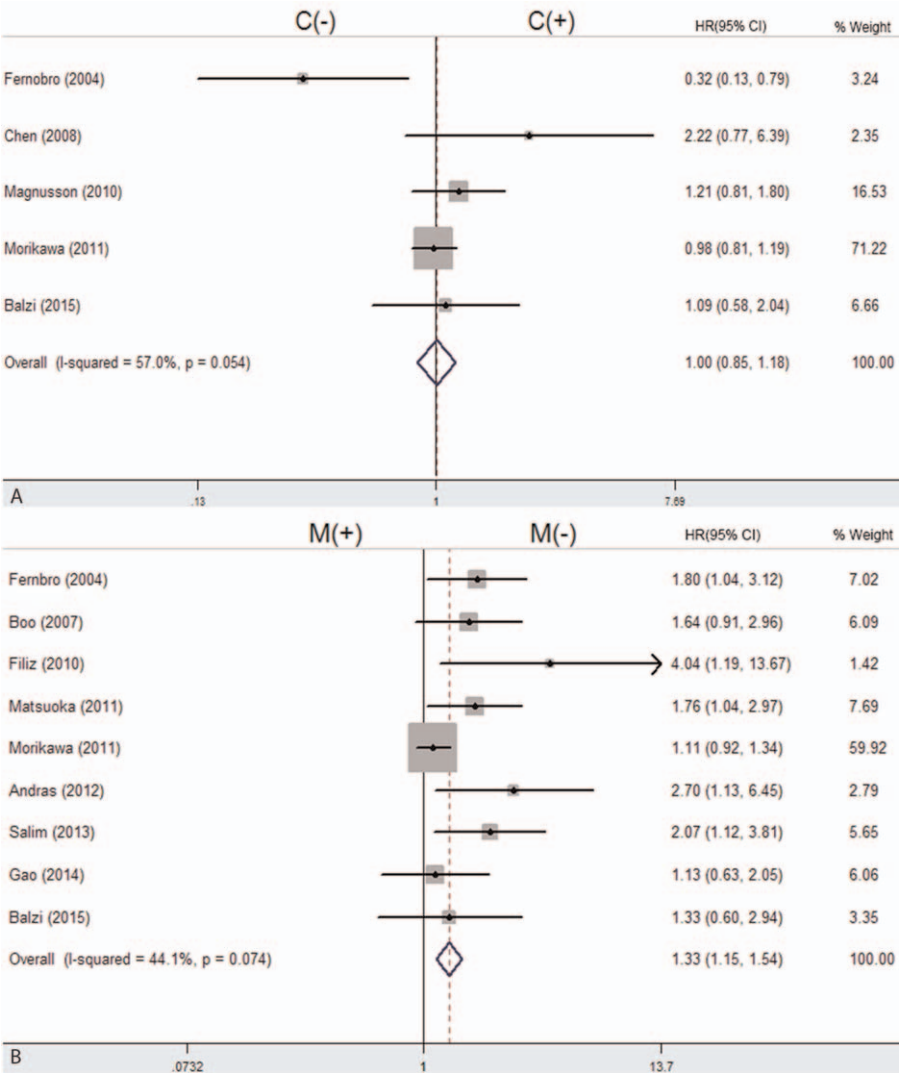

Figure 3. Forest plot of hazard ratio for the association of β-catenin expression in cytoplasm(A) and membrane(B) with 5-year OS.(C(−): β-catenin negative expression in the cytoplasm; C(+): β-catenin overexpression in the cytoplasm; M(−):reduced β-catenin expression in the membrane; M(+): β-catenin expression in the membrane). OS = overall survival.

95% confidence intervals (CIs) were used to evaluate the association between the different subcellular localizations of β-catenin expression and the prognosis or clinicopathological parameters. We pooled the statistical variables directly if they were depicted in articles. Otherwise, Kaplan–Meier curves were read by Engauge Digitizer to obtain the necessary data. The chi-square based Q statistical test was used to evaluate the heterogeneity among the outcomes of enrolled studies.[24] In addition, I^2 statistic represented the proportion of total variation caused by heterogeneity, and $I^2 > 50\%$ meant significant heterogeneity. According to the results of the Q statistical test, $P > 0.10$ indicated the outcomes of analysis among the results with low heterogeneity and a fixed-effects model was selected. In addition, the random-effects model was used for studies with $P < 0.05$. Egger's test and Begg's test were used to examine the potential risk of publication bias. Sensitivity analysis was performed following sequential omission of individual studies to evaluate the stability of the results.

3. Results

3.1. Studies description

A total of 445 eligible studies for inclusion based on the title was collected from different sources (Fig. 1). Then, 411 of them were excluded on the basis of inclusion and exclusion criteria. A total of 6216 cases, based on 29 studies that revealed a relationship between nuclear β-catenin expression and pathological features or DFS/5-year OS, were investigated and their major clinical characteristics are summarized in Table 1.[14,15,18–21,25–47] In addition, 9 studies showed a relationship between cytoplasmic β-catenin expression and pathological features or OS[17,19–21,25,33,34,37,43] (Table 2). Thirteen studies were selected for analysis of the prognostic value of reduced β-catenin expression in the membrane of CRC[17,19–21,26,31,34,35,38,43,48–50] (Table 3). There were also 3 studies included to examine the predictive role of nuclear β-catenin expression in the invasive front of tumor[17,41,51] (Table 4).

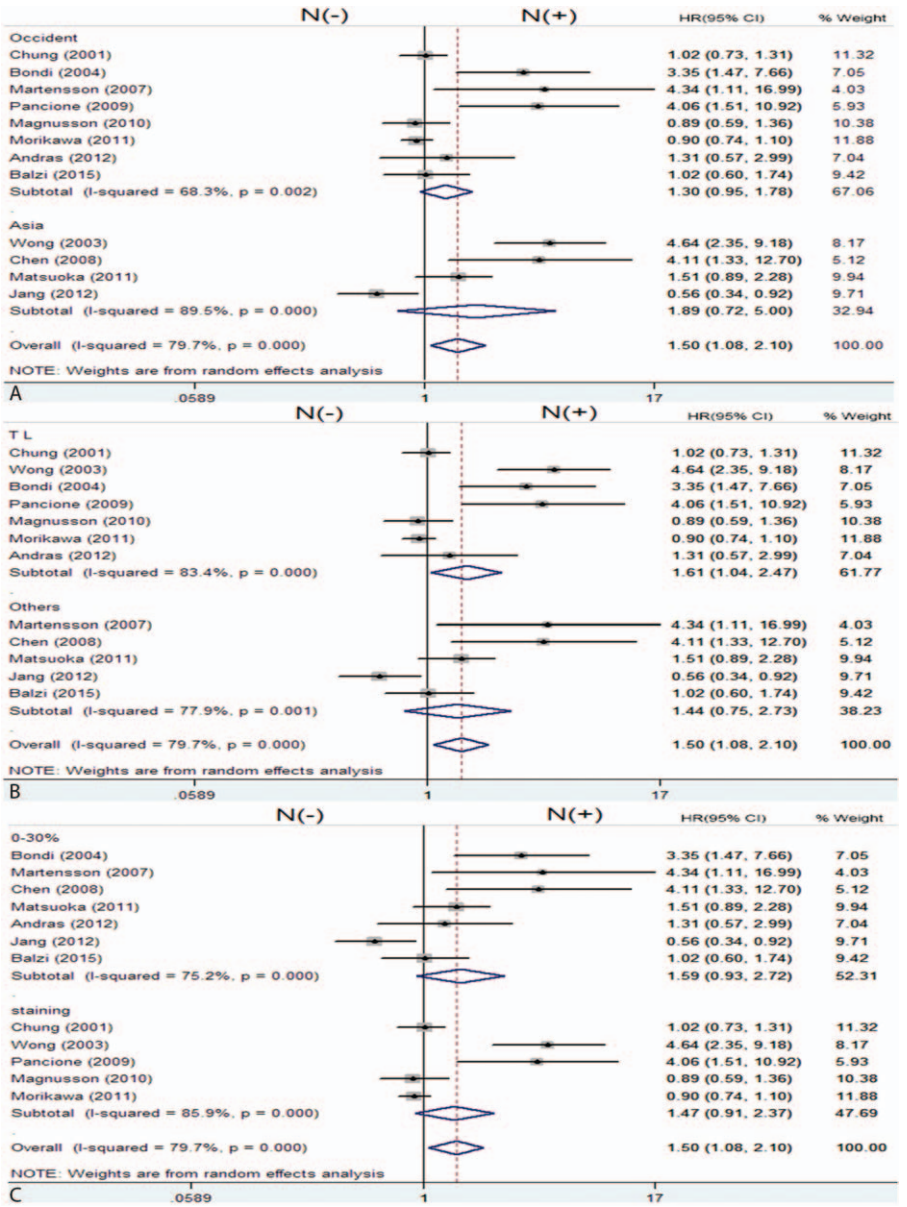

Figure 4. Forest plot of hazard ratio for the association of β-catenin expression in the nucleus with OS by subgroup analysis by study location (A), primary antibody sources (B), and evaluation standards(C). (N(−): β-catenin negative expression in the nucleus; N(+): β-catenin overexpression in the nucleus). OS = overall survival.

3.2. Quality of eligible studies

The Newcastle–Ottawa Scale (NOS) was conducted to assess the methodological quality of the studies. As described previously,[23] a score of 9 represented the highest quality and a score of 5 or more was considered as high quality. Twenty-seven studies included in our meta-analysis were of high quality with scores of 5 or more after quality assessment.

3.3. Prognostic value of β-catenin expression in colorectal cancer

Twelve enrolled studies provided the HRs and 95%CI directly or indirectly about the correlation between nuclear β-catenin overexpression and 5-year OS. The pooled HR of the β-catenin overexpression in nucleus with OS was 1.50 (95% CI: 1.08–2.10; Z = 2.40; $P = 0.016$) (Fig. 2A), but heterogeneity did exist ($I^2 = 79.7\%$ $P = 0.000$). The association of β-catenin overexpression in nucleus with DFS was analyzed based on 5 studies; the pooled HR was 1.17 (95% CI: 0.77–1.77; Z = 0.73; $P = 0.463$) (Fig. 2B). In addition, 3 studies assessed the association of nuclear β-catenin overexpression in the invasive front of tumor with OS; the pooled HR was 1.67 (95% CI: 0.73–3.82; Z = 1.22; $P = 0.221$) (Fig. 2C). Then, we evaluated the correlation between β-catenin overexpression in the cytoplasm and 5-year OS based on 5 studies, and the pooled HR was 1.00 (95% CI: 0.85–1.18; Z = 0.01; $P = 0.991$) (Fig. 3A). The pooled HR of the association of reduced membranous β-catenin expression and OS was 1.33 (95% CI: 1.15–1.54; Z = 3.81; $P = 0.0001$) based on 9 studies (Fig. 3B). The above results suggested that β-catenin overexpression in the nucleus was associated with lower OS, but not with DFS. In addition, reduced β-catenin expression in the membrane was correlated with a worse prognosis of CRC.

Table 5

Meta-analysis of β-catenin in the nucleus=cytoplasm and membrane.

Outcome of interest	Sublocation	No. of studies	Analytical model	OR/HR	95%CI	*P*	Isquared %	*P* value for heterogeneity
5-year OS	N	12	REM	1.5	1.08–2.10	0.016	79.7	0.000
	C	5	REM	1.00	0.71–1.42	0.991	57.0	0.054
	M	9	FEM	1.33	1.15–1.54	0.000	44.1	0.074
	Ni	3	REM	1.67	0.73–3.82	0.221	75.3	0.017
DFS	N	5	REM	1.17	0.77–1.77	0.463	55.0	0.064
Site (rectal vs colon)	N	10	REM	1.15	0.79–1.69	0.469	61.6	0.005
	C	4	FEM	1.85	1.42–2.44	0.000	0.0	0.751
Differentiation grade	N	13	FEM	0.72	0.54–0.96	0.003	34.8	0.104
(worse vs well=moderate)	C	5	FEM	0.59	0.41–0.86	0.006	33.8	0.196
	M	3	FEM	2.28	1.06–4.82	0.030	0.0	0.659
Lymph nodal status	N	9	REM	0.91	0.47–1.77	0.784	70.2	0.001
(N+ vs N-)	M	5	REM	1.16	0.49–2.74	0.739	79.9	0.001
Stage	N	17	REM	1.18	0.87–1.62	0.291	66.3	0.000
(III+IV vs I+II)	C	6	FEM	1.28	1.02–1.59	0.029	11.0	0.345
	M	5	REM	1.82	1.05–3.17	0.034	51.1	0.085
Metastasis	N	3	FEM	1.20	0.75–1.93	0.447	0.0	0.434
T (T3+T4 vs T1+T2)	N	5	FEM	1.76	1.16–2.68	0.007	0.0	0.771
Venous invasion	N	3	FEM	1.19	0.75–1.89	0.467	0.0	0.617
Lymphatic invasion	N	5	FEM	0.88	0.62–1.27	0.509	0.0	0.435

C=overexpression of β-catenin in cytoplasm, CI=confidence interval, N, overexpression of β-catenin in nucleus, DFS=disease-free survival, FEM=fixed-effects model, HR=hazard ratio, M=reduced expression of β-catenin in membrane, Ni=nuclear β-catenin overexpression in the invasive front of tumor, OR=odds ratio, OS=overall survival, REM=random-effects model.

However, β-catenin overexpressed in the cytoplasm or nuclear β-catenin overexpressed in the invasive front of tumor had no relationship with prognosis of CRC.

Subgroup analysis was performed by the publication year, study location, source of primary antibodies, and definition of β-catenin positive to explain the heterogeneity of the studies about the association of nuclear β-catenin with 5-year OS (Fig. 4). However, as indicated by subgroup analysis, a significant relationship between nuclear β-catenin overexpression and 5-year OS was shown only by an antibody sourced from the Transduction Laboratory (HR=1.61; 95%CI: 1.04–2.47; I^2= 83.4%, P=0.000). Other factors including study location and number of patients altered the significant prognostic impact of nuclear β-catenin expression. We could not identify the source of heterogeneity in this study by subgroup analysis; however, we inferred that the heterogeneity may have been caused by the different clinical features of patients or other factors we could not assess.

3.4. Correlations between β-catenin expression and clinicopathological factors

Next, we evaluated the correlations of the different subcellular localizations of β-catenin expression with clinicopathological characteristics in patients with CRC. As shown in Table 5, 13 studies were included for evaluation of the correlation between β-catenin expression in the nucleus and tumor differentiation grade. The pooled OR was 0.72 (95% CI: 0.54–0.96, Z=2.24, P=0.002) without heterogeneity (I^2 34.8% P=0.104) (Fig. 5A), which meansβ-catenin expression in the nucleus inversely correlated with differentiation grade. Patients with T3 and T4 CRC had significant nuclear β-catenin expression compared to patients with T1 and T2 CRC (OR=1.76, 95%CI: 1.16–2.68, Z=2.68, P=0.007) and without heterogeneity (I^2 0.0% P= 0.771) (Fig. 5B). However, other clinicopathological parameters, such as lymph nodal status (OR=0.91, 95%CI: 0.47–1.77), TNM stage (OR=1.18, 95%CI: 0.87–1.62), lymphatic invasion (OR=0.88, 95%CI: 0.62–1.27), venous invasion (OR=1.19, 95%CI: 0.75–1.89), and tumor site (OR=1.15, 95%CI: 0.79–1.69) had no relationship with β-catenin expression in the nucleus (Table 5). We also observed the significant association of β-catenin overexpression in the cytoplasm with tumor site, TNM stages, and differentiation grade (Table 5). The pooled ORs were 1.85 (95% CI: 1.42–2.44, Z=4.49, P=0.000), 1.28 (95% CI: 1.02–1.59, Z=2.18, P=0.029), and 0.59 (95% CI: 0.41–0.86, Z=2.16, P=0.030), respectively. All combined results were without heterogeneity ($I^2$0% P=0.751), (I^2 11.0% P=0.345) and (I^2 33.8% P=0.196), respectively. Furthermore, reduced β-catenin expression in membrane was significantly associated with differentiation grade and tumor stage, the pooled OR were 2.28 (95% CI: 1.06–4.82, Z=2.16, P=0.030) and 1.82 (95% CI: 1.05–4.82, Z=2.12, P=0.034), respectively (Table 5), but they were not associated with lymph nodal status (OR=1.16, 95%CI: 0.49–2.74, Z=0.33, P=0.739).

3.5. Publication bias

We assessed the publication bias by constructing a funnel plot (S1A Fig, S1B Fig, S2 Fig, http://links.lww.com/MD/B436) as more than 10 studies were included for meta-analysis. Egger's test indicated that publication bias existed when we evaluated the impact of β-catenin in the nucleus with 5-year OS, although Begg's Test showed no significant publications bias (P=0.064). However, with Egger's test, there is inadequate power of testing when the number of included studies is fewer than 20.[52] We performed sensitivity analysis and demonstrated that the pooled HRs were not significantly influenced by omitting any single study (S1C Fig, http://links.lww.com/MD/B436).

4. Discussion

Colorectal carcinogenesis is a complicated multistage process including multiple genetic alterations. The aberrant Wnt/β-catenin pathway has been proven to be involved in progression of CRC. Approximately 60% to 80% of CRCs development is

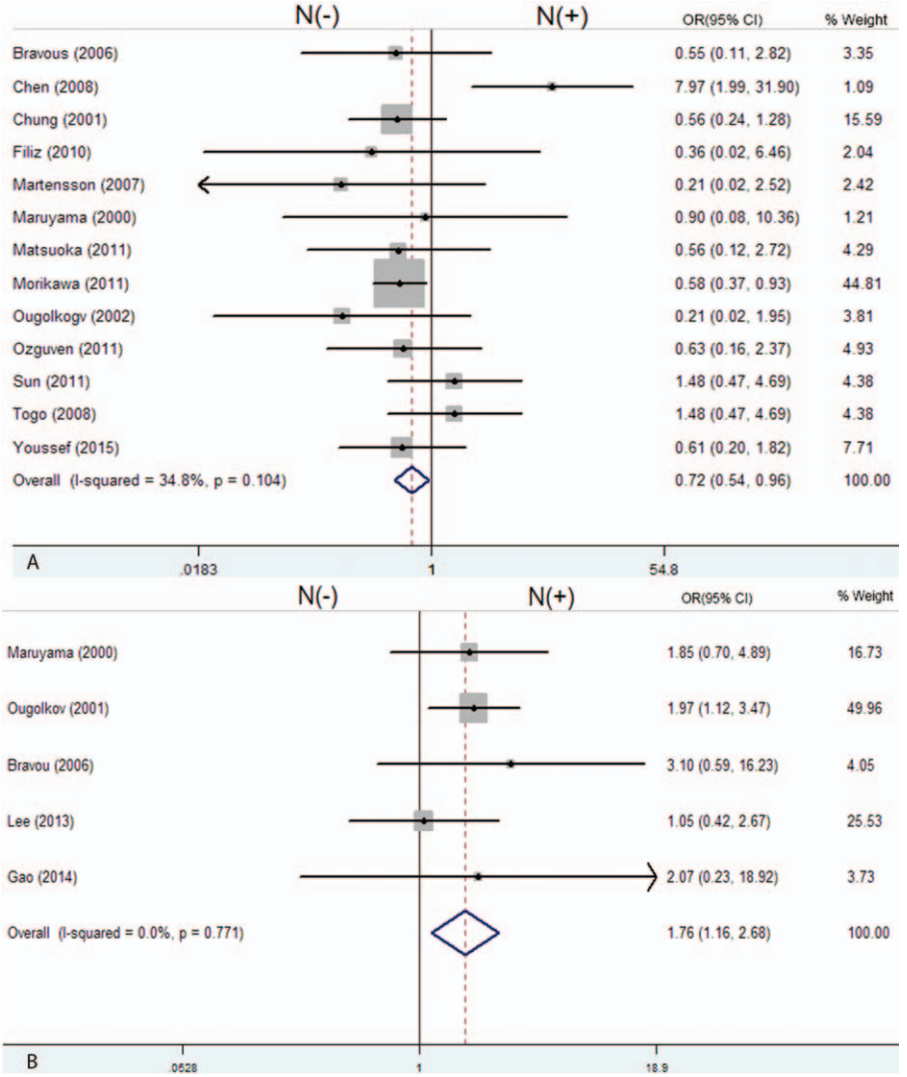

Figure 5. Forest plot of odd ratios for the association of nuclear β-catenin overexpression with differentiation grade (A) and depth of invasion (B). (N(−): β-catenin negative expression in the nucleus; N(+): β-catenin overexpression in the nucleus).

due to the aberrant activation of the Wnt/β-catenin signaling pathway.[53] Wnt signaling play a central role in both early colorectal tumorigenesis and later progression.[54] Activated Wnt/β-catenin signaling promotes EMT, migration, and invasion of CRC cells by targeting miR-150, BOP1, CKS2, and NFL3 genes, which induced a mesenchymal-like morphological change and experimental metastasis of CRC cells.[55,56] High Wnt/β-catenin signaling is also critical in the maintenance of the stem cell niche, which leads to tumor progression and metastasis.[10] β-catenin accumulation in the nucleus or cytoplasm was identified as a poor prognosis marker and nuclear β-catenin was implicated as a potential target for cancer therapy.[57,58] However, there were also contradictory results suggesting that β-catenin expression in the nucleus was associated with noninvasive tumors and a more favorable outcome.[59,60] Therefore, the prognostic significance of β-catenin expression in patients with CRC remains controversial and a systematic analysis is required to achieve a reliable conclusion. In this meta-analysis, we explored the prognostic significance of the different subcellular localizations of β-catenin expression for patients with CRC. The results indicated that nuclear expression of β-catenin or decreased expression of β-catenin in the membrane was associated with lower OS. However, no significant association was observed between β-catenin overexpression in the cytoplasm and 5-year OS, which was consistent with the previous results.[22] Unexpectedly, our results indicated that β-catenin overexpression in the nucleus and cytoplasm was negatively associated with differentiation grade, which needs further study to verify this conclusion.

Genetic mutations, such as mutation of APC or CTNNB1, are the main cause of accumulation of nuclear β-catenin.[61] Previous studies reported that the mutation rate of the CTNNB1 gene in CRC ranged from 10% to 50%.[62–64] Therefore, the β-cateninin the nucleus could be either mutant type or wild type; however, these 2 different typesof β-cateninin the nucleus are of functionally distinct. It is necessary to separate the wild-type

and mutant-type β-catenin proteins by expression staining and analyze their prognostic value. Here, we failed to distinguish whether the nuclear β-catenin was mutant type or wild type due to lack of relevant information. We inferred that the variable outcomes of the relationship between nuclear β-catenin expression and prognosis in CRC may be caused by the analysis of the mutant-type and wild-type β-catenin. Furthermore, such analysis may also contribute to inter-study heterogeneity.

In addition, we could not ignore the limitations in this meta-analysis. First, heterogeneity that would affect the results of meta-analysis does exist. The subjective evaluation of β-catenin expression, different source and dilution of primary antibodies, and different characteristics of patients in each study contributed to significant heterogeneity. However, we failed to identify the source of heterogeneity by stratified analysis. To eliminate variations across studies, the random-effects model was performed accordingly. Second, we did not include non-English studies, which might introduce potential language bias. In addition, publication bias existed as only studies performed with positive results or significant outcomes were suitable for publication. Another potential source of bias might have come from the less reliable data that were extrapolated from survival curves. As the presence of inevitable limitations exists in this meta-analysis, additional large and well-designed prospective studies are should to be conducted.

Our study is the first to meta-analyze the association between the reduced membranous expression of β-catenin and prognosis in CRC patients. The pooled data suggested that reduced expression of β-catenin in the membrane is significantly associated with poor survival in patients with CRC. In addition, nuclear β-catenin overexpression, rather thancytoplasmic β-catenin overexpression, could serve as a biomarker of poor prognosis on CRC. New approaches to therapeutically target Wnt/β-catenin pathway needs to be explored, and it is necessary to distinguish the differential subcellular localizations of β-catenin to develop different therapeutic strategies.

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