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Original Article

Clinicopathological and prognostic significance of beta-catenin expression in patients with bone and soft tissue sarcoma: a meta-analysis

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Abstract: The prognostic role of beta-catenin expression in bone and soft tissue sarcoma remains controversial. To investigate the impact of beta-catenin expression on survival outcomes and clinicopathological features in sarcoma patients, a meta-analysis was conducted. Comprehensive literature searches were performed in PubMed, Embase, Web of Science, and Cochrane Library for relevant studies. Pooled hazard ratio (HR) and relative risk (RR) were calculated to assess the correlations of beta-catenin expression with survival outcomes and clinicopathological features. In total, 14 studies with 1036 sarcoma patients were identified. Beta-catenin expression was found to be significantly associated with poor overall survival (HR=2.11, 95% CI: 1.65-2.69/P<0.001). Further, when the analysis was stratified by histological subtype (bone sarcoma including osteosarcoma and chondrosarcoma and soft tissue sarcoma including synovial sarcoma), subcellular staining position (nuclear staining and nuclear/cytoplasmic staining), and statistical analysis (multivariate analysis and univariate analysis), the significant correlation to poor survival was also observed except in chondrosarcoma group. For clinicopathological features, beta-catenin expression was significantly associated with higher rate of metastasis (RR=1.74, 95% CI=1.35-2.25; P<0.001) and local recurrence (RR=2.84, 95% CI=1.17-6.87; P=0.021), higher tumor grade (RR=1.38, 95% CI=1.02-1.86; P=0.039) and higher stage at diagnosis (RR=1.45, 95% CI=1.14-1.85; P=0.003), but not associated with tumor site and tumor size. In conclusion, beta-catenin expression may be an effective prognostic factor of poor survival and clinicopathological features in patients with bone and soft tissue sarcoma. Future studies are needed to validate our findings.

Keywords: Beta-catenin, sarcoma, prognosis, meta-analysis

Introduction

Sarcomas are a heterogeneous group of mesenchymal neoplasmas that can be divided into two general groups, primary bony sarcoma and soft tissue sarcoma [1]. Primary bony sarcomas mainly include osteosarcoma, Ewing's sarcoma and chondrosarcoma; soft tissue sarcomas mainly include leiomyosarcoma, synovial sarcoma, liposarcoma and angiosarcoma. The survival rate of sarcoma patients has increased with the development of surgical techniques and emergence of effective chemotherapy regimens [2]. However, metastasis still occurs in 20-55% of these patients, which remains the main cause of death [3]. For sarcoma patients with metastatic disease who have lost surgical intervention opportunity, effective systematic therapies are important to prolong life and improve life quality [4]. But efforts in the past 20 years including changes of the chemotherapy drugs, doses and administration schemes did not significantly improve survival [5]. Advanced treatment methods are urgently needed. Identification of prognostic oncological biomarkers could help to discover new targets and stratify patients for different treatments. Although have not entered into clinical application, a number of effective biomarkers of sarcoma have been discovered and could potentially contribute to the development of new treatment methods [6-8].

Beta-catenin is one of the essential molecules of E-cadherin-catenin complex, which plays a crucial role in cell-to-cell adhesion and main-

taining the integrity of cellular structure [9]. Besides, beta-catenin is also involved in Wnt signaling pathway, which could activate the transcription of Wnt target genes leading to tumor cell proliferation, invasion and metastasis [10, 11]. Indeed, beta-catenin expression was found to be related to poor prognosis in several cancers, including gastric, lung and colorectal cancers [12-14].

However, the prognostic role of beta-catenin expression in bone and soft tissue sarcoma has not reached a consensus with inconsistent results reported in previous studies [15-28]. To date, no comprehensive meta-analysis has been conducted to clarify this issue. Therefore, we performed the current meta-analysis to combine published studies and to comprehensively evaluate the prognostic significance of beta-catenin expression in bone and soft tissue sarcoma and its association to the clinicopathological features.

Materials and methods

Search strategies

Comprehensive electronic literature searches were conducted in PubMed, Web of Science, Embase and Cochrane Library with no restriction to language and date of publication. The last search was conducted on Dec 3, 2015. The search terms were as follows: ("β-catenin" OR "Beta-catenin" OR "CTNNB1") AND ("sarcoma" OR "soft tissue sarcoma" OR "bone sarcoma" OR "osteosarcoma" OR "chondrosarcoma" OR "Ewing sarcoma" OR "leiomyosarcoma" OR "angiosarcoma" OR "malignant fibrous histiocytoma" OR "liposarcoma" OR "rhabdomyosarcoma" OR "synovial sarcoma"). In addition, reference lists of identified studies were traced by Google Scholar for potential studies.

Inclusion and exclusion criteria

Studies were eligible for inclusion if they met the following criteria: (1) included patients with pathologically confirmed bone and soft tissue sarcoma; (2) analyzed the correlation of beta-catenin expression with clinical features and/or survival outcomes; (3) relevant data of clinical features and/or survival outcomes could be extracted; (4) were in language of English or Chinese. The following studies were excluded: (1) non-human research including animal experiments and cell research; (2) case reports, reviews, letters and conference abstracts; (3) not focused on patients with bone and soft tissue sarcoma; (4) not related to beta-catenin expression; (5) reported overlapping patients; (6) with insufficient information that the association to survival or clinical features cannot be extracted. When articles recruiting overlapping patients were identified, the most recent published article was included. The literatures were assessed independently by two authors for eligibility. Any disagreement was adjudicated by corresponding author.

Data extraction and quality assessment

Data of interest was extracted independently by two authors. The required data included: (1) basic information including first author, year of publication, study period, follow-up duration and study design; (2) data of patient and tumor including patient source, number of patient, age, gender, percentage of positive beta-catenin expression, histology type of tumor, grade, tumor site and tumor stage at diagnosis; (3) outcome measures including local recurrence, metastasis, overall survival and Kaplan–Meier curves; and (4) other variables including the methods of quantitative beta-catenin measurement, the definition of positivity (the cur-off value) and the antibody's source, type and dilution used for immunohistochemistry (IHC).

Newcastle-Ottawa Scale (NOS) (www.ohri.ca/programs/clinical_epidemiology/oxford.asp) was adopted to evaluate each included article's quality. Based on the quality of each study in selection, comparability and exposure, a score with a maximum of 9 points was appointed. Studies with 6 or more of the NOS scores were considered as high-quality and were included in the meta-analysis.

Statistical analysis

To evaluate the effect of beta-catenin expression on prognosis and pathological features, we calculated the pooled hazard ratio (HR) for overall survival and the pooled relative risk (RR) for clinicopathological variables (metastasis, local recurrence, grade, tumor site, tumor size and stage at diagnosis). Tumor staging was according to American Joint Committee on

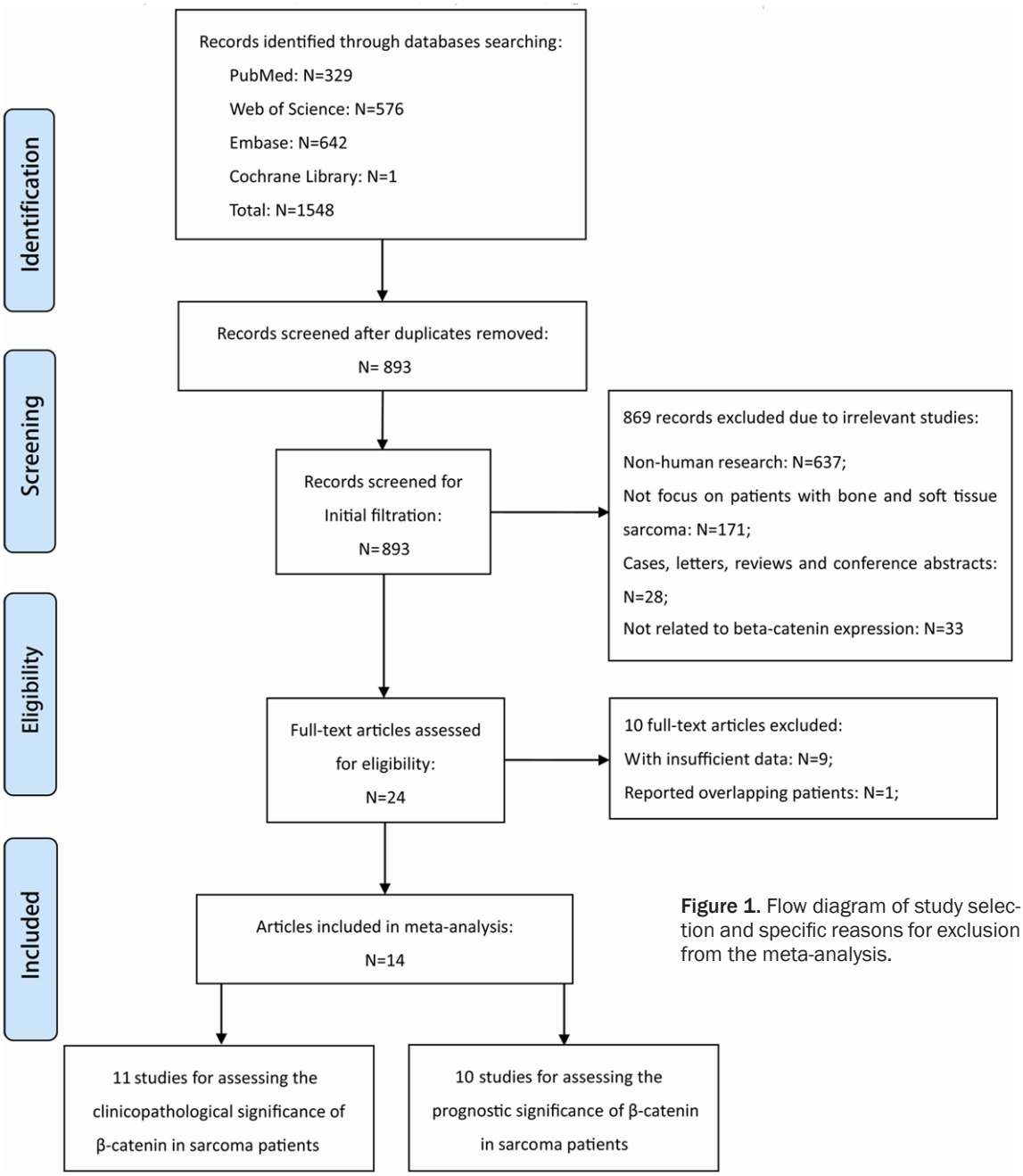

Figure 1. Flow diagram of study selection and specific reasons for exclusion from the meta-analysis.

Cancer (AJCC) System. If the HRs were given directly in the article, we used the original data. If the data were not given directly, we calculated the HRs with 95% CIs from outcome data available in the publications or from Kaplan-Meier curves through methods reported by Tierney et al [29-32]. Supplementary files or additional files of the included articles were also checked for available data.

Following recommendations of Cochrane Handbook (http://www.cochrane.org/training/cochrane-handbook), heterogeneity was assessed using I^2 statistic and Chi-squared test. Fine heterogeneity was defined as $I^2<50\%$ and $P>0.1$, suggesting that a fixed-effect model (Mantel-Haenszel method) would be performed. Otherwise, a random-effect model (DerSimonian and Laird method) would be applied if significant heterogeneity was observed ($I^2>50\%$ or $P<0.1$). As for publication bias, we conducted Begg's funnel plot test in which log (HRs) were plotted against their corresponding standard errors (SEs), and exam-

Table 1. Characteristics of eligible studies included in the meta-analysis

Study	Patient source	Study period	Follow-up duration (range), months	Histology type	Mean age (range), years	Number of patients	Percentage of beta-catenin expression, %	HR estimation	Study design	NOS score
Lu Y 2015 [15]	China	2007-2009	minimum 36	Osteosarcoma	20 (12-56)	96	61.5	Provided	S	9
Wan Y 2014 [16]	China	2001-2010	41.5 (8-85)	Osteosarcoma	18.6 (9-38)	37	40.5	–	S	7
Chen C 2014 [17]	China	NR	30 (4-98)	Chondrosarcoma	NR	63	54.0	Provided	M	7
Le Guellec 2013 [18]	France	1996-2006	77.4 (52.3-101.2)	Osteosarcoma	18 (8-57)	33	33.3	Available data	S	7
Kim 2013 [19]	Korea	1998-2011	NR	STS	NR	104	48.1	Provided	S	7
Deng Z 2013 [20]	China	2000-2008	NR	Osteosarcoma	18.3 (8-58)	90	60.0	Provided	S	8
Yang J 2010 [21]	China	2004-2008	26	Osteosarcoma	22.6 (12-49)	54	66.7	Available data	S	8
Wei X 2010 [22]	China	NR	66 (1-252)	Synovial sarcoma	36.5	98	53.1	–	S	7
Horvai 2006 [23]	USA	NR	40.2 (3-191)	Synovial sarcoma	34 (14-77)	43	55.8	Survival curve	S	7
Engellau 2005 [24]	Sweden	1988-2000	70 (30-165)	STS	69 (16-94)	140	16.4	–	S	7
Haydon 2002 [25]	USA	NR	60 (1-166)	Osteosarcoma	31.4	47	70.2	Provided	S	8
Hasegawa 2001 [26]	Japan	NR	61 (9-249)	Synovial sarcoma	34 (7-72)	44	56.8	Provided	S	7
Saito 2000 [27]	Japan	1955-1998	55.5 (1-232)	Synovial sarcoma	NR	72	38.9	Survival curve	S	8
Kuhnen 2000 [28]	Germany	1991-1998	28.6 (6-75)	STS	55.5 (21-85)	115	32.2	–	S	7

HR, hazard ratio; *NOS*, Newcastle-Ottawa Scale; *NR*, not reported; *STS*, soft tissue sarcoma; *S*, single center; *M*, multi-center.

Table 2. Methods of quantitative beta-catenin measurement of the included studies

Study	Method	Antibody type	Antibody dilution	Antibody Source	Definition of beta-catenin expression	
					Subcellular staining position	Cut-off value
Lu Y 2015 [15]	IHC	Polyclonal	NAG	Cell Signaling Technology	NAG	Score ≥4
Wan Y 2014 [16]	IHC	Monoclonal	1:1000	Cell Signaling Technology	NAG	Score ≥4
Chen C 2014 [17]	IHC	Monoclonal	1:500	BD Transduction Laboratories	Nuclear staining	Positive cells >10%
Le Guellec 2013 [18]	IHC	NAG	1:200	Dako	Membrane and Cytoplasm staining	Score ≥5
Kim 2013 [19]	IHC	Monoclonal	1:100	BD Transduction Laboratories	Nuclear staining	Score ≥3
Deng Z 2013 [20]	IHC	NAG	NAG	ZSGB-BIO	Nuclear and/or cytoplasmic staining	Score ≥6
Yang J 2010 [21]	IHC	NAG	NAG	ZSGB-BIO	NAG	Positive cells >10%
Wei X 2010 [22]	IHC	NAG	NAG	NAG	NAG	Positive cells >10%
Horvai 2006 [23]	IHC	Polyclonal	1:5000	BD Transduction Laboratories	Nuclear staining	Positive cells >10%
Engellau 2005 [24]	IHC	Monoclonal	1:5000	BD Transduction Laboratories	NAG	Positive cells >20%
Haydon 2002 [25]	IHC	NAG	1:200	BD Transduction Laboratories	Nuclear and/or cytoplasmic staining	NAG
Hasegawa 2001 [26]	IHC	Monoclonal	1:500	BD Transduction Laboratories	Nuclear staining	Positive cells >50%
Saito 2000 [27]	IHC	Monoclonal	1:200	BD Transduction Laboratories	Nuclear and/or cytoplasmic staining	Positive cells >75%
Kuhnen 2000 [28]	IHC	Monoclonal	1:300	BD Transduction Laboratories	Nuclear and/or cytoplasmic staining	Score ≥5

IHC, immunohistochemistry; *NAP*, not accurately given.

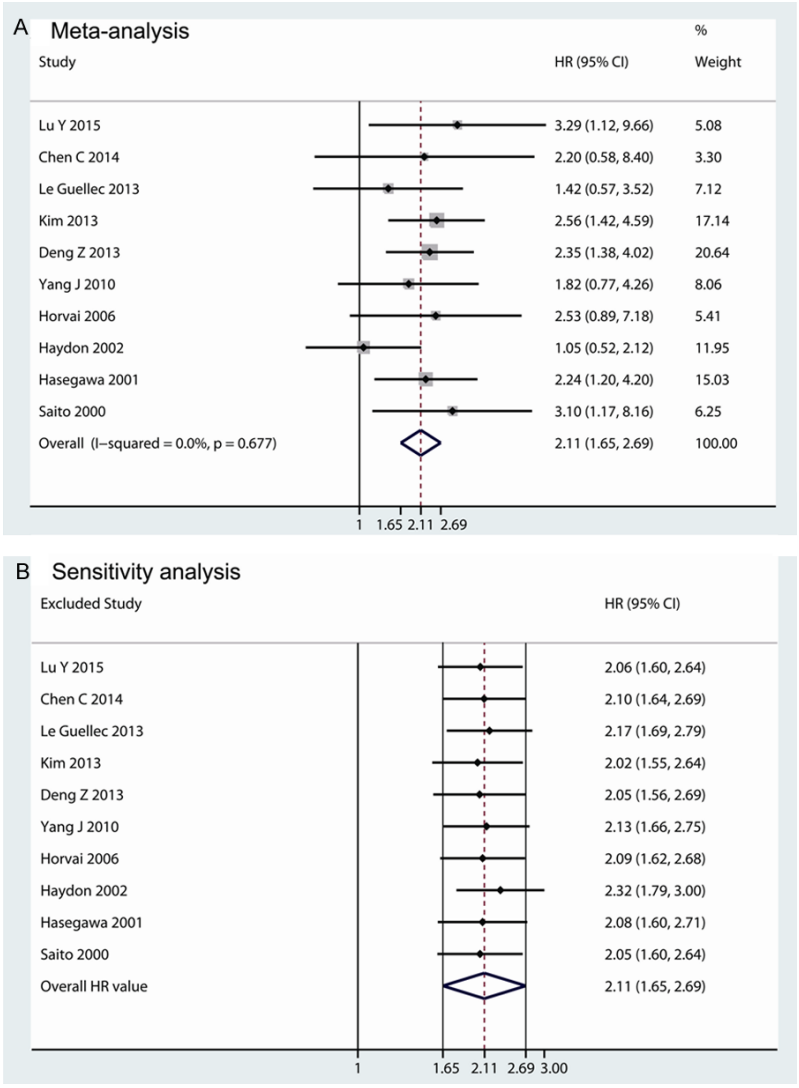

Figure 2. Forrest plots of meta-analysis and sensitivity analysis for the association between beta-catenin expression and overall survival in patients with bone and soft tissue sarcoma. A. Significant poor overall survival was observed in patients with beta-catenin expression; B. No statistically different results were observed when excluding every single study in sequence, indicating the stability of the results.

ined its asymmetry. In addition, we conducted Egger's test which could provide statistic estimation of publication bias (P>0.05 indicated no publication bias existed). Sensitivity analysis was performed to assess the stability of the results, in which the influence of a single study on the overall estimate could be detected by omitting individual study sequentially. All statistical analyses of the meta-analysis were conducted using STATA version 12.0 (STATA Corporation, College Station, TX).

Results

Searches results

At primary retrieval, a total of 1548 citations were identified by searching through 4 electronic databases, including 329 citations in PubMed, 576 citations in Web of Science, 642 citations in Embase and 1 citation in Cochrane Library. After removing 655 duplicates, the remaining 893 records were screened for initial filtration. Then, 869 records were excluded due to irrelevant studies, including 637 non-human studies, 171 studies not focusing on patients with bone and soft tissue sarcomas, 28 records of cases, reviews, letters or conference abstracts, and 33 studies not related to beta-catenin expression. Among the remaining 24 articles for full-text viewing, 10 were excluded, including 9 articles with insufficient data and 1 article with overlapping patients. Eventually, 14 articles published from 2000 to 2015 were included in the current meta-analysis [15-28] (**Figure 1**).

Characteristics of eligible studies

The basic characteristics of the included 14 studies are summarized in **Table 1**. Among them, 10 studies analyzed the prognostic significance of beta-catenin expression, and 11 studies focused on investigating clinicopathological significance. One study was multi-center designed and the rest were single-center designed. Osteosarcoma was the most studied histology subtype of all sarcomas, which was researched in 6 studies. Then were synovial sarcoma in 4 studies, and chondrosarcoma in

Table 3. Meta-analysis of the association between beta-catenin expression and overall survival

	No. of studies	Patients	Heterogeneity test (I^2, P)	Effect model	Combined HR (95% CI)/*P* value	Conclusion
Overall patients	10	644	0.0%, 0.677	Fixed	2.11 (1.65-2.69)/<0.001	Significant
Stratified by tumor histology						
Total bone sarcoma	6	383	0.0%, 0.447	Fixed	1.84 (1.33-2.54)/<0.001	Significant
Osteosarcoma	5	320	14.5%, 0.322	Fixed	1.82 (1.30-2.54)/<0.001	Significant
Chondrosarcoma	1	63	–	–	2.20 (0.58-8.37)/0.248	Not significant
Total soft tissue sarcoma	4	261	0.0%, 0.958	Fixed	2.51 (1.74-3.62)/<0.001	Significant
Synovial sarcoma	3	157	0.0%, 0.858	Fixed	2.48 (1.55-3.96)/<0.001	Significant
Stratified by subcellular staining position						
Nuclear staining	4	254	0.0%, 0.989	Fixed	2.40 (1.64-3.51)/<0.001	Significant
Nuclear and/or cytoplasmic staining	3	209	53.7%, 0.115	Random	1.91 (1.04-3.50)/0.036	Significant
Stratified by statistical analysis method						
Multivariate-analysis	6	444	1.0%, 0.410	Fixed	2.12 (1.60-2.82)/<0.001	Significant
Univariate-analysis	4	200	0.0%, 0.670	Fixed	2.06 (1.29-3.29)/0.002	Significant

HR, hazard ratio.

1 study. Particularly, the rest 3 studies included patients with a mixed type of soft tissue sarcomas, including leiomyosarcoma, liposarcoma, malignant fibrous histiocytoma and other subtypes. In total, 1036 sarcoma patients were included, and the patient samples of a single study ranged from 33 to 140. The overall rate of beta-catenin expression was 46.4% (481/1036). The NOS scores of the included studies are counted and shown in **Table 1**. All the studies have 6 or more of the NOS scores.

The methods of quantitative beta-catenin measurement of individual study are summarized in **Table 2**. All studies applied IHC to detect beta-catenin expression, with different sources, types and dilutions of antibody. The definition of beta-catenin expression also differed among them. Seven studies define the cut-off value by percentage of positive staining cells, and the rest seven studies define it by using a scoring system combining staining percentage and intensity.

Correlation between beta-catenin expression and overall survival

A total of 10 studies with 644 patients were included in the analysis of overall survival. The heterogeneity was not significant (I^2=0.0%, P=0.677). Under fixed-effect model, the pooled HR was 2.11 (95% CI: 1.65-2.69, P<0.001), suggesting that beta-catenin expression was an indicator for poor overall survival (**Figure 2A** and **Table 3**).

In the subgroup analysis stratified by histology subtype, the pooled HR for overall survival was 1.84 (95% CI: 1.33-2.54) for patients with bone sarcomas and 2.51 (95% CI: 1.74-3.62) for patients with soft tissue sarcomas. For individual histology subtype, the HR was 1.82 (95% CI: 1.30-2.54) for osteosarcoma, 2.20 (95% CI: 0.58-8.37) for chondrosarcoma and 2.48 (95% CI: 1.55-3.96) for synovial sarcoma (**Table 3**). The heterogeneity was not significant in all the analyses.

Then, a subgroup analysis stratified by subcellular staining position was conducted. Either positive nuclear staining (HR=2.40, 95% CI: 1.64-3.51; P<0.001) or nuclear and/or cytoplasmic staining (HR=1.91, 95% CI: 1.04-3.50; P=0.036) for beta-catenin was found to be significant associated with poor overall survival (**Table 3**). The heterogeneity was not significant in the subgroup of nuclear staining (I^2=0.0%, P=0.698), but significant in the subgroup of nuclear and/or cytoplasmic staining (I^2=53.7%, P=0.115).

We also conducted a subgroup analysis stratified by statistical analysis method. Six included studies reported HRs from multivariate analysis, which could reduce bias from some major confounders [33]. HRs from univariate analysis was available in another 4 studies. Correlations to poor overall survival were significant in both multivariate analysis group (HR=2.12, 95% CI: 1.60-2.82; P<0.001) and univariate analysis group (HR=2.06, 95% CI: 1.29-3.29; P=0.002) without significant heterogeneity (**Table 3**).

Correlation between beta-catenin expression and tumor clinicopathological features

The pooled RRs indicated that beta-catenin expression was significantly associated with

Table 4. Meta-analysis of the association between beta-catenin expression and tumor clinicopathological features

	No. of studies	Patients	Heterogeneity test (I^2, P)	Effect model	Combined RR (95% CI)/*P* value	Conclusion
Metastasis (yes vs. no)	6	419	0.0%, 0.605	Fixed	1.74 (1.35-2.25)/<0.001	Significant
Local recurrence (yes vs. no)	3	230	0.0%, 0.628	Fixed	2.84 (1.17-6.87)/0.021	Significant
Grade (high vs. low)	6	428	66.1%, 0.011	Random	1.38 (1.02-1.86)/0.039	Significant
Stage[a] at diagnosis (III-IV vs. I-II)	5	376	0.0%, 0.485	Fixed	1.45 (1.14-1.85)/0.003	Significant
Tumor site (femur/tibia vs. other)	6	359	0.0%, 0.937	Fixed	0.99 (0.86-1.14)/0.871	Not significant
Tumor size (>5 cm vs. <5 cm)	2	141	0.0%, 0.958	Fixed	1.03 (0.79-1.32)/0.849	Not significant

[a]According to AJCC stage system. *RR*, relative risk.

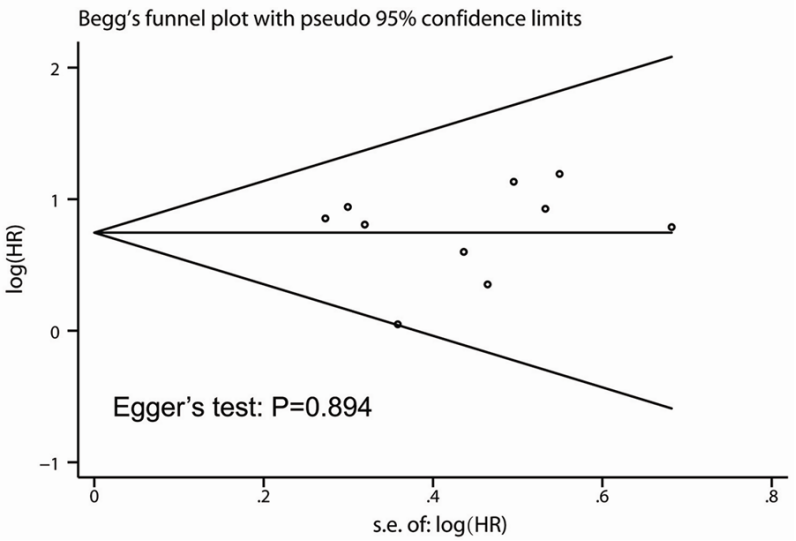

Figure 3. Publication bias of the association between beta-catenin expression and overall survival in patients with bone and soft tissue sarcoma detected by Begg's plot (no apparent asymmetry was found) and Egger's test (P=0.894), indicating no significant publication bias was observed.

increased risk of unfavorable clinicopathological outcomes in sarcoma patients, including higher risk of metastasis (RR=1.74, 95% CI: 1.35-2.25; P<0.001) and local recurrence (RR=2.84, 95% CI: 1.17-6.87; P=0.021), higher tumor grade (RR=1.38, 95% CI: 1.02-1.86; P=0.039) and higher stage at diagnosis (RR=1.45, 95% CI: 1.14-1.85; P=0.003), but not associated with tumor site (RR=0.99, 95% CI: 0.86-1.14; P=0.871) and tumor size (RR=1.03, 95% CI: 0.79-1.32; P=0.849). The heterogeneity was only observed within the association between beta-catenin expression and tumor grade (I^2=66.1%, P=0.011) (**Table 4**).

Sensitivity analysis and evaluation of publication bias

Sensitivity analysis was performed to assess the influence of a single study on the pooled HR value. For a more intuitive viewing, we put the forest plots from the meta-analysis (**Figure 2A**) and sensitivity analysis (**Figure 2B**) together. As the **Figure 2B** shows, when excluding individual study sequentially, we did not found any significant changes of the pooled HR, indicating that the meta-analysis was statistically stable and reliable.

Publication bias was evaluated by Begg's funnel plot test, in which log (HRs) were plotted against their corresponding standard errors (SEs). Visual assessment of the funnel plot found no apparent asymmetry (**Figure 3**). In addition, Egger's test which provided statistic estimation found no publication bias existed (P=0.894), indicating that the publication bias was not significant among the included studies.

Discussion

Beta-catenin is a central molecule of Wnt signaling pathway, which is expressed in three main forms: cell membrane, cytoplasm and nucleus localization. When it is expressed in the membrane, it plays an important role in maintaining cell-to-cell adhesion through forming cadherin-catenin complex with E-cadherin and actin filaments [9]. The complex is important for the integrity of cellular structure. Downregulation of any components of this complex could compromise the integrity of intercellular adhesion, thus facilitating tumor cell invasion and metastasis [34].

The other two forms are involved in the regulation of Wnt signaling pathway. Under normal circumstances, beta-catenin maintains at a low level of cytoplasmic concentration. In the absence of a Wnt ligand, beta-catenin is bound by a destruction complex that is composed of three proteins: scaffolding protein Axin, glycogen synthase kinase 3beta (GSK3beta) and casein kinase 1 (CK1) [35]. Then, beta-catenin is phosphorylated by GSK3beta and CK1, ubiquitinated by beta-transducing repeat-containing protein (beta-TrCP) and finally destructed by proteasome. However, when the Wnt pathway is activated, the Wnt ligand binds to receptors, which would make Axin translocates from cytoplasm to the transmembrane receptor complex, thus prohibiting the forming of the destruction complex [11]. As a result, beta-catenin accumulates in the cytoplasm and translocates to the nucleus, where it subsequently binds Lef/T-cell factor (TCF) transcription factors and activates Wnt target genes, leading to tumor cell proliferation, invasion and progression [10, 36].

Literatures have found that the expression of beta-catenin could contribute to proliferation, invasion and metastasis in several tumor cell lines [37, 38]. As for sarcoma, laboratory studies have also provided some details indicating a crucial role of beta-catenin in the development and progression of sarcoma cells. Kansara et al. [39] found that beta-catenin is overexpressed with the silence of tumor suppressor gene WIF1, which is correlated with the loss of differentiation and increased proliferation. Ma et al. [40] found that major components of Wnt/beta-catenin pathway, such as beta-catenin, Wnt3a and Lef1, were consistently up-regulated in human osteosarcoma cell line. Besides, the knock down of beta-catenin increased the sensitivity to methotrexate and induced cell death, which indicated a potential responsibility of beta-catenin for the invasiveness and chemo-resistance of osteosarcoma.

More recently, several systematic reviews indicated its prognostic significance of poor prognosis in several cancers, including gastric, lung and colorectal cancers [12-14]. However, inconsistent results were reported in previous studies about its prognostic significance in bone and soft tissue sarcoma and its association to the clinicopathological features.

In the current meta-analysis, we combined 14 studies related to prognosis and clinicopathology of beta-catenin expression in sarcoma patients. Firstly, for the clinicopathological significance, the pooled analysis showed that beta-catenin expression was significantly associated with some unfavorable tumor characteristics including metastasis, local recurrence, grade and stage; but the correlations to tumor size and site were not found. The summarized results were consistent with previously published laboratory studies [40, 41], and further verified the contribution of beta-catenin expression to sarcoma proliferation and invasion to some extent.

Then, to evaluate the prognostic value of beta-catenin expression, we integrated eligible survival outcomes from the included studies and calculated the pooled HR. We found that its expression was significantly associated with poor overall survival for the whole sarcoma group, and also in the subgroups of bone sarcoma and soft tissue sarcoma. For each individual histology subtype, the significant correlation was observed in patients with osteosarcoma and synovial sarcoma, but not found in chondrosarcoma patients. Although we attempt to search relevant articles completely, it should be noted that only one study was identified for patients with chondrosarcoma [17], which might lead to potential bias to the results. Considering we only included 63 patients with chondrosarcoma, the negative finding might be due to the insufficient patient samples. It also should be noted that, since several studies included patients with mixed types of soft tissue sarcomas [19, 24, 28], and the rest articles on soft tissue sarcoma all investigated synovial sarcoma, the HRs on other separate subtypes of soft tissue sarcoma other than synovial sarcoma were not able to be calculated. It is better to investigate the significance of beta-catenin expression in patients with chondrosarcoma and other subtypes of soft tissue sarcoma in future studies.

As we found that beta-catenin expression was significantly associated with unfavorable pathological characteristics of metastasis, local recurrence, grade and stage, the correlation between overall survival and its expression may be influence by these confounders. To validate whether its expression was an independent prognostic factor for poor overall survival,

we conducted a subgroup analysis stratified by statistical analysis method. Six studies provided HRs from multivariate analysis, and HRs from univariate analysis were available in another 4 studies. The results showed that HRs extracted from both multivariate and univariate analysis indicated an unfavorable survival. Since the multivariate analysis using Cox proportional hazards model or logistic regression model is an effective method in reducing bias from some major confounders [33], our findings suggested that beta-catenin expression might be an independent prognostic factor for poor overall survival. However, the results should still be prospectively validated by future studies.

Another concern that needs to be mentioned is the subcellular staining position, for which two major criteria were adopted among the included studies. One criterion is the nuclear staining, which means although beta-catenin is detected to be expressed in the cytomembrane, cytoplasm and nuclei, only nuclear expression was evaluated. Another criterion is the nuclear and/or cytoplasmic staining, which means that staining restricted to the cytomembrane or no staining at all was considered negative. It is generally acknowledged that intracytoplasmic and nuclear staining of beta-catenin has different meanings. In the presence of Wnt ligand, β-catenin would translocate to the nucleus, where it binds to transcription factors, displacing co-repressors and recruiting additional co-activators to target genes [11], thus acting as a transcriptional co-regulator to activate transcription of genes regulated by the Wnt/β-catenin pathway [42]. Besides, nuclear staining of beta-catenin is commonly used as a marker in some tumors like desmoid fibromatosis [43]. It is reasonable to suggest that nuclear beta-catenin is involved in the pathogenesis of sarcomas [44], and our findings that positive nuclear beta-catenin staining was significantly associated with poor overall survival was in consistent with the theory. However, the significance of cytoplasmic staining of beta-catenin remains unclear. Although some studies considered that cytoplasmic staining indicated the activation of the Wnt/beta-catenin signaling pathway, for that accumulation of beta-catenin in cytoplasm is prior to the translocation to nucleus [39, 45], whether the cytoplasmic staining has prognostic significance has not been recognized. In the meta-analysis, we could not separately evaluate the prognostic significance of cytoplasmic beta-catenin staining, since data of solely cytoplasmic staining could not be extracted. Nevertheless, we found that nuclear and/or cytoplasmic staining of beta-catenin was significantly associated with poor overall survival. The finding may have implications for future studies which would further investigate the significance of cytoplasmic beta-catenin expression.

On the basis of our findings, beta-catenin expression may be an effective prognostic factor of poor survival for bone and soft tissue sarcoma and may affect its progression and development. To our knowledge, it is the first time to systematically evaluate the correlation of beta-catenin expression with survival outcome and clinicopathological features in sarcoma patients.

Significant heterogeneity was observed in some analyses of the current study. The heterogeneity could be arisen from several aspects. Firstly, although all the included studies adopted IHC as the method of quantitative beta-catenin measurement, the type, dilution and source of the antibody were different among these trials. Since the concentration and type of the antibody could affect the sensitivity of IHC, the differences might lead to a potential bias. Besides, differences also existed in the cut-off value of positive beta-catenin expression. However, because of the small groups of studies adopting the same cut-off value and antibody, we could not conduct a subgroup analysis to clarify this technical problem.

Another potential source of bias may be related to the method to extract the HRs. If the HRs from multivariate survival analysis were given, we adopted them directly. If the HRs were not provided explicitly, we calculated them from outcome data provided in the publications; if this was impracticable, we extrapolated them from survival curves by univariate analysis [29-32]. The estimation might be less reliable than the HRs given directly in the papers. Therefore, the results of the meta-analysis should be cautiously interpreted and confirmed by well-designed prospective studies with appropriate multivariate analyses.

Publication bias is another major concern in meta-analysis, since positive results trend to

be published in journals. To prevent publication bias, we attempted to perform literature searches as complete as possible, using PubMed, Embase, Web of Science and Cochran Library. Although publication bias was not significant in the meta-analysis, it should be noted that we only included articles in English or Chinese. In addition, we excluded abstracts from conferences because it did not contain enough data for aggregation. The restrictions may potentially bring bias to the current meta-analysis.

In conclusion, the meta-analysis demonstrated that beta-catenin expression may be an effective prognostic factor of poor prognosis and clinicopathological features for patients with bone and soft tissue sarcoma. Future well-designed studies are needed to validate our findings and to further explore drug treatment of beta-catenin for bone and soft tissue sarcoma.

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Disclosure of conflict of interest

None.

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References

[1] Skubitz KM and D'Adamo DR. Sarcoma. Mayo Clin Proc 2007; 82: 1409-1432.

[2] Hwang JS, Mehta AD, Yoon RS and Beebe KS. From amputation to limb salvage reconstruction: evolution and role of the endoprosthesis in musculoskeletal oncology. J Orthop Traumatol 2014; 15: 81-86.

[3] Nakamura T, Matsumine A, Yamakado K, Matsubara T, Takaki H, Nakatsuka A, Takeda K, Abo D, Shimizu T and Uchida A. Lung radiofrequency ablation in patients with pulmonary metastases from musculoskeletal sarcomas [corrected]. Cancer 2009; 115: 3774-3781.

[4] Dai X, Ma W, He X and Jha RK. Review of therapeutic strategies for osteosarcoma, chondrosarcoma, and Ewing's sarcoma. Med Sci Monit 2011; 17: RA177-190.

[5] Luetke A, Meyers PA, Lewis I and Juergens H. Osteosarcoma treatment - where do we stand? A state of the art review. Cancer Treat Rev 2014; 40: 523-532.

[6] Hu T, Yang Q, Xu J, Zhang Z, He N and Du Y. Role of beta-isomerized C-terminal telopeptides (beta-CTx) and total procollagen type 1 amino-terminal propeptide (tP1NP) as osteosarcoma biomarkers. Int J Clin Exp Med 2015; 8: 890-896.

[7] Zhuang Y and Wei M. Impact of vascular endothelial growth factor expression on overall survival in patients with osteosarcoma: a meta-analysis. Tumour Biol 2014; 35: 1745-1749.

[8] Zhang M and Zhang X. Association of MMP-2 expression and prognosis in osteosarcoma patients. Int J Clin Exp Pathol 2015; 8: 14965-14970.

[9] Ivanov DB, Philippova MP and Tkachuk VA. Structure and functions of classical cadherins. Biochemistry (Mosc) 2001; 66: 1174-1186.

[10] Barker N and Clevers H. Mining the Wnt pathway for cancer therapeutics. Nat Rev Drug Discov 2006; 5: 997-1014.

[11] Nelson WJ and Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. Science 2004; 303: 1483-1487.

[12] Chen Z, He X, Jia M, Liu Y, Qu D, Wu D, Wu P, Ni C, Zhang Z, Ye J, Xu J and Huang J. beta-catenin overexpression in the nucleus predicts progress disease and unfavourable survival in colorectal cancer: a meta-analysis. PLoS One 2013; 8: e63854.

[13] Li LF, Wei ZJ, Sun H and Jiang B. Abnormal beta-catenin immunohistochemical expression as a prognostic factor in gastric cancer: a meta-analysis. World J Gastroenterol 2014; 20: 12313-12321.

[14] Mei XD, Su H, Song J and Dong L. Prognostic significance of beta-catenin expression in patients with non-small cell lung cancer: a meta-analysis. Biosci Trends 2013; 7: 42-49.

[15] Lu Y, Guan GF, Chen J, Hu B, Sun C, Ma Q, Wen YH, Qiu XC and Zhou Y. Aberrant CXCR4 and β-catenin expression in osteosarcoma correlates with patient survival. Oncol Lett 2015; 10: 2123-2129.

[16] Wan Y, Zhao W, Jiang Y, Liu D, Meng G and Cai Y. β-catenin is a valuable marker for differential diagnosis of osteoblastoma and osteosarcoma. Hum Pathol 2014; 45: 1459-1465.

[17] Chen C, Zhou H, Zhang X, Ma X, Liu Z and Liu X. Elevated levels of Dickkopf-1 are associated with beta-catenin accumulation and poor prog-

nosis in patients with chondrosarcoma. PLoS One 2014; 9: e105414.
[18] Le Guellec S, Moyal ECJ, Filleron T, Delisle MB, Chevreau C, Rubie H, Castex MP, De Gauzy JS, Bonnevialle P and Gomez-Brouchet A. The β5/focal adhesion kinase/glycogen synthase kinase 3β integrin pathway in high-grade osteosarcoma: A protein expression profile predictive of response to neoadjuvant chemotherapy. Hum Pathol 2013; 44: 2149-2158.
[19] Kim JR, Moon YJ, Kwon KS, Bae JS, Wagle S, Yu TK, Kim KM, Park HS, Lee JH, Moon WS, Lee H, Chung MJ and Jang KY. Expression of SIRT1 and DBC1 is associated with poor prognosis of soft tissue sarcomas. PLoS One 2013; 8: e74738.
[20] Deng Z, Niu G, Cai L, Wei R and Zhao X. The prognostic significance of CD44V6, CDH11, and β -Catenin expression in patients with osteosarcoma. Biomed Res Int 2013; 2013: 496193.
[21] Yang JZ, Zhang XH, Liu JR, Ding Y, Gao F and Wang Y. Expression and significance of N-cadherin and β-catenin protein in osteosarcoma. Zhonghua Zhong Liu Za Zhi 2010; 32: 586-589.
[22] Wei X, Sun Y, Zhao X, Cai W, Xia W, Lou D, Ban X and Sun B. The influence of SYT-SSX fusion gene, E-cadherin and β-catenin on the metastasis of synovial sarcoma. Chinese Journal of Clinical Oncology 2010; 37: 205-208.
[23] Horvai AE, Kramer MJ and O'Donnell R. β-catenin nuclear expression correlates with cyclin D1 expression in primary and metastatic synovial sarcoma: A tissue microarray study. Arch Pathol Lab Med 2006; 130: 792-798.
[24] Engellau J, Bendahl PO, Persson A, Domanski HA, Akerman M, Gustafson P, Alvegard TA, Nilbert M and Rydholm A. Improved prognostication in soft tissue sarcoma: independent information from vascular invasion, necrosis, growth pattern, and immunostaining using whole-tumor sections and tissue microarrays. Hum Pathol 2005; 36: 994-1002.
[25] Haydon RC, Deyrup A, Ishikawa A, Heck R, Jiang W, Zhou L, Feng T, King D, Cheng H, Breyer B, Peabody T, Simon MA, Montag AG and He TC. Cytoplasmic and/or nuclear accumulation of the β-catenin protein is a frequent event in human osteosarcoma. Int J Cancer 2002; 102: 338-342.
[26] Hasegawa T, Yokoyama R, Matsuno Y, Shimoda T and Hirohashi S. Prognostic significance of histologic grade and nuclear expression of β-catenin in synovial sarcoma. Hum Pathol 2001; 32: 257-263.
[27] Saito T, Oda Y, Sakamoto A, Tamiya S, Kinukawa N, Hayashi K, Iwamoto Y and Tsuneyoshi M. Prognostic value of the preserved expression of the E-cadherin and catenin families of adhesion molecules and of β-catenin mutations in synovial sarcoma. J Pathol 2000; 192: 342-350.
[28] Kuhnen C, Herter P, Muller O, Muehlberger T, Krause L, Homann H, Steinau HU and Muller KM. Beta-catenin in soft tissue sarcomas: expression is related to proliferative activity in high-grade sarcomas. Mod Pathol 2000; 13: 1005-1013.
[29] Tierney JF, Stewart LA, Ghersi D, Burdett S and Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007; 8: 16.
[30] Zheng X, Du XL and Jiang T. Prognostic significance of reduced immunohistochemical expression of E-cadherin in endometrial cancer-results of a meta-analysis. Int J Clin Exp Med 2015; 8: 18689-18696.
[31] Xu F, Xu L, Wang Q, An G, Feng G and Liu F. Clinicopathological and prognostic value of programmed death ligand-1 (PD-L1) in renal cell carcinoma: a meta-analysis. Int J Clin Exp Med 2015; 8: 14595-14603.
[32] Xie X, Wu W, Liang L, Han S, Chen T, Pan S, Xue M and Li S. Prognostic role of microRNA-210 in various carcinomas: a meta-analysis. Int J Clin Exp Med 2015; 8: 15283-15289.
[33] Jupiter DC. Causal diagrams and multivariate analysis III: confound it! J Foot Ankle Surg 2015; 54: 145-147.
[34] Saad N, Esa NM and Ithnin H. Suppression of beta-catenin and cyclooxygenase-2 expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA). Asian Pac J Cancer Prev 2013; 14: 3093-3099.
[35] Corrigan PM, Dobbin E, Freeburn RW and Wheadon H. Patterns of Wnt/Fzd/LRP gene expression during embryonic hematopoiesis. Stem Cells Dev 2009; 18: 759-772.
[36] He J, Huang Y, Weng J, Xiao L, Weng K and Ma X. Specific Inhibition of beta-Catenin in Jeko-1 Mantle Cell Lymphoma Cell Line Decreases Proliferation and Induces Apoptosis. Med Sci Monit 2015; 21: 2218-2224.
[37] Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, Wu J and Li M. MicroRNA-374a activates Wnt/beta-catenin signaling to promote breast cancer metastasis. J Clin Invest 2013; 123: 566-579.
[38] Lee KB, Ye S, Park MH, Park BH, Lee JS and Kim SM. p63-Mediated activation of the beta-catenin/c-Myc signaling pathway stimulates esophageal squamous carcinoma cell invasion and metastasis. Cancer Lett 2014; 353: 124-132.
[39] Kansara M, Tsang M, Kodjabachian L, Sims NA, Trivett MK, Ehrich M, Dobrovic A, Slavin J, Choong PF, Simmons PJ, Dawid IB and Thomas

DM. Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. J Clin Invest 2009; 119: 837-851.

[40] Ma Y, Ren Y, Han EQ, Li H, Chen D, Jacobs JJ, Gitelis S, O'Keefe RJ, Konttinen YT, Yin G and Li TF. Inhibition of the Wnt-beta-catenin and Notch signaling pathways sensitizes osteosarcoma cells to chemotherapy. Biochem Biophys Res Commun 2013; 431: 274-279.

[41] Chen C, Zhao M, Tian A, Zhang X, Yao Z and Ma X. Aberrant activation of Wnt/beta-catenin signaling drives proliferation of bone sarcoma cells. Oncotarget 2015; 6: 17570-17583.

[42] Jeanes A, Gottardi CJ and Yap AS. Cadherins and cancer: how does cadherin dysfunction promote tumor progression? Oncogene 2008; 27: 6920-6929.

[43] Montgomery E, Torbenson MS, Kaushal M, Fisher C and Abraham SC. Beta-catenin immunohistochemistry separates mesenteric fibromatosis from gastrointestinal stromal tumor and sclerosing mesenteritis. Am J Surg Pathol 2002; 26: 1296-1301.

[44] Ng TL, Gown AM, Barry TS, Cheang MC, Chan AK, Turbin DA, Hsu FD, West RB and Nielsen TO. Nuclear beta-catenin in mesenchymal tumors. Mod Pathol 2005; 18: 68-74.

[45] Kim I, Hur J and Jeong S. HuR represses Wnt/beta-catenin-mediated transcriptional activity by promoting cytoplasmic localization of beta-catenin. Biochem Biophys Res Commun 2015; 457: 65-70.