Original Paper

B7-H3 Overexpression Predicts Poor Survival of Cancer Patients: A Meta-Analysis

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Key Words

B7-H3 • Prognosis • Cancer • Meta-analysis

Abstract

Background: B7-H3 exhibits altered expression in various cancers. However, the correlation between B7-H3 expression and prognosis of cancer patients remains controversial. Therefore, we elicited a meta-analysis to investigate the potential value of B7-H3 in the prognostic prediction in human cancers. **Materials and Methods:** We searched PubMed (last update by June 15th, 2016) to identify studies assessing the effect of B7-H3 on survival of cancer patients. Hazard ratios (HRs) for overall survival (OS), recurrence free survival (RFS) and progression-free survival (PFS) from individual studies were calculated and pooled by using a random-effect or fix-effect model, and heterogeneity and publication bias analyses were also performed. **Results:** Data from 24 observational studies consisting of 4141 patients were summarized. An elevated baseline B7-H3 was significantly correlated with poor OS (pooled HR = 2.09; 95% CI = 1.60–2.74; $P < 0.001$). Differences across subgroups of tumor type ($P = 0.324$), year of publication ($P = 0.431$), ethnicity ($P = 0.940$), source of HR ($P = 0.145$), analysis type ($P = 0.178$) and sample size ($P = 0.909$) were not significant. Furthermore, high B7-H3 expression also predicted a significantly poor RFS (pooled HR = 1.39; 95% CI = 1.11–1.75; $P = 0.004$) but not PFS. **Conclusions:** This meta-analysis clarifies that elevated B7-H3 expression is significantly associated with poor survival in cancer patients.

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Introduction

The B7 family proteins presented on antigen-presenting cells where they are recognized by costimulatory receptors on T cells are important in T-cell proliferation, tolerance, and

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activation [1]. These proteins can be divided into three groups, according to the signals they transduce during T cell activation: I) co-stimulatory (e.g. B7-1 and B7-2); II) inhibitory (e.g. B7-H1); and III) co-stimulatory/inhibitory (e.g. B7-H3 and B7-H4) [2].

B7-H3 is a type I transmembrane protein that shares 20%~27% amino acid identity with other B7 family members [3]. The murine B7-H3 consists of a single extracellular variable-type IgV-IgC domain and a signature intracellular domain (2 Ig B7-H3), and the human B7-H3 possesses an additional isoform, 4Ig B7-H3, which contains a nearly exact tandem duplication of the IgV-IgC domain [4]. The receptor of B7-H3 still remains unknown, thus the role of B7-H3 in adaptive immune responses still remains elusive [5]. B7-H3 is expressed on tumor cells such as carcinomas of the colon, ovary, prostate, pancreas, kidney, prostate and urothelial carcinoma [6-10], but is not expressed, or expressed at low levels, in most normal cells or tissues [5, 11]. The exact function of B7-H3 remains unknown while both stimulatory and inhibitory effects on T-cell immunity have been identified [12]. The tumor cells expressed B7-H3 are also suggested to be correlated with the biological behaviors of cancer cells, such as migration, invasion, and metastatic capacity, and finally contributed to cancer progression [6-9, 13, 14]. B7-H3 overexpression has been observed to be associated with poor clinical outcome in some cancers [6, 8, 10]. However, studies investigating associations of B7-H3 expression with survival have shown conflicting results [5].

However, there has not been any pooled analysis on B7-H3 predicting the prognosis of cancer patients so far. Thus, a meta-analysis was conducted, including studies that assessing the role of B7-H3 in the clinical outcome of patients with various cancers to date.

Materials and Methods

Literature search strategy

The meta-analysis was performed following the Meta analysis of Observational Studies in Epidemiology group (MOOSE)[15]. A computerized search of MEDLINE was performed via PubMed from January 1990 to June 2016 to identify studies using the following search criteria: “B7-H3 OR CD276 OR B7H3” (all fields) AND “prognostic OR prognosis OR prognostication OR survive OR survived OR survival” (all fields) AND “tumor OR tumour OR neoplasm OR cancer OR carcinoma OR leukemia OR myeloma OR lymphoma” (all fields). Searches were limited to human articles published in the English or Chinese. Two investigators (Z. Ye and Y. Wu) inspected the title and abstracts of citations and obtained the full texts. The last search date was 15th June 2016.

Inclusion and exclusion criteria

Studies were eligible if they met the following initial inclusion criteria: (a) the expression of B7-H3 in tumor tissue or serum; (b) correlated survival outcomes with B7-H3 expression (Table 1) and provided sufficient data to estimate the hazard ratio (HR) and 95% confidence interval (CI); (c) clearly described B7-H3 detection; (d) clearly defined cut-off values. Studies were excluded if they were (a) review articles, case reports, experimental studies, conference abstracts, animal studies or letters; (b) unpublished data; (c) lacked essential data for the pooled calculation. Two reviewers independently evaluated titles and abstracts of the identified articles and subsequently excluded those that were irrelevant.

Data extraction

Eligible articles were reviewed independently by two authors (Y. Zhu and X. Li). Disagreements were resolved by consensus and consultation with a third investigator. Data were extracted independently by two authors (Z. Ye and Z. Zheng) using a standard protocol. The required information was collected: first author; year of publication; country of study belongs; number of patients; gender; tumor type; tumor stage; follow-up period; method of quantifying B7-H3 expression; definition of B7-H3 cut-off value to dichotomize patients into high and low groups; quality of study and HR for survival outcome, as well as their 95% CI and *P* values. Multivariate Cox analysis was priority of inclusion if available. Otherwise, univariate hazard analysis or Kaplan-Meier survival curves were conducted instead [16]. Parts of HRs were calculated using the graphical data. Rounding was avoided when performing these calculations.

First author [References]	Year	Country	Cancer	Male/ Female	Case number	Tumor stage (I/II/III/IV)	Follow-up (months)	Detected method	B7-H3 (+/-) NO.	Cut-off value	Multivariate analysis	Hazard Ratio	Outcome measures
Crispen[6] Zang[10]	2008	USA	RCC	476/267	743	366/123/161/93	1.2-200.4	IHC	129/614	Positive: >10% of tumor cells stained. The distribution score was multiplied by an intensity factor, which ranged from 1 to 3. This yielded the immunohistochemical score ranging from 0 to 300.	no	SC	OS
	2010	USA	ovarian carcinomas	NR	76	21/17/31/7	0.4-25.5	IHC	36/40	Reactivity was graded as: with 10–25% positive tumor cells; + with 25–50% positive tumor cells; ++ with 50–75% tumor cells; and +++ with 75–100% tumor cells. Positive: ++/+++	no	SC	OS
Gregorio[34]	2008	Italy	neuroblastoma	NR	53	15/11/1/30	NR	IHC	39/14	The signal of B7-H3 was graded according to the percent positive cells, and the level of IHC intensity was recorded as negative, low, medium, or high. Positive: medium and high. stained area: ≤33% of the tumor cells; 1, >33 to ≤66%; 2, >66% 3; intensity of staining: absent/weak: 1, moderate: 2, strong: 3. Each section had a final grade that derived from the multiplication of the area and intensity scores. Positive: final score of > 3.	no	SC	OS
Zhou[29]	2014	China	CRC	57/47	104	5/16/69/14	NR	IHC	59/45	0 = focal expression in <10%; + = 10–40%; ++ = 40–80%; +++ = >80%. Positive: ++/+++	no	SC	OS
Loos[7]	2009	Germany	pancreatic cancer	44/24	68	0/4/62/2	2–44	IHC	28/40		no	SC	OS
Ariagami[28] Xu[46]	2011	Japan	gastric cancer	64/31	95	52/43(41/II/III-IV)	1–74	qRT-PCR	48/47		yes	Reported	OS
	2010	China	Lung cancer	66/36	102	30/20/45/7	Patients were enrolled from January 2006; the date of last follow- up was March 2010.	IHC	71/31		yes	Reported	OS
Zang[47]	2007	USA	prostate cancer	823/0	823	NR	63.6-99.6	IHC	212/591	The percentage of tumor cells staining positive for B7-H3 was recorded in 10% intervals, and the level of IHC intensity was recorded as none, weak, moderate, or strong. Positive: strong.	yes	Reported	OS/RFS/PFS
Zhao[49]	2007	China	gastric cancer	25/13	38	5/12/13/8	3-23	IHC	15/23	Stained area: 53% of the tumor cells; 1, >33 to ≤66%; 2, >66% 3; intensity of staining: absent/weak: 1, moderate: 2, strong: 3. Each section had a final grade that derived from the multiplication of the area and intensity scores. Positive: final score of > 3.	no	SC	OS/DFS
Brunner[30]	2012	Austria	endometrial cancer	0/99	99	80/19(I/II-IV)	24-89	IHC	29/70	Staining intensity was scored as follows: 0–10% positive cells, 11–50% positive cells, 51–80% positive cells, and 81– 100% positive cells, respectively. Staining intensity was scored as faint (+), moderate (++), or strong (+++). respectively. The quantity score was multiplied by the intensity score, ranging from 1 to 12. Positive: final score ≥ 9.	no	SC	OS
Katayama[39]	2011	Japan	hypopharyngeal squamous cell carcinoma	35/2	37	3/5/3/26	6-133	IHC	8/29	The expression of B7-H3 was categorized in 4 grades: score 3 (strong): strong membranous expression of the molecule was present; score 2 (moderate): incomplete and discontinuous moderate membranous expression was present; score 1 (weak): cytoplasmic expression or rare membranous condensation was present; score 0 (none): <5% of tumor cells demonstrated expression. Positive: score 3.	Yes	Reported	OS
Sun[44]	2012	China	hepatocellular carcinoma	204/36	240	106/76/58/III/IIIA)	1.5-95	IHC	168/72	The immunostaining intensities were scored as follows: 0, negative; 1, weak; 2, moderate; 3, strong. Positive: scored 2 and 3.	yes	SC	OS/RFS
Ingebrigtsen[37]	2014	Norway	CRC	270/292	562	103/287/172/-	62.4-207.6	IHC	496/66	graded as 0 (no staining), 1 (less than 10% positive cells), 2 (10–49% positive cells) and 3 (more than 50% positive cells). Positive: scored 2 and 3.	no	SC	OS/RFS
Jin[38]	2015	China	Lung cancer	83/27	110	33/77(0/II-III)	NR	IHC	60/50	Intensity of cytoplasmic staining (-, negative; +, weak; ++, moderate; +++, strong). Positive: ++/+++.	no	SC	OS
Xu[48]	2013	China	pancreatic cancer	27/18	45	9/11/12/13	8-64	IHC	35/10	≤33% of the cancer cells; 1, >33 to ≤66% of the cancer cells; 2, >66% of the cancer cells; 3; intensity of staining: absent/weak: 1, moderate: 2, strong: 3. Each section had a final grade that derived from the multiplication of the area	no	SC	OS

continued

Table 1. Main characteristics of all studies included in the meta-analysis. RCC, renal cell carcinoma; CRC, Colorectal cancer; IHC, immunohistochemistry; SC, survival curve; NR, not report; OS, overall survival; RFS, recurrence free survival; DFS, disease free survival; PFS, progression-free survival

Quality assessment

The quality of each study was evaluated independently by two authors according to the Newcastle-Ottawa Quality Assessment Scale (NOS) [17]. For quality assessment, scores ranged from 0 to 9 (9 as best), and studies with scores of 6 or more were rated as high quality. The studies with at least scores of 4 were included and subsequent pooled analysis. None of the studies adhered in full to the REMARK (REporting recommendations for tumor MARKer prognostic studies) criteria for reporting studies on clinically prognostic tumor markers [18].

Statistical analysis

Meta-analysis was carried out using the STATA version 12.0 (Stata Corporation, College Station, TX, USA). Cut-off values of B7-H3 provided in the articles dichotomize patients into high and low groups. HRs and 95% CIs were combined to assess the effective value of B7-H3 expression on survival outcome. We extracted statistical variables directly if they were described in the study. The data from Kaplan-Meier survival curves were read by 2 independent researchers using Engauge Digitizer version 4.1. We also asked the corresponding authors of eligible articles for needed additional information and original data. HR greater than 1 indicated worse prognosis in patients and HR less than 1 suggested a better prognosis based on B7-H3 expression. Inter-study heterogeneity was assessed by visual inspection of forest plots, by performing the Chi-square test (assessing the P value), and by calculating the I^2 statistic [19, 20]. A random-effects model (the DerSimonian-Laird method) was used when heterogeneity present ($P < 0.05$ and/or $I^2 > 50\%$). Or the fixed-effects model (Mante-Haenszel method) was applied.

Publication bias assessment

Funnel plots with Egger's bias indicator test were used for assessing publication bias [21]. Scatterplots demonstrated each study's effect in relation to their sample size, and skewed indicate that bias exists. Pooled

First author [References]	Year	Country	Cancer	Male/ Female	Case number	Tumor stage (I/II/III/IV)	Follow-up (months)	Detected method	B7-H3 (+/-) No.	Cut-off value	Multivariate analysis	Hazard Ratio	Outcome measures
Yong[42]	2013	China	CRC	54/44	98	4/7/84/3	NR	IHC	45/53	and intensity scores: Positive: a final score of > 3 . According to the percentage and intensity of staining lymphocytes. Positive: the percentage of staining stained area $> 20\%$. 2. $> 66\%$; 3. intensity of staining: absent/weak: 1, moderate: 2, strong: 3. Each section had a final grade that derived from the multiplication of the area and intensity scores. Positive: final score of > 3 . Immunostaining intensities were scored: Grade 0, negative; grade 1, weak positive; grade 2, moderate positive and grade 3, strong positive. Positive: scored 2 and 3. Positive: 10% of blasts co-expressing B7-H3.	no	SC	OS
Maeda[40]	2014	Japan	breast cancer	0/90	90	NR	Median 67	IHC	52/38	Stained area $\geq 55\%$ of the tumor cells: 1. > 33 to $\leq 66\%$; 2. $> 66\%$; 3. intensity of staining: absent/weak: 1, moderate: 2, strong: 3. Each section had a final grade that derived from the multiplication of the area and intensity scores. Positive: final score of > 3 . Immunostaining intensities were scored: Grade 0, negative; grade 1, weak positive; grade 2, moderate positive and grade 3, strong positive. Positive: scored 2 and 3. Positive: 10% of blasts co-expressing B7-H3.	yes	Reported	OS/PFS
Sun[43]	2013	China	Lung cancer	77/28	105	11/45/31/18	NR	IHC	47/58	The staining intensity is defined as follows: grade 0, 10% cells stained; grade 1, 10%–30% cells stained; grade 2, 30%–60% cells stained; and grade 3, 60% cells stained. Positive: grade 2 and grade 3.	no	SC	OS
Hu[35]	2015	China	acute leukemia	73/61	134	NR	0.3-26	flow cytometer	60/74		yes	Reported	OS/PFS
Chen[32]	2014	China	pancreatic cancer	41/22	63	5/19/25/14	Patients were enrolled between December 2000 and August 2010. Follow-up to August 2013. 40.3-74.0	IHC	43/20		no	SC	OS
Mao[41]	2015	China	lung cancer	91/37	128	45/43/40/0	Patients were enrolled between 2007 and 2012. followed up until May 2015	IHC	89/39	The percentage of positively stained cells was scored as follows: 0 = no staining, 1 = 1%-10% of cells, 2 = 11%-50%, and 3 = more than 50%. Positive: scored 2 and 3. Scores representing the percentage of positive cells: 2, 26-50% positive cells; 3, 51-75%; and 4, 76-100%. Staining intensity was scored as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. These two scores multiplied to yield the final score: 0-2, (-); 3-4, (+); 5-8, (++); and 9-12, (+++). Positive: $+/++/+++$. H-score = (% tumor cells unstained $\times 0$) + (% tumor cells stained weak $\times 1$) + (% tumor cells stained moderate $\times 2$) + (% tumor cells stained strong $\times 3$). The H-scores ranged from 0 (100% negative tumor cells) to 300 (100% strong staining tumor cells). Positive: H-score > 185 . Positive: $> 45\%$ of cells co-expressing B7-H3.	yes	Reported	OS
Huang[36]	2016	China	cervical cancer	0/108	108	55/44/7/2	Patients were enrolled between 2007 and 2012. followed up until May 2015	IHC	78/30		yes	Reported	OS
Chen[35]	2015	China	esophageal cancer	128/46	174	14/102/37/21	NR	IHC	97/77		no	Reported	OS
Zhao[48]	2013	China	multiple myeloma	28/18	46	Not applicable	NR	flow cytometer	20/26		no	SC	OS/PFS

data of at least 3 studies (recommend more than 10) are required to calculate Egger's regression intercept. When publication bias was identified, sensitivity analysis was performed to assess the potential impact of missing studies. A two-tailed P value < 0.05 was considered statistically significant.

Results

Excluded and included studies

A total of 100 articles for consideration were yielded by using the described searching strategy. Title, publication types and abstract assessment identified 30 manuscripts appropriate for investigation of the correlation between B7-H3 expression and patient survival in various malignant tumors. Among these, 6 articles were excluded [22-27] because of lacking some important data or unable to extract data (Fig. 1). Finally, 24 studies fulfilled the criteria for meta-analysis [7, 10, 28-49].

Fig. 1. Flow chart of the search strategy and selected studies.

Study characteristics

The included studies analyzed a total of 4141 patients from P. R. China, USA, Italy, Germany, Austria, Japan and Norway. Details regarding the characteristics of the included studies are listed in Table 1. Patients from several regions were diagnosed with a variety of cancers, including renal cell carcinoma, colorectal cancer, ovarian carcinomas, neuroblastoma, pancreatic cancer, gastric cancer, lung cancer, prostate cancer, endometrial cancer, hypopharyngeal squamous cell carcinoma, hepatocellular carcinoma, breast cancer, hematological malignancies, cervical cancer, and esophageal cancer. The majority of studies (70.8%) reported on Asians, and 7 (29.2%) on Caucasians. Thirteen studies (54.2%) were published in 2013 or later. HRs were reported directly in 9 studies and estimated indirectly in the others. The cut-off values varied in these studies. The endpoints overall survival (OS) was addressed in all studies, while recurrences free survival (RFS) and progression-free survival (PFS) were addressed in 4 studies, respectively.

Quality assessment

Each of the 24 eligible studies included in our meta-analysis was assessed for quality according to NOS. The quality of all studies included varied from 4 to 9, with a mean of 5.7. A higher value indicated better methodology. Therefore, all studies were included in the subsequent analysis.

Meta-analysis results

Overall Survival. All included studies provided suitable data for OS analysis. The main results of this meta-analysis are listed in Table 2. Significant inter-study heterogeneity was found ($I^2 = 78.9\%$, $P < 0.001$), we applied the random-effects model to pool the HRs. The statistical results showed that B7-H3 upregulation was significantly associated with poor OS according to pooled analysis with a combined HR 2.09 (95% CI = 1.60–2.74; $P < 0.001$) (Fig. 2). Considering the inter-study heterogeneity, the significance of B7-H3 expression was further evaluated via subgroup analysis based on the tumor type, ethnicity, source of

Table 2. Pooled hazard ratios for overall survival according to subgroup analysis. CRC, Colorectal cancer; HR, hazard ratio; OS, overall survival

Outcome subgroup	No. of patients	No. of studies	Effects model	HR (95% CI) Figure	P value	Heterogeneity (Higgins I ² statistic)
OS	4141	24	Random	2.09 (1.60, 2.74)	Z = 5.38, P < 0.001	P < 0.001; I ² 78.9%
Tumor type						
CRC	764	3	Fix	1.21 (0.82, 1.79)	Z = 0.96, P = 0.336	P = 0.235; I ² 30.9%
Pancreatic	176	3	Random	1.37 (0.51, 3.64)	Z = 0.63, P = 0.530	P = 0.001; I ² 85.5%
Gastric	133	2	Random	0.66 (0.08, 5.23)	Z = 0.40, P = 0.692	P = 0.034; I ² 77.7%
Lung	445	4	Random	2.68 (1.55, 4.64)	Z = 3.53, P < 0.001	P = 0.030; I ² 66.4%
Hematological	180	2	Fix	2.13 (1.39, 3.27)	Z = 3.46, P = 0.001	P = 0.983; I ² 0%
other	2443	10	Random	2.86 (1.89, 4.31)	Z = 5.00, P < 0.001	P < 0.001; I ² 75.4%
Ethnicity						
Asian	1717	17	Random	2.09 (1.60, 2.74)	Z = 6.08, P < 0.001	P = 0.020; I ² 46.1%
Caucasian	2424	7	Random	2.11 (1.07, 4.18)	Z = 2.14, P = 0.032	P < 0.001; I ² 92.4%
HR obtain						
Reported in text	1691	9	Random	2.66 (1.80, 3.92)	Z = 4.92, P < 0.001	P = 0.018; I ² 56.8%
Survival curve extrapolated	2450	15	Random	1.80 (1.26, 2.57)	Z = 3.24, P = 0.001	P < 0.001; I ² 84.4%
Analysis type						
Multivariate	1480	8	Random	2.80 (1.81, 4.34)	Z = 4.61, P < 0.001	P = 0.049; I ² 52.5%
Univariate	2661	16	Random	1.86 (1.34, 2.58)	Z = 3.73, P < 0.001	P < 0.001; I ² 83.0%
Sample size						
>200	2368	4	Random	2.05 (0.94, 4.47)	Z = 1.81, P = 0.070	P < 0.001; I ² 94.8%
<200	1773	22	Random	2.07 (1.59, 2.70)	Z = 5.37, P < 0.001	P < 0.001; I ² 63.1%

Fig. 2. Forest plots of studies evaluating hazard ratios of elevated B7-H3 expression in cancer patients for overall survival.

HR, analysis type and sample size. Elevated B7-H3 as a negative predictor on OS was only confirmed in patients with lung cancer (pooled HR = 2.68; 95% CI = 1.55–4.64; $P < 0.001$), hematological malignancies (pooled HR = 2.13; 95% CI = 1.39–3.27; $P = 0.001$), and other cancers (pooled HR = 2.86; 95% CI = 1.89–4.31; $P < 0.001$), but not in patients with colorectal cancer, gastric cancer and pancreatic cancer (Fig. 3). Significant inter-study heterogeneity remained in lung cancer and other cancer subgroups ($I^2 = 66.4\%$, $P = 0.03$ and $I^2 = 75.4\%$, $P < 0.001$). Elevated B7-H3 expression was associated with poor OS in Asian (pooled HR = 2.09; 95% CI = 1.60–2.74; $P < 0.001$) and Caucasian populations (pooled HR = 2.11; 95% CI = 1.07–4.18; $P = 0.032$). Significant heterogeneity could be observed in subgroups ($I^2 = 46.1\%$, $P = 0.02$ and $I^2 = 92.4\%$, $P < 0.001$). The similar results were demonstrated in other subgroups of HR reported directly in articles (pooled HR = 2.66; 95% CI = 1.80–3.92; $P < 0.001$), HR estimated indirectly by survival curve extrapolated (pooled HR = 1.80; 95% CI = 1.26–2.57; P

Fig. 3. Forest plot of the relationship between elevated B7-H3 expression and overall survival in patients with a variety of cancers.

Fig. 4. Sensitivity analysis for meta-analysis of B7-H3 expression.

= 0.001), multivariate analysis (pooled HR = 2.80; 95% CI = 1.81–4.34; $P < 0.001$), univariate analysis (pooled HR = 1.86; 95% CI = 1.34–2.58; $P < 0.001$) and sample size < 200 (pooled HR = 2.07; 95% CI = 1.59–2.70; $P < 0.001$). Significant inter-study heterogeneity was detected in all such subgroups.

Heterogeneity analysis. Sensitivity analysis was performed by sequential omission of individual studies using the fixed-effects model, and the result pattern was not obviously impacted by any single study (Fig. 4). A meta-regression to explore the potential factors responsible for the heterogeneity was conducted. None of factors including tumor type ($P =$

Fig. 5. Funnel plots of publication biases on the relationships between B7-H3 expression and overall survival in cancer patients.

Fig. 6. Forest plots of studies evaluating hazard ratios of elevated B7-H3 expression in various cancers for recurrence free survival.

Fig. 7. Forest plots of studies evaluating hazard ratios of elevated B7-H3 expression in various cancers for progression-free survival.

0.324), year of publication ($P = 0.431$), ethnicity ($P = 0.940$), source of HR ($P = 0.145$), analysis type ($P = 0.178$) and sample size ($P = 0.909$) took responsibility for the heterogeneity. It revealed that heterogeneity was created under the action of various factors.

Table 3. Pooled hazard ratios for progression-free survival according to subgroup analysis. HR, hazard ratio; PFS, progression-free survival

Outcome subgroup	No. of patients	No. of studies	Effects model	HR (95% CI) Figure	P value	Heterogeneity (Higgins I ² statistic)
PFS	1041	4	Random	1.40 (0.62, 3.17)	Z = 0.81, P = 0.419	P < 0.001; I ² 83.3%
Analysis type						
Multivariate	957	2	Fix	2.43 (1.68, 3.51)	Z = 4.72, P < 0.001	P = 0.352; I ² 0%
Univariate	84	2	Random	0.69 (0.08, 6.03)	Z = 0.33, P = 0.419	P = 0.001; I ² 91.1%
Tumor type						
prostate cancer	823	1	-	2.79 (1.74, 4.46)	Z = 4.27, P < 0.001	-
Gastric	38	1	-	0.22 (0.11, 0.95)	Z = 2.75, P = 0.006	-
Hematological	180	2	Fix	1.97 (1.25, 3.11)	Z = 2.93, P = 0.003	P = 0.949; I ² 0%

Publication bias

The publication bias of all enrolled studies was evaluated using funnel plots, and Egger's and Begg's tests. The funnel plots were nearly symmetric by visual inspection as shown in Fig. 5, which revealed no evidence of publication bias. The conclusion was confirmed by Egger's tests ($P = 0.442$). Hence, publication bias was not presented in this meta-analysis.

Recurrences free survival and progression-free survival

Four studies comprising a total of 1715 patients provided suitable data for RFS analysis. No significant inter-study heterogeneity was detected ($I^2 = 36.6\%$; $P = 0.193$) and fixed-effects model was applied. It revealed that high expression of B7-H3 was statistically significant associated with poor RFS (pooled HR = 1.39; 95% CI = 1.11–1.75; $P = 0.004$). A forest plot of study-specific HRs for RFS is shown in Fig. 6.

Another 4 studies pooled for PFS analysis which comprising a total of 1041 patients. Significant inter-study heterogeneity was found ($I^2 = 83.3\%$, $P < 0.001$). However, expression of B7-H3 was not statistically significant associated with PFS (pooled HR = 1.40; 95% CI = 0.62–3.17; $P = 0.419$). All the HRs and corresponding 95% CIs are shown in Table 3. The forest plot is shown in Fig. 7. Subgroup analysis showed elevated B7-H3 only associated with poor PFS in multivariate analysis (HR = 2.43; 95% CI = 1.68–3.51; $P < 0.001$) with no heterogeneity among studies ($I^2 = 0\%$; $P = 0.352$). Subgroup analysis by tumor type indicated that adverse effect of elevated B7-H3 on PFS was in patients with prostate cancer (HR = 2.79; 95% CI = 1.74–4.46; $P < 0.001$), while favorable effect of elevated B7-H3 on PFS was in patients with gastric cancer (HR = 0.22; 95% CI = 0.11–0.95; $P = 0.006$).

Discussion

The prognostic value of B7-H3 expression remained inconclusive [5]. In this study, we performed a pooled analysis to explore the prognostic role of B7-H3 in 4141 patients with various cancers from 24 studies. To the best of our knowledge, there have not been any other meta-analyses evaluating the correlation between B7-H3 expression and clinical outcomes of cancer patients.

The pooled risk of elevated B7-H3 for OS in cancers was significant with a combined HR of 2.09 (95% CI 1.60–2.74, $P < 0.001$), which confirmed that B7-H3 was correlated with poor OS and might act as an independent risk factor. However, Zhao et al. [49] and Loos et al. [5] found the opposite conclusions. Both of them had very small sample sizes (38 for Zhao et al. and 68 for Loos et al.). Moreover, Loos et al. [7] only collected data of post-operation cases and they analyzed OS after operation. So their inconsistency is possibly due to the bias of sample selection and relatively small sample size. In the subgroup analysis, the B7-H3 showed the inconsistent prognostic effects in different tumor types. Elevated B7-H3 expression was correlated with poor OS in lung cancer, hematological malignancy and other cancer, but was not in colorectal cancer, pancreatic cancer or gastric cancer. The similar phenomenon was observed in subgroup analysis based on sample size as elevated B7-H3 in studies with < 200 patients accompanied with poor OS, while it was not statistically significant in studies with

> 200 patients. It seemed to suggest that the obvious heterogeneity among these 24 studies may chiefly induced by tumor type or sample size. But further meta-regression analysis disputed this conclusion. It revealed that heterogeneity was created under the action of various factors rather than single one. Besides, this meta-analysis also indicated that cancer patients with elevated B7-H3 have a significantly poor RFS but not PFS. But in subgroup of multivariate analysis, B7-H3 was found to be correlated with poor PFS. The more reliable result than univariate analysis suggested that B7-H3 could still be a negative factor for predicting PFS, which was similar to OS and RFS.

As detected by immunochemistry technique, over 60% and up to 93% of tumor tissues display aberrant expression of B7-H3 in the majority of cancer types [50]. Overexpression of B7-H3 has been associated with multiple cancers including esophageal cancer [41], pancreatic cancer [26], cervical cancer [36], non-small-cell lung cancer [38] and colorectal cancer [37]. However, high B7-H3 expression is also reported to be associated with better survival in acute myeloid leukemia [23]. This may be due to the different features of various cancers as well as the differences of sample sizes or measurement. In general, the conclusions from other researchers are consistent with ours.

As members of B7 family, higher B7-H1 expression in human cancer tissues of [51] renal cell carcinoma [52] ovarian cancer [53] non-small cell lung cancer [41], esophageal cancer [54], malignant pleural mesothelioma [55] and colorectal carcinoma [56] is significantly correlated with poor prognosis, while in breast cancer [57], non-small cell lung cancer [58], ovarian cancer [59], renal cell carcinoma [60], pancreatic cancer [26], oral squamous cell carcinoma [61], cholangiocarcinoma [62], hepatocellular carcinoma [63] and esophageal squamous cell carcinoma [64], aberrant expression of B7-H4 has been demonstrated to be associated with a poor clinical outcome. Therefore, we conclude that B7 family members play similar role as negative prognostic factors in tumor development.

Some limitations still exist and details need to be refined. Confined eligible studies resulted in relatively insufficiency data in the subgroup analyses, especially for RFS and PFS. Lack of a unified cut-off value in B7-H3 expression caused various cut-off values in eligible studies. The inaccurate cut-off values may affect the efficiency of B7-H3 as a prognostic biomarker. Most studies measured B7-H3 by immunohistochemistry, studies of hematological malignancy used flow cytometer because surface markers of mononuclear cells were more accurate than myeloid tissue. Identification of the most appropriate cut-off values are to be further explored. Several HRs were calculated from the data extracted from the survival curves, which inevitably caused small statistical errors. Finally, multivariate analyses may be more optimal to estimate effect size than univariate analyses only reported in few studies. Though pooled HRs based on univariate analyses does not show a notable difference in this study, it is possibly to overestimate the effect size in univariate analyses.

Conclusions

This meta-analysis clarifies that high B7-H3 expression was significantly associated with poor OS and RFS in cancer patients and can substantially improve prognosis prediction. Further prospective multi-center studies designed adequately with larger sample size are needed to confirm the prognosis value of B7-H3 in cancer patients, as well as to explore more effective therapy strategies.

Disclosure Statement

The authors declare that they have no conflicts of interest concerning this article.

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