B7-H4 Expression and Increased Death Risk of Cancer Patients: A Meta-Analysis

Jing-ting JIANG[1]
Chang-ping WU[1]
Xiao ZHENG[1]
Yao ZHAO[2]
Bin XU[1]
Bin-feng LU[3]
Yue-ping SHEN[2]

[1] Department of Tumor Biological Treatment, Key Laboratory of Biotechnology of Changzhou, the Third Affiliated Hospital, Soochow University, Changzhou 213003, Jiangsu Province, China.
[2] Department of Epidemiology and Biostatistics, Radiation Medicine and Public Health School of Soochow University, Suzhou 215123, Jiangsu Province, China.
[3] Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh PA 15261, USA.

Correspondence to: Binfeng LU
Tel: 412-648-9339
E-mail: binfeng@pitt.edu

Correspondence to: Yue-ping SHEN
Tel: 86-512-6588 0079
E-mail: syp_shen@yahoo.com.cn

OBJECTIVE The relationship between higher levels of B7-H4 expression and death risk of cancer patients remains to be clarified. In the current study, information from an ordinary scale and those from several outcome scales were combined to make a single estimate. PubMed databases were searched for survival studies on the hazard ratios (HR) of malignant tumors associated with higher B7-H4 expression from 1999 to 2010.
METHODS The fixed effect model was used to estimate the combined HRs of six studies. Sensitivity analysis was performed to assess the stability. Publication bias was also estimated. Six studies that meet the inclusion criteria were identified; these studies reported the associations between the higher B7-H4 expression and death risk of cancer patients.
RESULTS A 42% increase in death risk was observed in patients with higher B7-H4 expression (HR = 1.42; 95% confidence interval: 1.16–1.72). Sensitivity analyses found the results robust. The analysis shows that higher levels of B7-H4 expression are associated with the death risk of patients suffering from various cancers.
CONCLUSION B7-H4 may be a negative regulatory molecule for antitumor immune responses and a molecular target for tumor immunotherapy.

KEY WORDS: B7-H4, costimulatory molecules, malignant tumors, meta-analysis, gastric cancer.

Introduction

B7-H4 is a recently identified member of the B7 family and has been shown to inhibit T cell proliferation and cytokine secretion[1,2]. Experiments *in vivo* also support the function of B7-H4 as an inhibitor to T cell-mediated immunity[3–5]. B7-H4 is highly expressed in the human cancer microenvironment[6] and can mediate T-cell suppression[7–9], thereby likely to inhibit cancer immunotherapy. However, the effects of B7-H4 in various human cancers[8,10–14] are yet to be clarified. Therefore, combining the results of similar studies using meta-analysis is necessary.

Materials and Methods

Data Source

The electronic databases of PubMed from 1999–2010 were searched using the following key words: ''B7-H4,'' ''malignant tumors,'' and

Table 1. Characteristics of studies evaluating the association between the higher B7-H4 expression and increased death risk of patients with malignant tumors.

Authors	Year	Tumor Type	B7-H4(+)/total cases	Univariate HR (95% CI)	Multivariate HR (95% CI)
Krambeck AE[12]	2006	Renal cell carcinoma	153/259	3.05 (1.51–6.14)	1.78 (0.88–3.63)
Simon I[14]	2007	Ovarian cancer	148/233	1.47 (1.04–2.09)	1.02 (0.71–1.46)
Kryczek I[8]	2007	Ovarian cancer	56/70	3.9 (1.8–8.4)	2.7 (1.2–6.1)
Oikonomopoulou K[13]	2008	Ovarian cancer	-/98	2.04 (1.48–2.81)	1.69 (1.14–2.49)
Anderson G[10]	2010	Ovarian cancer	-/34	-	1.02 (0.61–1.69)
Jiang J[11]	2010	Gastric cancer	70/156	2.04 (1.35–3.06)	1.85 (1.15–2.96)

- not reported

''survival analysis''. In addition, a document retrospective method was used to find all detailed information.

Eligibility criteria

Abstracts of identified articles were screened to exclude studies that did not meet the eligibility criteria. The full text of those selected for further review was obtained and evaluated. Studies were included if (1) they were published between January 1999 and September 2010; (2) they are in full text form; (3) they contain survival analyses of patients with different levels of B7-H4 expression; (4) they clearly stated the total case numbers and numbers of B7-H4 positive specimens; and (5) they presented hazard ratio (HR) values and 95% confidence intervals (CI).

Exclusion criteria

Repetitive literature, review or abstract literature, literature whose design was not identified, and papers with low reliability and poor quality were excluded. In the end, six papers satisfied the inclusion criteria and were included in the meta-analysis. The total case number was 850.

Statistical analysis

The heterogeneity of the HRs in the six studies was assessed using a chi-square test. Taking the result from the heterogeneity test into account, the fixed effect model (Peto method[15]) was used to calculate the combined HR and 95% CIs for the higher B7-H4 expression associated with increased death risk from malignant tumors using the univariate and multivariate HR values from the original papers.

Sensitivity analyses were performed to assess the robustness of the primary analyses by repeating the original analyses; one or two studies were separately omitted at a time to assess whether the studies had extro-influence on the results.

Table 2. Heterogeneity test for HRs in the univariate and multivariate analyses.

	χ^2	Df	P
Univariate analysis	7.28	4	0.122
Multivariate analysis	9.61	5	0.087

Publication bias was assessed via visual inspection of the Begg funnel plot. Data were analyzed using the Stata 10.1 software.

Results

Basic situation of the document

After retrieving 35 articles, 6 studies (Table 1) met the inclusion criteria and were included in the primary analysis.

Heterogeneity analysis

Table 2 indicates the absence of a heterogeneity among the HRs from univariate or multivariate estimates ($P > 0.05$).

B7-H4 and malignant tumors

The meta-analysis indicates that patients with malignant tumors expressing higher levels of B7-H4 are at a greater risk of death compared with patients with tumors expressing lower B7-H4 levels. The combined HR and 95% CI of the univariate and multivariate analyses are 1.98 (1.64–2.40) and 1.42 (1.16–1.72), respectively (Table 3). The forest plots for the meta-analysis are shown in Figs. 1 and 2.

Sensitivity analysis

As can be seen in Table 4, after studies[11,12] on non-ovarian cancer were excluded, no more changes were found in the the combined HR compared with the

Table 3. Combined HR analysis.

	HR (95% CI)	Z	P
Univariate analysis	1.98 (1.64–2.40)	7.05	< 0.0001
Multivariate analysis	1.42 (1.16–1.72)	3.48	< 0.0001

Univariate analysis: one paper[10] did not provide the HR value of the univariate analysis; thus only the remaining five were analyzed.
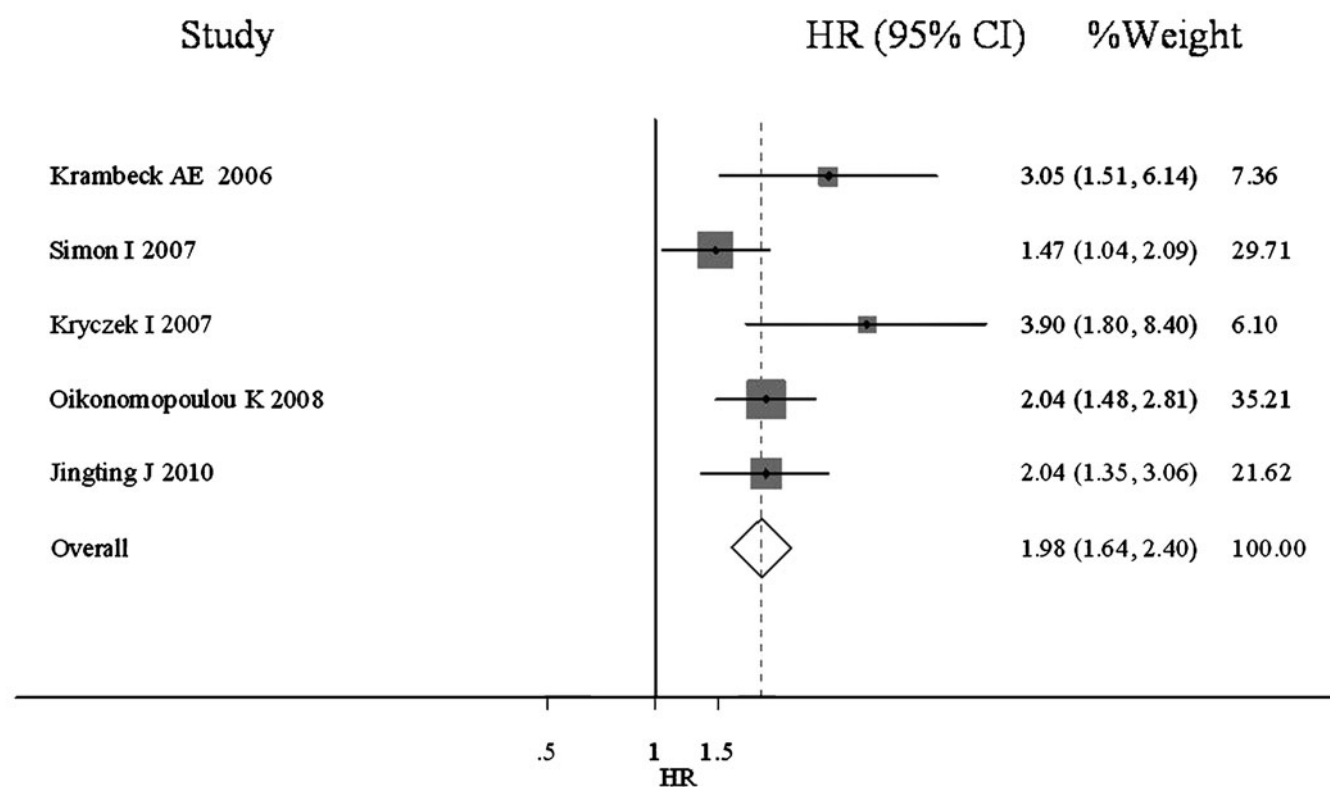
Fig.1. Meta-analysis of HRs from the univariate analysis of studies on higher B7-H4 expression and death risks of malignant tumors.
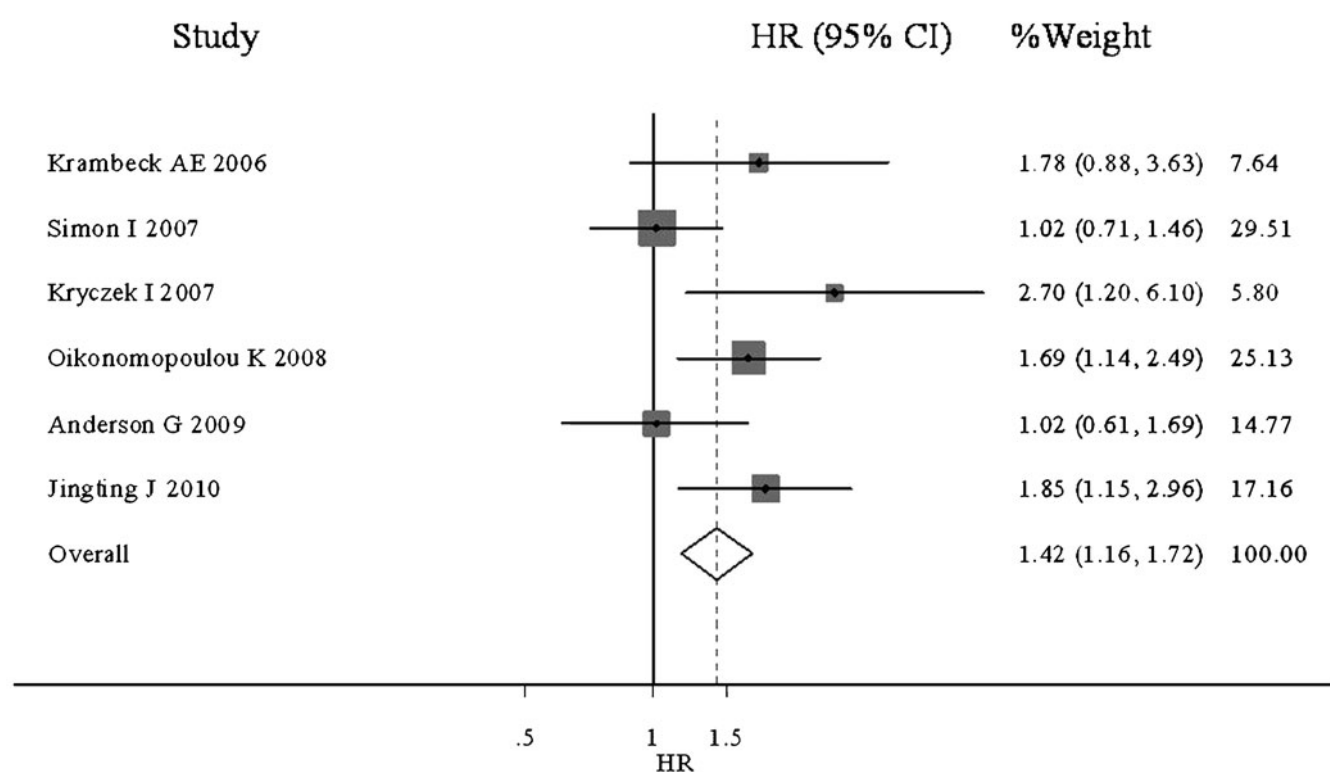

Fig.2. Meta-analysis of HRs from the multivariate analysis of studies on higher B7-H4 expression and death risks of malignant tumors.

Table 4. Sensitivity analysis.

	Study number	HR	95% CI	Heterogeneity test	
				x^2	P
Fixed effect model	6	1.42	1.16–1.72	9.61	0.087
After excluding non-ovarian cancer articles	4	1.37	0.93–1.99	2.94	0.455
After removing Chinese articles	5	1.41	1.02–1.96	4.08	0.290

previous analysis. The sensitivity analysis shows that the current meta-analysis is stable.

Publication bias

The Begg funnel plot does not indicate the presence of a publication bias, as shown by the rather symmetric distribution of the log HR vs. the standard error curves of both the univariate (Fig. 3) and multivariate analyses (Fig. 4). However, inferences on publication bias should be made with caution because of the small number of studies that reported on these associations.

Discussion

B7-H4 is highly expressed in many cancers, including lung, rectal, liver, gastric, kidney, pancreatic, breast, prostate, ovarian, and brain cancers. An initial study showed that a majority of ovarian carcinoma (22 out of 26) expressed high levels of B7-H4[16]. A follow-up study with large samples demonstrated that B7-H4 is highly expressed in ovarian papillary serous adenocarcinoma (88%), whereas mucinous and low-malignant potential ovarian cancers and normal tissues were negative for B7-H4[17]. Sun et al.[18] reported that 43% of the specimens expressed B7-H4 in non-small cell lung tumors and confirmed that B7-H4 is an important negative regulatory signal of T cells. In addition, B7-H4 expression was observed in lung cancers with lymph node metastasis[16], in renal cell carcinoma associated with poor survival, and in prostate cancer associated with disease spread, recurrence, and death[19]. A total of 5 out of 16 lung carcinomas were also found to express B7-H4[16], whereas all 17 melanoma specimens were found negative for B7-H4. Salceda[17] found that B7-H4 is highly expressed in breast cancer, but is low or not expressed in normal tissues. This study also supports the theory that B7-H4 plays a role in the malignant transformation of epithelial cells. Tringler[20] showed that B7-H4 is consistently expressed in most primary and metastatic breast carcinomas. B7-H4 is also highly expressed (80.0%) in colon and rectal cancers. Significantly fewer tumor-infiltrating lymphocytes (TILs) were identified in B7-H4-positive tumor specimens than in B7-H4-negative specimens[21]. Awadallah[22] reported that out of 36 pancreatic ductal adenocarcinoma specimens (21 via surgical ablation and 15 via EUS-guided fine needle aspiration), 33 cases (91.7%) expressed B7-H4. A recent study showed that high levels of B7-H4 expression are negatively correlated to the survival time of patients with gastric cancer[11]. The current study confirms that B7-H4 is a negative regulator molecule and can be used as an indicator of gastric cancer survival. It is also found preferentially expressed in non-dividing brain tumor cells and in a subset of brain tumor stem-like cells[23].

Evidence indicates that a receptor that can function on T cells can be induced[3,4,7]. The currently known functions of B7-H4 are exclusively inhibitory and its effect may be
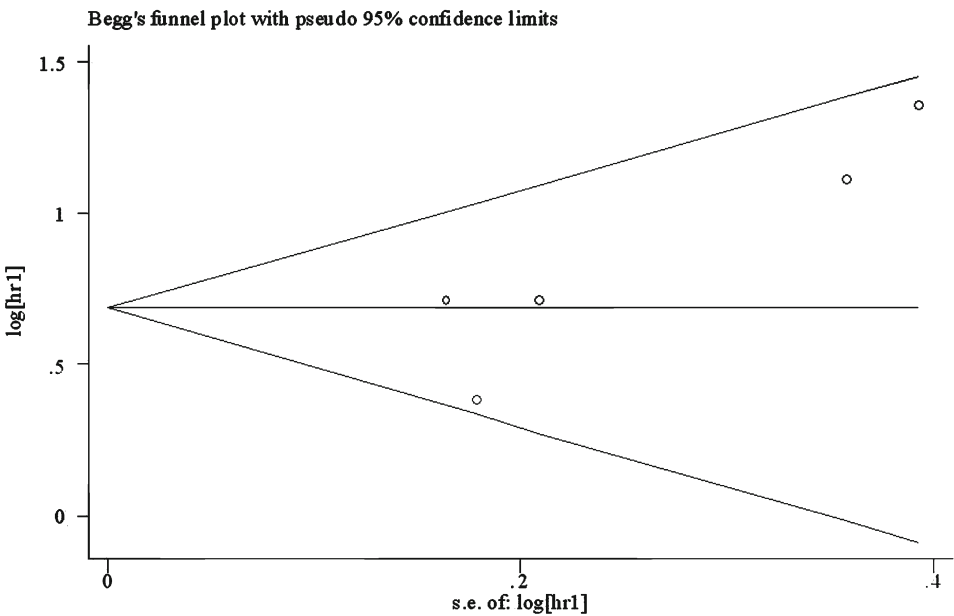

Fig.3. Funnel plots for the log HRs from the univariate analyses.

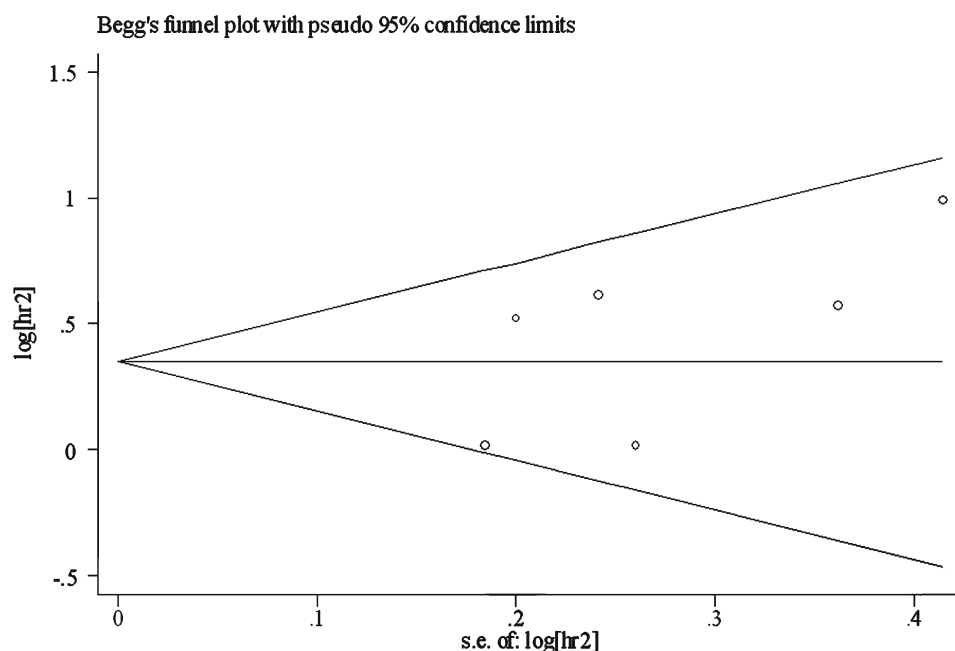Begg's funnel plot with pseudo 95% confidence limits

Fig.4. Funnel plots for the log HRs from the multivariate analyses.

mediated by a single receptor. Although the B- and T-lymphocyte attenuator (BTLA) was initially proposed as the receptor for B7-H4[24], recent studies showed that this is not the case[25–27]. High concentrations of the cytokines interleukin (IL)-6 and IL-10, which can stimulate macrophage B7-H4 expression, are found in the tumor microenvironment[7]. By contrast, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-4, which have low concentrations in the tumor microenvironment, inhibit B7-H4 expression.

Although high levels of B7-H4 expression are observed in human cancers, articles on cancer survival analysis are limited. Three studies[10,13,14] in the current meta-analysis showed that despite its expression in cancer cells, B7-H4 may not statistically increase the population risk of human cancer patients [HR and 95% CIs are 1.78 (0.88–3.63), 1.02 (0.71–1.46), and 1.02 (0.61–1.69)]. Three more studies[7,11,13] showed that B7-H4 expression is significantly associated with poor patient survival [HR and 95% CIs are 2.7 (1.2–6.1), 1.69 (1.14–2.49), and 1.85 (1.15–2.96)]. Thus, agreement on the effects of B7-H4 in various cancers is lacking. Some results may not be reliable because of the limited number of patient samples in the studies. Therefore, meta-analysis was used to reanalyze these published results. In the current study, the survival risk between B7-H4 expression and cancers was summarized and generalized. The results show that the risk of death of patients with malignant tumors increased by an average of 42% with higher levels of B7-H4 (HR = 1.42; 95% CI, 1.16–1.72).

However, the current study has some limitations. The small number of studies used for the meta-analysis is the most critical because of the low numbers of studies with positive survival results. In addition, the existence of a publication bias may have had an effect on the outcome of the analysis.

In conclusion, as more clinical studies on the relationship between B7-H4 expression and the survival risk of malignant tumors become available, evidence that support the negative regulatory function of B7-H4 in tumors will increase. Furthermore, B7-H4 may become a diagnostic marker for cancers and is a potential therapeutic target.

Acknowledgments

Conflict of Interest Statement

No potential conflicts of interest were disclosed.

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