Association between B7-H1 expression and bladder cancer: a meta-analysis

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ABSTRACT. B7 homolog 1 (B7-H1), which is also known as programmed death-L1, is an important member of the B7/CD28 costimulatory factor superfamily, which are emerging as important mediators of various host immune responses. B7-H1 is differentially expressed in various cell subsets and to different extents in human and murine cells. Human B7-H1 is constitutively expressed at low levels in dendritic cells and activated T cells (compared with high expression in activated murine T cells) and is highly expressed in monocytes and tumor cells. We conducted a meta-analysis to explore the association between B7-H1 expression and bladder cancer risk. Two groups were examined, including 352 bladder cancer cases and 60 healthy controls. Meta-analysis results revealed that B7-H1 expression is positively associated with bladder cancer and is strongly associated with the clinical stage of bladder cancer. However, no significant difference was found with respect to gender and the pathological grade of bladder cancer.

Key words: B7-H1 expression; Bladder cancer; Meta-analysis

INTRODUCTION

Bladder cancers have been characterized into a tumor group in which immunological responses are relatively well preserved (Tsujihashi et al., 1988, 1989; Lipponen et al., 1993). Thus, bladder cancer has been shown to be sensitive to immunotherapy with *Bacillus* Calmette-Guerin (Patard et al., 1998). T cells play an integral role in mediating antitumoral immunity. Expression of inhibitory T-cell coregulators by tumor cells has been suggested as a mechanism by which cancers may evade the host immune response (Knutson et al., 2007; Mougiakakos et al., 2010; Nishikawa and Sakaguchi, 2010; Girardin et al., 2012). Inhibitory T-cell coregulators, which are typically expressed only in lymphoid cells, may downregulate the antigen-specific T-cell response by inducing T-cell anergy or apoptosis (Dong et al., 2002; Zang et al., 2003). The B7 family of immune coregulatory proteins has been reported to play an important role in urologic malignancies such as renal cell carcinoma, affecting disease recurrence as well as cancer-specific and overall survival (Thompson et al., 2004, 2006, 2007).

B7 homolog 1 (B7-H1) is a recently discovered T-cell costimulatory molecule that has been implicated in tumor immune escape. It can inhibit immune responses by inducing T-cell apoptosis, impairing cytokine production, and diminishing the cytotoxicity of activated T cells (Dong et al., 2002; Hori et al., 2006; Tsushima et al., 2006). An initial study demonstrated that tumor-associated B7-H1 cells promoted apoptosis of effector cytotoxic T lymphocytes and escaped lysis caused by these cells (Blank et al., 2004). In murine syngeneic tumor models, a B7-H1 blockade using an anti-B7-H1 monoclonal antibody enhanced antitumor immunity and inhibited tumor growth (Iwai et al., 2002; Curiel et al., 2003). An association between tumor-associated B7-H1 expression and variable clinicopathological features has been recently reported in lung (Konishi et al., 2004), esophageal (Ohigashi et al., 2005), and renal cell cancers (Thompson et al., 2005). Based on these findings, we investigated the B7-H1 status in bladder cancer. We hypothesized that B7-H1 expression was positively associated with bladder cancer. We performed a meta-analysis of the most recent and relevant articles to examine this hypothesis.

MATERIAL AND METHODS

Literature search

We performed an electronic search of the PubMed, Cochrane Library, Embase, Web of Science, Springer Link, and CBM databases to identify relevant studies available through December 11, 2013. The search terms included ['bladder cancer' or 'bladder tumor' or 'bladder neoplasms' (Mesh)] and ['B7-H1' or 'PD-L1' (Mesh)]. References in the eligible studies or textbooks were also reviewed by manual searching to identify other potentially eligible studies.

Inclusion and exclusion criteria

The studies included were required to meet the following criteria: i) the type of study should be a case-control study; ii) these case-control studies should focus on the association between B7-H1 expression and bladder cancer; iii) all patients were diagnosed with bladder cancer (all bladder cancer samples had been identified pathologically); iv) included control

group tissue microarrays specimens of normal urothelium; and v) the publication was in English. Studies were excluded if they reported incomplete, useless, or overlapping data, or if they were meta-analyses, letters, reviews, or editorial articles.

Data extraction

Using a standardized form, data from published studies were independently extracted by 2 reviewers (Yu Wang and Ang Liu) to tabulate the information. The following information was extracted from each article: first author, year of publication, language, study design, source of cases and controls, number of cases and controls, mean age, sample, clinical symptom, diagnostic criteria, genotype methods, polymorphism genotype frequency, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In case of conflicting evaluations, an agreement was reached following discussion with a third reviewer (Shan Zhao).

Quality assessment of studies included

Two reviewers (Yu Wang and Ang Liu) independently assessed the quality of papers according to modified STROBE quality score systems (von Elm et al., 2007; Zhang et al., 2011). Forty assessment items related to quality appraisal were used in this meta-analysis, with scores ranging from 0 to 40. Scores of 0-20, 20-30, and 30-40 were defined as low, moderate, and high quality, respectively. Disagreement was resolved by discussion.

Statistical analysis

The odds ratio (OR) and 95% confidence interval (95%CI) were calculated using the Review Manager Version 5.1.6 (provided by Cochrane Collaboration; http://ims.cochrane.org/revman/download [accessed August 9, 2012]) and the STATA Version 12.0 (Stata Corp, College Station, TX, USA). Between-study variations and heterogeneities were estimated using Cochran's Q-statistic (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005) ($P \leq 0.05$ was considered a manifestation of statistically significant heterogeneity). We also quantified the effect of heterogeneity by using the I^2 test, which ranged from 0 to 100% and represented the proportion of inter-study variability that can be attributed to heterogeneity rather than to chance. When a significant Q test ($P < 0.05$) or an I^2 of >50% indicated that heterogeneity among studies existed, the random-effects model was used for meta-analysis. Otherwise, the fixed effects model was used. We tested whether the genotype frequencies of controls were in HWE using the chi-squared test. Sensitivity analysis was mainly performed by sequential omission of individual studies. Funnel plots are often used to detect publication bias. However, because of the limitations related to varied sample sizes and subjective reviews, Egger's linear regression test, which measures funnel plot asymmetry by using a natural logarithm scale of OR, was used to evaluate publication bias (Peters et al., 2006). When the P-value was <0.05, publication bias was considered to be statistically significant. All P values were 2-sided. To ensure the reliability and accuracy of the results, 2 reviewers (Yu Wang and Ang Liu) tabulated the data in the statistical software programs independently and obtained the same results.

RESULTS

Characteristics of studies included

The search strategy retrieved 96 potentially relevant studies. According to the inclusion criteria, 2 studies (Wang et al., 2009; Xylinas et al., 2014) were included in the meta-analysis and 94 were excluded. The flow chart of study selection is shown in Figure 1. The 2 case-control studies selected included 352 bladder cancer cases and 60 healthy controls and evaluated the relationship between B7-H1 expression and bladder cancer. The publication years of the 2 involved studies were 2009 and 2014. All specimens of bladder cancer had been identified pathologically. The source of controls was normal bladder tissue. The HWE test was performed on the genotype distribution of the controls in all studies included and all were found to be in HWE (P > 0.05). All quality scores of the studies included were >20 (moderate-high quality). The characteristics and methodological quality of the studies included are summarized in Table 1.
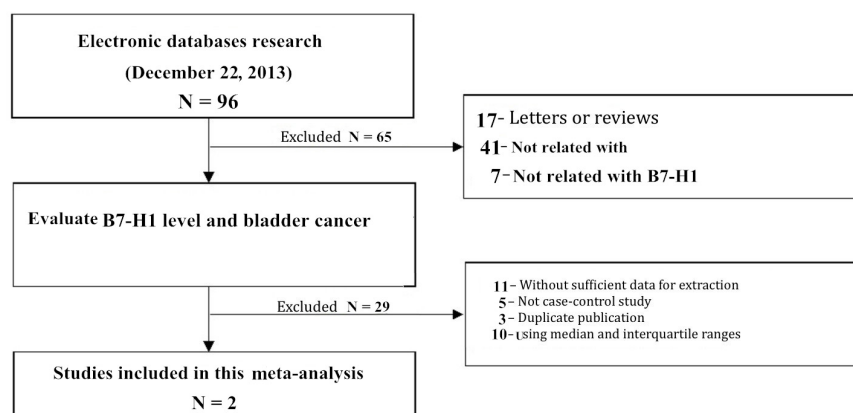

Figure 1. Flow chart showing the study selection procedure.

Table 1. Summary of study characteristics.

Author	Y. Wang	E. Xylinas
Year	2009	2014
Case number	The 50 patients included 40 males and 10 females aged 42-78 years (average 61.7 years). According to WHO and UICC standards, tumor grade was G1-G2 (low-grade group) in 23 patients and was G3 (high-grade group) in 27 patients, and the clinical stage was Ta-1 (superficial group in 19 patients and was T2-4 (invasive group) in 31 patients.	The 302 patients included 244 males and 58 females aged 60-72 years (average 65.6 years). According to WHO and UICC standards, tumor grade was G1-G2 (low-grade group) in 60 patients and G3 (high-grade group) in 242 patients, and the clinical stage was Ta-1 (superficial group) in 33 patients and was T2-4 (invasive group) in 293 patients.
Control group (N)	10	76
Sample Case	Bladder cancer tissue	Bladder cancer tissue
Control	Normal bladder tissue	Normal bladder tissue
Method	Immunostaining	Immunostaining
Clinical characteristics	B7-H1 expression	B7-H1 expression
Quality score	27	31

Association between B7-H1 expression and bladder cancer risk

A summary of the meta-analysis findings regarding the association between B7-H1 expression and bladder cancer risk is shown in Figure 2. The meta-analysis results showed that B7-H1 expression was positively associated with bladder cancer (OR = 11.29, 95%CI = 1.33-95.70, P < 0.05). The significance of the pooled OR in all individual analyses was not influenced excessively by omitting any single study.
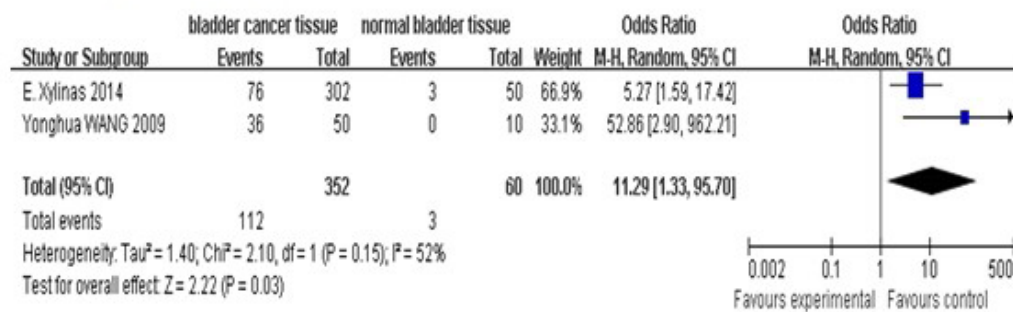
B7-H1 expression positive

Study or Subgroup	bladder cancer tissue Events	Total	normal bladder tissue Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
E. Xylinas 2014	76	302	3	50	66.9%	5.27 [1.59, 17.42]
Yonghua WANG 2009	36	50	0	10	33.1%	52.86 [2.90, 962.21]
Total (95% CI)		352		60	100.0%	11.29 [1.33, 95.70]
Total events	112		3			

Heterogeneity: Tau² = 1.40; Chi² = 2.10, df = 1 (P = 0.15); I² = 52%
Test for overall effect: Z = 2.22 (P = 0.03)

Odds Ratio M-H, Random, 95% CI — 0.002 0.1 1 10 500
Favours experimental   Favours control

Figure 2. Association between B7-H1 expression and bladder cancer risk. 95%CI = 95% confidence interval; d.f. = degrees of freedom.

Difference in B7-H1 expression between male and female patients with bladder cancer

A summary of the meta-analysis findings of differences in B7-H1 expression between male and female patients with bladder cancer is shown in Figure 3. No significant difference was observed between male and female patients with bladder cancer regarding B7-H1 expression (P = 0.49). Sensitivity analysis was conducted by omitting single studies, and no influence was observed in the significance of the pooled OR.
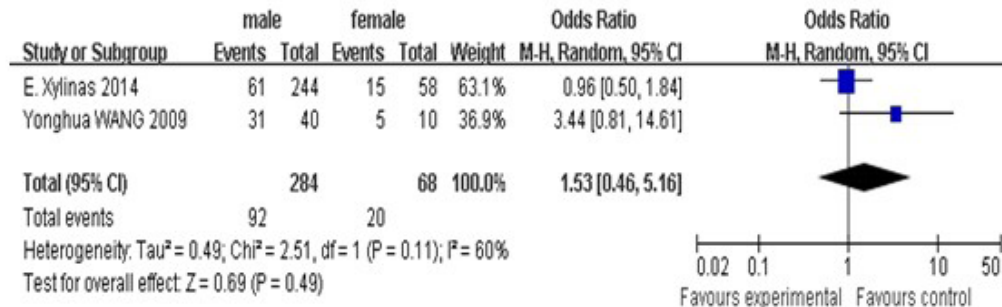
B7-H1 expression positive

Study or Subgroup	male Events	Total	female Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
E. Xylinas 2014	61	244	15	58	63.1%	0.96 [0.50, 1.84]
Yonghua WANG 2009	31	40	5	10	36.9%	3.44 [0.81, 14.61]
Total (95% CI)		284		68	100.0%	1.53 [0.46, 5.16]
Total events	92		20			

Heterogeneity: Tau² = 0.49; Chi² = 2.51, df = 1 (P = 0.11); I² = 60%
Test for overall effect: Z = 0.69 (P = 0.49)

Odds Ratio M-H, Random, 95% CI — 0.02 0.1 1 10 50
Favours experimental   Favours control

Figure 3. B7-H1 expression in males and females with bladder cancer. 95%CI = 95% confidence interval; d.f. = degrees of freedom.

Difference in B7-H1 expression between patients with different stages of bladder cancer

A summary of the meta-analysis findings of difference in B7-H1 expression between patients with low-grade bladder cancer (tumor grade G1-G2) and high-grade bladder cancer (tumor grade G3) is shown in Figure 4. The meta-analysis revealed no significant difference between patients with low-grade bladder cancer (tumor grade G1-G2) and high-grade bladder cancer (tumor grade G3) for B7-H1 expression (P = 0.25). Sensitivity analysis was conducted by omitting single studies, and no influence was observed in the significance of the pooled OR.
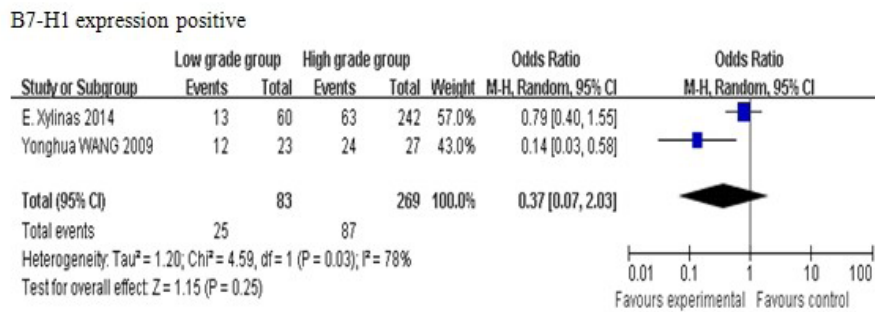
Figure 4. B7-H1 expression in the low-grade bladder cancer group (tumor grade G1-G2) and high-grade bladder cancer group (tumor grade G3). 95%CI = 95% confidence interval; d.f. = degrees of freedom.

Difference in B7-H1 expression between patients with superficial and invasive bladder cancer

A summary of the meta-analysis results regarding the difference in B7-H1 expression between patients with superficial bladder cancer (clinical stage Ta-1) and invasive bladder cancer (clinical stage T2-4) is shown in Figure 5. The meta-analysis result showed that patients with superficial bladder cancer (clinical stage Ta-1) had lower B7-H1 expression than those with invasive bladder cancer (clinical stage T2-4) (OR = 0.41, 95%CI = 0.18-0.91, P < 0.05). Sensitivity analysis was conducted by omitting single studies, and no influence was found in the significance of the pooled OR.
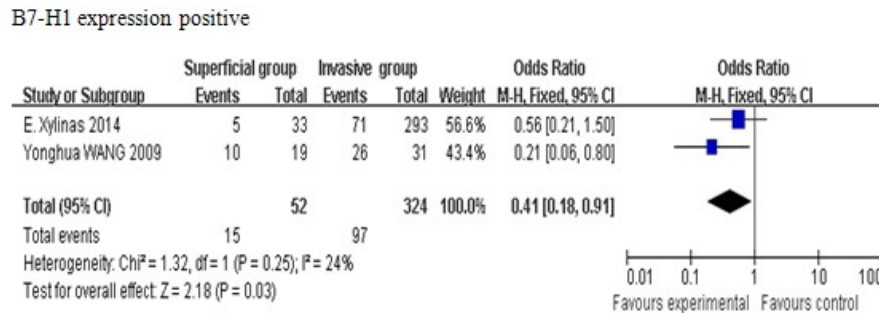

Figure 5. B7-H1 expression in the superficial bladder cancer group (clinical stage Ta-1) and invasive bladder cancer group (clinical stage T2-4) 95%CI = 95% confidence interval; d.f. = degrees of freedom.

Publication bias

Publication bias was assessed by Begger's funnel plot and the Egger linear regression test. Egger's linear regression test was used to measure the asymmetry of the funnel plot. The graphical funnel plots of the studies included appeared to be symmetrical (Figure 6 and 7). Egger's test also revealed no publication bias.
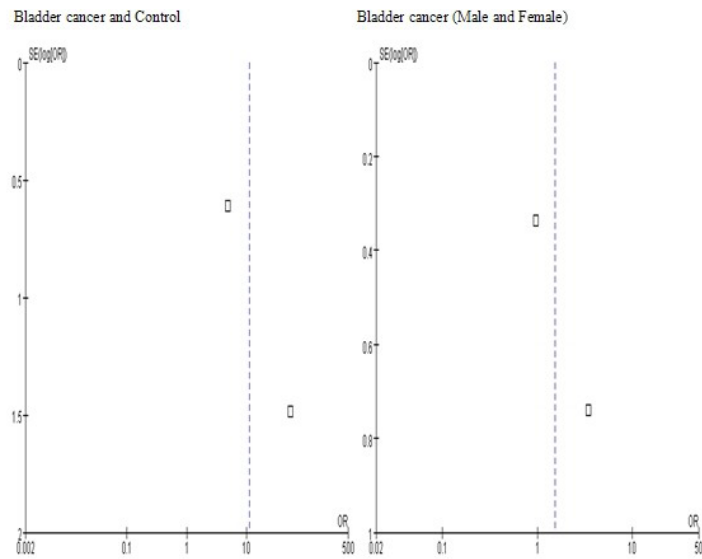

Figure 6. Begger's funnel plot of publication bias based on B7-H1 expression. SE = standard error; OR = odds ratio.
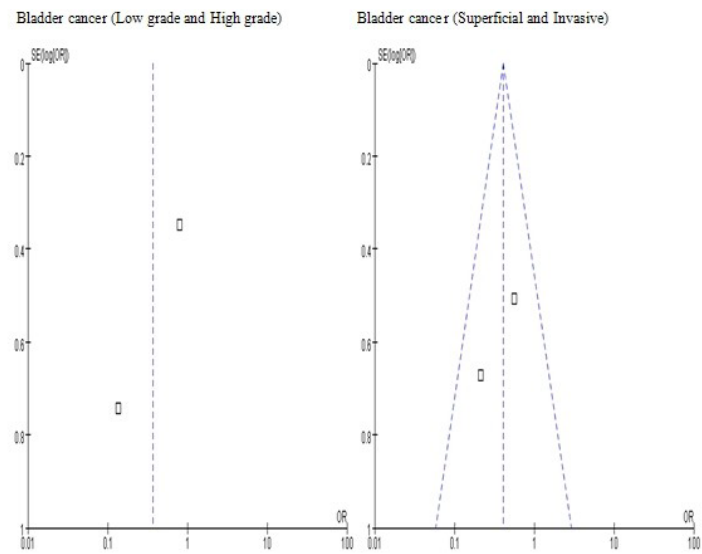

Figure 7. Begger's funnel plot of publication bias based on B7-H1 expression. SE = standard error; OR = odds ratio.

DISCUSSION

Interactions between the immune system and malignant cells play an important role in tumorigenesis. Failure of the immune system to detect and reject transformed cells may lead to tumor development, and tumors can use multiple mechanisms to escape immune-mediated rejection (Wintterle et al., 2003; He et al., 2005; Okazaki and Honjo, 2007). B7-H1 is a newly discovered costimulatory molecule that is closely related to tumor immune escape; it has been found to be overexpressed on a variety of tumor cell surfaces (Liu et al., 2003; Pulko et al., 2009). B7-H1 expression was detected on the cell membrane and in the cytoplasm of bladder cancer cells. Although the precise mechanism regulating B7-H1 expression in tumor cells is unknown, a previous report showed that B7-H1 was expressed more frequently in freshly isolated cancer tissue specimens than in cultured tumor cell lines (Dong et al., 2002). Several cytokines, including interferon-γ, tumor necrosis factor-α, and interleukin-2, have been implicated as possible regulators of B7-H1 expression on the surface of several tumor cells (Wintterle et al., 2003). T cells and natural killer cells infiltrate tumor lesions and secrete various cytokines, including interferon-γ, tumor necrosis factor-α, and interleukin-2. In addition, the degree of effector lymphocyte infiltration in bladder cancer has been associated with the World Health Organization grade and T classification (Lipponen et al., 1993). Therefore, induction of B7-H1 in bladder cancer may be mediated by secreted cytokines from tumor-infiltrating effector lymphocytes. Interferon-γ is a potential candidate as the inducer of B7-H1.

In this meta-analysis, we examined a total of 352 bladder cancer cases and 60 healthy controls from 2 independent studies. We examined the association between B7-H1 expression and bladder cancer. Similarly to previous study results, we found that B7-H1 expression was positively associated with bladder cancer (OR = 11.29, 95%CI = 1.33-95.70, P < 0.05). In addition, we found that patients with superficial bladder cancer (clinical stage Ta-1) showed lower B7-H1 expression than those with invasive bladder cancer (clinical stage T2-4) (OR = 0.41, 95%CI = 0.18-0.91, P < 0.05). However, we found no significant difference between male and female patients with bladder cancer regarding B7-H1 expression (P = 0.49). We also found no significant difference between patients with low-grade bladder cancer (tumor grade G1-G2) and high-grade bladder cancer (tumor grade G3) for B7-H1 expression (P = 0.25).

Similar to the results of other meta-analyses, there were some limitations to our study. First, the eligible number of studies in this meta-analysis was small and the sample size of this meta-analysis was not large. In addition, some relevant studies could not be included in our analysis because of incomplete raw data. Third, although all cases and controls of each study were well-defined using similar inclusion criteria, there may have been potential factors that were not taken into account, which would influence our results. Fourth, meta-analysis is a retrospective method that is subject to methodological limitations. Most importantly, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs or presented ORs that were not adjusted by the same potential confounders such as age, ethnicity, and exposure. Based on these results, additional studies are needed, and our conclusions should be interpreted with caution.

In conclusion, this meta-analysis of 2 case-control studies revealed the remarkable expression of B7-H1 in bladder cancer. B7-H1 expression in tumor cells was strongly associated with the clinical stage of bladder cancer. However, we observed no significant difference between male and female patients with bladder cancer regarding B7-H1 expression. We also found no significant difference between patients with low-grade bladder cancer (tumor grade

G1-G2) and high-grade bladder cancer (tumor grade G3) in B7-H1 expression. The basis for these associations may be related to the recognized role of B7-H1 in tumor immune escape by inducing T-cell apoptosis. Therefore, the manipulation of B7-H1 may become a beneficial target for immunotherapy in human bladder cancer. Because few studies are available in this field, current evidence remains limited. Therefore, large studies with adequate methodological quality and proper controls for confounding factors are necessary to validate our results.

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