

Implications of B7-H1 Expression in Clear Cell Carcinoma of the Kidney for Prognostication and Therapy

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Abstract B7-H1 encompasses a recently discovered cell surface glycoprotein within the B7 family of T-cell coregulatory molecules. B7-H1 expression can be induced on activated T lymphocytes and is normally expressed by macrophage lineage cells. In addition, some human tumors acquire the ability to aberrantly express B7-H1. Tumor-associated B7-H1, as well as B7-H1 on activated lymphocytes, has been shown to impair antigen-specific T-cell function and survival *in vitro*. In contrast, *in vivo* monoclonal antibody-mediated blockade of B7-H1 has been shown to potentiate antitumoral responses in several murine cancer models. Consequently, tumor-associated B7-H1 has garnered much attention in the recent literature as a potential inhibitor of host antitumoral immunity. Our group has recently reported that B7-H1 is aberrantly expressed in both primary and metastatic renal cell carcinoma (RCC) as revealed via immunohistochemical staining of both fresh-frozen and paraffin-embedded nephrectomy specimens. In addition, we have shown that B7-H1 expression by clear cell RCC tumors (or infiltrating mononuclear cells) correlates with aggressive pathologic features, including advanced tumor-node-metastasis stage, tumor size, higher nuclear grade, and coagulative necrosis. In one study of 306 patients, with a median clinical follow-up of 11 years, we reported that RCC B7-H1 expression correlates with increased risk of disease progression, cancer-specific death, and overall mortality even after multivariate adjustment. Five-year cancer-specific survival rates in this study were 42% and 83% for patients harboring B7-H1⁺ versus B7-H1⁻ RCC tumors, respectively. Such associations may relate to the recognized ability of B7-H1 to inhibit T-cell-mediated antitumoral immunity. In summary, B7-H1 encompasses a potent independent predictor of prognosis for patients with RCC and an extremely promising target to facilitate immunotherapeutic responses during the management of this treatment-refractory tumor.

Despite the fact that the incidence of renal cell carcinoma (RCC) continues to increase, stage migration and advances in the surgical management of this form of cancer have culminated in notable improvements in RCC patient survival (1). A sobering reality, however, is that the number of patients who present with regional or systemic dissemination has also increased within the contemporary era (1). At present, it is estimated that ~60% of RCC patients will ultimately experience metastatic progression during the course of their disease (1, 2). Median survival for patients with metastatic dissemination remains only 6 to 10 months (3). Based on this, it is alarmingly clear that more effective systemic therapies for treatment of advanced RCC are desperately needed.

As is the case with melanoma and other immunogenic tumors, subsets of advanced RCC patients exhibit objective responses to cytokine-based immunotherapy (3–5). It has been reported that allogeneic stem cell transplantation may also induce sustained regression in patients with disseminated RCC (6). Spontaneous and complete regression of RCC metastases has been reported to occur, albeit on very rare occasions, (7) and a few studies have reported profuse infiltration of RCC tumors by infiltrating lymphocytes and other mononuclear cells (8, 9). It is these observations that have collectively implicated RCC as an immunogenic tumor. Unfortunately, it is also apparent that mononuclear infiltrates within RCC are frequently composed of dysfunctional lymphocytes that have been rendered incapable of effectively mediating tumor regression (10–14). Thus, an exhaustive effort has been pursued to understand the mechanism whereby RCC tumors acquire the ability to undermine or impair host antitumoral immunity. To date, this mechanism has remained frustratingly elusive.

Recent basic science observations have suggested that the fate and activity of T cells (i.e., activation, proliferation, acquisition of memory, and cytolytic function versus induction of anergy, ignorance, and programmed death) are ultimately governed by the balance between positive and negative signaling within T cells conferred through interactions between various T-cell coregulatory receptors and ligands (15). Related to this, we have recently reported strong associations between tumor expression

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of a particular T-cell coregulatory molecule and cancer progression in RCC patients. Specifically, B7-H1 (also known as PD-L1) has recently been introduced as a novel T-cell coregulatory molecule that is capable of inhibiting generalized and antitumoral T-cell-mediated immunity (16, 17). Cell surface expression of B7-H1 has been reported to be primarily restricted to macrophage lineage cells (16). Aberrant B7-H1 expression, however, has also been reported to be exhibited by several human malignancies, including RCC (17–23). Perhaps, most interesting is the observation that blockade of tumor-associated B7-H1 has been shown to promote *in vivo* tumor regression in several murine cancer models (17, 24, 25). Thus, we surmise that B7-H1, along with other negative regulators of T-cell activation, may act in concert to thwart host antitumoral immunity in patients with RCC and other forms of malignancy as well. In this report, we provide a comprehensive review of the literature and describe important clinical observations that suggest that B7-H1 overexpression by RCC tumors may encompass a mechanism whereby RCC tumors impair host T-cell-mediated antitumoral immunity before and during immunotherapeutic treatment.

Materials and Methods

A Medline search was done using the keywords “B7-H1,” “PD-L1,” and “carcinoma, renal cell.” Mapping the keywords “B7-H1” or “PD-L1” produced 130 articles, which were reviewed for background information on mechanism and function of this molecule. Combining these 130 articles with the keyword “carcinoma, renal cell” produced four peer-reviewed articles that specifically addressed the clinical role of B7-H1 in RCC (23, 26–28). These articles are reviewed herein.

Results

B7-H1 is a coregulatory inhibitor of antitumoral T-cell responses. Recently, it has become clear that T-cell responses are ultimately governed by a balance between positive and negative coregulatory signals that are generated within the T cell (15). It has been shown that highly orchestrated T-cell coregulatory receptor-ligand interactions act to direct, modulate, and fine tune T-cell receptor signaling to either promote or suppress T-cell activation; depending on timing and location, costimulatory molecules can control T-cell priming, growth, differentiation, and functional maturation (15). More simply stated, T-cell coregulatory receptor-ligand interactions can provide either positive “go” signals to amplify antigen-specific T-cell activation or “stop” signals that can inactivate or kill T cells (Fig. 1). For example, OX-40 (CD134; ref. 29) and 4-1BB (CD137; ref. 30) represent positive coregulators (also referred to as “accessory costimulators”) that generally function to bolster antigen-specific T-cell stimulation, thereby intensifying immune assaults against specific antigens that are formally presented to the T cell. In contrast, CTLA-4 and B7-H1 represent negative coregulators (also referred to as “coinhibitors”) that can down-regulate T-cell function and truncate T-cell survival presumably to preempt self-antigen recognition and, thus, the genesis and evolution of autoimmune disease (15, 31).

Hence, beginning in late 2003, our group initiated an exhaustive survey of T-cell phenotypes, coregulatory receptors and

ligands, and markers of T-cell activation expressed within human RCC tumors. This survey included immunohistochemical analyses of RCC tumors for levels of CD3⁺, CD4⁺, CD8⁺ T cells, T-cell accessory costimulators, including OX-40 and 4-1BB, activation markers, such as high-affinity interleukin-2 receptor (CD25), cytolytic T-cell perforins, and granzymes, and, finally, T-cell coinhibitors, such as B7-H1. Of all of the T-cell phenotypes, markers, receptors, and ligands studied, only B7-H1 immediately showed a robust association with RCC disease progression and patient outcome even despite the preliminary nature of our earliest studies.

B7-H1 was first discovered by Dong et al. (16) in 1999. B7-H1 is a cell surface glycoprotein within the B7 family of T-cell coregulatory molecules. B7-H1 mRNA is expressed in nearly all human tissues, but cell membrane protein expression is largely restricted to a fraction of macrophage lineage cells and subsets of activated T lymphocytes (16, 17). Several human malignancies, including cancers of the breast, colon, and lung, can also exhibit cell surface B7-H1 expression (17–22). Thus, these and other molecular studies indicate that B7-H1 protein expression is posttranscriptionally regulated perhaps in response to proinflammatory cytokines, such as IFN- γ , which have been shown to up-regulate B7-H1 expression (15, 17). Various studies have also reported that B7-H1 can both positively and negatively coregulate T-cell responses depending on the location and context of expression (15–17, 32). Although the significance of this apparent duality in B7-H1 function is not well understood, some evidence exists that B7-H1 may facilitate naive T-cell activation in lymphoid tissues and within areas of acute inflammation, whereas in peripheral tissues, including solid tumors, B7-H1 may primarily function to inhibit activated or memory T-cell phenotypes to down-regulate and, thereby, stem immune responses (15).

Cancer cell expression of B7-H1 inhibits antitumor immunity. Experimental studies to date uniformly suggest that B7-H1 acts to down-regulate antitumoral T-cell-mediated immunity. Tumor-associated B7-H1 has been shown to inhibit antitumoral T-cell-mediated immunity by interacting with T-cell PD-1 (or a not-yet-identified non-PD-1 receptor), resulting in tumor-specific T-cell apoptosis or impairments in cytokine production and cytotoxicity of activated antitumoral T cells (17, 20, 24, 33). B7-H1 expressed by murine tumors has been reported to abrogate immune-mediated tumor regression after both adoptive transfer of tumor antigen-specific CD8⁺ T-cell clones and treatment with agonistic costimulatory antibodies (i.e., 4-1BB) that typically promote T-cell activation (25). On the other hand, *in vivo* monoclonal antibody-mediated blockade of B7-H1 has been shown to potentiate antitumoral T-cell responses against immunogenic murine cancers that express B7-H1 either endogenously or after gene transduction (17, 24, 25). Thus, preclinical studies support that B7-H1 blockade can be immunotherapeutically exploited to facilitate antitumor immunity in murine cancer models (22, 25). Because several human cancers have now been reported to aberrantly express B7-H1 (Table 1), it is hypothesized that B7-H1 may facilitate human cancer progression by impairing host antitumoral immunity.

B7-H1 is expressed in clear cell RCC. In December 2004, we reported the first investigation of B7-H1 expression in RCC (23). Specifically, we immunohistochemically stained 196 clear cell RCC nephrectomy specimens for B7-H1 expression levels using an anti-B7-H1 monoclonal antibody (clone 5H1). All

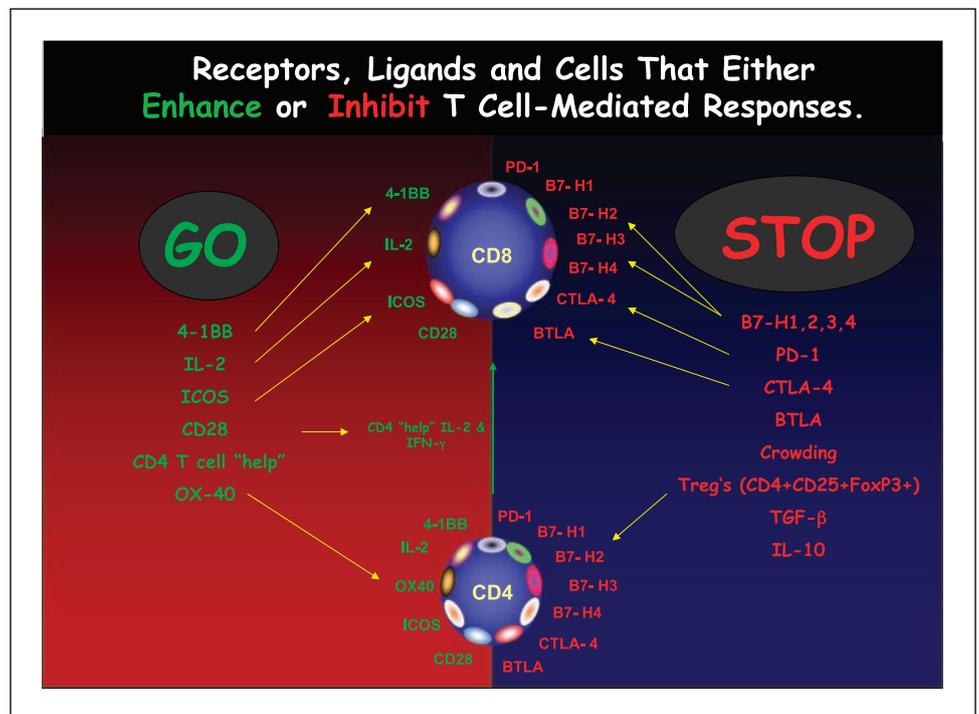


Fig. 1. Various T-cell – associated receptors, ligands, cytokines, conditions, and cell types that have been implicated in the coregulatory inhibition or further activation of antigen-specific T-cell function.

tissue slides in this study were centrally reviewed by a single urologic pathologist (Dr. John C. Cheville, Division of Anatomic Pathology, Mayo Clinic, Rochester, MN), who was blinded to patient outcome. At the time of these studies, the immunohistochemical staining method for B7-H1 was only applicable to fresh-frozen tissue specimens. Consequently, the duration of follow-up in our initial study was somewhat limited (median, 2 years) because all patients with available fresh-frozen tissue specimens had undergone nephrectomy beginning in 2000 and beyond. Despite the somewhat limited interval of follow-up, several important findings were revealed by this study. First, we observed that B7-H1 protein is expressed by both clear cell RCC tumor cells (present in 66% of specimens) and tumor-infiltrating mononuclear cells (present in 59% of specimens; Fig. 2; ref. 23). This finding in itself was deemed novel because B7-H1 was not observed to be expressed in normal human kidney tissues (16, 17). The observation that malignant cells of the kidney acquire the ability to express surface B7-H1 protein and that RCC tumors are infiltrated by B7-H1⁺ mononuclear cells suggested the possibility that, perhaps, B7-H1 might modulate the immunogenicity of this tumor. Second, we noted that patients with high levels of B7-H1, present on either their tumor cells or tumor-infiltrating mononuclear cells, were significantly more likely to exhibit adverse pathologic features within their tumors, including (a) higher nuclear grade ($P < 0.001$), (b) positive lymph node metastatic involvement ($P < 0.001$), (c) distant metastases ($P = 0.022$), and (d) increased levels of histologic tumor necrosis ($P < 0.001$; ref. 23). For this study, we defined high levels of B7-H1 as specimens containing $\geq 10\%$ of B7-H1⁺ tumor cells or specimens with an adjusted lymphocyte count of >100 (moderate to marked lymphocytic infiltration with $\geq 50\%$ of the lymphocytes staining positive for B7-H1; ref. 23). These observations were generally consistent with our overall hypothesis that B7-H1⁺ RCC tumors are associated

with a more aggressive tumor phenotype. Third, we observed that RCC patients with high tumor-associated or lymphocyte-associated B7-H1 were at significantly increased risk of cancer-specific death in a univariate statistical model [risk ratio, 4.53; 95% confidence interval (95% CI), 1.94-10.56; $P < 0.001$; ref. 23]. However, risk of death for B7-H1⁺ patients did not achieve statistical significance in multivariate analysis (risk ratio, 2.19; $P = 0.079$). Based on this, we speculated that the statistical power of our study was somewhat constrained by limited follow-up and low number of events (cancer-specific deaths) and that statistical significance would eventually be achieved with additional follow-up. Based on the notion that RCC is an immunogenic tumor that commonly harbors high levels of dysfunctional lymphocytes, we proposed that B7-H1 may function at the clinical level to impair host T-cell-mediated immunity, thereby promoting cancer progression. Hence,

Table 1. Human tumors reported to express the costimulatory molecule B7-H1

Thymic carcinoma
Bladder transitional cell carcinoma
Ductal and lobular breast carcinoma
Cervix squamous cell carcinoma
Colon adenocarcinoma
Endometrial carcinoma
Larynx squamous cell carcinoma
Hepatocellular carcinoma
Lung squamous and adenocarcinoma
Ovarian carcinoma
Stomach adenocarcinoma
Salivary carcinoma
Thyroid follicular carcinoma
RCC, clear cell type
Esophageal carcinoma
Melanoma
Glioblastoma

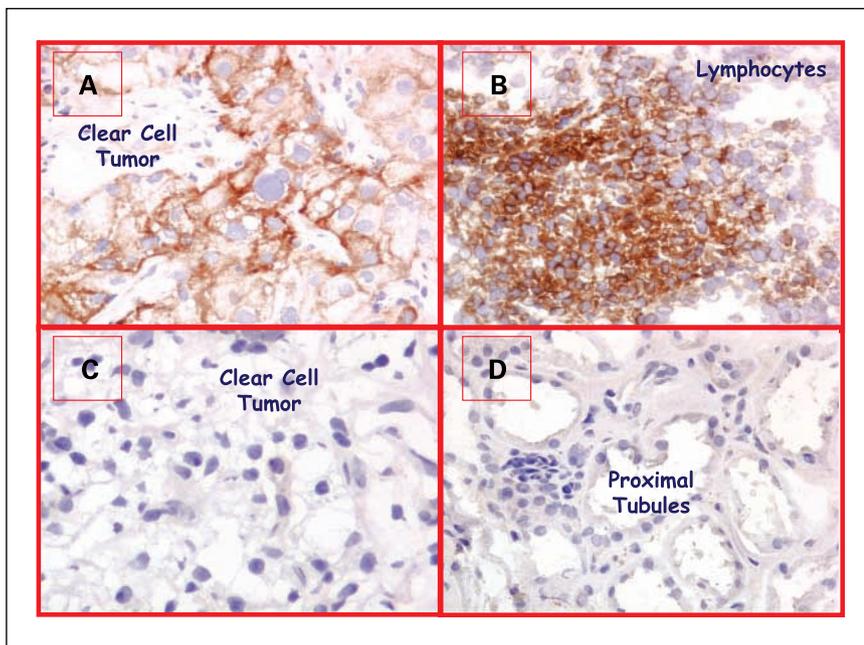


Fig. 2. Photomicrographs at $\times 400$ magnification. *A.* RCC specimen with high tumor B7-H1 expression. *B.* RCC specimen with high lymphocyte B7-H1 expression. *C.* RCC specimen with no B7-H1 staining in either the tumor cells or lymphocytes. *D.* normal kidney specimen with no B7-H1 staining in the proximal tubules. Reprinted with permission from Thompson et al. (23).

additional studies with longer intervals of patient follow-up seemed warranted.

B7-H1 is expressed in metastatic RCC. With 1 additional year of follow-up, we reported a second clinical investigation of B7-H1 in RCC (26). Using the same cohort of patients mentioned above, we confirmed that patients with high tumor- or lymphocyte-associated B7-H1 remained at a significantly increased risk of death (risk ratio, 2.63; 95% CI, 1.23-5.64; $P = 0.013$) even after multivariate adjustment for prognostic indices encompassed in the Mayo Clinic tumor-node-metastasis stage, tumor size, grade, and necrosis score, a prognostic algorithm specifically developed for patients with the clear cell subtype of RCC and one that has been externally validated (34, 35). Additionally, in this second study, we examined metastatic clear cell RCC specimens for B7-H1 expression and observed that both disseminated tumor cells and infiltrating lymphocytes express B7-H1 at rates similar to what was observed in primary clear cell RCC tumor specimens (Fig. 3;

ref. 26). This finding is important because immunotherapy is often rendered after cytoreductive nephrectomy. Prompted by the observation that clear cell RCC metastases also express B7-H1, we surmised that advanced RCC patients may still derive a benefit from B7-H1 targeted therapy even after surgical primary tumor extirpation (26). One critique of these translational studies, however, was the definition of high B7-H1 levels. The cut-point levels that we used to define high B7-H1 levels in our earliest studies (which were somewhat underpowered because of limited patient follow-up) were elucidated using statistical analyses of scatter plots and survival differences in the Cox proportional hazards regression model. However, we observed that a significant number of patients harbored RCC tumors that contained 5% tumor-associated B7-H1 (29%), and these patients were thus excluded from the 10% or more high tumor B7-H1 group. Moreover, the reproducibility of discerning 5% versus 10% B7-H1⁺ tumor staining remained unknown. We speculated that if B7-H1 functions to protect cancer cells from

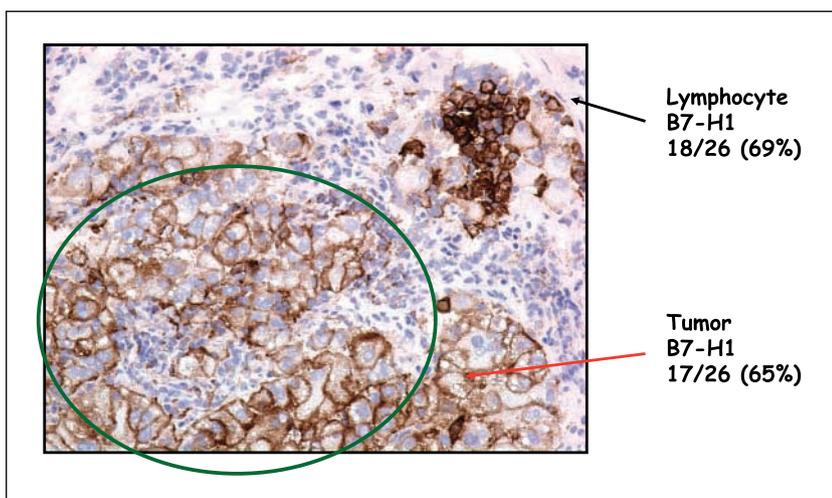


Fig. 3. Photomicrograph at $\times 400$ magnification. Metastatic RCC specimen to lung with high B7-H1 expression on both tumor cells (circled, red arrow) and infiltrating lymphocytes (black arrow). Reprinted with permission from Thompson et al. (26).

T-cell-mediated immune destruction, then patients with any level of B7-H1 protein, including 5%, should be at risk of poor outcome.

To address this concern, we conducted another clinical investigation using the same cohort of patients mentioned above (27). Now, with a median follow-up of 2.7 years, the clinical effect of B7-H1⁺ RCC tumors ($\geq 5\%$ of tumor cells) was simply compared against negative RCC tumors that essentially lacked B7-H1 expression ($< 5\%$ of tumor cells); that is, RCC patients with 5% to 100% of tumor cells expressing B7-H1 (positive tumor) were compared against RCC patients with $< 5\%$ of tumor cells expressing B7-H1 expression (negative tumors). The results of this study confirmed that patients with B7-H1⁺ tumors remained at a significantly increased risk of cancer-specific death relative to patients with B7-H1⁻ tumors even after a multivariate adjustment (risk ratio, 3.52; $P = 0.010$; ref. 27). Thus, this study was congruent with our previous thoughts that B7-H1⁺ RCC tumors are more aggressive tumors and likely to disseminate and kill the patient. These associations were also consistent with the recognized ability of tumor-associated B7-H1 to inhibit T-cell-mediated immunity as was reported in the basic science literature. However, as we noted, confirmatory reports with longer follow-up were needed to more rigorously validate these conclusions.

Long-term clinical follow-up of B7-H1 expression in RCC patients. Until 2006, B7-H1 immunohistochemical staining was only feasible using fresh-frozen RCC nephrectomy specimens that we began collecting in 2000. Other mRNA-based methods, such as *in situ* hybridization, to assess B7-H1 levels in formalin-fixed, paraffin-embedded specimens were ruled out to extend our studies because expression of mRNA does not necessarily correlate with the presence or absence or absolute levels of encoded protein (15). Hence, after roughly 6 months of arduous and systematic testing, Susan Kuntz in our laboratory was able to generate a reproducible antigen retrieval method that permitted B7-H1 to be immunohistochemically stained in paraffin-embedded specimens (28). Shortly thereafter, we immunohistochemically stained 306 paraffin-embedded clear cell RCC tumor specimens recovered from patients who underwent nephrectomy during the years 1990 to 1994 (28). Our results obtained using paraffin-embedded tissues were consistent with our earlier observations made using fresh-frozen specimens. That is, RCC tumors expressing B7-H1 (24%) were significantly more likely to exhibit a higher nuclear grade, larger tumor size, higher tumor-node-metastasis stage, and coagulative tumor necrosis ($P < 0.001$ for all) than tumors lacking B7-H1 expression (28). Similarly, RCC patients with B7-H1⁺ tumors remained at significantly higher risk of cancer-specific death (risk ratio, 3.92; 95% CI, 2.61-5.88; $P < 0.001$) and overall mortality (risk ratio, 2.37; 95% CI, 1.72-3.28; $P < 0.001$) compared against patients whose tumors scored negative for B7-H1 expression. This increased risk of death from RCC persisted in multivariate models that adjusted for tumor-node-metastasis stage, grade, and performance status and even after adjusting for the Mayo Clinic tumor-node-metastasis stage, tumor size, grade, and necrosis score (28). In this study, 5-year cancer-specific survival rate was 42% for patients with B7-H1⁺ tumors versus 83% for patients with B7-H1⁻ tumors (Fig. 4). Moreover, for the subset of RCC patients with completely localized forms of disease (pT_xN₀M₀), patients with B7-H1⁺ tumors were at significantly increased risk of experiencing

metastatic disease progression (risk ratio, 3.46; 95% CI, 2.11-5.69; $P < 0.001$; Fig. 5) and death from RCC (risk ratio, 4.13; 95% CI, 2.49-6.86; $P < 0.001$) relative to patients with B7-H1⁻ tumors. Thus, these data support the finding that B7-H1 independently portends a poorer prognosis for RCC patients (even for patients with seemingly localized disease) and encompass some of the most compelling evidence to date that B7-H1 may be clinically operational in RCC patients impairing host immunity and fostering tumor progression.

Discussion

Our understanding of the fundamental regulatory mechanisms that govern host immune cell activation, with a particular emphasis on T-cell coregulation, has expanded exponentially within the last decade. B7-H1 encompasses the first T-cell coregulatory molecule reported to be significantly and independently associated with disease progression and patient survival for any solid human malignancy. Our studies show that B7-H1⁺ clear cell RCC tumors typically exhibit more severe pathologic features. In addition, patients who harbor B7-H1⁺ tumors are at significantly increased risk of experiencing cancer progression, cancer-specific death, and overall mortality. Although the precise role of B7-H1 in the regulation of T-cell responses in inflammation and autoimmunity remains a topic for further investigation, B7-H1 has consistently been reported as an immunoinhibitory molecule in the context of tumor immunity (15, 25). In fact, we are now aware of two additional recent studies that suggest that tumor B7-H1 expression is associated with a poorer outcome for patients with malignancies of the breast and colon (36, 37). Hence, it seems that B7-H1 may well represent a general promoter of tumor progression. However, further studies will need to be conducted to establish this notion.

The basic science and translational investigations of B7-H1 in RCC now strongly support the development of a fully humanized monoclonal antibody for use in phase 1 clinical trials. At present, it is anticipated that clinical grade antibody for B7-H1 blockade in humans may be available within 18 to 24 months. Until then, B7-H1 still encompasses a potent independent predictor of outcome for clear cell RCC patients and may ultimately help determine which patients will benefit from adjuvant therapy.

A compelling precedent for abrogating inhibitory T-cell signaling to potentiate antitumor immunity has been established by the experience with CTLA-4 blockade immunotherapy (31). Similar to B7-H1, CTLA-4 is an inducible cell surface glycoprotein that participates in T-cell coregulation, functioning as a potent inhibitor of T-cell-mediated immunity (31). In fact, CTLA-4 knockout mice have been shown to survive for < 1 month, secondary to aggressive polyclonal multiorgan lymphocytic infiltration (38). CTLA-4 blockade is currently being tested in clinical trials for patients with advanced melanoma, prostate cancer, and several other forms of malignancy (39). There is, however, one distinct difference between CTLA-4 and B7-H1; B7-H1 is aberrantly expressed on human cancer cells, whereas CTLA-4 is present on all forms of activated T cells irrespective of the source of antigenic stimulation. Thus, B7-H1 blockade may, by definition, potentiate T-cell-mediated responses in a more tumor-specific fashion, thereby limiting autoimmune side effects.

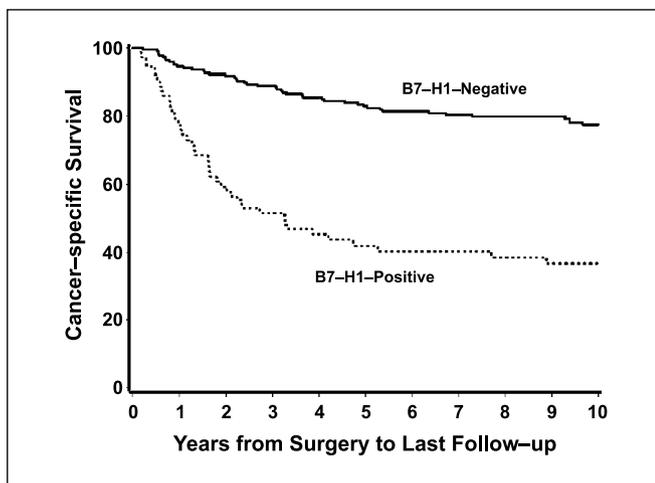


Fig. 4. Association of tumor B7-H1 expression with death from RCC for 306 patients with clear cell RCC (risk ratio, 3.92; 95% CI, 2.61-5.88; $P < 0.001$). Cancer-specific survival rates at 5 and 10 yrs after nephrectomy were 42% and 37%, respectively, for patients with positive tumor B7-H1 expression compared with 83% and 77%, respectively, for patients with negative tumor B7-H1 expression. Reprinted with permission from Thompson et al. (28).

Current applications of immunotherapy and vaccination for human malignancies are often capable of eliciting strong and functionally intact T-cell responses; however, these responses against tumor-associated antigens do not often culminate in tumor regression in clinical trials (25, 40). These findings suggest a tumor escape mechanism whereby cancer cells are capable of evading antitumor immunity in a microenvironment of immune resistance. For patients with clear cell RCC, B7-H1 may contribute to the profile of immunosuppression by forming a “molecular shield” to prevent tumor destruction (25). Analogous to this, it has been recently shown that B7-H1 seems to facilitate states of chronic viral infection and, conversely, that B7-H1 blockade is capable of reviving “exhausted” memory CD8⁺ cytotoxic T cells that subsequently may participate in clearing viral burden (41). That B7-H1 is present on numerous other human malignancies suggests that it may facilitate tumor evasion of host immunity. Although it is tempting to attribute immune dysfunction in RCC and other tumors to aberrant B7-H1 expression, it is likely that multiple other host factors also contribute. Nevertheless, further investigation of B7-H1 and other T-cell coregulatory molecules is clearly warranted. Such investigations should include (a) evaluation of PD-1, a known receptor of B7-H1, in relationship to RCC outcome; (b) investigation of B7-H1 in non-clear cell RCC histologic subtypes; and (c) investigations of other T-cell coregulatory molecules that may potentially collaborate with B7-H1 to help RCC militate against host immunity. Obviously, a fully humanized antibody to block B7-H1 needs to be developed and tested for toxicity and efficacy. RCC has garnered much attention in modern oncology due to major recent advances in molecular genetics and targeted therapies (42). We believe that B7-H1 is a potent prognostic marker and one of the most promising and targetable RCC molecules reported to date. We are confident that further investigations of B7-H1 will not only improve our understanding of tumor-induced immune dysfunction but also broaden the availability of useful therapeutic options for patients afflicted with advanced RCC.

Conclusion

B7-H1 is aberrantly expressed in clear cell RCC and is associated with increased aggressive pathologic features and risk of cancer progression, cancer-specific death, and overall mortality. The basis for these associations may relate to the recognized ability of tumor-associated B7-H1 to inhibit T-cell-mediated immunity. Therefore, blockade of B7-H1 represents a realistic potential immunotherapeutic treatment approach for patients with RCC. Finally, it is clear that B7-H1, alone or in combination with other cancer biomarkers, may prove extremely useful to identify high-risk patients who may require and respond to standard-of-care adjunctive therapy or newly emerging treatments that are to be tested in the context of clinical trials.

Open Discussion

Dr. Atkins: One would think that if B7-H1 expression correlated with poor prognosis that you would see an enrichment of B7-H1 expression in metastatic disease compared with primary rather than the expression being essentially equivalent.

Dr. Kwon: One possibility is that there is another molecule like B7-H1 that also does the same thing and it is on the metastases. Another possibility is that B7-H1 does not necessarily stick to the cells and is actually shed into the serum. Yet, another possibility is that B7-H1 is not relevant at all to renal cell carcinoma or its ability to metastasize.

Dr. Ochoa: Why would B7-H1 be present in T cells that infiltrate those tumors?

Dr. Kwon: The observation thus far has been that, when a T cell gets activated, a subset of T cells will express B7-H1 on the surface. The reason the B7-H1 appears on those activated T cells is not yet elucidated.

Dr. Sosman: Would you predict that the B7-H1-expressing tumors might respond better to anti-CTLA-4?

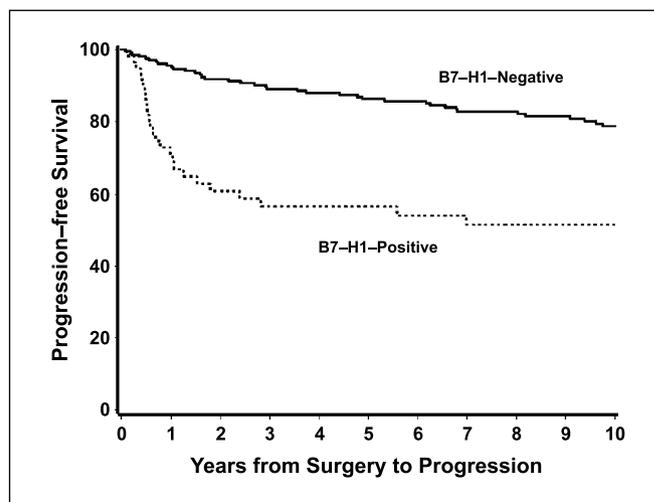


Fig. 5. Association of tumor B7-H1 expression with progression to distant metastases for 268 patients with localized clear cell RCC (risk ratio, 3.46; 95% CI, 2.11-5.69; $P < 0.001$). Progression-free survival rates at 5 and 10 yrs after nephrectomy were 57% and 51%, respectively, for patients with positive tumor B7-H1 expression compared with 86% and 79%, respectively, for patients with negative tumor B7-H1 expression. Reprinted with permission from Thompson et al. (28).

Dr. Kwon: There are at least 10 different ways that these tumors are impairing immune responses. Anti-CTLA-4 monoclonal antibody is a good strategy to use in combination with B7-H1 blockade. B7-H1 blockade has an advantage in that it is intrinsically specific to the tumor in this case, unlike anti-CTLA-4, which is a generalized way of preventing down-regulation of T-cell responses where autoimmunity might easily prevail. I like the B7-H1 approach, but it can be used in combination with anti-CTLA-4.

Dr. Vieweg: With your B7-H1 studies, you have an opportunity to look at knockdown or down-regulation of your target molecule, which was not the case in the trials using the CTLA-4 antibody. What frustrated me the most with the CTLA-4 studies is that there was no proof that you have actually impacted CTLA-4 signaling. Therefore, I presume B7-H1 is a better target.

Dr. Atkins: Data were presented at ASCO last year showing that, in melanoma patients receiving CTLA-4 antibody, the patients who developed autoimmunity but did not have tumor response were reportedly patients who had PD-1 expression on their lymphocytes. The hypothesis is that the melanoma cells that express B7-H1 are able to evade the immune system, suggesting that a combination of CTLA-4 antibody and B7-H1 antibody might be able to overcome the resistance in this group of patients and produce responses in all the patients who are predisposed to develop autoimmunity with CTLA-4 antibody administration.

Dr. Kwon: We are naive if we think that one bullet will bring down these cancers. It is going to have to be multiple agents, and then multiple agents may be used in combination with certain kinds of drugs or a surgical approach.

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