Tumor Biol. (2016) 37:7583–7587
DOI 10.1007/s13277-015-4652-z

ORIGINAL ARTICLE

Prognostic investigations of B7-H1 and B7-H4 expression levels as independent predictor markers of renal cell carcinoma

Hamid Reza Safaei[1] · Ayoob Rostamzadeh[2] · Omid Rahmani[3] · Mohsen Mohammadi[4] · Omar Ghaderi[5] · Hamid Yahaghi[6] · Koroosh Ahmadi[7]

Received: 21 September 2015 / Accepted: 14 December 2015 / Published online: 18 December 2015

Abstract In order to evaluate the correlation of B7-H4 and B7-H1 with renal cell carcinoma (RCC), we analyzed B7-H1 and B7-H4 expressions and their clinical significance by immunohistochemical method. Our result indicated that B7-H4-positive staining was detected in 58.13 % of RCC tissues (25 tissues tumors), and there were 18 tissues of patients without detectable B7-H4. Furthermore, 21 cases (48.83 %) were B7-H1-positive. Positive tumor expressions of B7-H4 and B7-H1 were markedly related to advanced TNM stage ($P=0.001$; $P=0.014$), high grade ($P=0.001$; $P=002$), and larger tumor size ($P=0.002$; $P=024$) in RCC tissues than patients with B7-H4-negative and B7-H1-negative in RCC tissues. The patients with B7-H1 and B7-H4-positive expressions were found to be markedly correlated with the overall survival of the patients ($P<0.05$) and tended to have an increased risk of death when compared with negative expression groups. Univariate analysis showed that B7-H4 and B7-H1 expressions, TNM stage, high grade, and tumor size were significantly related to the prognosis of RCC. Furthermore, multivariate analysis showed that B7-H4 and B7-H1 expressions decreased overall survival. The adjusted HR for B7-H1 was 2.83 (95 % CI 1.210–2.971; $P=0.031$) and also was 2.918 (95 % CI 1.243–3.102, $P=0.006$) for B7-H4 that showed these markers were independent prognostic factors in RCC patients. The expressions of B7-H1 and B7-H4 in RCC patients indicate that these markers may be as a predictor of tumor development and death risk. Further investigations can be helpful to confirm B7-H1 and B7-H4 roles as an independent predictor of clinical RCC outcome.

Keywords B7-H1 and B7-H4 · Renal cell carcinoma · Analysis · Prognosis · Immunohistochemistry

✉ Koroosh Ahmadi
ahmadik@mums.ac.ir

1 Department of Pediatric Nephrology, AJA University of Medical Sciences, Tehran, Iran

2 Department of Anatomical Sciences, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

3 Department of Pathology, Be'sat Hospital, AJA University of Medical Sciences, Tehran, Iran

4 Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran

5 Department of Pharmaceutical Biotechnology, Tehran University of Medical Sciences, Tehran, Iran

6 Department of Molecular Biology, Baqiyatallah University of Medical Sciences, Tehran, Iran

7 Department of Emergency Medicine, Alborz University of Medical Sciences, Karaj, Iran

Introduction

Renal cell carcinoma (RCC) is the eighth most common cancer with the highest mortality rate, over 40 % [1, 2]. It has been shown that many kinds of biomarker alterations and subsequent downstream pathways are involved in the development and progression of tumor. Understanding more about molecular mechanisms is very important to identify biomarkers and therapeutic targets, especially in the era of targeted therapies [3, 4]. Despite different prognostic biomarkers for the disease, the behavior of RCC remains difficult to predict. B7-H1 (PD-L1), member of the B7 ligand family, is a co-stimulatory molecule that negatively regulates antitumor cell-mediated immunity [5, 6], and the aberrant expression of B7-H1 has been previously reported in many kinds of malignancies including RCC [7–12].

Moreover, the overexpression of B7-H1 was reported in carcinomas of the lung, ovary, breast, colon and renal cells, and also in melanoma and glioblastoma, and has been observed to impair antitumor T cell immunity [6, 11–14]. B7-H1 has been reported to have a stimulator function in vivo tumor regression in different models of murine cancer [15, 16]. B7-H4 is known as a member of the B7 ligand family that is a negative regulator of T cell cell-mediated immunity [17]. It has been previously indicated that B7-H4 protein ligand expression was increased in the lung, breast, and ovarian cancer [17–19]. A Previous study has reported the cytoplasmic and membranous staining of B7-H4 in invasive carcinomas in different kinds of ovarian tumors [20]. It has been reported that the expression levels of both B7-H4 and B7-H1 are associated with higher risk of death in RCC patients [21].

Therefore, the aim of this investigation was to assess the clinical significance of B7-H1 and B7-H4 in renal cell carcinoma.

Materials and methods

In this study, the medical records of 43 RCC tissues were collected from patients who underwent radical or partial nephrectomy at Tehran and Shiraz hospitals between 2008 and 2013 (Fig. 1). Furthermore, adjacent normal tissue specimens were evaluated. The pathologic characteristics studied included tumor size, distant metastases at nephrectomy (M), the 2002 TNM stage groupings, and nuclear grade. Moreover, the clinical factors studied included age and sex. The clinical features were summarized in Table 1. The overall survival of patients was defined as the time elapsed from surgery to death.

Immunohistochemistry

Immunohistochemistry was done using 4-μm formalin-fixed paraffin-embedded tissue sections and then dewaxed in xylene, rinsed in graded ethanol, and followed by rehydration by using distilled water. Antigen retrieval was performed using heating tissue slides in ethylenediaminetetra acetic acid (EDTA) 1 mmol/L (pH 8) to 121 °C using a Digital Decloing Chamber (Biocare Medical, Concord, CA, USA), after cooling to 90 °C (incubation was done for 5 min). Then, the sections were treated with a peroxidase blocking solution to block endogenous peroxidase activity. The sections' incubation was done in 1:100 dilution of mouse anti-B7-H1 monoclonal antibody (clone 5H1) and mouse antihuman B7-H4 monoclonal antibody (clone hH4.1), respectively. The slides were incubated with a horseradish peroxidase-conjugated anti-biotin antibody. DAB was used as the chromogen, and slides were counterstained with hematoxylin. The tumor cell percentages (positive B7-H4 and B7-H1 staining) were quantified in 5 % increments. Tumors with <5 % of tumor staining were assigned into negative expression.

Statistical analysis

All variables were analyzed using the SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The correlation of expression with clinicopathological features in patients was studied using χ^2. Survival curves were plotted using the Kaplan-Meier method and analyzed by the log-rank test. Univariate and multivariate Cox regression analyses were applied to assess the survival. Statistical analysis was considered to be statistically significant $P < 0.05$.

Results

Immunohistochemical staining results

B7-H4 and B7-H1 staining displayed a heterogeneous staining pattern with a median level of staining of 20 and 25 % (range 10–90 %; 5–90 %). The patients were assigned into positive or negative groups based on whether B7-H1 and B7-H4 were present or absent. Our result indicated that B7-H4-positive staining was detected in 58.13 % of RCC tissues (25 tissue tumors), and there were 18 tissues of patients without detectable B7-H4. Furthermore, 21 cases (48.83 %) were B7-

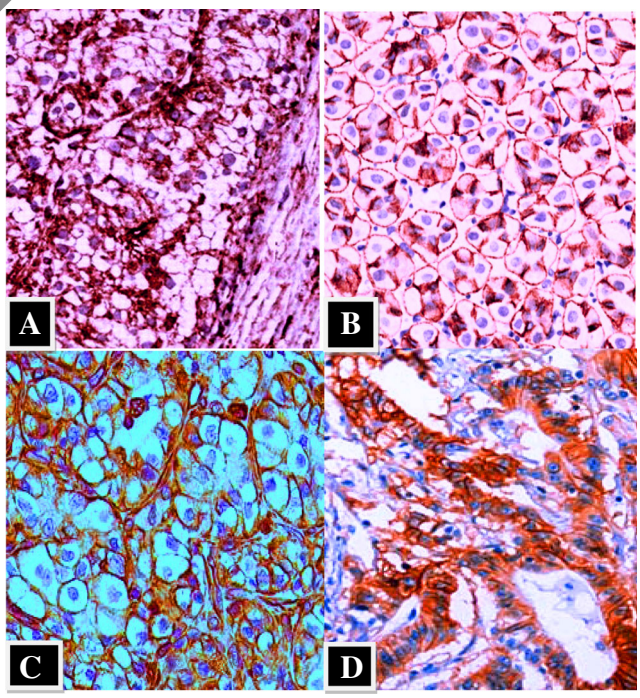

Fig. 1 Immunohistochemical staining of B7-H1 and B7-H4 and their correlation with survival in RCC. **a**–**d** Representative immunohistochemical staining of the positive and negative expressions of RCC

Table 1 Clinical and pathologic features by tumor B7-H4 and B7-H1 expressions

Parameter	Number	B7-H4		*P* value of B7-H4	B7-H1		*P* value of B7-H1
	43	Positive ($n=25$)	Negative ($n=18$)		Positive ($n=21$)	Negative ($n=22$)	
Age				0.591			0.325
<55	20	12	8		7	13	
≥55	23	13	10		14	9	
Sex				0.542			0.641
Male	21	10	11		8	13	
Female	22	15	7		13	9	
Primary tumor size, centimeter				0.002			0.024
<5	18	8	10		7	11	
5 to <7	14	8	6		6	8	
≥7	11	9	2		8	3	
Distant metastases at nephrectomy				0.129			0.251
pM0	28	15	13		16	12	
Pm1	15	10	5		5	10	
TNM stage groupings				0.001			0.014
I/II	25	11	14		10	15	
III/IV	18	14	4		11	7	
Nuclear grade							
1	14	6	8	0.001	3	11	0.002
2	8	5	3		4	4	
3	10	6	4		7	3	
4	11	8	3		7	4	

H1-positive. Only 6 tissues of 20 adjacent tissues showed positive B7-H4 expression. There was no or very weak B7-H1 staining in the adjacent normal tissue specimens. The positive tumor expression of B7-H4 and B7-H1 were significantly correlated with advanced TNM stage ($P=0.001$; $P=0.014$), high grade ($P=0.001$; $P=002$), and larger tumor size ($P=0.002$; $P=024$) in RCC tissues compared with patients with B7-H4-negative and B7-H1-negative in RCC tissues (Table 1).

Combination of B7-H1 and B7-H4-positive staining

Our result indicated that tumors with B7-H1-positive staining were more likely to be B7-H4-positive in comparison with tumor tissues that showed to be B7-H1-negative ($P=0.006$). B7-H1/B7-H4-positive staining tissues were markedly more likely to have adverse clinical and pathological characteristics.

Positive B7-H1 and B7-H4 expressions are correlated with poor overall survival

Kaplan-Meier survival and log-rank analysis were done to evaluate the relationship of B7-H4 and B7-H1 expressions with the survival of patients. The patients with B7-H1 and B7-H4-positive expressions were found to be markedly correlated with the overall survival of the patients ($P<0.05$) and tended to have an increased risk of death when compared with negative expression groups. Univariate analysis indicated that B7-H4 and B7-H1 expressions, TNM stage, high grade, and tumor size were significantly correlated with the prognosis of RCC (Table 2). Furthermore, multivariate analysis showed that B7-H4 and B7-H1 expressions decreased overall survival. The adjusted HR for B7-H1 was 2.83 (95 % CI 1.210–2.971; $P=0.031$) and also was 2.918 (95 % CI 1.243–3.102; $P=0.006$) for B7-H4, that showed these markers were independent prognostic factors in RCC patients (Table 2).

Discussion

The aberrant expression of B7-H1 has been previously observed in various malignancies including RCC [7–12]. Furthermore, the overexpression of B7-H1 has been indicated in carcinomas of the lung, ovary, breast, colon, renal cells, melanoma, and glioblastoma, and has been observed to impair antitumor T cell immunity [6, 11–14]. B7-H1 has been found to have stimulator function in vivo tumor regression in

Table 2 Cox regression analysis of prognostic factors for overall survival in RCC patients

	Univariate			Multivariate		
	HR	95 % CI	*P* value	HR	95 % CI	*P* Value*
B7-H4 expression	2.832	(1.134–3.723)	0.003	2.918	(1.243–3.102)	0.006
Age	0.732	(0.534–1.521)	0.513	0.910	(0.623–1.742)	0.647
Sex	1.03	(0.892–2.071)	0.315	1.142	(0.723–1.891)	0.352
Primary tumor size, centimeter	2.791	(1.035–3.631)	0.006	2.723	(1.127–3.04)	0.002
TNM stage	2.90	(1.09–3.537)	0.011	2.562	(1.190–2.971)	0.02
Distant metastases at nephrectomy	1.573	(0.871–2.21)	0.231	1.152	(0.712–1.623)	0.512
Nuclear grade	2.811	(1.321–3.629)	0.007	2.623	(1.318–3.024)	0.1
B7-H1 expression	2.781	(1.125–3.621)	0.008	2.83	(1.210–3.071)	0.031

95 % CI indicates 95 % confidence interval

*$P \leq 0.05$

different models of murine cancer [15, 16]. The expression of tumor-associated B7-H1 is related to poor prognosis and high grade of malignancy. The blockade of tumor-related B7-H1 has been reported to elevate tumor regression in vivo in different kinds of murine tumor transplants [6, 14–16]. In the present study, we found that 21 cases (48.83 %) were B7-H1-positive. There was no or very weak B7-H1 staining in the adjacent normal tissue specimens. The positive tumor expression of B7-H1 was significantly linked to advanced TNM stage, high grade, and larger tumor size in RCC tissues compared with patients with B7-H1-negative in RCC tissue.

The patients with B7-H1-positive expression tended to have an increased risk of death when compared with negative expression groups. Univariate analysis indicated that B7-H1 expressions, TNM stage, high grade, and tumor size were significantly correlated with the prognosis of RCC. Furthermore, multivariate analysis showed that B7-H1 expressions decreased overall survival, and this marker was an independent prognostic factor in RCC patients.

In this study, we provide evidence that the positive expression of B7-H1 is associated with adverse clinical and pathologic characteristics in renal cell carcinoma. This finding is in agreement with a previous finding that indicated TNM stage and nuclear grade are clinical predictors of outcome in patients suffering from RCC [22, 23]. The aberrant expression of B7-H1 has been reported in human RCC and low expression of B7-H1 in overall survival [12, 24]. Survival rate by traditional predictive indices among patients with RCC, but tends to be variable, indicating the heterogeneous behavior of RCC [24]. Hence, our result indicated that tumor B7-H1 expression is independently associated with the risk of cancer progression. The expression of tumor-associated B7-H1 is related to poor prognosis and high grade of malignancy [14–16]. Moreover, it has been shown that B7-H1 expression could be a prognostic factor independent in many kinds of malignancy such as colorectal cancer and RCC [12, 23, 25]. It has been reported that the expression levels of B7-H1 are associated with greater risk of death in patients with RCC tumors [21].

Moreover, our result indicated that B7-H4-positive staining was detected in 58.1 % of RCC tissues. Only 6 tissues of 20 adjacent normal tissues showed positive B7-H4 expression. The positive tumor expression of B7-H4 was significantly related with advanced TNM stage, high grade, and larger tumor size in RCC tissues compared with patients with B7-H4-negative in RCC tissues.

In the present study, the patients with B7-H4-positive expression was found to be markedly correlated with the overall survival of the patients and tended to have an increased risk of death when compared with negative expression groups. Univariate analysis indicated that B7-H4 expression, TNM stage, high grade, and tumor size were significantly correlated with the prognosis of RCC. Furthermore, multivariate analysis showed that high expression of B7-H4 is related to the decrease of overall survival. The adjusted HR for B7-H4 showed that this marker was an independent prognostic factor in RCC patients. B7-H4 has been shown to be overexpressed in various kinds of tumors, including, non-small cell lung breast, ovarian cancers, and lobular breast cancer, etc., compared to normal tissues [19, 26, 27]. Previous study has reported that the cytoplasmic and membranous patterns of B7-H4 staining were detected only in invasive carcinomas in various forms of ovarian tumors [20]. Thus, the overexpression of B7-H4 may make it an effective target for facilitating antitumoral immunotherapeutic responses in malignant tissues. Previous studies indicated that B7-H4 may negatively regulate T cell responses [17]. Furthermore, it has been suggested that B7-H4 has a direct role in preventing apoptosis in tumor cell. The overexpression of B7-H4 can elevate tumor development in SCID in ovarian cancer cell lines. It was shown that the knockdown of B7-H4 mRNA and expression of protein can elevate intracellular caspase activity in the SKBR3 cell line of breast cancer

and promote tumor cell apoptosis [19]. It has been reported that B7-H4 expression was linked to adverse clinical and pathologic characteristics in RCC tissues. It was indicated that the expression level of B7-H4 is linked to greater risk of death in patients with RCC tumors [21]. The correlation between the tumor expression of B7-H4 and outcome observed in our study is consistent with those reported by the abovementioned study. We found that B7-H4 expression is correlated with an increased risk of death and disease progression in RCC patients. Current evidence indicates that B7-H4 functions as antitumoral immunity inhibitor or extends tumor cell survival. The correlation of B7-H4 expression with the progression and survival of RCC patients may implicate for future therapy. The expressions of B7-H1 and B7-H4 in RCC patients indicate that these markers may be as predictor of tumor development and death risk.

Compliance with ethical standards

Conflicts of interest None

References

1. Howlader N, Noone AM, Krapcho M. SEER cancer statistics review, 1975–2011. Bethesda: National Cancer Institute; 2014.
2. Campbell SC, Novick AC, Bukowski RM. Renal tumors. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, editors. Campbell-Walsh urology. 9th ed. Philadelphia: Saunders; 2007. 1567–637.
3. McGuire BB, Fitzpatrick JM. Biomarkers in renal cell carcinoma. Curr Opin Urol. 2009;19:441–6.
4. Dutcher JP. Recent developments in the treatment of renal cell carcinoma. Ther Adv Urol. 2013;5:338–5
5. Dong H, Zhu G. Tamada K.B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999;5:1365–9.
6. Dong H, Strome SE, Salomao DR. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793–
7. Brown JA, Dorfman DM, FR. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol. 2003;170:1257–66.
8. Konishi J, Yamazaki K, Azuma M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res. 2004;10:5094–100.
9. Wintterle S, Schreiner B, Mitsdoerffer M. Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. Cancer Res. 2003;63:7462–7.
10. Ohigashi Y, Sho M, Yamada Y. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res. 2005;11:2947–53.
11. Curiel TJ, Wei S, Dong H. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med. 2003;9:562–7.
12. Thompson RH, Gillett MD, Cheville JC. Costimulatory B7-H1 in renal cell carcinoma patients: indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A. 2004;101:17174–9.
13. Dong H, Chen L. B7-H1 pathway and its role in the evasion of tumor immunity. J Mol Med (Berl). 2003;81:281–7.
14. Strome SE, Dong H, Tamura H, Voss SG, Flies DB, Tamada K, Salomao D, Cheville J, Hirano F, Lin W, Kwon ED, Sharma P, Chen L. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res. 2003;63:6501–5.
15. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, Tamada K, Chen L. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res. 2005;65:1089–96.
16. Iwai Y, Ishida M, Tanaka Y. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A. 2002;99:12293–7.
17. Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. Immunity. 2003;18:849–61.
18. Choi IH, Zhu G, Sica GL, Strome SE, Cheville JC, Lau JS, et al. Genomic organization and expression analysis of B7-H4, an immune inhibitory molecule of the B7 family. J Immunol. 2003;171:4650–
19. Salceda S, Tang T, Kmet M, Munteanu A, Ghosh M, Macina R, et al. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. Exp Cell Res. 2005;306:128–41.
20. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, et al. B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. J Exp Med. 2006;203(4):871–81.
21. Krambeck AE, Thompson RH, Dong H, Lohse CM, Park ES, Kuntz SM, et al. B7-H4 expression in renal cell carcinoma and tumor vasculature: associations with cancer progression and survival. Proc Natl Acad Sci U S A. 2006;103(27):10391–6.
22. Zisman A, Pantuck AJ, Dorey F. Improved prognostication of renal cell carcinoma using an integrated staging system. J Clin Oncol. 2001;19:1649–57.
23. Thompson RH, Kuntz SM, Leibovich BC, Dong H, Lohse CM, Webster WS, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res. 2006;66(7):3381–5.
24. Thompson RH, Dong H, Kwon ED. Implications of B7-H1 expression in clear cell carcinoma of the kidney for prognostication and therapy. Clin Cancer Res. 2007;13:709s–15s.
25. Shi SJ, Wang LJ, Wang GD, Guo ZY, Wei M, Meng YL, et al. B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. PLoS One. 2013;8(10):e76012.
26. Sun Y, Wang Y, Zhao J, Gu M, Giscombe R, Lefvert AK, et al. B7-H3 and B7-H4 expression in non-small-cell lung cancer. Lung Cancer. 2006;53:143–51.
27. Tringler B, Zhuo S, Pilkington G, Torkko KC, Singh M, Lucia MS, et al. B7-h4 is highly expressed in ductal and lobular breast cancer. Clin Cancer Res. 2005;11(5):1842–8.