Immunomodulatory molecules in renal cell cancer: CD80 and CD86 are expressed on tumor cells

Floercken, A., Johannsen, M., Nguyen-Hoai, T., Gerhardt, A., Miller, K., Doerken, B., Pezzutto, A., Westermann, J., Joehrens, K.

This is the original version of the work, which was first published in:

International Journal of Clinical and Experimental Pathology
2017 FEB ; 10(2): 1443-1454
URL: http://www.ijcep.com/files/ijcep0028400.pdf

Publisher: e-Century Publishing Corporation

Int J Clin Exp Pathol 2017;10(2):1443-1454
www.ijcep.com /ISSN:1936-2625/IJCEP0028400

Original Article

Immunomodulatory molecules in renal cell cancer: CD80 and CD86 are expressed on tumor cells

Anne Flörcken[1], Manfred Johannsen[2], Tam Nguyen-Hoai[3], Anne Gerhardt[1], Kurt Miller[4], Bernd Dörken[1], Antonio Pezzutto[5], Jörg Westermann[1*], Korinna Jöhrens[6*]

[1]Department of Hematology, Oncology and Tumor Immunology, Charité-University Medicine, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353, Berlin, Germany; [2]Facharztpraxis Urologie, Carl-Schurz-Str. 31, 13597, Berlin, Germany; [3]Max-Delbrück Center for Molecular Medicine, Robert-Rössle-Str. 10, 13125, Berlin, Germany; [4]Department of Urology, Charité-University Medicine, Campus Mitte, Charitéplatz 1, 10117, Berlin, Germany; [5]Department of Hematology, Oncology and Tumor Immunology, Charité-University Medicine Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12200, Berlin, Germany; [6]Institute of Pathology, Charité-University Medicine, Campus Mitte, Charitéplatz 1, 10117, Berlin, Germany. [*]Equal contributors.

Received March 16, 2016; Accepted June 21, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Despite modern therapies with tyrosine kinase inhibitors (TKI), the management of patients with metastatic renal cell carcinoma (mRCC) remains a challenge. Significant immunosuppression has been described in patients with mRCC. Therefore, immunotherapeutic strategies such as checkpoint inhibitors have been developed. To further elucidate the underlying mechanisms of immunosuppression and response to therapy, different features of the immune microenvironment (expression of HIF-1-α, VEGFR-1, FOXP3, TGF-ß1, CD80, CD86, PD-1, and PD-L1) were analyzed in tumor tissues within different subgroups of mRCC patients (responders vs. non-responders to therapy). Results: The most interesting finding was low-level CD80 and CD86-expression on tumor tissue samples (n = 18) of nearly all mRCC patients. This finding was in line with CD86 expression, which could also be found in renal carcinoma cell lines. To the best of our knowledge, this is the first report on CD80/CD86 expression in human renal cell carcinoma-possibly reflecting an immunomodulatory mechanism of the tumor.

Keywords: Renal cell carcinoma, expression, CD80, CD86

Introduction

Over the last decade, potent targeted therapies have evolved for patients with mRCC [1-6]. Sequential use of these agents has led to an improvement in progression-free survival and (partially) overall survival as compared to cytokine therapy [7-9]. However, despite the successful development of novel targeted therapies, the management of patients with mRCC remains challenging and relevant subgroups of patients are refractory to TKI treatment [10, 11]. Recently, immune checkpoint pathways have been increasingly recognized and their inhibition has been incorporated into the treatment strategies of several tumors, including mRCC [12-15]. Immune checkpoints pose an important immune evasion mechanism utilized by cancer cells. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1) protein are the key receptors on T cells mediating inhibitory interactions [16]. CTLA-4 is solely expressed on T cells and its inhibition leads to enhanced T cell activation via increased co-stimulation by CD28 [14, 16-19]. PD-1 is an immune-checkpoint receptor mediating immunosuppression and is expressed by activated and follicular T cells, B cells, and NK-cells [20]. In tumors, these activated T cells may bind to their immunosuppressive ligands PD-L1 and PD-L2 [14], that are expressed by tumor cells and in the tumor microenvironment. PD-L1 may also be expressed on macrophages, myeloid-derived suppressor cells (MDSC), dendritic cells (DC), B and T cells [21, 22]. It has recently been shown, that blockade of PD-1 or PD-L1 has significant antitumor effects in RCC [12, 15, 23]. Multiple efforts have been made to correlate the expression of PD-L1 in tumor tissue with the clinical response to anti-PD-1 directed therapy [12, 13, 24, 25]. So far, no clear relationship could be established. However, the rele-

Table 1. Patients' characteristics

Patient	Age in years	Histologic subtype	Manifestations	Therapy	PFS in weeks
1) CR after TKI therapy					
1	55	Clear cell	Adrenal, Bone	Sunitinib	106
2	74	Clear cell	Pulmonary	Sunitinib	308
3	62	Clear cell	Pulmonary	Sorafenib	472
4	63	Clear cell	Pulmonary, Bone	Sunitinib	100
5	69	Clear cell	Pulmonary	Sunitinib	60
6	64	Clear cell	Pulmonary, Lymphogenic	Sunitinib	316
2) Aggressive disease/refractory to therapy					
1	41	Sarcomatoid/chromophob	Pulmonary, hepatic	None	8
2	54	Clear cell	Bone	Sorafenib	5
3	61	Sarcomatoid	Pulmonary, hepatic, lymphogenic	Temsirolimus	12
3) Cytokine treatment					
1	58	Clear cell	Pulmonary, Lymphogenic	Cytokines	33*
2	40	Papillary	Pulmonary, Adrenal, Spleen, Muscle, Local recurrence	Cytokines	4*
3	59	Not classified	Bone, Adrenal, Lymphogenic	Cytokines	30*
4	59	Clear cell	Pulmonary, Bone	Cytokines	109
5	62	Chromophob	Pulmonary, Lymphogenic	Cytokines	23
6	60	Clear cell	Pulmonary	Cytokines	79
7	47	Clear cell	Pulmonary	Cytokines	23

Patients (n = 16) included the following subgroups: 1) Complete remission (CR) after tyrosine kinase inhibitor (TKI) therapy: n = 6. 2) Aggressive disease/refractory to therapy: n = 3. 3) Cytokine treatment (IL-2/IFN-α/5-FU/GM-CSF): n = 7. *lost to follow-up after the weeks stated.

vance of PD-L1 expression of tumor infiltrating lymphocytes remains interesting, particularly in the context of other immunomodulatory factors in the tumor microenvironment [16, 24]. Interestingly, in mRCC PD-L1 expression on tumor cells and lymphocytes has been more clearly correlated with an adverse outcome [26-31]. To further elucidate the underlying mechanisms of immunosuppression and response to therapy, we analyzed the expression of immunomodulatory molecules on tumor tissue samples within different subgroups of mRCC patients: patients with aggressive or TKI-refractory disease, patients with TKI-responsive disease, and patients treated with cytokines, in whom a negative correlation between CD80/CD86 expression on T cells and prognosis had previously been described [32]. Different molecules associated with tumor cell proliferation (VEGFR-1, HIF-1-α) or immunomodulatory functions (CD-80, CD86, PD-1, PD-L1, TGF-ß1, and FOXP3) [14, 33] were included in our histological analyses.

Material and methods

Patients

In our retrospective analysis, tissue samples (n = 18) from different subgroups of mRCC patients (n = 16) were included: 6 patients had been highly responsive to TKI therapy (complete remission under TKI therapy +/- metastasectomy), 3 patients had been refractory to TKI-therapy, and 7 patients had been treated with cytokines [32]. Clinical information as well as follow-up reports were obtained through medical record review. The following parameters were evaluated: histological subtype of the tumor, age at study entry, tumor manifestations, therapy regimen and time of progression-free survival (see **Table 1**). The study was performed in accordance with local ethical guidelines.

Immunohistological analysis

Immunohistological analyses were performed on 18 tumor tissue samples of 16 mRCC patients. Samples included nephrectomy samples (n = 13) and metastasectomy samples (n = 5). Four-micrometer-thick sections from paraffin-embedded samples were performed for immunohistological staining. The following antibodies were used:

The mouse monoclonal antibody against PD-1 (clone MRQ-22), (Zytomed Systems GmbH, Berlin, Germany) was used in a dilution of 1:50. The antibodies against PD-L1, FOXP3, HIF-1-alpha, TGF-beta 1, CD80 and CD86 were purchased from Abcam (Abcam plc 330 Cambridge, UK). For PD-L1 detection, a rabbit monoclonal antibody was used in a dilution of 1:300 (clone EPR1161 (2), FOXP3 (clone 236A/E7) was chosen in a dilution of 1:100, HIF-1-alpha (mouse monoclonal antibody clone H1alpha67) in a dilution of 1:100, CD80 (clone EP1155Y) in a dilution of 1:2000, and CD86 (clone EP1158Y) in a dilution of 1:50. VEGFR-1 (polyclonal) was used in a dilution of 1:50 from Acris Antibodies (Acris Antibodies GmbH, Herford, Germany). All these stains were performed using the Bond-MaxTM device (Leica Biosystems GmbH, Wetzlar, Germany). Antigen retrieval and visualization of bound antibodies were performed employing the manufacturer's protocols and reagents (Bond Polymer Refine, DAB; Leica). The sections were dewaxed and subjected to an antigen retrieval protocol within a BenchMark Ultra (Ventana) followed by incubation with the primary antibody. Bound antibodies were visualized using the streptavidin-biotin-peroxidase method and diaminobenzidine as chromogen (UltraviewKit, also obtained from Ventana). The expression level was quantified as follows: (-) = Negative/single cells, (+) ≤ 25% expression, (++) = 25-50% expression, (+++) ≥ 50% expression, (n.a.) = not available.

Flow cytometric analyses

For FACS analysis, fluorochrome-labeled antibodies against CD80, CD86, and isotype controls (all BD Biosciences, Heidelberg, Germany) were used according to the manufacturer's instructions. 60.000 cells were analyzed on a BD FACSCanto using BDFACSDiva software, (BD Biosciences). Analysis was performed using Flow Jo software version 10 (FLOWJO LLC, Asland, OR, USA). Flow cytometric analyses were performed on two renal carcinoma cell lines (CAKI1-HTB46 and CAKI2-HTB47), (ATCC/LGC Standards, Wesel, Germany).

Results

Expression of immunomodulatory molecules on renal cell carcinoma

Low-level expression of CD80 was shown in 89% (16/18) of tumor tissue samples (n = 18)

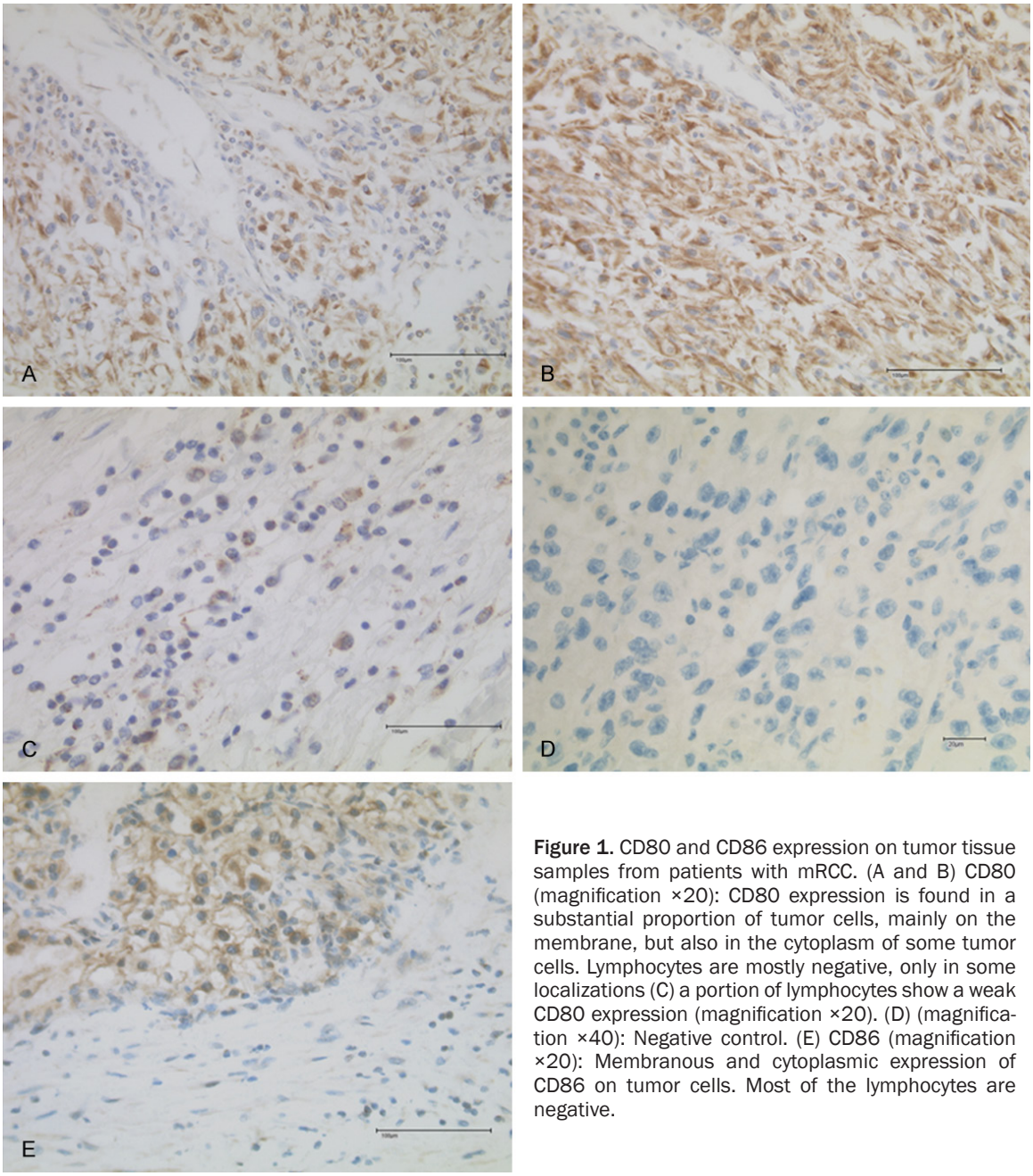

Figure 1. CD80 and CD86 expression on tumor tissue samples from patients with mRCC. (A and B) CD80 (magnification ×20): CD80 expression is found in a substantial proportion of tumor cells, mainly on the membrane, but also in the cytoplasm of some tumor cells. Lymphocytes are mostly negative, only in some localizations (C) a portion of lymphocytes show a weak CD80 expression (magnification ×20). (D) (magnification ×40): Negative control. (E) CD86 (magnification ×20): Membranous and cytoplasmic expression of CD86 on tumor cells. Most of the lymphocytes are negative.

of mRCC patients. CD80 was expressed in the cytoplasm as well as on the cell membrane of the tumor cells (**Figure 1A**, **1B**). Tumor infiltrating lymphocytes only scarcely expressed CD80 (**Figure 1C**, **1D**). CD86 expression could also be demonstrated in 89% (16/18) of patients' tumor tissue samples. For CD86, both cytoplasmic and membranous expression could be observed in the tumor cells. Tumor infiltrating lymphocytes were mostly negative (**Figure 1E**). In the different subgroups of mRCC patients, no significant differences were observed concerning CD86 or CD80 expression (**Table 2**).

To support the finding of CD80/CD86 expression on RCC, additional flow cytometric analyses were performed with renal carcinoma cell lines (CAKI1-HTB46 and CAKI2-HTB47). Low-level CD86 expression could be demonstrated on both cell lines, whereas CD80 was negative (**Figure 2**). Furthermore, PD-L1 expression was analyzed on the available tumor tissue samples within the different subgroups. Interestingly, 67% (2/3) of patients refractory to TKI-therapy showed PD-L1 expression on their tumors. In contrast, in the group of TKI-responsive patients, PD-L1 expression was only found in

Table 2. Expression of PD-1 and PD-L1 on samples of different subgroups of mRCC patients

Patient	CD86	CD80	PD-L1		PD-1	
	Tumor	Tumor	Tumor	Lymphocytes	Tumor	Lymphocytes
CR after TKI therapy						
1	+	++	-	-	-	-
2	+++	++	-	+	-	-
3	n.a.	++	n.a.	n.a.	n.a.	n.a.
4-1	++	++	-	-	-	-
4-2	+++	++	-	-	-	-
4-3	+++	+	-	-	-	-
5	+	+	-	-	n.a.	-
6	+++	++	-	-	-	-
Aggressive disease/refractory to therapy						
1	++	+	+	-	-	-
2	++	-	-	-	-	-
3	++	+	+	-	-	-
Cytokine treatment						
1	-	++	-	-	-	-
2	+	+	-	-	-	+
3	-	-	-	-	-	-
4	++	+	-	-	-	+
5	++	+	-	-	-	-
6	++	++	-	-	-	-
7	++	++	-	-	-	-

Expression of immunomodulatory markers was observed in tissue samples of different subgroups of mRCC patients and was quantified as follows: (-) = Negative / single cells, (+) ≤ 25% expression, (++) = 25-50% expression, (+++) ≥ 50% expression, (n.a.) = not available. The group contains three samples from one patient (patient 4). PD-1 = programmed cell death protein 1, PD-L1 = programmed death protein ligand 1.

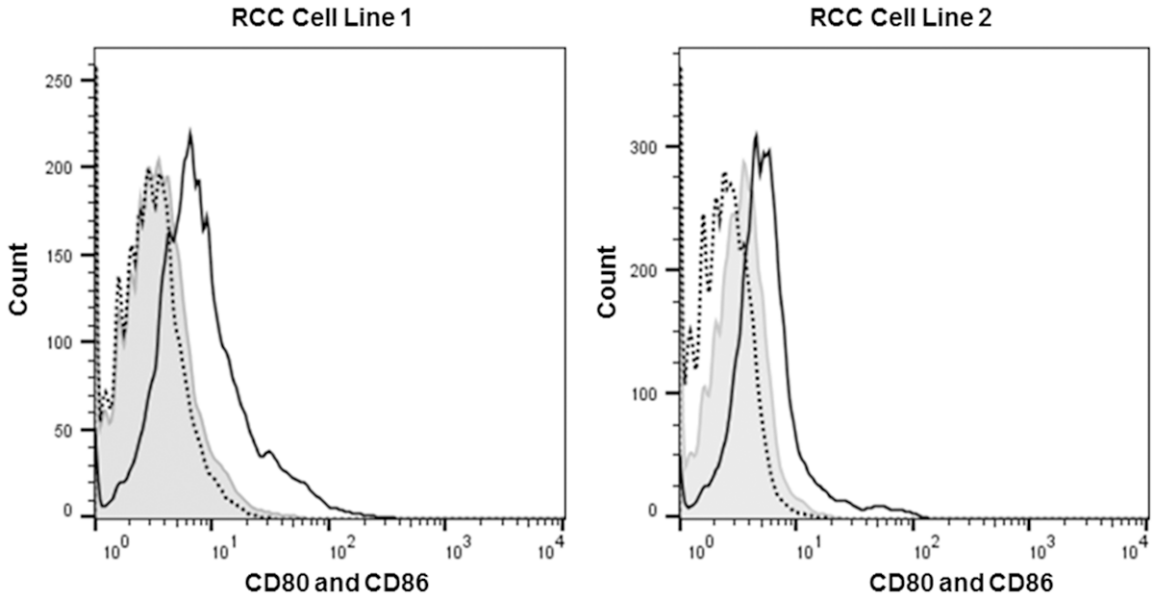

Figure 2. CD86 and CD80 expression on renal carcinoma cell lines. Flow cytometric analyses demonstrated weak CD86 expression on both RCC cell lines, whereas CD80 is not expressed (RCC cell line 1 = CAKI1-HTB46 and RCC cell line 2 = CAKI2-HTB47). (__) = CD86, (....) = CD80, (■) = Isotype control.

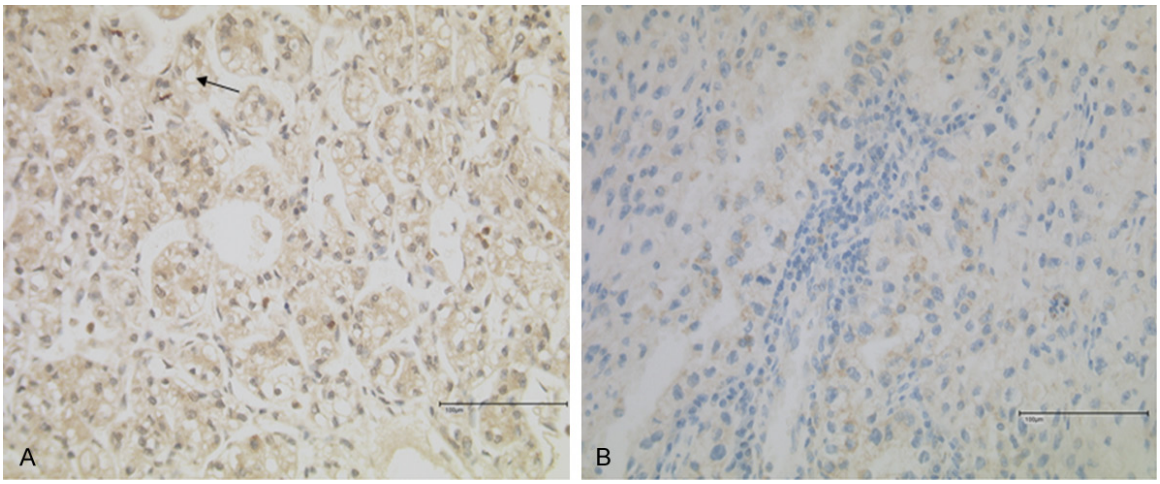

Figure 3. Expression of immunomodulatory markers on samples of different subgroups of mRCC patients. A: TGF-ß1 (magnification ×10): TGF-ß1 is mostly expressed in the cytoplasm of the tumor cells but also a membranous staining could be observed (arrow). B: VEGFR-1 (magnification ×10): Weak cytoplasmic expression of VEGFR-1 was observed in a portion of tumor cells. TGF-ß1 = transforming growth factor beta 1, VEGFR-1 = vascular endothelial growth factor receptor 1.

12.5% (1/8 samples) (**Table 2**). In our cohort of patients, only scarce lymphocyte infiltrations could be demonstrated in tumor tissue. Therefore, no significant PD-1 expression could be observed on tumor-infiltrating lymphocytes (TIL) in the different subgroups (n = 18).

Since molecules influencing tumor growth or immunological responses may be expressed both on tumor cells themselves and in the tumor microenvironment, we have also studied the expression of different other molecules that are associated with tumor progression and/or immunological tolerance. TGF-ß1 was only weakly expressed on tumor cells in samples of TKI-responsive patients (62.5% = 5 of 8 samples with expression on < 25% of tumor cells), whereas the expression level was markedly higher in patients refractory to therapy (67% = 2 of 3 cases with expression on 25-50% of tumor cells, 33% = 1 of 3 cases with expression on > 50% of tumor cells) (**Figures 3A** and **4A**). The same distribution was observed for FOXP3: TKI-responsive patients showed only weak expression (in 100% = 8 of 8 cases with expression in only single lymphocytes or negative expression pattern), whereas higher expression could be observed in TKI-refractory patients (33% = 1 of 3 cases with expression on 25-50% of lymphocytes, 67% = 2 of 3 cases with expression in only a few cells) (**Figure 4B**). Surprisingly, a different pattern was observed for HIF-1-α on tumor cells: in TKI-responsive patients, a higher expression was found (62.5% = 3 of 8 cases with expression on > 50% of tumor cells, 12.5% = 1 of 8 cases with expression on 25-50% of tumor cells, 25% = 2 of 8 cases with expression on < 25% of tumor cells). In contrast, TKI-refractory patients showed a lower expression (100% = 3 of 3 cases with expression on only a few tumor cells or negative expression pattern) (**Figure 4C**). The expression pattern for VEGFR-1 was not substantially different: in TKI-responsive patients only a weak expression was found (62.5% = 5 of 8 cases with expression on some tumor cells or negative expression, 25% = 2 of 8 cases with expression on < 25% of tumor cells, and 12.5% = 1 of 8 cases expression on 25-50% of tumor cells). TKI-refractory patients also showed weak expression (100% = 3 of 3 cases with expression on < 25% of tumor cells (**Figures 3B** and **4D**).

Discussion

In patients with mRCC, targeted therapies have led to improvement in progression-free survival and (partially) overall survival. These therapies mainly mediate tumor cell death, however, multiple additional immunomodulatory mechanisms have been described in RCC [34-40]. To further identify the underlying mechanisms of tumor-induced immune suppression and its correlation with response to therapy, we examined the expression pattern of molecules that

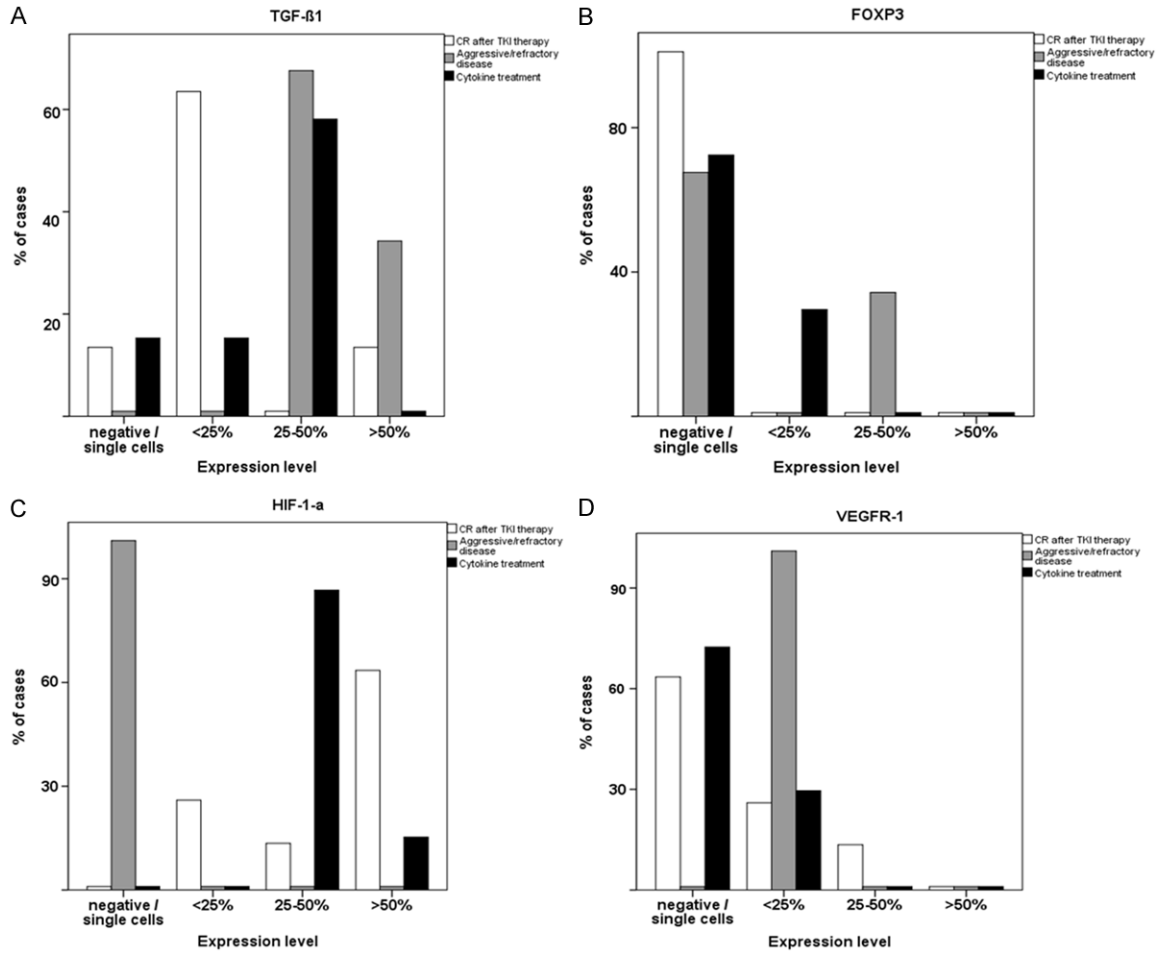

Figure 4. Expression of immunomodulatory markers on samples of different subgroups of mRCC patients. Expression of immunomodulatory markers was observed in tissue samples of different subgroups of mRCC patients and was quantified as seen above. Expression in tumor infiltrating lymphocytes (TIL): FOXP3 = forkhead box P3, Expression in tumor cells: HIF-1-α = hypoxia inducible factor 1-alpha, TGF-ß1 = transforming growth factor beta 1, VEGFR-1 = vascular endothelial growth factor receptor 1.

have been implicated in both tumor progression and immune responses in tumor tissue of patients with mRCC.

HIF-1-α expression was particularly found in the subgroup of patients responding to TKI therapy. HIF-1-α is a driver of several pathways associated with disease progression in RCC [41]. Since some to these pathways are targeted by TKI therapy, it could be hypothesized that high HIF-1-α expression is predictive for response to TKI therapy. However, this issue remains controversial, since no correlation between HIF-1-α expression and response to VEGF-directed targeted therapies has been established so far [41, 42].

The most interesting finding was that CD86 and CD80 are expressed in mRCC. CD80/86 expression could be demonstrated throughout the different subgroups of mRCC patients, however, no clear correlation with clinical outcome could be established. This finding was supported by CD86 expression on RCC tumor cell lines, which could be detected by flow cytometry. PD-L1 expression was associated with aggressive/refractory disease. This is in line with previous reports describing a correlation between PD-L1 expression and an adverse outcome [26-30]. Additionally, further immunosuppressive molecules such as TGF-ß1 and FOXP3 were predominantly found in patients with aggressive/refractory disease. Since CD80/CD86 expression was repetitively demonstrated on tumor samples within different subgroups of mRCC patients and- in the case of CD86- also on renal carcinoma cell lines, we

can exclude technical artifacts leading to non-specific staining.

In the literature, CD80 and CD86 expression have been described on both tumor cells and tumor infiltrating lymphocytes (TIL).

In nasopharyngeal carcinoma [43], CD80 and CD86 expression was associated with improved clinical outcome [44]. In contrast, CD86 expression on myeloma cells and acute myeloid leukemia has been linked to an adverse prognosis [45-47]. Expression on melanoma cells could not be correlated with clinical outcome [48, 49]. Additionally, expression of CD80 in various murine cancer cell lines has previously been demonstrated [50]. To the best of our knowledge, this is the first report demonstrating expression of CD80 and CD86 in human renal cell cancer.

Additionally, there are reports on CD80-expressing TIL in renal cell carcinoma. It has been hypothesized that these CD80+ TIL pose an example of self-co-stimulation between T cells, which may finally lead to unresponsiveness [51]. Furthermore, there are conflicting results on the relevance and prognostic significance of TIL in RCC [52]. Some recent observations demonstrate that TIL in RCC have very heterogeneous profiles with regard to the expression of immune checkpoint molecules and the presence of mature DC within the tumor, leading to a different clinical outcome [52, 53]. It has been reported that particularly tumor infiltration with CD8+ T cells expressing high levels of PD-1 in the absence of mature DC may lead to immunosuppression [53]. Ultimately, we have previously reported on CD80/CD86+ T cells in peripheral blood of renal cell carcinoma patients under cytokine therapy. These CD80/CD86+ T cells were associated with an adverse outcome, possibly reflecting an inhibitory function. These different studies show that CD80/CD86 expression may mediate different immunological functions, depending on the immunological environment [32].

Thus, it seems very likely that the functional consequences of CD80 and CD86 expression on different cell types are not clear-cut with respect to stimulation or inhibition. It seems much more reasonable that they rather depend on the particular immunological context. Therefore, CD80/86 expression on RCC cells might balance both stimulatory and inhibitory signals, e.g. via CTLA-4 and CD28 on T cells.

Interestingly, expression of CD80 and PD-L1 on T cells has recently been linked and it has been shown that they may lead to impaired T cell proliferation and decreased cytokine production. It has also been suggested, that CD80 may have a higher affinity for PD-L1 than for CD28, resulting in a predominantly inhibitory signal in the presence of PD-L1 [54]. Furthermore, tumor-infiltrating immune effector cells selectively expressing CTLA-4 (and not CD28) have been observed [55] and CTLA-4+ T cells have been described particularly in undifferentiated RCC with an adverse prognosis [56]. Thus, CD80/86 expression on the tumor might- at the same time-prevent T cell activation via CTLA-4 and inhibit effector T cells via PD-L1 interaction [14]. This may even occur in T cells, that express both CTLA-4 and CD28 since the binding affinity of CD80/CD86 for CTLA-4 is higher than for CD28 [57, 58], with CD80 being the dominant CTLA-4-ligand and CD86 the dominant ligand of CD28 [59]. Therefore, it seems reasonable that expression of CD80 on RCC cells is a mechanism to prevent T cell activation within the tumor. These immunosuppressive interactions via CD80/CD86-CTLA-4 and CD80-PD-L1 might outweigh immunostimulatory interactions via CD80/CD86-CD28. CD86 expression on tumor cells is an additional mechanism contributing to immune evasion since CD86 may lead to proliferation and homeostasis of Treg [60]. Additionally, it has been shown that co-stimulation via CD86 results in a TH2-polarization of T cells with predominant secretion of IL-4 and IL-10 which inhibit cytotoxic lymphocyte (CTL) responses [61, 62].

Interestingly, low-level CD80-expression was also observed in a murine colon carcinoma mouse model. In this mouse tumor model, low-level CD80 expression- in contrast to high-level CD80-expression- was associated with immune escape due to high affinity binding to CTLA-4 [50]. Therefore, the expression level of CD80/CD86 within the tumor might be a further determinant of immunological outcome.

In summary, we have demonstrated CD80 and CD86 expression in human renal cell carcinoma. In the context of recent literature, it seems very likely that these molecules are part of a network that may protect the tumor from being

attacked by T cells. It was beyond the scope of this study to unravel the exact mechanism, however, and to the best of our knowledge, this is the first report demonstrating CD80 and CD86 expression inhuman renal cell cancer. Our finding might be relevant for future immunotherapeutic strategies.

Acknowledgements

AF, MJ, and AG were responsible for primary patient care. AF, MJ, KM, BD, AP, BD, JW and KJ were responsible for patient selection and detailed planning of the analysis. Flow cytometry was performed by TN. KJ performed immunohistological staining. The manuscript was prepared and written by AF, JW and KJ.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Anne Flörcken, Department of Hematology, Oncology and Tumor Immunology, Charité-University Medicine, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353, Berlin, Germany. Tel: +49-30-450553827; Fax: +49-30-450553914; E-mail: anne.floercken@charite.de

References

[1] Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Bajetta E, Gorbunova V, Bay JO, Bodrogi I, Jagiello-Gruszfeld A and Moore N. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 2007; 370: 2103-2111.

[2] Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebwohl D and Ravaud A. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008; 372: 449-456.

[3] Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM and Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356: 115-124.

[4] Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, Barrios CH, Salman P, Gladkov OA, Kavina A, Zarba JJ, Chen M, McCann L, Pandite L, Roychowdhury DF and Hawkins RE. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol 2010; 28: 1061-1068.

[5] Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R and Bukowski RM. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007; 356: 125-134.

[6] Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, Michaelson MD, Gorbunova VA, Gore ME, Rusakov IG, Negrier S, Ou YC, Castellano D, Lim HY, Uemura H, Tarazi J, Cella D, Chen C, Rosbrook B, Kim S and Motzer RJ. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. Lancet 2011; 378: 1931-1939.

[7] Kroeger N, Xie W, Lee JL, Bjarnason GA, Knox JJ, Mackenzie MJ, Wood L, Srinivas S, Vaishamayan UN, Rha SY, Pal SK, Yuasa T, Donskov F, Agarwal N, Kollmannsberger CK, Tan MH, North SA, Rini BI, Choueiri TK and Heng DY. Metastatic non-clear cell renal cell carcinoma treated with targeted therapy agents: characterization of survival outcome and application of the International mRCC Database Consortium criteria. Cancer 2013; 119: 2999-3006.

[8] Soerensen AV, Donskov F, Hermann GG, Jensen NV, Petersen A, Spliid H, Sandin R, Fode K and Geertsen PF. Improved overall survival after implementation of targeted therapy for patients with metastatic renal cell carcinoma: results from the Danish Renal Cancer Group (DARENCA) study-2. Eur J Cancer 2014; 50: 553-562.

[9] Li P, Wong YN, Armstrong K, Haas N, Subedi P, Davis-Cerone M and Doshi JA. Survival among patients with advanced renal cell carcinoma in the pretargeted versus targeted therapy eras. Cancer Med 2016; 5: 169-81.

[10] Busch J, Seidel C, Weikert S, Wolff I, Kempkensteffen C, Weinkauf L, Hinz S, Magheli A, Miller K and Grunwald V. Intrinsic resistance to tyrosine kinase inhibitors is associated with poor clinical outcome in metastatic renal cell carcinoma. BMC Cancer 2011; 11: 295.

[11] Heng DY, Mackenzie MJ, Vaishampayan UN, Bjarnason GA, Knox JJ, Tan MH, Wood L, Wang Y, Kollmannsberger C, North S, Donskov F, Rini BI and Choueiri TK. Primary anti-vascular endothelial growth factor (VEGF)-refractory metastatic renal cell carcinoma: clinical characteristics, risk factors, and subsequent therapy. Ann Oncol 2012; 23: 1549-1555.

[12] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming

PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM and Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443-2454.

[13] Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A and Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012; 366: 2455-2465.

[14] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12: 252-264.

[15] Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, Castellano D, Choueiri TK, Gurney H, Donskov F, Bono P, Wagstaff J, Gauler TC, Ueda T, Tomita Y, Schutz FA, Kollmannsberger C, Larkin J, Ravaud A, Simon JS, Xu LA, Waxman IM and Sharma P. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 2015; 373: 1803-13.

[16] Topalian SL, Drake CG and Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015; 27: 450-461.

[17] Chambers CA, Kuhns MS, Egen JG and Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 2001; 19: 565-594.

[18] Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H and Mak TW. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 1995; 270: 985-988.

[19] Lenschow DJ, Walunas TL and Bluestone JA. CD28/B7 system of T cell costimulation. Annu Rev Immunol 1996; 14: 233-258.

[20] Keir ME, Butte MJ, Freeman GJ and Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26: 677-704.

[21] Okazaki T and Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007; 19: 813-824.

[22] Tang Y, Jiang Q, Ou Y, Zhang F, Qing K, Sun Y, Lu W, Zhu H, Gong F, Lei P and Shen G. BIP induces mice CD19(hi) regulatory B cells producing IL-10 and highly expressing PD-L1, FasL. Mol Immunol 2016; 69: 44-51.

[23] Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, Vaishampayan UN, Drabkin HA, George S, Logan TF, Margolin KA, Plimack ER, Lambert AM, Waxman IM and Hammers HJ. Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. J Clin Oncol 2015; 33: 1430-1437.

[24] Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS and Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL32-80A in cancer patients. Nature 2014; 515: 563-567.

[25] Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, Chen L, Pardoll DM, Topalian SL and Anders RA. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 2014; 20: 5064-5074.

[26] Thompson RH, Dong H and Kwon ED. Implications of B7-H1 expression in clear cell carcinoma of the kidney for prognostication and therapy. Clin Cancer Res 2007; 13: 709s-715s.

[27] Thompson RH, Kuntz SM, Leibovich BC, Dong H, Lohse CM, Webster WS, Sengupta S, Frank I, Parker AS, Zincke H, Blute ML, Sebo TJ, Cheville JC and Kwon ED. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res 2006; 66: 3381-3385.

[28] Choueiri TK, Fay AP, Gray KP, Callea M, Ho TH, Albiges L, Bellmunt J, Song J, Carvo I, Lampron M, Stanton ML, Hodi FS, McDermott DF, Atkins MB, Freeman GJ, Hirsch MS and Signoretti S. PD-L1 expression in nonclear-cell renal cell carcinoma. Ann Oncol 2014; 25: 2178-2184.

[29] Shin SJ, Jeon YK, Kim PJ, Cho YM, Koh J, Chung DH and Go H. Clinicopathologic Analysis of PD-L1 and PD-L2 Expression in Renal Cell Carcinoma: Association with Oncogenic Proteins Status. Ann Surg Oncol 2016; 23: 694-702.

[30] Leite KR, Reis ST, Junior JP, Zerati M, Gomes Dde O, Camara-Lopes LH and Srougi M. PD-L1 expression in renal cell carcinoma clear cell type is related to unfavorable prognosis. Diagn Pathol 2015; 10: 189.

[31] Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, Chen L, Zincke H, Blute ML, Leibovich BC and Kwon ED. Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. Cancer 2005; 104: 2084-2091.

[32] Westermann J, Hecker AC, Florcken A, Dorken B and Pezzutto A. Granulocyte macrophage-colony stimulating factor plus interleukin-2 plus alpha-interferon plus 5-fluorouracil in the

treatment of metastatic renal cell cancer: induction of CD80/86+ T cells indicates adverse outcome. J Immunother 2009; 32: 667-675.
[33] Vinay DS, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, Lichtor T, Decker WK, Whelan RL, Kumara HM, Signori E, Honoki K, Georgakilas AG, Amin A, Helferich WG, Boosani CS, Guha G, Ciriolo MR, Chen S, Mohammed SI, Azmi AS, Keith WN, Bilsland A, Bhakta D, Halicka D, Fujii H, Aquilano K, Ashraf SS, Nowsheen S, Yang X, Choi BK and Kwon BS. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Semin Cancer Biol 2015; 35 Suppl: S185-198.
[34] Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, Kavanaugh D and Carbone DP. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996; 2: 1096-1103.
[35] Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED, Carbone DP and Gabrilovich DI. Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res 2000; 6: 1755-1766.
[36] Gabrilovich D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. Nat Rev Immunol 2004; 4: 941-952.
[37] Ghiringhelli F, Puig PE, Roux S, Parcellier A, Schmitt E, Solary E, Kroemer G, Martin F, Chauffert B and Zitvogel L. Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. J Exp Med 2005; 202: 919-929.
[38] Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, Divino CM and Chen SH. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res 2006; 66: 1123-1131.
[39] Pan PY, Wang GX, Yin B, Ozao J, Ku T, Divino CM and Chen SH. Reversion of immune tolerance in advanced malignancy: modulation of myeloid-derived suppressor cell development by blockade of stem-cell factor function. Blood 2008; 111: 219-228.
[40] Cao M, Xu Y, Youn JI, Cabrera R, Zhang X, Gabrilovich D, Nelson DR and Liu C. Kinase inhibitor Sorafenib modulates immunosuppressive cell populations in a murine liver cancer model. Lab Invest 2011; 91: 598-608.
[41] Schodel J, Grampp S, Maher ER, Moch H, Ratcliffe PJ, Russo P and Mole DR. Hypoxia, Hypoxia-inducible Transcription Factors, and Renal Cancer. Eur Urol 2016; 69: 646-57.
[42] Choueiri TK, Fay AP, Gagnon R, Lin Y, Bahamon B, Brown V, Rosenberg JE, Hutson TE, Baker-Neblett KL, Carpenter C, Liu Y, Pandite L and Signoretti S. The role of aberrant VHL/HIF pathway elements in predicting clinical outcome to pazopanib therapy in patients with metastatic clear-cell renal cell carcinoma. Clin Cancer Res 2013; 19: 5218-5226.
[43] Agathanggelou A, Niedobitek G, Chen R, Nicholls J, Yin W and Young LS. Expression of immune regulatory molecules in Epstein-Barr virus-associated nasopharyngeal carcinomas with prominent lymphoid stroma. Evidence for a functional interaction between epithelial tumor cells and infiltrating lymphoid cells. Am J Pathol 1995; 147: 1152-1160.
[44] Chang CS, Chang JH, Hsu NC, Lin HY and Chung CY. Expression of CD80 and CD86 costimulatory molecules are potential markers for better survival in nasopharyngeal carcinoma. BMC Cancer 2007; 7: 88.
[45] Pope B, Brown RD, Gibson J, Yuen E and Joshua D. B7-2-positive myeloma: incidence, clinical characteristics, prognostic significance, and implications for tumor immunotherapy. Blood 2000; 96: 1274-1279.
[46] Graf M, Reif S, Hecht K, Pelka-Fleischer R, Kroell T, Pfister K and Schmetzer H. High expression of costimulatory molecules correlates with low relapse-free survival probability in acute myeloid leukemia (AML). Ann Hematol 2005; 84: 287-297.
[47] Tamura H, Dan K, Tamada K, Nakamura K, Shioi Y, Hyodo H, Wang SD, Dong H, Chen L and Ogata K. Expression of functional B7-H2 and B7.2 costimulatory molecules and their prognostic implications in de novo acute myeloid leukemia. Clin Cancer Res 2005; 11: 5708-5717.
[48] Hersey P, Si Z, Smith MJ and Thomas WD. Expression of the co-stimulatory molecule B7 on melanoma cells. Int J Cancer 1994; 58: 527-532.
[49] Bernsen MR, Hakansson L, Gustafsson B, Krysander L, Rettrup B, Ruiter D and Hakansson A. On the biological relevance of MHC class II and B7 expression by tumour cells in melanoma metastases. Br J Cancer 2003; 88: 424-431.
[50] Tirapu I, Huarte E, Guiducci C, Arina A, Zaratiegui M, Murillo O, Gonzalez A, Berasain C, Berraondo P, Fortes P, Prieto J, Colombo MP, Chen L and Melero I. Low surface expression of B7-1 (CD80) is an immunoescape mechanism of colon carcinoma. Cancer Res 2006; 66: 2442-2450.
[51] Thurnher M, Radmayr C, Hobisch A, Bock G, Romani N, Bartsch G and Klocker H. Tumor-infiltrating T lymphocytes from renal-cell carcinoma express B7-1 (CD80): T-cell expansion by T-T cell co-stimulation. Int J Cancer 1995; 62: 559-564.

[52] Baine MK, Turcu G, Zito CR, Adeniran AJ, Camp RL, Chen L, Kluger HM and Jilaveanu LB. Characterization of tumor infiltrating lymphocytes in paired primary and metastatic renal cell carcinoma specimens. Oncotarget 2015; 6: 24990-25002.

[53] Giraldo NA, Becht E, Pages F, Skliris G, Verkarre V, Vano Y, Mejean A, Saint-Aubert N, Lacroix L, Natario I, Lupo A, Alifano M, Damotte D, Cazes A, Triebel F, Freeman GJ, Dieu-Nosjean MC, Oudard S, Fridman WH and Sautes-Fridman C. Orchestration and Prognostic Significance of Immune Checkpoints in the Microenvironment of Primary and Metastatic Renal Cell Cancer. Clin Cancer Res 2015; 21: 3031-3040.

[54] Butte MJ, Keir ME, Phamduy TB, Sharpe AH and Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity 2007; 27: 111-122.

[55] Stojanovic A, Fiegler N, Brunner-Weinzierl M and Cerwenka A. CTLA-4 is expressed by activated mouse NK cells and inhibits NK Cell IFN-gamma production in response to mature dendritic cells. J Immunol 2014; 192: 4184-4191.

[56] Geissler K, Fornara P, Lautenschlager C, Holzhausen HJ, Seliger B and Riemann D. Immune signature of tumor infiltrating immune cells in renal cancer. Oncoimmunology 2015; 4: e985082.

[57] Collins AV, Brodie DW, Gilbert RJ, Iaboni A, Manso-Sancho R, Walse B, Stuart DI, van der Merwe PA and Davis SJ. The interaction properties of costimulatory molecules revisited. Immunity 2002; 17: 201-210.

[58] Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA and Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity 1994; 1: 793-801.

[59] Pentcheva-Hoang T, Egen JG, Wojnoonski K and Allison JP. B7-1 and B7-2 selectively recruit CTLA-4 and CD28 to the immunological synapse. Immunity 2004; 21: 401-413.

[60] Zeng M, Guinet E and Nouri-Shirazi M. B7-1 and B7-2 differentially control peripheral homeostasis of CD4(+)CD25(+)Foxp3(+) regulatory T cells. Transpl Immunol 2009; 20: 171-179.

[61] Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N and Glimcher LH. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell 1995; 80: 707-718.

[62] Kronfeld K, Abken H and Seliger B. B7-1 and B7-2 act differentially in the induction of a T cell response: their impact for a HLA-matched and HLA-mismatched anti-tumor immunotherapy. Int J Cancer 2005; 117: 794-799.