

# HLA class I expression predicts prognosis and therapeutic benefits from tyrosine kinase inhibitors in metastatic renal-cell carcinoma patients

Jiajun Wang<sup>1</sup> · Li Liu<sup>1</sup> · Yang Qu<sup>1</sup> · Wei Xi<sup>1</sup> · Yu Xia<sup>1</sup> · Qi Bai<sup>1</sup> · Ying Xiong<sup>1</sup> · Qilai Long<sup>1</sup> · Jiejie Xu<sup>2</sup>  · Jianming Guo<sup>1</sup>

Received: 19 March 2017 / Accepted: 12 September 2017 / Published online: 16 September 2017  
© Springer-Verlag GmbH Germany 2017

## Abstract

**Purpose** Classical HLA class I antigen is highly involved in antigen presentation and adaptive immune response against tumor. In this study, we explored its predictive value for treatment response and survival in metastatic renal-cell carcinoma (mRCC) patients.

**Experimental design** A TKI cohort of 111 mRCC patients treated with sunitinib or sorafenib and a non-TKI cohort of 160 mRCC patients treated with interleukin-2 or interferon- $\alpha$ -based immunotherapy at a single institution were retrospectively enrolled. HLA class I expression and cytotoxic T lymphocyte (CTL) density was assessed by immunohistochemistry on tissue microarrays. Association between HLA class I and CTL was also assessed in the TCGA KIRC cohort.

**Results** In the TKI cohort, down-regulated HLA class I was associated with lower objective response rate of TKI therapy ( $P = 0.004$ ), shorter overall survival (OS) ( $P = 0.001$ ), and shorter progression free survival (PFS) ( $P < 0.001$ ).

Multivariate Cox regression model defined HLA expression as an independent prognostic factor for both OS [hazard ratio 1.687 (95% CI 1.045–2.724),  $P = 0.032$ ] and PFS [hazard ratio 2.139 (95% CI 1.376–3.326),  $P = 0.001$ ]. In the non-TKI cohort, HLA class I was not significantly associated with survival. HLA class I expression was associated with CTL infiltration and function, and its prognostic value was more predominant in CTL high-density tumors ( $P < 0.001$ ) rather than CTL low-density tumors ( $P = 0.294$ ).

**Conclusions** Classical HLA class I expression can serve as a potential predictive biomarker for TKI therapy in mRCC patients. Its predictive value was restricted in CTL high-density tumors. However, further external validations and functional investigations are still required.

**Keywords** Metastatic renal-cell carcinoma · Tyrosine kinase inhibitors · Human leukocyte antigen class I · Cytotoxic T lymphocyte · Survival · Biomarker

## Abbreviations

IMDC	International Metastatic Renal-Cell Carcinoma Database Consortium
KIRC	Kidney renal clear cell carcinoma
mRCC	Metastatic renal-cell carcinoma
RCC	Renal-cell carcinoma
TCGA	The Cancer Genome Atlas
TKI	Tyrosine kinase inhibitor

## Introduction

Renal-cell carcinoma (RCC) accounts for approximately 3% of all new cancer cases in the United States [1]. Patient with metastatic renal-cell carcinoma (mRCC) usually receives targeted agents like tyrosine kinase inhibitors (TKIs) and

Jiajun Wang, Li Liu, and Yang Qu contributed equally to this work.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00262-017-2064-1) contains supplementary material, which is available to authorized users.

✉ Jiejie Xu  
jjxu@fudan.edu.cn

✉ Jianming Guo  
guo.jianming@zs-hospital.sh.cn

<sup>1</sup> Department of Urology, Zhongshan Hospital, Fudan University, No. 180 Fenglin Road, Shanghai 200032, China

<sup>2</sup> Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, No. 138 Yixueyuan Road, Shanghai 200032, China

mechanistic target of rapamycin inhibitors [2]. Recently, immune checkpoint inhibitors have also emerged as an option [3]. Patient selection for TKI therapy is currently based on eligibility criteria in pivotal phase III trials, patient preferences, toxicity profiles, comorbidities, and costs. However, given the modest increments provided by TKIs coupled with their toxicities and comorbidities, optimal patient selection depending on reliable predictive biomarkers is necessary to maximize treatment benefits [4].

Activation of the immune system has long been observed in tumor elimination. However, tumor cells also acquire properties to evade the host immune system, which is called immune escape [5, 6]. Human leukocyte antigen (HLA) class I molecules are involved in the recognition, antigen presentation, and lysis of tumor cells by cytotoxic T lymphocytes (CTLs), and its defects could facilitate the immune escape of tumor [6]. Down-regulated HLA class I expression has been reported in various human malignancies including breast, lung, kidney cancer, and so on [7–9]. For non-metastatic RCC, down-regulated HLA class I expression has been identified as an adverse prognostic factor [10, 11]. As HLA class I loss was more frequently found in RCC metastases [12], we hypothesized that it may be involved in the development of mRCC. Moreover, TKIs showed immunomodulatory effects in mRCC patients, including a reduction of myeloid-derived suppressor cells and regulatory T cells as well as increased trafficking of CD8<sup>+</sup> CTLs, indicating that the preexisting tumor microenvironment may influence TKI therapy response [13, 14].

In this study by immunohistochemistry (IHC), we evaluated HLA class I in tumor specimens of 111 mRCC patients receiving TKI therapy and 160 mRCC patients treated with interleukin-2 or interferon- $\alpha$  based immunotherapy. The potential role of HLA class I expression as a predictive biomarker for TKI therapy was evaluated. We further investigated the relationship between HLA class I and CTL infiltration and function in three independent RCC cohorts.

## Materials and methods

### Study population

The study involved two cohort of mRCC patients, a TKI cohort and a non-TKI cohort. The TKI cohort included 111 mRCC patients treated with TKIs at Zhongshan Hospital of Fudan University between Mar 2005 and Jun 2014. The inclusion criteria were mRCC patients receiving TKIs as first-line therapy, with available archived primary tumor tissue and no history of other malignancies. The exclusion criteria were other former systemic therapy, no available tissue sample, tumor necrosis area larger than 80% or loss of follow-up. The non-TKI cohort included 160 mRCC patients

who received interleukin-2 or interferon- $\alpha$  based immunotherapy at Zhongshan Hospital of Fudan University between Jan 2005 and Dec 2011. No patient in the non-TKI cohort ever received TKIs, mTOR inhibitors or immune checkpoint inhibitors. This study was approved by the institutional ethical review boards of Zhongshan Hospital, Fudan University (Registration No. B2015-030). Informed consent was obtained from all individual participants included in the study. The study was reported following the REMARK criteria [15].

Baseline clinicopathologic data were collected retrospectively from medical records. Initial tumor stage was reclassified according to the 2010 AJCC TNM classification [16]. Metastasis was clinically diagnosed by experienced urologists according to the imaging data. Data on treatment, response, and survival were also collected retrospectively from medical records and disease progression was defined according to the RECIST 1.1 criteria [17]. All the medical records were reviewed by a professional urologist. All tumor sections underwent pathologic re-review by an experienced genitourinary pathologist, and tumor histology and nucleus grade were determined [18, 19]. International Metastatic Renal-Cell Carcinoma Database Consortium (IMDC) model was applied for patient risk stratification of the TKI cohort [20].

Clinicopathologic and genomic data of The Cancer Genome Atlas (TCGA) kidney renal clear cell carcinoma (KIRC) cohort were downloaded from the UCSC cancer browser (<https://genome-cancer.ucsc.edu>). Five hundred and thirty-two patients with integrated clinicopathologic and genomic data were defined as the TCGA KIRC cohort. Tumor-infiltrating immune cell proportion was defined using the CIBERSORT method [21], a computational approach for inferring leukocyte proportion according to bulk tumor transcriptomes.

### Tissue microarray and IHC staining

Two representative tumor cores were taken from each formalin-fixed paraffin-embedded tumor block for tissue microarray construction. Each tumor core was 3 mm in diameter, 5  $\mu$ m thick. IHC staining was carried out as previously described [22]. We used monoclonal HLA class I antibody EMR8-5 against the heavy chains of HLA-A, -B, and -C (dilution 1:200; ab70328, Abcam) as the primary antibody for HLA staining. Anti-CD8 antibody (C8/144B, DAKO) was used for CTL identification. Anti-mouse IgG1 antibody (dilution 1:100, ab91353, Abcam) was used as negative control.

Microscopic analysis of tumoral HLA class I expression and CTL infiltration density was performed independently by two genitourinary pathologists in a blinded manner. HLA class I was evaluated under three different areas at 200 $\times$

magnification for each tumor core. The membrane immunoreactivity level for HLA class I was categorized as previously described [10]. A score of 0 was defined as undetectable staining. A score of +1 was defined as faint, incomplete membrane staining in more than 20% of the tumor cells, or as moderate to complete staining in cytoplasm but negative membrane staining. A score of +2 was defined as complete membrane staining in more than 80% of the tumor cells. As only seven samples were defined as score 0, HLA class I expression was finally classified as down-regulated (scores 0 and +1) or positive (score +2) (Fig. 1). The number of tumoral CD8<sup>+</sup> CTLs on both cores of each patient's tumor sample was counted. Tumoral CTL infiltration was defined as high density (CTL/tumor cell  $\geq$  5%) and low density (CTL/tumor cell < 5%). Cohen's Kappa test was performed for inter-observer agreement evaluation, and the kappa value was > 0.75 for both HLA class I and CTL evaluation.

### Statistical analysis

Connection between HLA class I expression and clinicopathologic characteristics was tested by Chi-square test, Fisher's exact analysis or Cochran–Mantel–Haenszel test. Overall survival (OS) was defined as the time from initiation of systemic therapy to death or was censored at last follow-up. Progression free survival (PFS) was defined as the time from initiation of systemic therapy to disease progression or was censored at last follow-up. Relationship between HLA class I and OS/PFS was estimated by Kaplan–Meier method and log-rank test. Univariate and multivariate Cox regression analyses were also used for assessing prognostic value of HLA class I expression. Correlation between HLA class I expression and CTL infiltration and function was assessed by Pearson's or Spearman's correlation analysis. Risk stratification models were assessed using receiver operating characteristic analysis. All data analyses were done using GraphPad Prism 6 (GraphPad Software Inc.), SPSS 19.0

(SPSS Inc.), and R software version 3.1.2 with the “bioconductor” package (R Foundation for Statistical Computing). Pathway analyses were conducted using DAVID Bioinformatics Resources 6.7 (NIAID, NIH). Two tailed  $P < 0.05$  was considered statistically significant.

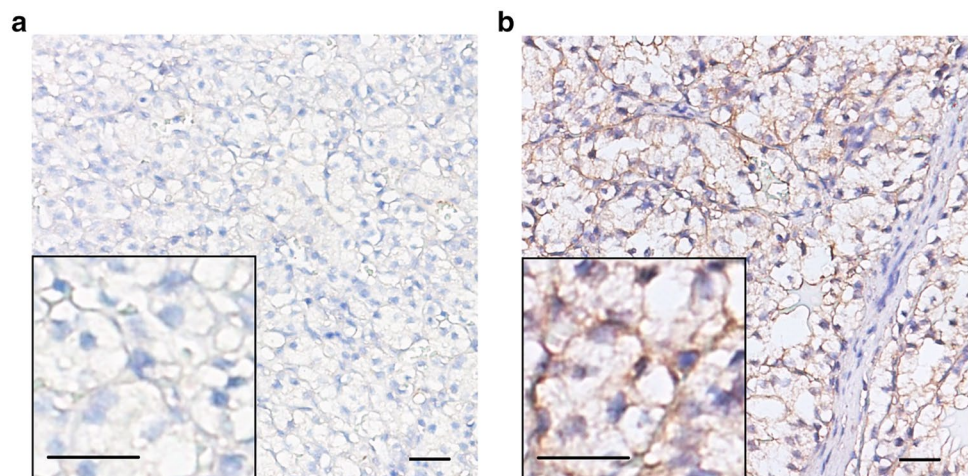
## Results

### Clinicopathologic characteristics and association with HLA class I expression

Clinicopathologic characteristics of the TKI cohort are listed in Table 1 and those of the non-TKI cohort are listed in Supplementary Table S1. In the TKI cohort, 74 (66.7%) patients received sunitinib as first-line therapy, while the other 37 (33.4%) patients received sorafenib. In the non-TKI cohort, all patients received interleukin-2 or interferon- $\alpha$ -based immunotherapy. Eighty-nine (80.2%) patients in the TKI cohort and 131 (81.9%) patients in the non-TKI cohort were diagnosed of ccRCC. Median survival time was 23.5 (95% CI 15.2–31.8) months for the TKI cohort and was 12.0 (95% CI 9.9–14.1) months for the non-TKI cohort.

In the TKI cohort, 45 (40.5%) patients were grouped as HLA class I down-regulated and 66 (59.5%) as positive expression. In the non-TKI cohort, 70 (43.8%) patients were classified as HLA class I down-regulated and 90 (56.3%) as HLA positive expression. Supplementary Table S2 and Supplementary Table S3 show the correlation between clinical characteristics and HLA expression in the two cohorts. Down-regulated HLA expression was found associated with non-clear cell type ( $P = 0.028$ ) and smaller primary tumor size ( $P = 0.021$ ) in the non-TKI cohort, but not statistically significant in the TKI cohort. Positive correlation was also found between HLA expression and CTL infiltration ( $P = 0.021$  for the non-TKI cohort and  $P = 0.097$  for the TKI cohort).

**Fig. 1** Representative images of HLA class I staining in mRCC samples. **a** Down-regulated HLA class I expression in the tumor tissue; **b** Positive HLA class I expression in the tumor tissue. Scale bars 25  $\mu$ m



**Table 1** Baseline demographics and clinical characteristics of the TKI cohort

Characteristics	Patients ( <i>n</i> = 111)
Age (years) <sup>a</sup>	59 (53–65)
Sex	
Male	79 (71.2)
Female	32 (28.8)
Nephrectomy	111 (100)
Histology	
Clear cell type	89 (80.2)
Papillary	15 (13.5)
Chromophobe	2 (1.8)
Collecting duct	2 (1.8)
Unclassified	3 (2.7)
Tumor nucleus grade <sup>b</sup>	
I	2 (1.9)
II	54 (51.9)
III	41 (39.4)
IV	7 (6.7)
Tumor size <sup>c</sup>	6.5 (5.0–9.0)
TNM stage at first diagnosis	
Stage I–III	52 (46.8)
Stage IV	59 (53.2)
Site of metastatic disease	
Only lung	51 (45.9)
Other sites involved <sup>d</sup>	60 (54.1)
Number of organ sites with metastases	
1	78 (70.3)
2 or more	33 (29.7)
IMDC risk factors	
0 (favorable)	23 (20.7)
1–2 (intermediate)	60 (54.1)
3 or more (poor)	28 (25.2)
Systemic therapy	
Sunitinib	74 (66.7)
Sorafenib	37 (33.4)
HLA class I expression status	
Positive	66 (59.5)
Down-regulated	45 (40.5)
Tumoral CTL infiltration	
CTL/tumor cell ≥ 5%	50 (45.0)
CTL/tumor cell < 5%	61 (55.0)

Data are *n* (%) or median (IQR)

IMDC International Metastatic Renal-Cell Carcinoma Database Consortium

<sup>a</sup>Age at initiation of systemic therapy

<sup>b</sup>Missing for seven patients

<sup>c</sup>Determined after radical or palliative nephrectomy

<sup>d</sup>Other metastatic sites include bone, liver, brain, lymph nodes, etc

## HLA class I expression predicts treatment response and survival in the TKI cohort

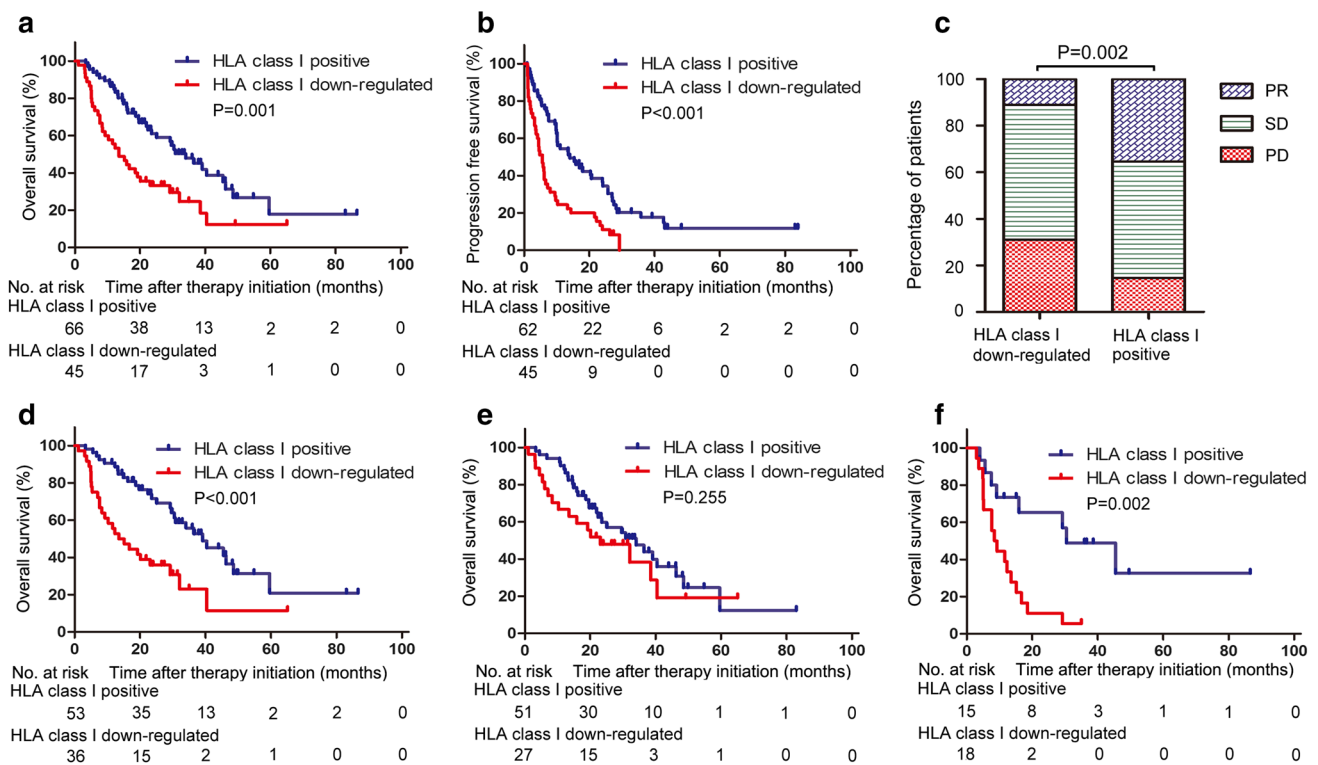
We assessed the correlation between HLA expression and patients' survival in both cohorts. In the TKI cohort, down-regulated HLA class I expression was associated with shorter OS ( $P = 0.001$ , Fig. 2a) and PFS ( $P < 0.001$ , Fig. 2b) by log-rank test. Four patients were excluded for PFS analysis because of incomplete imaging data during follow-up. Patients with HLA class I down-regulated tumor showed worse TKI treatment response ( $P = 0.002$ , Fig. 2c). In the non-TKI cohort, HLA expression showed no significant correlation with survival ( $P = 0.164$ , Supplementary Figure S1).

We then focused on the prognostic value of HLA expression in the TKI cohort. We identified histologic type ( $P = 0.006$ ), TNM stage at diagnosis ( $P = 0.002$ ), number of organ sites with metastases ( $P = 0.019$ ), IMDC risk group ( $P < 0.001$ ), systemic therapy ( $P = 0.052$ ), and HLA class I expression ( $P < 0.001$ ) as prognostic factors for OS using univariate Cox regression analysis, and similar results were also found for PFS (Supplementary Table S4). Multivariate Cox regression analysis including these parameters identified down-regulated HLA class I expression as an independent predictive factor for both OS (hazard ratio 1.687, 95% CI 1.045–2.724,  $P = 0.032$ ) and PFS (hazard ratio 2.139, 95% CI 1.376–3.326,  $P = 0.001$ ) (Table 2). These results suggested HLA class I expression as a predictive marker for TKI treatment in mRCC patients.

We further assessed predictive value of HLA class I in different patient subgroups of the TKI cohort. Down-regulated HLA expression could predict shorter OS in ccRCC patients ( $P < 0.001$ , Fig. 2d). In patients with only one organ involved by RCC metastasis, HLA expression showed unsatisfactory predictive value ( $P = 0.255$ , Fig. 2e). However, in patients with more than one organs involved by RCC metastases, down-regulated HLA class I predicted poor survival, in spite of the small sample size ( $P = 0.002$ , Fig. 2f).

## HLA class I associates with tumoral CTL infiltration and function

To investigate the possible functional pathways of HLA class I, we made gene profile investigation in the TCGA KIRC cohort. Top 1000 co-expression genes of HLA-A were enrolled in the gene enrichment analysis. HLA-A was found involved in multiple immune-related pathways including antigen processing and presentation, T cell receptor signaling pathway, and interferon- $\gamma$  mediated signaling pathway (Supplementary Table S5). These pathways are closely related to CTLs. Gene enrichment analysis on HLA-B and HLA-C revealed similar results (Supplementary Table S5).



**Fig. 2** HLA class I expression status predicting treatment response and survival in mRCC patients under TKI treatment. **a, b** Kaplan–Meier survival analysis of **a** OS, and **b** PFS according to the HLA class I expression. *P* values, log-rank test. **c** TKI treatment response in different HLA class I expression groups. *PR* partial response, *SD*

stable disease, *PD* progressive disease. *P* value, Cochran–Mantel–Haenszel test. **d–f** Kaplan–Meier survival analysis of OS in **d** ccRCC patients, **e** patients with only one organ involved by metastasis and **f** patients with more than one organs involved by metastases. *P* values, log-rank test

**Table 2** Multivariate analyses of prognostic and predictive factors for OS and PFS

Variables	OS (111 patients)		PFS (107 patients) <sup>b</sup>	
	HR (95% CI) <sup>c</sup>	<i>P</i> value <sup>c</sup>	HR (95% CI) <sup>c</sup>	<i>P</i> value <sup>c</sup>
Histologic type		<b>0.001</b>		<b>0.044</b>
Non-clear cell vs clear cell type	2.609 (1.473–4.622)		1.713 (1.015–2.891)	
TNM stage at diagnosis		0.358		0.547
IV vs I–III	1.283 (0.754–2.181)		1.157 (0.719–1.862)	
Number of organ sites with metastases <sup>a</sup>		0.355		<b>0.020</b>
≥ 2 vs 1	1.286 (0.755–2.190)		1.776 (1.096–2.879)	
IMDC risk factors		<b>&lt;0.001</b>		<b>0.003</b>
0 (favorable)	Reference		Reference	
1–2 (intermediate)	1.827 (0.824–4.055)		1.204 (0.629–2.306)	
3 or more (poor)	6.281 (2.510–15.720)		2.882 (1.336–6.218)	
Systemic therapy		0.129		0.098
Sorafenib vs sunitinib	1.477 (0.893–2.444)		1.464 (0.932–2.299)	
Tumoral HLA class I expression		<b>0.032</b>		<b>0.001</b>
Down-regulated vs positive	1.687 (1.045–2.724)		2.139 (1.376–3.326)	

*P* values < 0.05 were marked in bold font

*HR* hazard ratio, *CI* confidence interval, *OS* overall survival, *PFS* progression free survival, *IMDC* International Metastatic Renal-Cell Carcinoma Database Consortium

<sup>a</sup>Number of organs involved by metastases at the initiation of systemic therapy

<sup>b</sup>Four patients were excluded in PFS analysis because of incomplete imaging data during follow-up

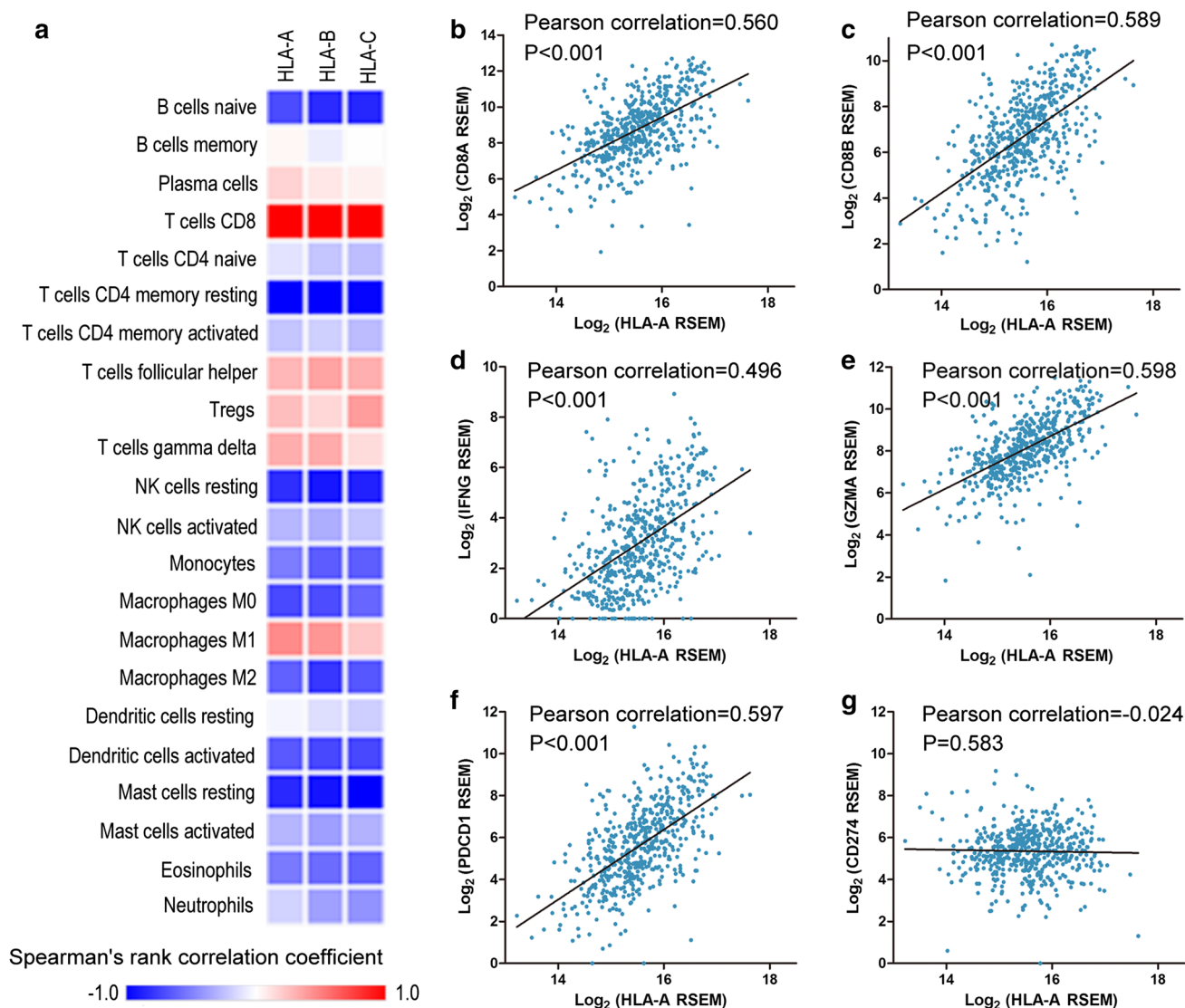
<sup>c</sup>Data obtained from the Cox proportional hazards model

We further investigated the relationship between HLA class I and tumor-infiltrating immune cells. Correlation between HLA class I mRNA expression and immune cell infiltration was evaluated in the TCGA KIRC cohort (Fig. 3a). Positive correlation was found between HLA class I expression and CD8<sup>+</sup> CTL infiltration (Spearman correlation coefficient  $r_s = 0.581, 0.619, 0.515$  for HLA-A, -B, -C, respectively; Fig. 3a). Positive correlation was also found between HLA-A and CTL infiltration markers CD8A and CD8B (Pearson correlation  $r = 0.560$  and  $0.589$ , respectively; Fig. 3b, c), as well as CTL function markers IFNG and GZMA (Pearson correlation  $r = 0.496$  and  $0.598$ , respectively; Fig. 3d, e). We also found that HLA-A

was associated with PD-1 expression (Pearson correlation  $r = 0.597$ , Fig. 3f). However, HLA-A did not correlate with PD-L1 expression (Pearson correlation  $r = -0.024$ ,  $P = 0.583$ , Fig. 3g). Similar results were also found for HLA-B and HLA-C (Supplementary Figure S2).

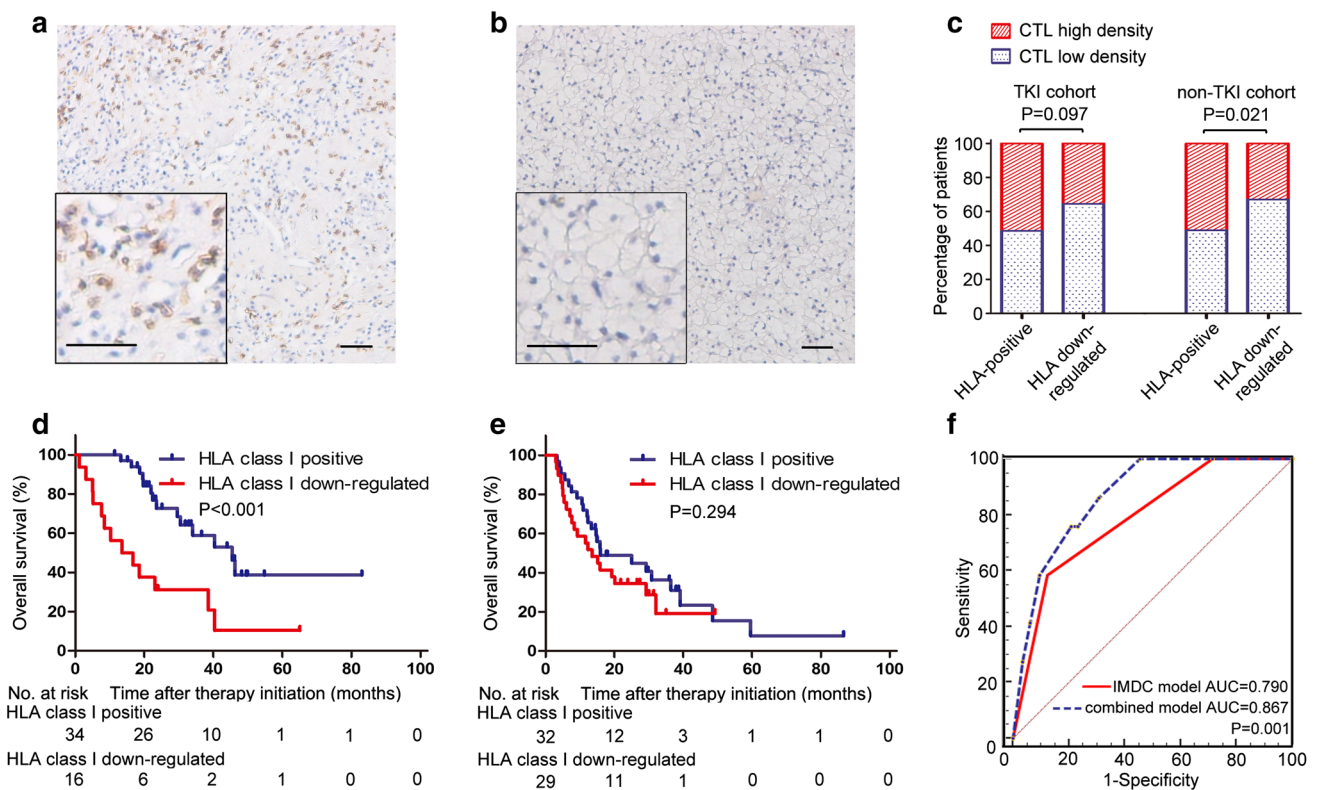
### Predictive value of HLA class I expression depends on CTLs

We performed IHC staining of CD8 for CTL identification in both the TKI cohort and the non-TKI cohort (Fig. 4a, b). Tumor-infiltrating CTL/tumor cell ratio  $\geq 5\%$  was defined as high CTL infiltration. We found positive correlation between



**Fig. 3** HLA class I expression is associated with CTL infiltration and function. **a** Heatmap of Spearman's correlation coefficients calculated from the comparison of the HLA-A, -B, and -C expression and tumor-infiltrating immune cell proportion in the TCGA KIRC cohort. Red squares indicate positive correlations, while blue squares indi-

cate negative correlations. **b–g** Co-expression between HLA-A and CD8A (**b**), CD8B (**c**), IFN- $\gamma$  (**d**), GZMA (**e**), PD-1 (**f**), and PD-L1 (**g**) according to the RNA-seq RSEM data in the TCGA KIRC cohort.  $P$  values, Pearson's correlation analysis



**Fig. 4** Predictive value of HLA class I expression depending on tumoral CTL infiltration. **a, b** Representative of **a** high CTL infiltration density and **b** low CTL infiltration density in the RCC tissue. Scale bars 50  $\mu$ m. **c** CTL density in different HLA class I expression groups in the TKI cohort and the non-TKI cohort. *P* value, Chi-

square test. **d, e** OS in **d** high CTL density and **e** low CTL density mRCC patients. *P* values, log-rank test. **f** Receiver operating characteristic analysis for predicting 1-year survival. Red curve, IMDC risk criteria alone. Blue curve, IMDC risk criteria combined with HLA class I expression and CTL density. *P* value, Z test

HLA expression and CTL infiltration ( $P = 0.097$  for the TKI cohort,  $P = 0.021$  for the non-TKI cohort, Fig. 4c). Since CTL infiltration and function was closely related to HLA class I molecules, we assessed prognostic value of HLA class I expression in CTL high-density and low-density mRCC. Significant predictive value was found in CD8 high-density RCC ( $P < 0.001$ , Fig. 4d), rather than CD8 low-density tumors ( $P = 0.294$ , Fig. 4e). We further combined HLA class I expression, CTL density, and the IMDC risk criteria to generate a new predictive model for OS. Receiver operating characteristic analysis showed better survival prediction using the combined model (AUC = 0.867, 95% CI 0.789–0.924) than the IMDC model alone (AUC = 0.790, 95% CI 0.702–0.862) ( $P = 0.001$ , Fig. 4f).

## Discussion

In this study, we explored the relationship between HLA class I antigen expression and TKI treatment response in mRCC patients. The results showed that down-regulated HLA class I expression predicted worse treatment response

and shorter survival. We also found significant correlation between HLA class I and CTL infiltration. HLA class I expression predicted treatment benefit of TKIs more efficiently in CTL high infiltration tumors.

A proper constitutive HLA class I surface expression is necessary for the presentation of self/non-self antigens [23]. Although HLA class I antigen is constitutively expressed in almost all adult-nucleated cells under physiological conditions, its expression could also be regulated by cytokines like interferon (IFN)- $\gamma$  and interleukin (IL)-10 [23]. The published data on the HLA-I expression in RCC are controversial. HLA class I loss has been reported in a number of cancers [7–11]. However, in RCC, the percentage is much lower than in the other types of malignancy. Our study identified the positive correlation between HLA class I expression and tumoral CTL infiltration and function in RCC (Figs. 3, 4c), suggesting that even not completely loss, relatively down-regulated HLA class I expression could also lead to tumor immune escape. In addition, the prognostic significance of HLA class I was found more predominant in mRCC with CTL high infiltration (Fig. 4d, e), suggesting that tumor-infiltrating

CTLs contribute to disease limitation especially in HLA class I positive mRCC. Moreover, there are also publications demonstrating that RCC tumor cells express higher levels of HLA-I than healthy adjacent renal tissue, both on protein and on RNA levels [11, 24]. Because of the immunogenic nature and abundant immune cell infiltration in RCC, the elevated HLA class I expression might represent the local activation of anti-tumor immunity in RCC.

In our study, we observed close relationship between HLA class I status and TKI treatment response of mRCC patients. Tumors with positive HLA class I expression showed an objective response rate of 35.5%, compared with 11.1% in those with down-regulated HLA class I ( $P = 0.004$ ). A significantly prolonged OS ( $P = 0.001$ , Fig. 2a) and PFS ( $P < 0.001$ , Fig. 2b) was also observed in patients with HLA class I positive mRCC. Moreover, in the integrated model combining HLA class I expression, CD8<sup>+</sup> CTL infiltration, and IMDC score, we observed significantly improved predictive value than IMDC score alone (AUC = 0.867 vs AUC = 0.790,  $P = 0.001$ , Fig. 4f). These results suggested that HLA class I expression could serve as a potential predictive biomarker for patient selection of TKI therapy.

The mechanism underlying better response rate of HLA class I positive mRCC remains obscure, since HLA class I antigen has not been reported to involve in angiogenesis. However, although TKIs were usually considered as anti-angiogenic agents, recent studies have also revealed their immunomodulatory effects. TKIs have been shown to decrease the number and effectiveness of regulatory T cells and MDSCs in various cancer models including RCC [13, 25, 26]. Clinical trials are still testing the potential benefits of immunotherapy combined with targeted agents for treating mRCC [27]. These studies implied that the effect of TKIs at least partially relied on their immunomodulatory effects. On the other hand, once the tumor successfully escaped from the immune surveillance system, the effect of TKIs might be unsatisfactory. In our study, we identified the association between down-regulated HLA class I expression and TKI resistance, which also indicated the immunomodulatory effect of TKI agents. Data mining into the possible related biological processes of HLA also supported the theory, together with the observation that the predictive value of HLA class I was restricted in CTL high-density tumors.

In our study, we observed statistically significant correlation between HLA-A and PD-1 expression (Pearson's correlation = 0.597,  $P < 0.001$ , Fig. 3f). However, HLA-A and PD-L1 expression seemed to be independent (Pearson's correlation = 0.024,  $P = 0.583$ , Fig. 3g). Similar results were also found for HLA-B and HLA-C (Supplementary Figure S2e, S2f, S2k, S2l). PD-1 could be regarded as a T cell activation marker, but the interaction of PD-1 and PD-L1 contributes to T cell exhaustion. The

lack of association between PD-L1 and HLA-I indicated that tumoral HLA-I expression was independent from PD-L1 related immune suppression.

The major limitations of this study are its retrospective design and relatively small sample size mostly from East Asia. Future prospective external validations are still required. Moreover, we used HLA class I antibody EMR8-5 against all alleles of HLA-A, -B and -C in this study, but the function of HLA class I complex also relies on  $\beta_2$ -microglobulin. Distinct functions of HLA-A, -B, and -C, and their functional interaction with  $\beta_2$ -microglobulin should be evaluated in further studies. In addition, HLA polymorphism may be involved in anti-tumor immunity, which also needs further investigations. Finally, patients in this study received TKIs as first-line therapy. As HLA class I antigen is strongly involved in the adaptive immune response against tumor, its predictive value for immune checkpoint inhibitors needs further investigations.

## Conclusions

Our study indicated that down-regulated HLA class I expression could serve as a predictive biomarker for low response rate and dismal survival in mRCC patients under TKI therapy. HLA class I expression was associated with tumoral CTL infiltration and function, and its predictive value was restricted in CTL high infiltration tumors.

**Acknowledgements** This study was funded by grants from National Natural Science Foundation of China (31270863, 81471621, 81472227, 81472376 and 81671628), Program for New Century Excellent Talents in University (NCET-13-0146), Shanghai Municipal Natural Science Foundation (14ZR1406300) and Zhongshan Hospital Science Foundation (2016ZSQN30). All these study sponsors have no roles in the study design, in the collection, analysis, and interpretation of data.

**Author contributions** JX and JG conceived and designed the study. JW, LL, and YQ contributed to the acquisition, analysis, and interpretation of data. JW, LL, and YQ performed the statistical analysis. JW wrote the paper. JW, LL, YQ, JX, and JG reviewed and edited the manuscript. WX, YX, QB, YX, and QL provided technical and material support. JX and JG contributed to funding obtaining and study supervision. All authors read and approved the manuscript.

## Compliance with ethical standards

**Ethical approval** This study was approved by the institutional ethical review boards of Zhongshan Hospital, Fudan University (Registration No. B2015-030).

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Conflict of interest** The authors declare that they have no conflict of interest.



## References

1. Siegel RL, Miller KD, Jemal A (2015) Cancer statistics, 2015. *CA Cancer J Clin* 65:5–29
2. Ljungberg B, Bensalah K, Canfield S et al (2015) EAU guidelines on renal cell carcinoma: 2014 update. *Eur Urol* 67:913–924
3. Motzer RJ, Escudier B, McDermott DF et al (2015) Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 373:1803–1813
4. Sonpavde G, Choueiri TK (2012) Biomarkers: the next therapeutic hurdle in metastatic renal cell carcinoma. *Br J Cancer* 107:1009–1016
5. Schreiber RD, Old LJ, Smyth MJ (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 331:1565–1570
6. Khong HT, Restifo NP (2002) Natural selection of tumor variants in the generation of “tumor escape” phenotypes. *Nat Immunol* 3:999–1005
7. Vitale M, Rezzani R, Rodella L et al (1998) HLA class I antigen and transporter associated with antigen processing (TAP1 and TAP2) down-regulation in high-grade primary breast carcinoma lesions. *Cancer Res* 58:737–742
8. Ramnath N, Tan D, Li Q et al (2006) Is downregulation of MHC class I antigen expression in human non-small cell lung cancer associated with prolonged survival? *Cancer Immunol Immunother* 55:891–899
9. Seliger B, Atkins D, Bock M et al (2003) Characterization of human lymphocyte antigen class I antigen-processing machinery defects in renal cell carcinoma lesions with special emphasis on transporter-associated with antigen-processing down-regulation. *Clin Cancer Res* 9:1721–1727
10. Kitamura H, Honma I, Torigoe T, Asanuma H, Sato N, Tsukamoto T (2007) Down-regulation of HLA class I antigen is an independent prognostic factor for clear cell renal cell carcinoma. *J Urol* 177:1269–1272
11. Yuan J, Liu S, Yu Q et al (2013) Down-regulation of human leukocyte antigen class I (HLA-I) is associated with poor prognosis in patients with clear cell renal cell carcinoma. *Acta Histochem* 115:470–474
12. Buszello H, Ackermann R (1994) Immunohistochemical studies on the expression of HLA class I antigens in renal cell carcinoma: comparison of primary and metastatic tumor tissue. *Eur Urol* 25:158–163
13. Terme M, Colussi O, Marcheteau E, Tanchot C, Tartour E, Taieb J (2012) Modulation of immunity by antiangiogenic molecules in cancer. *Clin Dev Immunol* 2012:492920
14. Finke JH, Rini B, Ireland J et al (2008) Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. *Clin Cancer Res* 14:6674–6682
15. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2005) Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Cancer Inst* 97:1180–1184
16. Edge SB, Compton CC (2010) The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 17:1471–1474
17. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 45:228–247
18. Srigley JR, Delahunt B, Eble JN et al (2013) The international society of urological pathology (ISUP) Vancouver classification of renal neoplasia. *Am J Surg Pathol* 37:1469–1489
19. Delahunt B, Cheville JC, Martignoni G et al (2013) The international society of urological pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters. *Am J Surg Pathol* 37:1490–1504
20. Heng DY, Xie W, Regan MM et al (2009) Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. *J Clin Oncol* 27:5794–5799
21. Newman AM, Liu CL, Green MR et al (2015) Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 12:453–457
22. Liu H, Xu J, Zhou L et al (2011) Hepatitis B virus large surface antigen promotes liver carcinogenesis by activating the Src/PI3K/Akt pathway. *Cancer Res* 71:7547–7557
23. Bukur J, Jasinski S, Seliger B (2012) The role of classical and non-classical HLA class I antigens in human tumors. *Semin Cancer Biol* 22:350–358
24. Stickel JS, Stickel N, Hennenlotter J et al (2011) Quantification of HLA class I molecules on renal cell carcinoma using Edman degradation. *BMC Urol* 11:1
25. Yuan H, Cai P, Li Q et al (2014) Axitinib augments antitumor activity in renal cell carcinoma via STAT3-dependent reversal of myeloid-derived suppressor cell accumulation. *Biomed Pharmacother* 68:751–756
26. Desar IM, Jacobs JH, Hulsbergen-vandeKaa CA et al (2011) Sorafenib reduces the percentage of tumour infiltrating regulatory T cells in renal cell carcinoma patients. *Int J Cancer* 129:507–512
27. Carlo MI, Voss MH, Motzer RJ (2016) Checkpoint inhibitors and other novel immunotherapies for advanced renal cell carcinoma. *Nat Rev Urol* 13:420–431