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ORIGINAL ARTICLE

Development of Response Classifier for Vascular Endothelial Growth Factor Receptor (VEGFR)-Tyrosine Kinase Inhibitor (TKI) in Metastatic Renal Cell Carcinoma

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Abstract Vascular endothelial growth factor receptor (VEGFR)-targeted therapy improved the outcome of metastatic renal cell carcinoma (mRCC) patients. However, a prediction of the response to VEGFR-tyrosine kinase inhibitor (TKI) remains to be elucidated. We aimed to develop a classifier for VEGFR-TKI responsiveness in mRCC patients. Among 101 mRCC patients, ones with complete response, partial response, or ≥24 weeks stable disease in response to VEGFR-TKI treatment were defined as clinical benefit group, whereas patients with <24 weeks stable disease or progressive disease were classified as clinical non-benefit group. Clinicolaboratory-histopathological data, 41 gene mutations, 20 protein expression levels and 1733 miRNA expression levels were compared between clinical benefit and non-benefit groups. The classifier was built using support vector machine (SVM). Seventy-three patients were clinical benefit group, and 28 patients were clinical non-benefit group. Significantly different features between the groups were as follows: age, time from diagnosis to TKI initiation, thrombocytosis, tumor size, pT stage, ISUP grade, sarcomatoid change, necrosis, lymph node metastasis and expression of pAKT, PD-L1, PD-L2, FGFR2, pS6, PDGFRβ, HIF-1α, IL-8, CA9 and miR-421 (all, $P < 0.05$). A classifier including necrosis, sarcomatoid component and HIF-1α was built with 0.87 accuracy using SVM. When the classifier was checked against all patients, the apparent accuracy was 0.875 (95% CI, 0.782–0.938). The classifier can be presented as a simple decision tree for clinical use. We developed a VEGFR-TKI response classifier based on comprehensive inclusion of clinicolaboratory-histopathological, immunohistochemical, mutation and miRNA features that may help to guide appropriate treatment in mRCC patients.

Keywords Metastatic renal cell carcinoma · Vascular endothelial growth factor signaling · Tyrosine kinase inhibitors · Response classifier · Machine learning

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Introduction

Vascular endothelial growth factor receptor (VEGFR)-targeting therapy has improved survival compared to either interferon-alpha or placebo for patients with metastatic renal cell carcinoma (mRCC). Recently, tyrosine kinase inhibitors (TKIs) targeting the VEGF/VEGFR axis including sunitinib and sorafenib have become the referral standard for the first-line treatment of mRCC [1]. Although VEGFR-TKIs have shown promising activity and tolerable toxicities, the clinical benefit in individual patients is highly unpredictable, and sustained complete responses remain the exception [2].

Between 20% and 30% clear cell RCC patients derive no benefit from first-line TKI treatment [3].

Several factors have been suggested as potential predictive markers for VEGFR-targeted therapy [4–6]. VEGFR, HIF-1α, interlukin-6, HGF and osteopontin proposed as predictive or prognostic biomarkers for VEGFR-targeted therapy. The expression of miRNAs, including miR-192 and miR-424*-C, was associated with response to sunitinib in advanced RCC patients [7–9]. We also previously reported histopathological features such as sarcomatoid features, necrosis and grade to be prognostic factors of them [5]. However, no validated biomarkers or comprehensive predictive response classification model to VEGFR-targeted therapy and prognosis has yet been established.

We analyzed the relationship of the response to VEGFR-TKI with various clinicopathologic data, gene mutations, and protein and miRNA expressions in mRCC patients and developed a well-defined classifier for VEGFR-TKI response.

Materials and Methods

Patients

101 VEGFR-TKI treated metastatic clear cell RCC patients from June 2006 to March 2011 at Asan Medical Center (AMC), having the formalin-fixed paraffin-embedded (FFPE) tissues of their primarily resected renal masses were retrospectively collected. Clinical data were retrieved from the medial records. Response to VEGFR-TKI was assessed according to the Revised Response Evaluation Criteria in Solid Tumors guidelines (version 1.1) [10]. Based on a previous study [11], patients showing complete response, partial response, or ≥24 weeks stable disease in response to treatment were defined as clinical benefit group; patients showing <24 weeks stable disease or progressive disease were defined as clinical non-benefit group by an oncologist at this institution. This study was approved by the Institutional Review Board (2012–0788).

Histopathology

Histopathological features of each patient were reviewed according to the 2016 WHO Classification [12]. pT stage according to the 7th AJCC cancer staging, International Society of Urological Pathology (ISUP) nuclear grade [13], lymphovascular invasion, sarcomatoid components, tumor necrosis, resection margin status, lymph node (LN) metastasis and degrees of inflammation were evaluated. The extent of sarcomatoid components and the extent of tumor necrosis were estimated semiquantitatively by two uropathologists (Supplementary Methods).

Immunohistochemistry (IHC)

Immunohistochemical detection of various cancer-related proteins was conducted with tissue microarrays (Supplementary Methods). The number of PD-L1-expressed tumor infiltrative lymphocytes (TIL) was counted. The average tumoral microvascular densities (MVD) per unit area (mm^2) of VEGFR2 and PDGFRβ were calculated. Each protein expression in the tumor cytoplasm, membrane or nucleus in clinical benefit group was compared with the corresponding expression in non-benefit group. The detailed antibody information and staining conditions were summarized in Table S1.

Mutation Status

OncoMap version 4.4-Core was used to assess the mutation status of several tumor-related genes (Supplementary Methods).

MiRNA Expression

MiRNA expression was screened the signatures between clinical benefit and non-benefit groups by microarray. To confirm the expression levels of the selected miRNAs on microarray, quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) were performed (Supplementary Methods). A list of miRNAs and primer sequences was provided in Table S2.

Statistical Analysis and Model Building

Statistical analyses were performed using R3.0.2. The relationships between and among groups were compared using the Fisher's exact test, or Student's *t*-test. The Kaplan-Meier method with the log-rank test was used to evaluate the implication of the classifier on patient survival. To assess model accuracy (discrimination) for patient survival, Harrell's bias-corrected concordance index (C-index) was calculated. Models were refit 1000× with the bootstrap resampling. All statistical tests were two-sided, and statistical significance was defined as $P < 0.05$.

Machine learning approaches were applied to establish a predictive classifying model for VEGFR-TKI response. A ten-fold-cross-validated support vector machine (SVM) method and decision tree analysis were used for modeling.

Results

Clinical Characteristics

The median follow-up period for the patients was 40.9 months (range, 2.3–171.7 months). The median time between the date of diagnosis and the date of TKI

treatment initiation was 3.8 months (0–134.3 months). Before TKI therapy, nine patients (8.9%) had undergone immunotherapy, three (3.0%) had received cytotoxic chemotherapy, and four (4.0%) had received both types of therapy. The most common TKI used was sunitinib (n = 78; 77.2%), followed by sorafenib (n = 20; 19.8%) and pazopanib (n = 3; 3%). Ninety-two patients (91.1%) had a Karnofsky performance status ≥80, and 88 (87.1%) showed favorable or intermediate Memorial Sloan Kettering cancer center scores [14]. The median time between the date of diagnosis and the date of death was 29.9 months (2.3–142.8 months). The median time between TKI treatment initiation and the date of death was 22.2 months (0.9–75.4 months).

Compared to TKI clinical benefit group, non-benefit group were younger (P = 0.010) and showed shorter the time from diagnosis to TKI initiation (P = 0.048) and more frequent presence of thrombocytosis (P = 0.005).

Histopathological and Immunohistochemical Characteristics

The mean tumor size of clinical non-benefit group was larger than that of benefit group (P = 0.002). pT stage (P < 0.001) and ISUP grade (P = 0.007) were also higher in clinical non-benefit group than in benefit group. Sinus fat invasion (P = 0.038), pherinephric fat invasion (P = 0.026), the presence of sarcomatoid component (P = 0.007) and its extents (P < 0.001), the presence of necrosis (P < 0.001) and its extent (P < 0.001), and LN metastasis (P = 0.033) were also more frequent in clinical non-benefit group than in benefit group (Table 1).

Among 20 investigated proteins, the expression of VEGF (P = 0.024), pAKT (p = 0.008), PD-L1 (P < 0.001), PD-L2 (P = 0.049), FGFR4 (P = 0.033), and pS6 in tumors were stronger in clinical non-benefit group than in benefit group (P = 0.002). Meanwhile, HIF-1α (P < 0.001), IL-8 (P = 0.023) and CA9 (P = 0.022) showed higher expression in clinical benefit group. The MVD of PDGFRβ was higher in clinical non-benefit group (P = 0.024) (Table 1).

Molecular Alterations

Somatic mutations of tumor-related genes were evaluated using OncoMap. Fifteen patients (14.9%) harbored somatic mutations (Table 1); among them, *MLH1_V384D* showed polymorphism in the Koreans. *VHL* gene (n = 4; 4.0%) was the most frequently mutated. The mutation rates did not differ between clinical benefit and non-benefit groups (P = 0.115), and distinctively different mutated genes between the two groups were not identified.

Thirty-one miRNAs were differentially expressed between the groups (false discovery rate < 0.05) on microarray: 18 were up-regulated and 13 were down-regulated in clinical non-benefit group. Among these, six, i.e., miR-138-5p, miR-34a-5p, miR-1301, miR-4791, miR-1275 and miR-421, showed more than a 2-fold change and a P < 0.01 between two groups. The expression levels of miR-138-5p, miR-34a-5p, miR-1301, miR-1275 and miR-421 were evaluated by qRT-PCR. To minimize individual variation, miRNA expression level of the tumor was calibrated by the matched non-tumoral cortex in each patient. The expressions of miR-34a-5p, miR-421 and miR-708-5p were upregulated, and the expression of miR-138-5p was downregulated in tumors compared to non-tumors, although miR-1275 showed similar expression. In addition, all evaluated miRNAs except miR-1275 showed the same expression pattern as in the microarray. Notably, miR-421 was significantly upregulated in clinical non-benefit group compared to clinical benefit group, based on either ddCt calibrated by non-tumor or dCt using tumor only (P = 0.006 and P = 0.008, respectively) (Table 1). The differences in expression between the groups were not statistically significant in the other miRNAs. Unfortunately, the expression of miR-4791 could not be validated due to failure to make appropriate primers for qRT-PCR.

Development of a Predictive Classifier for TKI Response

To develop a predictive classifier, features that showed the statistical differences with P < 0.01 between the groups were selected and the most appropriate cut-off for each feature was calculated. Tumor size, pT stage, ISUP grade, necrosis, sarcomatoid component, pAKT, PD-L1, pS6, miR-421, CA9 and HIF-1α were primarily selected (Table 2). The first nine of these features were associated with poor response to TKI, and the last two were associated with good response if their status was higher than the cut-off. Secondary feature selection was performed using SVM to develop the most efficient model, showing the highest accuracy with the least number of features. Necrosis, sarcomatoid component and HIF-1α expression were finally selected for the classifier, and the accuracy by 10-fold-CV was 0.870. When we applied this classifier to 89 patients with all data for the selected features, the apparent accuracy was high (0.875). The sensitivity was 0.852, specificity was 0.887, positive predictive value was 0.793 and negative predictive value was 0.922. We also presented this classifier as decision tree for clinical utility. According to this classifier, mRCC patients could be categorized into two groups: “good-responder” and “poor-responder” (Fig. 1).

Table 1 Histopathologic, immunohistochemical and molecular characteristics of patients

Features		Total ($N = 101$)	Clinical benefit group ($N = 73$)	Clinical non-benefit group ($N = 28$)	P
Histopathology					
Tumor size	cm	8.44 (1.3–19)	7.82 (1.3–16)	10 (3.5–19)	0.002
pT stage	1, 2	48 (47.5)	43 (58.9)	5 (17.9)	<0.001
	3, 4	53 (52.5)	30 (41.1)	23 (82.1)	
ISUP grade	2, 3	55 (54.5)	46 (63.0)	9 (32.1)	0.007
	4	46 (45.5)	27 (37.0)	19 (67.9)	
Sinus fat invasion	Absent	64 (63.4)	51 (69.9)	13 (46.4)	0.038
	Present	37 (36.6)	22 (30.1)	15 (53.6)	
Perinephric fat invasion	Absent	72 (71.3)	57 (78.1)	15 (53.6)	0.026
	Present	29 (28.7)	16 (21.9)	13 (46.4)	
Lymphovascular invasion	Absent	55 (54.5)	43 (58.9)	12 (42.9)	0.183
	Present	46 (45.5)	30 (41.1)	16 (57.1)	
Sarcomatoid change	Absent	55 (54.5)	46 (63.0)	9 (32.1)	0.007
	Present	46 (45.5)	27 (37.0)	19 (67.9)	
Sarcomatoid component	Extent, %	14.3 (0–90)	7.62 (0–80)	31.6 (0–90)	<0.001
Tumor necrosis	Absent	42 (41.6)	38 (52.1)	4 (14.3)	<0.001
	Present	59 (58.4)	35 (47.9)	24 (85.7)	
Necrosis extent	Extent, %	16.2 (0–90)	9.44 (0–90)	33.9 (0–85)	<0.001
Resection margin	Not involved	92 (91.1)	69 (94.5)	23 (82.1)	0.111
	Involved	9 (8.9)	4 (5.5)	5 (17.9)	
Inflammation degree	None	8 (7.9)	6 (8.2)	2 (7.2)	0.245
	Mild	21 (20.8)	15 (20.6)	6 (21.4)	
	Moderate	33 (32.7)	20 (27.4)	13 (46.4)	
	Severe	39 (38.6)	32 (43.8)	7 (25.0)	
LN metastasis[a]	Absent	51 (78.5)	35 (87.5)	16 (64.0)	0.033
	Present	14 (21.5)	5 (12.5)	9 (36.0)	
Protein expression via IHC[b]					
VEGF	Intensity	2.22 (0–3)	2.11 (0–3)	2.46 (1–3)	0.024
PTEN	Intensity	0.61 (0–3)	0.49 (0–3)	0.85 (0–3)	0.159
pAKT	Intensity	1.17 (0–3)	0.95 (0–3)	1.63 (0–3)	0.008
EGFR	Intensity	1.71 (0–3)	1.57 (0–3)	2.00 (0–3)	0.062
PD-L1	Intensity	0.83 (0–3)	0.63 (0–2)	1.29 (0–3)	<0.001
PD-L2	Intensity	1.27 (0–3)	1.16 (0–3)	1.54 (0–3)	0.049
c-MET	Intensity	1.17 (0–3)	1.16 (0–3)	1.19 (0–3)	0.917
FGFR1	Intensity	0.07 (0–2)	0.056 (0–2)	0.11 (0–2)	0.464
FGFR2	Intensity	0.591 (0–3)	0.49 (0–2)	0.82 (0–3)	0.096
FGFR3	Intensity	0	0	0	NA
FGFR4	Intensity	0.63 (0–3)	0.52 (0–3)	0.93 (0–2)	0.033
FGF-basic	No expression	99 (99.0)	71 (98.6)	28 (100)	1.000
	Expression	1 (1.0)	1 (1.4)	0 (0)	
HIF-1α[c]	Extent, %	42.6 (0–220)	52 (0–220)	22 (0–80)	<0.001
HIF-2α[d]	H-score	29.8 (0–270)	28 (0–195)	34.8 (0–270)	0.424
IL-8	Intensity	0.478 (0–3)	0.591 (0–3)	0.192 (0–1)	0.023
pS6	Intensity	0.886 (0–3)	0.689 (0–2)	1.33 (0–3)	0.002
mTOR	Extent, %	38.3 (0–100)	42.2 (0–100)	29.3 (0–100)	0.085
CA9	Extent, %	78.3 (0–150)	84.5 (0–100)	62.9 (0–150)	0.022
PDGFRβ[e]	MVD	45.9 (0–399)	35.9 (0–188)	70.2 (0–399)	0.024
VEGFR2[e]	MVD	64.6 (0–368)	62.2 (0–368)	70.6 (0–336)	0.633

Table 1 (continued)

Features		Total ($N = 101$)	Clinical benefit group ($N = 73$)	Clinical non-benefit group ($N = 28$)	P
PD-L1+ TIL[f]	No/mm^2	1.16 (0–25)	0.781 (0–5)	2.04 (0–25)	0.082
OncoMap	No mutation	86 (85.1)	65 (89.0)	21 (75.0)	0.115
	Mutation[g]	15 (14.9)	8 (11.0)	7 (25.0)	
MicroRNA expression[h]					
miR-34a-5p	dCt	−1.2 (−8.9–8.5)	−0.88 (−6.8–8.5)	−1.9 (−8.9–2.3)	0.300
miR-138-5p	dCt	2.94 (−7.7–12.5)	3.24 (−7.7–12.5)	2.26 (−6.5–8.4)	0.361
miR-421	dCt	−1.78 (−9.9–4.7)	−2.45 (−9.9–4.1)	−0.31 (−4.6–4.7)	0.008
miR-1275	dCt	−0.003 (−7.8–6.1)	−0.01 (−7.8–6.1)	0.01 (−3.9–6.1)	0.817
miR-1301	dCt	−0.30 (−9.2–9.8)	0.11 (−9.2–9.8)	−1.18 (−5.9–4.2)	0.208

Values are presented as n (%) or mean (range), unless otherwise indicated

ISUP international society of urological pathology, *LN* lymph node, *TIL* tumor infiltrating lymphocyte

[a] Lymph node metastasis evaluation was available in 65 cases

[b] The mean intensity was evaluated when ≥5% of the tumor cells expressed primary antibodies on VEGF, PTEN, pAKT, EGFR, PD-L1, PD-L2, C-MET, FGFR1, FGFR2, FGFR3, FGFR4, IL-8 and pS6 immunohictochemical staining (IHC)

[c] The intensity of nuclear expression was evaluated in case of HIF-1α IHC

[d] HIF-2α IHC expression was expressed by which H-score and H-score was defined as percent of primary antigen positive tumor cells x staining intensity

[e] PDGFRβ and VEGFR2 were evaluated using microvessel density (MVD) defined the number of primary antigen expressed tumor vessel per mm^2

[f] The number of PD-L1-positive tumor infiltrating lymphocytes (TIL) was evaluated per mm^2

[g] Mutatuon includes VHL_F148fs*11, VHL_L158Q, VHL_L89H, STK11_P281L, CDKN2A_R58*, KRAS_A59T, TP53_R248Q, CTNNB1_S37A and MLH1_V384D

[h] dCt defined as $Ct_{(miRNA)} - Ct_{(U6)}$

Performances of the VEGFR-TKI Response Classifier for Survival Prediction

The progression-free survival (PFS) and overall survival (OS) of necrosis and sarcomatoid component were significantly shorter in higher extent groups (≥15% and ≥40%, each) than in lower extent groups (<15% and <40%, each). The high HIF-1α expression group showed a trend of longer PFS than did the low expression group, but OS did not show significant differences between the groups. The C-index values of necrosis, sarcomatoid component and HIF-1α were 0.6426, 0.6061 and 0.5762 for PFS and 0.6264, 0.5752 and 0.5278 for OS, respectively.

When this analysis was performed using the response classifier, the six-month PFS was 88.2% for patients included in the good-responders and 20.7% for patients in the poor-responders (Fig. 2a). In the poor-responders, the median time to progression was 3.1 months and the OS was 9.4 months, whereas in the good-responders, these values were 22.2 and 35.4 months, respectively (Fig. 2a–b). The C-index of the response classifier was 0.7001 for PFS and 0.6552 for OS,

Table 2 Features which p-value was less than 0.01 between clinical benefit and non-benefit groups of tyrosine kinase inhibitors and their criteria and cut-offs

Feature	Criteria	Cut-off
Tumor size	cm	< 7 cm vs. ≥ 7 cm
pT stage		1, 2 vs. 3, 4
ISUP grade		1–3 vs. 4
Necrosis	Extent, %	< 15% vs. ≥ 15%
Sarcomatoid component	Extent, %	< 40% vs. ≥ 40%
pAKT	Intensity in ≥5% of tumor cells	< 2+ vs. ≥ 2+
PD-L1	Intensity in ≥5% of tumor cells	< 1+ vs. ≥ 1+
CA9	Extent, %	< 10% vs. ≥ 10%
pS6	Intensity in ≥5% of tumor cells	< 2+ vs. ≥ 2+
HIF-1α	Nuclear extent, %	≥ 75% vs. < 75%
miR-421	dCt	> −3.55 vs. ≤ −3.55

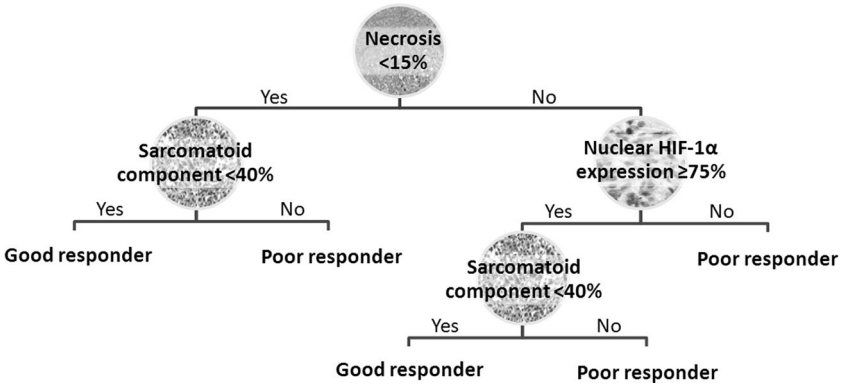

Fig. 1 Decision tree for predicting response to VEGFR-TKI using a primary tumor of mRCC patients

which were higher than those of each classifier components analyzed in single.

Discussion

We developed a VEGFR-TKI response classifier composed of the extent of sarcomatoid components, extent of necrosis and HIF-1α expression by comprehensive inclusion of various clinicopathological features using machine learning methods. The poor-responders are particularly relevant from the clinical perspective because their data might help identify patients to whom alternative managements should be offered.

Several biomarkers for a response prediction to VEGFR-targeted therapies in RCC patients have been suggested [7]. Unfortunately, no biomarker has yet been used in a clinical setting, perhaps due to a lack of validated markers, lack of application protocol for biomarkers or methods that are difficult to use clinically. Thus, we investigated features previously reported to be associated with anti-angiogenic therapies, although we could not cover all suggested biomarkers and tried to provide clinically accessible methods and a practically applicable algorithm. Previous studies reported sarcomatoid features and necrosis as poor predictive or prognostic parameters in RCC patients receiving VEGFR-targeted therapy [5, 15, 16]. HIF-1α was reported to be associated with good response to VEGFR-TKI therapy and good prognosis, although the cut-off levels differed from study to study [17, 18]. Methodologically, dCt of miR-421 was chosen as a criteria rather than ddCt because using a tumor alone is clinically easier than a procedure requiring both tumor and non-tumor tissues. Moreover, the statistical significance of miR-421 was maintained regardless of whether calibration was performed using the matched renal cortex. Two machine learning methods were employed because SVM is known to exhibit much higher accuracy than decision tree, but decision tree approach can be implemented easily on clinical practice [19, 20].

VHL gene mutation was found in 39.6% to 66% of clear cell RCC and was associated with a good response to VEGFR-targeted therapy or with a favorable prognosis [21–23]. However, in this study, only four patients showed the *VHL* gene mutation, perhaps due to using FFPE tissues and the OncoMap method, which can detect only limited types of genetic mutation. VHL complex inactivation was found in most RCC patients due to mutation, loss of heterozygosity or promoter methylation, and the correlation between

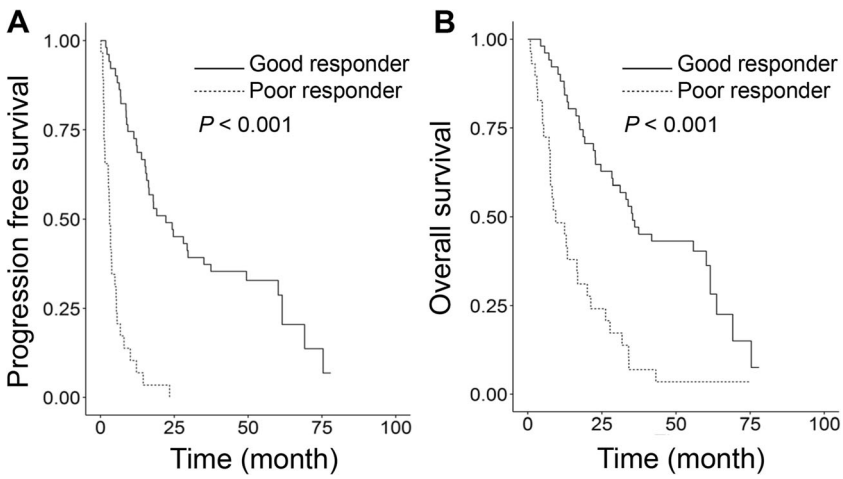

Fig. 2 Implications of the VEGFR-TKI response classifier on patients' survival. **a** Progression-free survival. **b** Overall survival via Kaplan-Meier analysis

VHL alteration and molecular-targeted therapies is uncertain [21, 23, 24]. Therefore, VHL alteration seems not to be a potential biomarker for VEGFR-targeted therapy.

Small size of patients and retrospective design are an obvious limitation in this study Moreover, an independent cohort for validation is necessary to confirm the clinical utility of this response classifier.

Conclusions

We built a VEGFR-TKI response classifier incorporating clinicopathological and molecular features that was able to discriminate poor responders from good responders in mRCC patients. The machine learning methods may be helpful in establishing appropriate therapeutic strategies.

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Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (2012–0788) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

References

1. Srinivasan R, Ricketts CJ, Sourbier C, Linehan WM (2015) New strategies in renal cell carcinoma: targeting the genetic and metabolic basis of disease. Clinical cancer research : an official journal of the American Association for Cancer Research 21(1):10–17. https://doi.org/10.1158/1078-0432.CCR-13-2993
2. Rini BI (2007) Vascular endothelial growth factor-targeted therapy in renal cell carcinoma: current status and future directions. Clinical cancer research : an official journal of the American Association for Cancer Research 13(4):1098–1106. https://doi.org/10.1158/1078-0432.ccr-06-1989
3. 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, Bukowski RM (2007) Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 356(2):125–134. https://doi.org/10.1056/NEJMoa060655
4. Maroto P, Rini B (2014) Molecular biomarkers in advanced renal cell carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 20(8):2060–2071. https://doi.org/10.1158/1078-0432.CCR-13-1351
5. Park JY, Lee JL, Baek S, Eo SH, Go H, Ro JY, Cho YM (2014) Sarcomatoid features, necrosis, and grade are prognostic factors in metastatic clear cell renal cell carcinoma with vascular endothelial growth factor-targeted therapy. Hum Pathol. https://doi.org/10.1016/j.humpath.2014.02.019
6. Sun M, Shariat SF, Cheng C, Ficarra V, Murai M, Oudard S, Pantuck AJ, Zigeuner R, Karakiewicz PI (2011) Prognostic factors and predictive models in renal cell carcinoma: a contemporary review. Eur Urol 60(4):644–661. https://doi.org/10.1016/j.eururo.2011.06.041
7. Funakoshi T, Lee CH, Hsieh JJ (2014) A systematic review of predictive and prognostic biomarkers for VEGF-targeted therapy in renal cell carcinoma. Cancer Treat Rev 40(4):533–547. https://doi.org/10.1016/j.ctrv.2013.11.008
8. Gamez-Pozo A, Anton-Aparicio LM, Bayona C, Borrega P, Gallegos Sancho MI, Garcia-Dominguez R, de Portugal T, Ramos-Vazquez M, Perez-Carrion R, Bolos MV, Madero R, Sanchez-Navarro I, Fresno Vara JA, Espinosa Arranz E (2012) MicroRNA expression profiling of peripheral blood samples predicts resistance to first-line sunitinib in advanced renal cell carcinoma patients. Neoplasia 14(12):1144–1152
9. Garcia-Donas J, Leandro-Garcia LJ, Gonzalez Del Alba A, Morente M, Alemany I, Esteban E, Arranz JA, Climent MA, Gallardo E, Castellano DE, Bellmunt J, Mellado B, Puente J, Moreno F, Font A, Hernando S, Robledo M, Rodriguez-Antona C (2013) Prospective study assessing hypoxia-related proteins as markers for the outcome of treatment with sunitinib in advanced clear-cell renal cell carcinoma. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 24(9):2409–2414. https://doi.org/10.1093/annonc/mdt219
10. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45(2):228–247. https://doi.org/10.1016/j.ejca.2008.10.026
11. Di Leo A, Jerusalem G, Petruzelka L, Torres R, Bondarenko IN, Khasanov R, Verhoeven D, Pedrini JL, Smirnova I, Lichinitser MR, Pendergrass K, Garnett S, Lindemann JP, Sapunar F, Martin M (2010) Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. Journal of clinical oncology : official Journal of the American Society of Clinical Oncology DDDD (30):4594–4600. doi:https://doi.org/10.1200/jco.2010.28.8415
12. Moch H, Humphrey PA, Ulbright TM, Reuter VE (2016) WHO Classification Tumours of the Urinary system and Male Genital Organs, 4th edn. IARC Press, Lyon
13. Delahunt B, Cheville JC, Martignoni G, Humphrey PA, Magi-Galluzzi C, McKenney J, Egevad L, Algaba F, Moch H, Grignon DJ, Montironi R, Srigley JR (2013) The International Society of Urological Pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters. Am J Surg Pathol 37(10):1490–1504. https://doi.org/10.1097/PAS.0b013e318299f0fb
14. Mekhail TM, Abou-Jawde RM, Boumerhi G, Malhi S, Wood L, Elson P, Bukowski R (2005) Validation and extension of the Memorial Sloan-Kettering prognostic factors model for survival in patients with previously untreated metastatic renal cell carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 23(4):832–841. https://doi.org/10.1200/jco.2005.05.179
15. Golshayan AR, George S, Heng DY, Elson P, Wood LS, Mekhail TM, Garcia JA, Aydin H, Zhou M, Bukowski RM, Rini BI (2009) Metastatic sarcomatoid renal cell carcinoma treated with vascular endothelial growth factor-targeted therapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 27(2):235–241. https://doi.org/10.1200/jco.2008.18.0000
16. Molina AM, Tickoo SK, Ishill N, Trinos MJ, Schwartz LH, Patil S, Feldman DR, Reuter VE, Russo P, Motzer RJ (2011) Sarcomatoid-variant renal cell carcinoma: treatment outcome and survival in advanced disease. Am J Clin Oncol 34(5):454–459. https://doi.org/10.1097/COC.0b013e3181f47aa4

17. Dornbusch J, Zacharis A, Meinhardt M, Erdmann K, Wolff I, Froehner M, Wirth MP, Zastrow S, Fuessel S (2013) Analyses of potential predictive markers and survival data for a response to sunitinib in patients with metastatic renal cell carcinoma. PLoS One 8(9):e76386. https://doi.org/10.1371/journal.pone.0076386
18. Saez MI, Trigo Perez JM, Perez-Rivas LG, Perez-Villa L, Villatoro R, Montesa A, Cid JI, Leon M, de Luque V, Fernandez C, Quero C, Pajares B, Alba E (2012) Hypoxia-inducible factor (HIF) 1α and 2α as predictive markers of outcome to VEGFR tyrosine kinase inhibitors (TKI) in renal cell carcinoma (RCC). J Clin Oncol 30(suppl; abstr 4630):309S
19. Krishnan VG, Westhead DR (2003) A comparative study of machine-learning methods to predict the effects of single nucleotide polymorphisms on protein function. Bioinformatics (Oxford, England) 19(17):2199–2209
20. Cruz JA, Wishart DS (2006) Applications of machine learning in cancer prediction and prognosis. Cancer Informat 2:59–77
21. Brugarolas J (2014) Molecular genetics of clear-cell renal cell carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 32(18):1968–1976. https://doi.org/10.1200/jco.2012.45.2003
22. Rini BI, Jaeger E, Weinberg V, Sein N, Chew K, Fong K, Simko J, Small EJ, Waldman FM (2006) Clinical response to therapy targeted at vascular endothelial growth factor in metastatic renal cell carcinoma: impact of patient characteristics and Von Hippel-Lindau gene status. BJU Int 98(4):756–762. https://doi.org/10.1111/j.1464-410X.2006.06376.x
23. Sato Y, Yoshizato T, Shiraishi Y, Maekawa S, Okuno Y, Kamura T, Shimamura T, Sato-Otsubo A, Nagae G, Suzuki H, Nagata Y, Yoshida K, Kon A, Suzuki Y, Chiba K, Tanaka H, Niida A, Fujimoto A, Tsunoda T, Morikawa T, Maeda D, Kume H, Sugano S, Fukayama M, Aburatani H, Sanada M, Miyano S, Homma Y, Ogawa S (2013) Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet 45(8):860–867. https://doi.org/10.1038/ng.2699
24. Cowey CL, Rathmell WK (2009) VHL gene mutations in renal cell carcinoma: role as a biomarker of disease outcome and drug efficacy. Curr Oncol Rep 11(2):94–101