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ORIGINAL ARTICLE

Prognostic tissue biomarker exploration for patients with metastatic renal cell carcinoma receiving vascular endothelial growth factor receptor tyrosine kinase inhibitors

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Abstract In metastatic renal cell carcinoma (mRCC), the prognostic role of several tumor tissue biomarkers has been evaluated, but the results were controversial. This study aims to verify the prognostic importance of selected tumor tissue biomarkers in patients with mRCC. The clinicopathological features, immunohistochemical staining and scoring for select tissue biomarkers, treatment, and outcome of patients with mRCC treated with vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors (TKIs) between July 2006 and March 2011 at Asan Medical Center in Seoul, South Korea, were reviewed. In total, 123 patients met the inclusion criteria. Most patients had clear-cell carcinoma (107 patients, 87.0 %). First-line VEGFR TKIs were sunitinib (97 patients, 78.9 %), sorafenib (23 patients, 18.7 %), and pazopanib (3 patients, 2.4 %). With a median follow-up period of 60.0 months (95 % confidence interval (CI), 56.3–63.6), median overall survival (OS) and progression-free survival (PFS) were 25.6 months (95 % CI, 19.2–32.0) and 12.2 months (95 % CI, 8.1–16.3), respectively. In the multivariable analysis for OS, carbonic anhydrase IX (CAIX;

47.5 % or less vs. more than 47.5 %, $p=0.014$), sarcomatoid change (40 % or less vs. more than 40 %, $p<0.001$), tumor necrosis (20 % or less vs. more than 20 %, $p=0.006$), and Heng's risk group (good vs. intermediate vs. poor, $p=0.011$) were identified as independent prognostic factors. In the multivariable analysis for PFS, CAIX ($p<0.001$), phosphatase and tensin homolog (PTEN; 45 % or less vs. more than 45 %, $p=0.004$), sarcomatoid change ($p=0.002$), and tumor necrosis ($p=0.001$) were identified as independent factors affecting PFS. CAIX and PTEN had prognostic importance for mRCC patients receiving first-line VEGFR TKI. Future validation and mechanistic studies are required.

Keywords Renal cell carcinoma · Biomarker · Prognosis · Carbonic anhydrase IX · PTEN

Introduction

Until early 2000, immunotherapy using interferon or interleukin was the de facto standard treatment for metastatic renal cell carcinoma (mRCC). Unfortunately, immunotherapy achieved only occasional responses, whereas substantial toxicities occurred invariably [1]. Limited treatment options and nonspecific mechanisms of action behind immunotherapy retarded vigorous biomarker investigations, preventing discrimination between patients who are likely to respond and those who are unlikely to respond.

Recent advances in understanding the molecular pathobiology underlying RCC has lead to the introduction of agents that target the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) signaling pathways, resulting in huge changes in the management of this disease. There are currently several effective approaches for treating metastatic RCC [2–7]. Although VEGF receptor (VEGFR)

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tyrosine kinase inhibitors (TKIs) are rationally designed and are most commonly used as first-line treatment for mRCC, biomarkers that predict or prognosticate the treatment outcome have not been well elucidated.

In addition to VEGF and mTOR signaling pathway, several molecules have been investigated for their role in RCC pathogenesis. *Von Hippel–Lindau* (*VHL*) gene mutation leads to hypoxia-inducible factor (HIF) disregulation and resultant overexpression of VEGF that can lead to tumor angiogenesis [8]. Hepatocyte growth factor receptor (HGFR, also known as MET) is a receptor tyrosine kinase functions for cell proliferation, differentiation, angiogenesis, and tissue repair. It is also a proto-oncogene with implication in various cancers [9]. MET expression was higher in all RCC subtypes than in adjacent normal renal tissues in a series of more than 300 patients [10]. Carbonic anhydrases are metalloenzymes whose function in solid tumors is a neutralization of the intracellular milieu with a concomitant acidification of the extracellular milieu [11]. Although carbonic anhydrase IX (CAIX) is not expressed in healthy renal tissue, it is expressed in almost all clear cell RCCs and its expression is influenced by activation of the HIF-1α and the inactivation of the VHL gene [12, 13].

On the other hand, some proteins are shown to be involved in inducing resistance to VEGF inhibitors. Interleukin-8 (IL-8) is a member of the CXC family of chemokines and is a potent proangiogenic factor. In a recent study, IL-8 was upregulated in sunitinib-resistant tumor xenograft and inhibition of IL-8 re-sensitized these resistant tumors to sunitinib. Also, IL-8 expression was elevated in human clear cell RCC having intrinsic resistance to sunitinib therapy [14]. There are some evidences that upregulation of fibroblast growth factor (FGF) and FGF receptor (FGFR) may have a role in introducing resistance to anti-VEGF therapy [15]. FGF2 (FGF-basic) can suppress sunitinib-induced retraction of endothelial tubules and thus regulates endothelial sensitivity to sunitinib [16].

Several tumor tissue biomarkers such as von Hippel–Lindau protein, HIF-1α/HIF-2α, VEGF (and related proteins), or CAIX have been evaluated, but the results were controversial and defective with respect to retrospective design, small sample size, and lack of validation [17].

Some prognostic models comprising several clinical characteristics are widely used in practice [18, 19], but they are population-based models that categorize patients into a few prognostic groups (good, intermediate, or poor risk group); are composed of only clinical and laboratory characteristics, which are indicators of the general condition of the patients and the burden of the disease; and are missing molecular pathologic characteristics reflecting tumor biology. Therefore, to provide precision treatment, avoid futile treatment, and educate patients, the development of reliable biomarkers, which can be integrated into established prognostic models, is eagerly required.

To verify the prognostic importance of selected tumor tissue biomarkers in patients with mRCC, we performed immunohistochemical (IHC) staining in tumor samples and statistical analyses of the results.

Patients and methods

Patient selection

The medical records of 520 consecutive patients with histologically confirmed recurrent or metastatic RCC who were treated with VEGFR TKIs at the Department of Oncology, Asan Medical Center (Seoul, South Korea) from January 2006 to December 2013 were retrospectively reviewed. Among these patients, the selection criteria for study inclusion were as follows: patients who received VEGFR TKI as a first-line systemic treatment, patients for whom pre-treatment tissue samples were available, and patients who had undergone proper imaging, including baseline imaging with at least one follow-up image. We set liberal inclusion criteria in terms of performance status, prognostic risk group, and histology. Patients were excluded if the patients received immunotherapy, cytotoxic chemotherapy, or molecular targeted agents other than VEGFR TKI prior to VEGFR TKI; patients had inadequate clinicopathological data; insufficient tissue amount to perform pre-specified IHC; patients cannot be included in survival information due to lost to follow-up.

Clinical data collection

Clinicopathological data, including age, sex, Karnofsky performance status (KPS), tumor histology, pathological stage, laboratory data, date of the initial diagnosis, disease status (recurrent or metastatic), metastatic site, surgery (if performed), and survival status, were collected. A comprehensive review was performed on baseline and follow-up radiographic images, which were obtained every 6–8 weeks during treatment. Target lesion selection and response evaluations were performed retrospectively according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The study was approved by the institutional review board of Asan Medical Center.

Pathology review

Pre-treatment pathologic materials from each patient were reviewed for diagnostic reassessment and histologic subtyping according to the 2004 World Health Organization Tumor Classification. Each tumor was graded according to the Fuhrman grading system [20]. Pathologic materials, including immunostained tissue microarray (TMA) slides, were

assessed by a single pathologist (YMC) who was unaware of the clinical variables and therapeutic outcomes.

TMA construction

A TMA construct with 1-mm-diameter cores was generated from formalin-fixed paraffin-embedded tissue blocks of renal cell carcinoma specimens using a tissue microarrayer (Beecher Instruments, Silver Spring, MD). Three representative cores were used to access each case.

IHC

The expression of FGFR 1, 2, 3, and 4, FGF-basic, HIF-1α and HIF-2α, CAIX, mTOR, phosphatase and tensin homolog (PTEN), IL8, phosphor-S6 (pS6), and HGFR/MET was analyzed by IHC staining, which was performed using an automated staining system (BenchMark XT, Ventana Medical Systems, Tucson, AZ) and the ultraView universal DAB detection kit (Ventana Medical Systems). The primary antibodies used in the study, their dilutions, and the subcellular localization of each antigen are summarized in supplementary Table 1. Nuclei were counterstained with hematoxylin.

Assessment of IHC results

IHC staining was assessed using the semiquantitative "eyeball" measurement, and each core was scored based on the percentage of positive cell staining and the intensity, which was scored as negative, weak, moderate, or strong. Cases with moderate staining (FGFR1, FGF base, and HIF-1α) or strong staining (CAIX, mTOR, PTEN, FGFR2, FGFR3, FGFR4, IL8, pS6, HIF2α, and HGFR/MET) were regarded as positive, and the average percentage of positive cells in all cores was recorded.

Statistical analysis

The primary and secondary objectives of the present study were to identify prognostic biomarkers for overall survival (OS) and progression-free survival (PFS). OS was defined as the time from the date of initiation of the first-line line treatment to the date of death from any cause, and PFS was defined as the date of disease progression or death from any cause, whichever comes first. Patient characteristics, histologic findings, and IHC results were summarized descriptively, and continuous clinical variables were dichotomized for convenience. Maxstat, a maximally selected rank statistics in R 3.0.3 (R Development Core Team, Vienna, Austria, http://www.R-project.org), was used to identify optimal cutting points for each marker [21]. We performed univariate analyses for survival using the log-rank test,

and the Cox proportional hazard model was performed for factors that were significant in univariate analysis. Statistical analyses were performed using PASW statistics (version 20; IBM Co., Armonk, NY) and R. p values less than 0.05 (two-sided) were considered statistically significant.

Results

Patient characteristics

In total, 123 patients met the inclusion criteria (Table 1). The median age was 57 years (range 17–85), and 88 patients (71.5 %) were male. Most patients had clear-cell carcinoma (107 patients, 87.0 %). Fuhrman's nuclear grade (NG) was 2 in 21 (17.1 %), 3 in 49 (39.8 %), and 4 in 52 patients (42.3 %). Sarcomatoid change and coagulative necrosis were found in 51 patients (41.5 %) and 58 patients (47.2 %), respectively. Seventy-two patients (57.7 %) had initially metastatic disease, whereas 52 patients (42.3 %) had recurrent disease. Nephrectomy was performed in 120 patients (97.6 %), and metastasectomy in 33 patients (26.8 %). Most patients had a relatively good performance status, as 11 (8.9 %), 51 (41.5 %), and 50 patients (40.7 %) had 100, 90, and 80 % of KPS. Common sites of metastases were the lung (74.8 %), lymph node (24.4 %), bone (20.3 %), and liver (11.4 %). Using Heng's criteria, 20 (16.3 %), 87 (70.7 %), and 16 patients (13.0 %) belonged to the favorable, intermediate, and poor risk groups, respectively. First-line VEGFR TKIs prescribed were sunitinib (97 patients, 78.9 %), sorafenib (23 patients, 18.7 %), and pazopanib (3 patients, 2.4 %).

The best responses to first-line VEGFR TKI were complete response (CR) in 1 patient (0.8 %), partial response (PR) in 44 (35.8 %), stable disease (SD) in 58 (47.2 %), and progressive disease (PD) in 20 patients (16.3 %). Thus, the objective response rate (CR or PR) was 36.6 %.

Impact of biomarkers on OS

For the primary endpoint, we analyzed OS with known clinical prognostic factors and IHC staining results. Cutoff values for statistical analyses were obtained by the maximally selected rank statistics. With a median follow-up duration of 60.0 months (95 % confidence interval (CI), 56.3–63.6 months), OS for all patients was 25.6 months (95 % CI, 19.2–32.0 months, Fig. 1). In the univariate analysis (Table 2), CAIX (less than 47.5 vs. 47.5 % or more, $p=0.001$), HIF-2α (negative vs. positive, $p<0.001$), mTOR (20 % or less vs. more than 20 %, $p=0.0032$), Heng's risk group (good vs. intermediate vs. poor, $p<0.001$), sarcomatoid change (40 % or less vs. more than 40 %, $p<0.001$), tumor necrosis (20 % or less vs. more than 20 %, $p<0.001$),

Table 1 Patient characteristics

Characteristics	n (%)	Characteristics	n (%)
Age, years, median (range)	57 (17–85)	No. of metastasized organ	
Sex		1	56 (45.5)
Male	88 (71.5)	2	44 (35.8)
Female	35 (28.5)	3	15 (12.2)
Histology	4$\geq$		8 (6.4)
Clear cell carcinoma	107 (87.0)	Heng et al. risk group[a]	
Papillary carcinoma	11 (8.9)	Favorable (0 factor)	20 (16.3)
Mixed histology	4 (3.2)	Intermediate (1–2 factors)	87 (70.7)
chromophobe	1 (0.8)	Poor (3–6 factors)	16 (13.0)
Unclassified carcinoma	1 (0.8)	1st line VEGFR TKI	
Fuhrman's nuclear grade		Sunitinib	97 (78.9)
2	21 (17.1)	Sorafenib	23 (18.7)
3	49 (39.8)	Pazopanib	3 (2.4)
4	52 (42.3)	IHC staining	Positive/negative
% sarcomatoid change, median (range)	0 (0–95)	FGF-basic	1/122
% Tumor necrosis, median (range)	0 (0–90)	FGFR1	8/115
Disease status		FGFR2	25/96
Recurrent	52 (42.3)	FGFR3	0/123
Initially metastatic	71 (57.7)	FGFR4	39/83
Nephrectomy	120 (97.6)	HGFR/MET	5/117
Metastasectomy before TKI	33 (26.8)	PTEN	61/60
Karnofsky performance status		CAIX	93/27
100	11 (8.9)	pS6	42/74
90	51 (41.5)	HIF-1α	7/115
80	50 (40.7)	HIF-2α	1/122
<80	11 (8.9)	IL-8	18/102
Sites of metastases		mTOR	98/24
Lung	92 (74.8)		
Lymph node	30 (24.4)		
Bone	25 (20.3)		
Liver	14 (11.4)		
Adrenal	10 (8.1)		

VEGFR TKI vascular endothelial growth factor receptor tyrosine kinase inhibitor, *IHC* immunohistochemical staining, *FGF* fibroblast growth factor, *FGFR* fibroblast growth factor receptor, *HGFR* hepatocyte growth factor receptor, *PTEN* phosphatase and tensin homolog, *CAIX* carbonic anhydrase IX, *pS6* phospho S6, *HIF* hypoxia-inducible factor, *IL* interleukin, *mTOR* mammalian target of rapamycin

[a] The Heng risk group was defined by six risk factors: neutrophilia, anemia, thrombocytosis, corrected hypercalcemia, poor Karnofsky performance status, and a time from diagnosis to treatment of less than 1 year

and Furhman NG (2 vs. 3 vs. 4, $p=0.037$) were statistically significant. In the multivariable analysis (Table 3), CAIX, Heng's risk group, sarcomatoid change, and tumor necrosis were identified as independent prognostic factors related to OS.

Impact of biomarkers on PFS and response

Median PFS was 12.2 months (95 % CI, 8.1–16.3 months, Fig. 1) for all patients. Using the same cutoff value as in OS analyses, we performed univariate analysis on PFS (Table 4). PTEN (40 % or less vs. more than 40 %, $p=0.012$), CAIX (less than 47.5 % vs. 47.5 % or more, $p=0.001$), Heng's risk group (good vs. intermediate vs. poor, $p<0.014$), sarcomatoid change (40 % or less vs more than 40 %, $p<0.001$), tumor necrosis (20 % or less vs more than 20 %, $p<0.001$), and Furhman NG (2 vs. 3 vs. 4, $p=0.003$) were statistically significantly associated with longer PFS. In the multivariable analysis (Table 5), CAIX, PTEN, sarcomatoid change, and tumor necrosis were identified as independent factors affecting PFS.
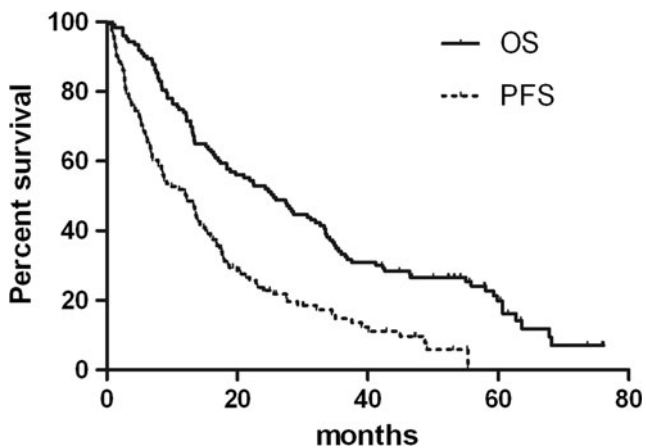
Fig. 1 Kaplan–Meier curves for overall survival (OS) and progression-free survival (PFS) with first-line VEGFR TKI (n=123). With a median follow-up period of 60.0 months (95 % CI, 56.3–63.6 months), median OS and PFS were 25.6 months (95 % CI, 19.2–32.0 months) and 12.2 months (95 % CI, 8.1–16.3 months), respectively

Discussion

In the present study, we examined the prognostic role of tissue biomarkers in patients with mRCC treated with first-line VEGFR TKIs. We found that a lower expression of CAIX, a higher percentage of sarcomatoid change, abundant tumor necrosis, and belonging to the poor Heng's risk group were independent adverse prognostic factors for OS. In addition, lower expression of CAIX, higher expression of PTEN, higher percentage of sarcomatoid change, and abundant tumor necrosis were independent adverse prognostic factors for PFS.

Heng's risk group [18], sarcomatoid change [22], and tumor necrosis [23] are well-known prognostic factors in mRCC. In our study, all three factors were statistically significant in multivariable analysis for OS. However, CAIX, which was identified as a statistically significant prognostic factor for OS in our study, has been controversial. CAIX is a cytosolic transmembrane protein that regulates cell proliferation under hypoxic conditions and is expressed in the vast majority of clear-cell RCCs but is absent in non-tumor kidney tissue [24]. CAIX expression is mediated by the HIF transcriptional complex and correlates with *von Hippel–Lindau* gene (*VHL*) inactivation, which has a central role in the pathogenesis of clear-cell RCC [25]. In the pre-VEGFR TKI era, high CAIX expression (>85 %) as determined by IHC was initially reported to be associated with a higher response rate and a better survival in mRCC patients undergoing immunotherapy [26, 27], but in a prospective validation study, the SELECT trial failed to validate its role as a predictive factor [28]. In the VEGFR TKI era, CAIX was shown to be neither a predictive factor for response nor a prognostic factor for survival in patients with mRCC receiving VEGFR targeted therapy (mainly sunitinib or sorafenib), in both retrospective [29] and prospectively maintained datasets [30, 31] from the TARGET or renal EFFECT trial [2, 32].

Contrary to those studies that evaluated CAIX in the VEGFR TKI era [29–31], CAIX was an independent prognostic factor for both OS and PFS in the present study. It is uncertain why the prognostic role of CAIX differs between studies, but there were some differences between our study and previous studies. Our study only included patients who were administered VEGFR TKI as a first-line systemic treatment, whereas many patients in the study by Choueiri et al. (2010) had received previous systemic therapy. In total, 40 % of patients in the retrospective dataset received cytokines, vaccines, chemotherapy, or thalidomide, and all patients in the prospectively maintained dataset received cytokines [30]. In addition, the cutoff value for dichotomizing patients was different. Both studies by Choueiri et al. used 85 % as the cutoff point based on a previous study [27], in which the cutoff value was calculated by using survival tree analysis. Because the 85 % cutoff point was derived from patients who underwent immunotherapy [27], it might be unreliable to adopt this cutoff point for patients receiving VEGFR TKI. To solve this problem, we determined a new cutoff value of 47.5 % by using the maximally selected rank statistics which can estimate and provide a simple cutoff point, even for censored or tied observations [21]. Recently, a more complicated scoring system of CAIX, such as individual scores (percent of tumor expression, predominant intensity, focal score, and focal intensity) and composite scores, was used, and four-group categorization also failed to show any statistical and clinical significance [31].

In an analysis for PFS, we demonstrated that lower CAIX expression, abundant PTEN expression, sarcomatoid change, and abundant tumor necrosis were independent adverse prognostic factors for PFS. PTEN inhibits phosphoinositide 3-kinase (PI3K)/Akt signaling via its lipid phosphatase activity, thereby controlling cell growth, survival, and metabolic processes [33]. In addition, the PI3K/Akt/mTOR signaling pathway positively regulates HIF-1α protein in certain cancer cells [34–36]. Therefore, PTEN theoretically inhibits HIF-1α via inhibition of PI3K/Akt/mTOR pathway. In patients with advanced RCC, positive PTEN was indicative of a good prognosis with sunitinib (PFS, 15.1 vs. 6.5 months; p= .003) [37]. Therefore, our result that higher PTEN expression (>45 %) is an independent adverse prognostic factor for PFS is contradictory to that reported previously.

Although canonical role of PTEN with relation to inhibition of PI3K/Akt/mTOR pathway is plain, it is not clear how PTEN correlates with HIFs. In recent years, it has emerged that among HIFs, HIF-2α is more relevant in the development and progression of RCC [38]. However, most studies have focused on the relationship between the PI3K/Akt/mTOR pathway and HIF-1α [34, 35]. On the other hand, the PI3K/Akt/mTOR pathway has not been clearly shown to correlate with HIF-2α [36]. One study suggested that HIF-2α is upregulated by PTEN at both the transcriptional and posttranscriptional levels under hypoxic conditions in the embryonic

Table 2 Log-rank test for overall survival

Variables	Category	No. of patients		Median OS (95 % CI)	*p* value
		Death	Total		
FGF-basic	Negative	100	122	25.2 (18.3–32.1)	0.728
	Positive	1	1	55.9	
FGFR1	Negative	96	115	25.6 (19.4–31.8)	0.847
	Positive	5	8	11.8 (0.0–50.7)	
FGFR2	Negative	77	96	26.0 (16.3–35.6)	0.337
	Positive	22	25	24.8 (13.7–35.9)	
FGFR3	Negative	101	123	25.6 (19.2–32.0)	
	Positive	0	0		
FGFR4	Negative	68	83	28.7 (20.6–36.7)	0.248
	Positive	32	39	13.4 (0.9–25.9)	
HGFR/MET	Negative	96	117	26.0 (18.3–33.6)	0.270
	Positive	4	5	7.9 (1.4–14.3)	
PTEN	≤45 %	78	97	28.0 (20.6–35.3)	0.101
	>45 %	21	24	15.6 (8.3–22.8)	
CAIX	≤47.5 %	28	31	13.1 (10.6–15.7)	0.001
	>47.5 %	70	89	30.8 (23.0–38.6)	
pS6	≤45 %	87	103	22.6 (15.8–29.4)	0.092
	>45 %	8	13	55.9 (16.5–95.2)	
HIF-1α	Negative	95	115	25.6 (18.4–32.8)	0.805
	Positive	5	7	33.7 (11.8–55.7)	
HIF-2α	Negative	100	122	25.6 (18.1–33.1)	<0.001
	Positive	1	1	3.0	
IL-8	Negative	86	102	22.6 (14.7–30.4)	0.172
	Positive	12	18	34.2 (25.5–42.9)	
mTOR	≤20 %	49	55	18.4 (8.9–28.0)	0.032
	>20 %	51	67	30.8 (19.8–41.7)	
Heng risk group	Favorable	14	20	41.3 (31.3–51.2)	<0.001
	Intermediate	81	87	26.0 (20.0–31.9)	
	Poor	16	16	10.1 (2.6–17.5)	
Sarcomatoid change	≤40 %	82	103	30.8 (23.9–37.6)	<0.001
	>40 %	19	19	9.3 (5.7-12.9)	
Tumor necrosis	≤20 %	78	98	30.8 (24.2–37.3)	<0.001
	>20 %	23	24	10.8 (5.5–16.1)	
Furhman NG	2	15	21	33.7 (14.7–52.8)	0.037
	3	40	49	34.2 (25.1–43.3)	
	4	46	52	17.1 (9.6–24.6)	

OS overall survival, *95 % CI* 95 % confidence interval, *FGF* fibroblast growth factor, *FGFR* fibroblast growth factor receptor, *HGFR* hepatocyte growth factor receptor, *PTEN* phosphatase and tensin homolog, *CAIX* carbonic anhydrase IX, *pS6* phospho S6, *HIF* hypoxia-inducible factor, *IL* interleukin, *mTOR* mammalian target of rapamycin, *NG* nuclear grade

Table 3 Cox proportional hazard modeling for overall survival

Variables	*B*	Exp(B)	95 % CI for Exp(B)	*p* value
CAIX	0.598	1.818	1.131–2.922	0.014
Sarcomatoid change	1.055	2.871	1.607–5.130	<0.001
Tumor necrosis	0.709	2.031	1.225–3.368	0.006
Heng risk group				0.011
Good versus intermediate	0.260	1.298	0.704–2.390	0.403
Good versus poor	1.081	2.949	1.348–6.453	0.007

95 % CI 95 % confidence interval, *CAIX* carbonic anhydrase IX

Table 4 Log-rank test for progression-free survival

Variables	Category	No. of patients		Median PFS (95 % CI)	P value
		Death	Total		
FGF-basic	Negative	104	122	12.2 (8.1–16.3)	0.778
	Positive	1	1	22.9	
FGFR1	Negative	97	115	12.3 (8.1–16.4)	0.367
	Positive	8	8	7.0 (3.7–10.4)	
FGFR2	Negative	78	96	12.0 (7.1–17.0)	0.178
	Positive	25	25	13.2 (10.4–16.0)	
FGFR3	Negative	105	123	12.2 (8.1–16.3)	
	Positive	0	0		
FGFR4	Negative	71	83	14.0 (11.2–16.8)	0.145
	Positive	33	39	8.0 (4.1–11.9)	
HGFR/MET	Negative	99	117	13.2 (9.1–17.3)	0.268
	Positive	5	5	5.9 (0.0–11.9)	
PTEN	≤45 %	83	97	13.7 (11.0–16.3)	0.012
	>45 %	22	24	5.9 (3.0–8.7)	
CAIX	≤47.5 %	28	31	5.0 (2.6–7.4)	0.001
	>47.5 %	75	89	14.9 (11.1–18.6)	
pS6	≤45 %	88	103	12.0 (7.8–16.3)	0.395
	>45 %	11	13	17.8 (3.2–32.5)	
HIF-1α	Negative	97	115	12.2 (8.2–16.2)	0.968
	Positive	7	7	16.9 (0.0–39.8)	
HIF-2α	Negative	104	122	12.3 (8.0–16.5)	0.081
	Positive	1	1	3.0	
IL-8	Negative	87	102	11.1 (6.5–15.8)	0.160
	Positive	15	18	16.3 (11.4–21.2)	
mTOR	≤20 %	46	55	11.1 (4.3–17.9)	0.989
	>20 %	58	67	13.4 (7.8–18.9)	
Heng risk group	Favorable	17	20	18.8 (5.7–31.9)	0.014
	Intermediate	74	87	12.3 (7.7–16.8)	
	Poor	14	16	5.3 (1.8–8.8)	
sarcomatoid change	≤40 %	86	103	14.0 (10.7–17.3)	<0.001
	>40 %	18	19	3.4 (1.8–5.0)	
Tumor necrosis	≤20 %	82	98	14.9 (11.8–17.9)	<0.001
	>20 %	22	24	3.8 (1.0–6.6)	
Furhman NG	2	14	21	21.4 (11.7–31.0)	0.003
	3	42	49	15.9 (10.6–21.2)	
	4	48	52	5.9 (2.1–9.6)	

PFS progression-free survival, *95 % CI* 95 % confidence interval, *FGF* fibroblast growth factor, *FGFR* fibroblast growth factor receptor, *HGFR* hepatocyte growth factor receptor, *PTEN* phosphatase and tensin homolog, *CAIX* carbonic anhydrase IX, *pS6* phospho S6, *HIF* hypoxia-inducible factor, *IL* interleukin, *mTOR* mammalian target of rapamycin, *NG* nuclear grade

Table 5 Cox proportional hazard modeling for progression-free survival

Variables	B	Exp(B)	95 % CI for Exp(B)	p value
CAIX	1.125	3.079	1.877–5.051	<0.001
Sarcomatoid change	0.869	2.385	1.364–4.169	0.002
Tumor necrosis	1.124	3.077	1.854–5.109	<0.001
PTEN	0.718	2.049	1.254–3.349	0.004

95 % CI 95 % confidence interval, *CAIX* carbonic anhydrase IX, *PTEN* phosphatase and tensin homolog

stem cell–derived embryoid body [39]. Although the exact mechanisms underlying the regulation of HIF-2α by PTEN have not been elucidated, yin yang 1 (YY1), a noble corepressor of HIF-2α, may play a role in this pathway [40]. Thus, the role of PTEN with respect to its relationship with HIF-1α and HIF-2α under VHL inactivation conditions in the prognosis of mRCC patients receiving VEGFR TKI needs further evaluation.

Despite the considerable amount of effort that has been expended by clinicians and researchers investigating tissue biomarkers for RCC, unfortunately, no tissue biomarker has

been universally accepted as a predictive or prognostic factor for mRCC [17]. Recently, the concept of intratumoral heterogeneity and branched evolution has arisen [41]. This concept purports that genetic alterations or the protein expression profile of only a part of a tumor might not be shared with and representative of the whole cancer burden in the patient. This constitutes a major challenge for the development of tissue biomarkers, which usually reflect a limited part of the tumor. For the time being at least, perhaps the priority should not be tissue biomarker development or validation but, rather, the invention of novel techniques that overcome intratumoral heterogeneity and clonal evolution.

There are some limitations in this study of note. First, this is a retrospective study which included a limited number of patients who were treated at single institution over several years and had available pre-treatment tissue sample. Therefore, there could be potential biases inherent to this type of study, such as information or selection bias. Second, pathologic examination and immunohistochemical staining were performed on only limited part of tumor mass. Some of them were prior nephrectomy specimen for the patients with recurrent disease, while others are debulking nephrectomy specimen or core needle biopsy specimen for the patients with initially metastatic patients. Considering intratumoral heterogeneity and branched evolution, our result may not reflect overall characteristics of whole tumor burden.

Conclusions

Lower expression of CAIX, higher expression of PTEN, higher percentage of sarcomatoid change, and abundant tumor necrosis were independent adverse prognostic tissue biomarkers for OS and/or PFS. Future validation and mechanistic studies are required, and consideration should be given to novel techniques for overcoming intratumoral heterogeneity and branched evolution.

Compliance with ethical standards

Conflicts of interest None

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