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Prospective study assessing hypoxia-related proteins as markers for the outcome of treatment with sunitinib in advanced clear-cell renal cell carcinoma

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: Previous studies suggest that expression of hypoxia markers may be associated with response to antiangiogenic drugs. Thus, we aimed to identify predictors of sunitinib outcome in clear-cell renal cell carcinoma (ccRCC).

Patients and methods: The expression of eight key proteins related to hypoxia (CAIX, HIF1A, HIF2A, VEGFA, VEGFR1, VEGFR2, VEGFR3 and PDGFRB) and P-glycoprotein were assessed by immunohistochemistry in 67 primary ccRCC samples from prospectively recruited patients treated with first-line sunitinib. The proteins expression, VHL inactivation and EGLN3 mRNA content were compared with the patients' response to sunitinib.

Results: High expression of HIF2A and PDGFRB was associated with better sunitinib RECIST objective response (P = 0.024 and P = 0.026; respectively) and increased VEGFR3 expression was associated with longer progression-free survival (P = 0.012). VEGFR3 overexpression showed a negative correlation with VEGFR3 polymorphism rs307826 (P = 0.002), a sunitinib resistance predictor. With respect to overall survival (OS), high VEGFA was associated with short (P = 0.009) and HIF2A with long (P = 0.048) survival times. High EGLN3 mRNA content was associated with shorter OS (P = 0.023).

Conclusions: We found an association between several proteins involved in hypoxia and sunitinib efficacy. In addition, low VEGFR3 expression was associated with worse outcome and with VEGFR3 rs307826 variant allele, reinforcing VEGFR3 as a marker of sunitinib resistance.

Key words: renal carcinoma, EGLN3, hypoxia, immunohistochemistry, predictive marker, sunitinib

introduction

More than 260 000 new renal cancer cases are diagnosed around the world every year and kidney cancer incidence seems to be rising ~2%–3% per decade [1]. Advanced disease remains a lethal condition, but the development of new-targeted therapies, such as VEGF/VEGFR and mTOR inhibitors, has dramatically improved survival and daily management [2]. In fact, now that several therapeutic options are available, attending physicians face the challenge of selecting drugs to treat kidney cancer patients without reliable predictors of response that could help prioritizing one drug over another. Currently, this selection relies on personal experience and the anticipated toxic effect profile of the drug, without support from molecular characteristics of the tumor and the patient, which could be critical for drug

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outcome. Therefore, predictors of efficacy for targeted treatments in kidney cancer are urgently needed.

Sunitinib (Sutent; Pfizer, New York, NY) is an approved tyrosine kinase inhibitor (TKI) of VEGFR1-3, PDGFR, KIT, FLT3 and CSF-1, and is widely used for first-line treatment of advanced clear-cell renal cell carcinoma (ccRCC) [2]. Recently, several studies have investigated single-nucleotide polymorphisms [3], plasma factors and circulating endothelial cells [4, 5] as potential markers of response to antiangiogenic treatment in renal cancer with promising results. With respect to molecular events and expression of proteins related to tumor hypoxia, a crucial mechanism for renal cancer development and antiangiogenic drug therapy, some studies have investigated the association between Von Hippel–Lindau (VHL) inactivation and antiangiogenic drug response, mainly obtaining negative results [6–10]. For Carbonic Anhydrase IX (CAIX) expression, no clear conclusions have been reached [11, 12], and for hypoxia-inducible factors (HIFs), a recent study suggested that low HIF1A expression might be associated with better progression-free survival (PFS) of patients treated with sunitinib [9], while high HIF2A expression has been suggested to improve response to sunitinib [12]. However, the tumor mRNA expression of prolyl hydroxylase EGLN3, an excellent marker of hypoxia in RCC [13], has not been investigated in relation to response to antiangiogenic drugs. Assessment of these markers in prospective studies is a crucial step to determine their predictive value and to facilitate their integration into the clinic. In this exploratory study, we aimed to identify new biomarkers of sunitinib efficacy and to validate others previously reported, focusing on hypoxia-related proteins using 71 ccRCC samples collected in an observational prospective study [14].

patients and methods

study population

The current work is a pre-planned observational prospective study aimed at the identification of molecular tumor markers of sunitinib efficacy in ccRCC, carried out in a population that was reported in an earlier study [14]. In this previous study, 101 patients with RCC treated with first-line sunitinib were included to investigate the association between SNPs and sunitinib efficacy and toxic effect. Drug schedule, dose reduction policy, and timing of radiological assessments were up to the discretion of the individual physicians, in accordance with current local practice guidelines. The present study used a subset of 71 consecutive patients of whom formalin-fixed paraffin-embedded tumor material was available.

immunohistochemical study

The detection of CAIX, HIF1A, HIF2A, VHL, VEGFA, VEGFR1, VEGFR2, VEGFR3, PDGFRB and P-glycoprotein (Pgp) was carried out by immunohistochemistry (IHC) in tissue microarrays (supplementary Methods, available at *Annals of Oncology* online) using specific antibodies (supplementary Table S1, available at *Annals of Oncology* online). The IHC scoring was based on the average percentage of cells with positive staining, as in the Human Protein Atlas (http://www.proteinatlas.org): 0%–5%: absent (0), 5%–25%: weak (1), 25%–75%: moderate (2) and >75%: strong (3). Supplementary Table S2, available at *Annals of Oncology* online shows the staining score for each protein marker analyzed and the number of tumors included in each category.

assessment of *VHL* inactivation

VHL mutations were detected through PCR (supplementary Methods, available at *Annals of Oncology* online), and tumors harboring *VHL* frameshifts, stop gains, stop losses, splicing defects, mutations previously described as pathogenic and mutations in codons with previously described pathogenic mutations, were considered pathogenic ($n = 17$, supplementary Table S3, available at *Annals of Oncology* online). In addition, c.338G>A (R113Q) was considered pathogenic based on Polyphen2 predictions (http://genetics.bwh.harvard.edu/pph2/). Cases carrying a pathogenic *VHL* mutation and cases without VHL protein expression were classified as *VHL* inactivated ($n = 20$). Only tumors successfully sequenced for all three exons, with a wild type *VHL* sequence and a positive staining of VHL protein were considered to have a non-altered VHL function ($n = 11$); otherwise, VHL inactivation status was defined as non-assessable.

EGLN3 mRNA quantification

The mRNA content of *EGLN3* was quantified through qRT-PCR using specific primers and probes (supplementary Methods, available at *Annals of Oncology* online). Normalization was carried out with the internal standard β-glucuronidase and the ΔΔCt method was used for the calculation of mRNA content. In 65 of the 67 primary ccRCC samples, *EGLN3* mRNA was successfully quantified.

statistical analyses

This study defined objective response according to RECIST criteria, and PFS and overall survival (OS) as previously described [14]. Protein expression and VHL inactivation status were tested as dichotomous variables and *EGLN3* mRNA content was recorded as a continuous variable. These variables were tested against progression of the disease (PD) as best objective response using logistic regression, and against PFS and OS using Cox regression. Proteins with a $P < 0.1$ in the univariate analyses were selected for multivariable analyses, including as covariates the MSKCC prognostic classification and gender [14]. Given the explorative nature of this study, the *P*-values were not corrected for multiple testing. The correlation between the expression of the different proteins, VHL status and *EGLN3* mRNA content was evaluated using the Spearman test. Bilateral *P*-values <0.05 were considered significant. SPSS version 19.0 was used for statistical analysis.

results

study population

Of the initial 71 patients that had undergone nephrectomy, four were excluded from the analysis (two corresponded to primary RCCs with a histology different from ccRCC and two to patients with no primary tumor material available, only metastases). The main characteristics of the 67 patients included in the analysis are presented in Table 1. The median PFS of the patients after sunitinib treatment was 12.7 months and 46 (69%) were alive at the time of the analysis. Objective response was assessed in 60 patients with measurable disease: 31 (52%) had partial response, 20 (33%) had stabilization of the disease and 9 (15%) had PD. Patients with high MSKCC risk factor scores had worse OS (HR = 2.2, 95% confidence interval (CI) 1.0–4.7, $P = 0.045$) and showed a tendency of higher risk of PD as best response ($P = 0.066$). Women showed a trend for higher PD risk ($P = 0.078$). These clinical characteristics are similar to those described previously for the whole series [14].

Table 1. Patient and clinical characteristics

Characteristic	No.	%
Age at sunitinib (years)	66	
Min–max (IQR[a])	46–82 (55–72)	
Sex		
Male	45	67
Female	22	33
ECOG score		
0	19	28
1	37	55
2	6	9
Unknown	5	7
No. of metastatic sites		
1	17	25
2	35	52
3	11	16
4	4	6
Common metastasis sites		
Lung	49	73
Lymph nodes	34	51
Bone	16	24
Kidney	12	18
Liver	9	13
MSKCC risk factors[b]		
0 (favorable)	36	54
1–2 (intermediate)	30	45
≥3 (poor)	1	1

[a]Interquartile range.

[b]Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic factors: ECOG performance status >1, high LDH levels (>1.5 times upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dl) and no nephrectomy.

proteins involved in hypoxia and angiogenesis are associated with outcome of sunitinib treatment

We compared the expression of nine different proteins related to hypoxia, angiogenesis or sunitinib transport in the tumors. As shown in supplementary Table S2, available at *Annals of Oncology* online, there was a substantial variability in the expression of these proteins in the primary ccRCC tumors and the expression of several of these proteins was correlated. The strongest correlation was between VEGFA and VEGFR2 ($r = 0.60$ and $P = 2 \times 10^{-7}$, supplementary Table S4, available at *Annals of Oncology* online).

When we studied the association between the expression of specific proteins and sunitinib response, we found that high expression of HIF2A and PDGFRB was associated with protection against PD [odds ratio (OR) = 0.11, 95% CI 0.02–0.75, $P = 0.024$ and OR = 0.04, 95% CI 0.002–0.68, $P = 0.026$, respectively, Table 2]. With respect to PFS, high VEGFR3 expression was associated with longer times to progression (HR = 0.40, 95% CI 0.20–0.82, $P = 0.012$; Figure 1). Regarding OS, high VEGFA expression was associated with short survival times (HR = 4.29, 95% CI 1.43–12.8, $P = 0.0092$; Figure 1), and high HIF2A expression with long survival times (HR = 0.39, 95% CI 0.15–0.99, $P = 0.048$; Figure 1).

Interestingly, we had previously found that the missense variants of *VEGFR3* rs307826 and rs307821, in high linkage disequilibrium, were associated with short PFS in sunitinib-treated patients [14]. When we compared VEGFR3 protein expression with rs307826 and rs307821, we found a significant correlation, with the variant alleles showing a significantly lower expression of VEGFR3 protein ($r = -0.38$, $P = 0.0019$ and $r = -0.32$, $P = 0.011$, respectively, Figure 2). Other polymorphisms in *VEGFA* (rs699947, rs2010963, rs1570360) and *VEGFR2* (rs699947, rs2010963, rs1570360) did not show a correlation with the expression of their respective proteins.

Table 2. Proteins associated with sunitinib efficacy in ccRCC

Protein	Univariate analyses[a]			Multivariable analyses[b]		
	HR[c]	95% CI	*P*-value	HR[c]	95% CI	*P*-value
PD as best response						
HIF2A	0.20	0.04–0.91	**0.037**	0.11	0.017–0.75	**0.024**
PDGFRB	0.13	0.014–1.08	0.059	0.04	0.002–0.68	**0.026**
Pgp	8.00	0.93–69.2	0.059	7.08	0.76–66.3	0.086
PFS						
VEGFR3	0.39	0.20–0.80	**0.0093**	0.403	0.20–0.82	**0.012**
VEGFA	2.56	1.04–6.29	**0.041**	2.560	0.99–6.63	0.053
HIF2A	0.52	0.26–1.04	0.064	0.537	0.26–1.13	0.100
OS						
VEGFA	3.58	1.38–9.33	**0.0089**	4.29	1.43–12.8	**0.0092**
HIF2A	0.34	0.14–0.83	**0.017**	0.39	0.15–0.99	**0.048**
VEGFR2	2.14	0.90–5.09	0.086	2.05	0.85–4.91	0.108
Pgp	2.41	0.88–6.57	0.087	2.21	0.81–6.07	0.123

P-values <0.05 are shown in bold.

[a]Only proteins with $P < 0.1$ are presented; proteins with $P < 0.1$ in the univariate analyses were selected for multivariable analyses.

[b]Multivariable analyses included as covariates the MSKCC prognostic classification and gender.

[c]HR <1.0 indicates that high expression of the protein associates with better outcome, HR >1.0 indicates association with worse outcome.

EGLN3 mRNA content is associated with the overall survival of the patients

VHL inactivation and hypoxia, which in RCC correlate with *EGLN3* mRNA content [13], could be relevant markers of response to antiangiogenic drugs. In fact, we found that *EGLN3* mRNA content, quantified in 65 cases, showed a positive correlation with CAIX ($r = 0.44$, $P = 0.0007$) and with *VHL* inactivation ($r = 0.42$, $P = 0.02$; supplementary Table S4, available at *Annals of Oncology* online). In addition, *EGLN3* mRNA content was negatively correlated with the expression of PDGFRB ($r = -0.46$, $P = 0.0002$).

To determine whether VHL inactivation and *EGLN3* mRNA content were associated with PD as best response, PFS and OS, we carried out a multivariable analysis. We did not find an association between *VHL* inactivation and sunitinib efficacy, but we found that high *EGLN3* mRNA content was associated with a shorter OS ($P = 0.023$). When the RCCs were divided into high and low *EGLN3* expression groups, using the mean expression as the cutoff value rather than using *EGLN3* mRNA as a continuous variable, cases with high expression had an HR of 2.89 (95% CI 1.04–8.05, $P = 0.042$, data not shown).

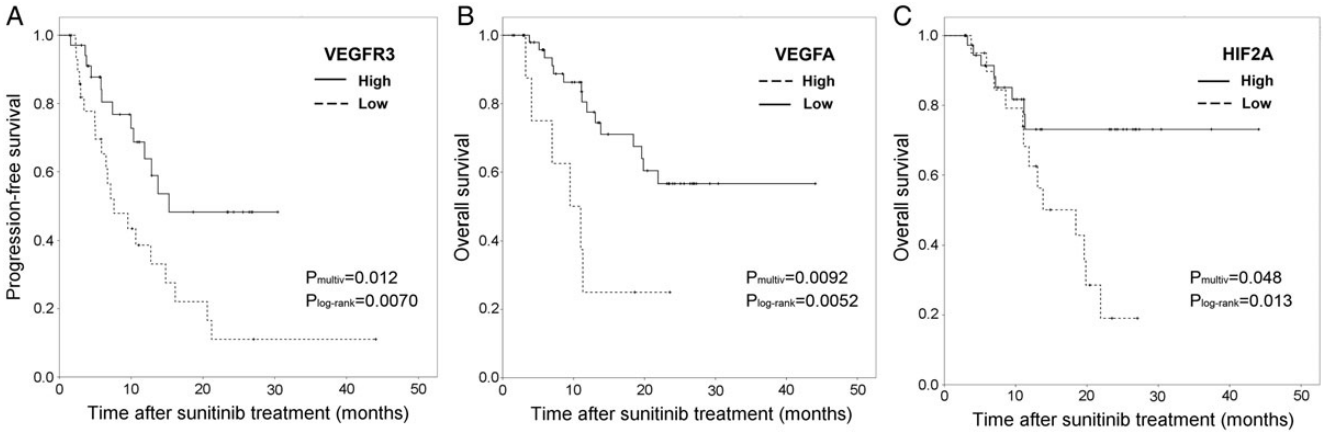

Figure 1. Progression-free survival and overall survival in ccRCC patients after treatment with sunitinib. (A) Progression-free survival of patients grouped according to VEGFR3 protein expression; median progression-free survival for patients with tumors with low and high VEGFR3 expression was 7.6 and 15.3 months, respectively. (B) Overall survival of patients grouped according to VEGFA protein expression; median overall survival for patients with tumors with low VEGFA expression was not reached and for patients with tumors with high VEGFA expression it was 9.5 months. (C) Overall survival of patients grouped according to HIF2A protein expression; median overall survival for patients with tumors with low HIF2A expression was 18.4 months and, for patients with tumors with high HIF2A, it was not reached. *P*-values are from multivariable Cox regression analysis and from the univariate log-rank test.

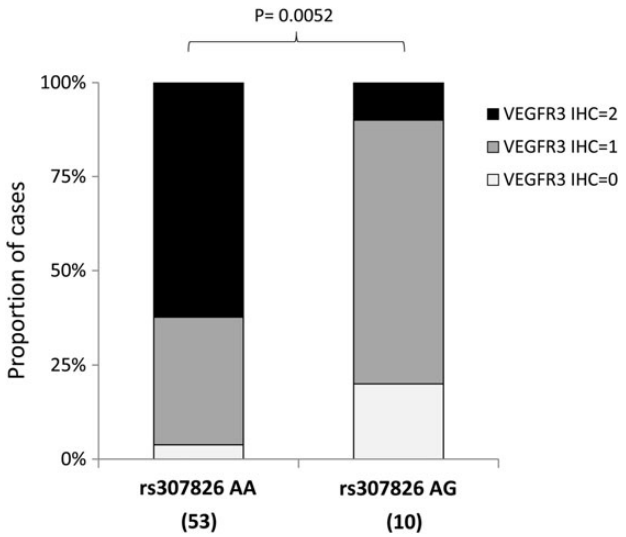

Figure 2. Immunohistochemical staining of VEGFR3 protein according to *VEGFR3* rs307826 genotype. Of the 53 cases with the rs307826 AA genotype, 2 (4%), 18 (34%) and 33 (62%) had a VEGFR3 staining of 0, 1 and 2, respectively. Of the 10 cases with the rs307826 AG genotype, 2 (20%), 7 (70%) and 1 (10%) had a VEGFR3 staining of 0, 1 and 2, respectively. The *P*-value shown was calculated by the χ^2 test.

discussion

In this study, we focused on the identification of tumor markers of response to sunitinib, by using a prospective series of patients receiving sunitinib for advanced ccRCC as first-line treatment [14]. We found that *EGLN3* mRNA content and VEGFA expression were associated with worse outcome, while overexpression of HIF2A, PDGFRB and VEGFR3 were associated with better response and survival.

VEGFR3 is a transmembrane tyrosine kinase receptor traditionally associated with lymphangiogenesis; however, recent studies have shown expression of VEGFR3 in the tumor blood vasculature [15]. VEGFR3 has been proposed as a pharmacodynamic marker for sunitinib response [16, 17]. In addition, we previously found that VEGFR3 was associated with worse outcome of sunitinib treatment through two missense polymorphisms in high linkage disequilibrium (rs307826, T494A; rs307821, R1324L) [14]. In the present study, we found a large variability in VEGFR3 expression in tumors and a statistically significant association between a high tumor expression of VEGFR3 protein and longer PFS of the patients treated with sunitinib ($P = 0.012$, Table 2). Interestingly, we also found a strong correlation between VEGFR3 protein expression in the tumor and rs307826 and rs307821 *VEGFR3* variant alleles. The mechanisms by which these polymorphisms exert their effect is unknown; however, these results may imply a low stability of the variant VEGFR3 protein, which in turn could lead to decreased sunitinib effects.

VEGFA has been studied in relation with sunitinib treatment as a dynamic marker [17, 18] and SNPs in *VEGFA* have been evaluated as predictors of sunitinib response [14, 19]; however, the results obtained are inconclusive. Regarding its expression, a study suggested a better outcome in tumors with high VEGFA content [20], but our study showed a significant association between high VEGFA expression and worse OS ($P = 0.0092$, Table 2), similar to other studies investigating RCC prognostic factors [21, 22].

HIFs are oxygen-sensitive transcription factors, which regulate biological processes that promote adaptation to oxygen deprivation. In our study, overexpression of HIF2A was significantly associated with clinical benefit, longer OS and a tendency for longer PFS of the patients (Table 2). In agreement, there are some studies that have associated high HIF2A levels with improved response to sunitinib [12]. Concerning VHL, a key regulator of the hypoxia response pathway and the gene most frequently mutated in ccRCC, our study did not find an association between VHL inactivation and sunitinib efficacy, in

line with previous works [7, 23]. However, the number of samples evaluated was relatively small ($n = 31$). To get an accurate assessment of hypoxia in the tumors, we quantified the mRNA content of *EGLN3* [13], and found a positive correlation with CAIX ($P = 0.00072$) and VHL inactivation ($P = 0.02$). Our data support previous studies in which CAIX failed to predict response and survival in ccRCC [9, 11, 12]; however, we found that high *EGLN3* mRNA content was associated with short OS ($P = 0.023$). This suggests that highly hypoxic tumors, although might have a better initial response to antiangiogenic drugs, could have a worse prognosis, as evidenced by the shorter OS found for tumors with high VEGFA or high *EGLN3* expression.

Some limitations of this study are caused by intrinsic characteristics of the techniques used, such as IHC scoring and *EGLN3* mRNA measurement, which can vary among institutions since these determinations are not used in daily practice and no consensus criteria for quantification have been established yet. However, the IHC scoring was carried out independently by two pathologists and *EGLN3* expression was measured using a quantitative technique. Other caveats are related to the observational nature of the study, which cannot distinguish among predictive and prognostic markers, but can only suggest associations. In addition, subsequent anticancer treatments, that could potentially impact OS, were not recorded. There is also the need to validate the results of this exploratory study in an independent series of patients, to precisely determine the predictive value of the proposed markers.

In conclusion, we have confirmed HIF2A and VEGFR3 as potential predictors of sunitinib efficacy in ccRCC, and found an association between high VEGFA expression and shorter OS. In addition, significant correlations between rs307826 and rs307821 and VEGFR3 protein content was identified for the first time, suggesting a possible biological mechanism for the worse outcome of sunitinib treatment associated with these variant alleles. Finally, a new marker of ccRCC hypoxia, *EGLN3* expression, was associated with short OS. If these finding are validated in independent series, prospective interventional trials based on molecular biomarkers and aiming at the identification of new therapeutic strategies for ccRCC should be undertaken to ultimately improve RCC outcome.

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disclosure

JG-D and JB receive consultant and advisory board fees from Pfizer. The other authors declare that they have no conflicts of interest.

references

1. Gupta K, Miller JD, Li JZ et al. Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev 2008; 34: 193–205.
2. Escudier B, Szczylik C, Porta C et al. Treatment selection in metastatic renal cell carcinoma: expert consensus. Nat Rev Clin Oncol 2012; 9: 327–337.
3. Rodriguez-Antona C, Garcia-Donas J. Constitutional genetic variants as predictors of antiangiogenic therapy outcome in renal cell carcinoma. Pharmacogenomics 2012; 13: 1621–1633.
4. Perez-Gracia JL, Prior C, Guillen-Grima F et al. Identification of TNF-alpha and MMP-9 as potential baseline predictive serum markers of sunitinib activity in patients with renal cell carcinoma using a human cytokine array. Br J Cancer 2009; 101: 1876–1883.
5. Gruenwald V, Beutel G, Schuch-Jantsch S et al. Circulating endothelial cells are an early predictor in renal cell carcinoma for tumor response to sunitinib. BMC Cancer 2010; 10: 695.
6. Rini BI, Jaeger E, Weinberg V et al. 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 2006; 98: 756–762.
7. Choueiri TK, Vaziri SA, Jaeger E et al. von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. J Urol 2008; 180: 860–865; discussion 865–866.
8. Pena C, Lathia C, Shan M et al. Biomarkers predicting outcome in patients with advanced renal cell carcinoma: results from sorafenib phase III Treatment Approaches in Renal Cancer Global Evaluation Trial. Clin Cancer Res 2010; 16: 4853–4863.
9. Motzer RJ, Hutson TE, Hudes GR et al. Novel circulating proteins, single nucleotide polymorphisms, and molecular tumor markers as potential biomarkers of sunitinib efficacy in treatment-naive patients with advanced renal cell carcinoma. Abstract, ESMO 2012 Congress 2012.
10. Gossage L, Eisen T. Alterations in VHL as potential biomarkers in renal-cell carcinoma. Nat Rev Clin Oncol 2010; 7: 277–288.
11. Choueiri TK, Regan MM, Rosenberg JE et al. Carbonic anhydrase IX and pathological features as predictors of outcome in patients with metastatic clear-cell renal cell carcinoma receiving vascular endothelial growth factor-targeted therapy. BJU Int 2010; 106: 772–778.
12. Patel PH, Chadalavada RS, Ishill NM et al. Hypoxia-inducible factor (HIF) 1alpha and 2alpha levels in cell lines and human tumor predicts response to sunitinib in renal cell carcinoma (RCC). J Clin Oncol 2008; 26: Abstract 5008.
13. Dalgliesh GL, Furge K, Greenman C et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 2010; 463: 360–363.
14. Garcia-Donas J, Esteban E, Leandro-Garcia LJ et al. Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicentre, observational, prospective study. Lancet Oncol 2011; 12: 1143–1150.
15. Smith NR, Baker D, James NH et al. Vascular endothelial growth factor receptors VEGFR-2 and VEGFR-3 are localized primarily to the vasculature in human primary solid cancers. Clin Cancer Res 2010; 16: 3548–3561.
16. Rini BI, Michaelson MD, Rosenberg JE et al. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. J Clin Oncol 2008; 26: 3743–3748.
17. Deprimo SE, Bello CL, Smeraglia J et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. J Transl Med 2007; 5: 32.
18. Kontovinis LF, Papazisis KT, Touplikioti P et al. Sunitinib treatment for patients with clear-cell metastatic renal cell carcinoma: clinical outcomes and plasma angiogenesis markers. BMC Cancer 2009; 9: 82.
19. Kim JJ, Vaziri SA, Rini BI et al. Association of VEGF and VEGFR2 single nucleotide polymorphisms with hypertension and clinical outcome in metastatic clear cell renal cell carcinoma patients treated with sunitinib. Cancer 2012; 118: 1946–1954.

20. Paule B, Bastien L, Deslandes E et al. Soluble isoforms of vascular endothelial growth factor are predictors of response to sunitinib in metastatic renal cell carcinomas. PLoS One 2010; 5: e10715.
21. Phuoc NB, Ehara H, Gotoh T et al. Prognostic value of the co-expression of carbonic anhydrase IX and vascular endothelial growth factor in patients with clear cell renal cell carcinoma. Oncol Rep 2008; 20: 525–530.
22. Jacobsen J, Grankvist K, Rasmuson T et al. Expression of vascular endothelial growth factor protein in human renal cell carcinoma. BJU Int 2004; 93: 297–302.
23. Gad S, Sultan-Amar V, Meric J-B et al. Somatic von Hippel-Lindau (VHL) gene analysis and clinical outcome under antiangiogenic treatment in metastatic renal cell carcinoma: preliminary results. Target Oncol 2007; 2: 3–6.

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Germline single nucleotide polymorphisms associated with response of urothelial carcinoma to platinum-based therapy: the role of the host†

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Background: Variations in urothelial carcinoma (UC) response to platinum chemotherapy are common and frequently attributed to genetic and epigenetic variations of somatic DNA. We hypothesized that variations in germline DNA may contribute to UC chemosensitivity.
Patients and methods: DNA from 210 UC patients treated with platinum-based chemotherapy was genotyped for 80 single nucleotide polymorphisms (SNPs). Logistic regression was used to examine the association between SNPs and response, and a multivariable predictive model was created. Significant SNPs were combined to form a SNP score predicting response. Eleven UC cell lines were genotyped as validation.
Results: Six SNPs were significantly associated with 101 complete or partial responses (48%). Four SNPs retained independence association and were incorporated into a response prediction model. Each additional risk allele was associated with a nearly 50% decrease in odds of response [odds ratio (OR) = 0.51, 95% confidence interval 0.39–0.65, $P = 1.05 \times 10^{-7}$). The bootstrap-adjusted area under the curves of this model was greater than clinical prognostic factors alone (0.78 versus 0.64). The SNP score showed a positive trend with chemosensitivity in cell lines ($P = 0.115$).
Conclusions: Genetic variants associated with response of UC to platinum-based therapy were identified in germline DNA. A model using these genetic variants may predict response to chemotherapy better than clinical factors alone.
Key words: biomarkers, carboplatin, cisplatin, genetics, germline, urothelial

introduction

Neoadjuvant cisplatin-based chemotherapy is the standard of care for the treatment of muscle-invasive urothelial carcinoma (UC) [1–3], while both cisplatin- and carboplatin-based therapy have efficacy in patients with metastatic disease [4, 5]. Unfortunately, despite being considered a chemotherapy-sensitive disease, only 32%–38% of patients with muscle-invasive disease and 40%–50% of patients with metastatic disease have a major response to platinum-based chemotherapy [1, 2, 4, 5]. Other than clinical stage of the primary tumor, pretreatment factors predictive of response in the neoadjuvant setting are lacking. In contrast, Karnofsky performance status (KPS) <80% and the presence of visceral (lung, liver, or bone) metastases have been used in the MSKCC clinical risk score [5, 6], which correlates with both response and survival in the

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