ANTICANCER RESEARCH *41*: 4875-4883 (2021)
doi:10.21873/anticanres.15301

CD44 Is Involved in Sunitinib Resistance and Poor Progression-free Survival After Sunitinib Treatment of Renal Cell Carcinoma

YOHEI SEKINO[1], KENSHIRO TAKEMOTO[1], DAIKI MURATA[1], TAKASHI BABASAKI[1,2], KOHEI KOBATAKE[1], HIROYUKI KITANO[1], KENICHIRO IKEDA[1], KEISUKE GOTO[1], SHOGO INOUE[1], TETSUTARO HAYASHI[1], DAIKI TANIYAMA[2], MASANOBU SHIGETA[3], KAZUYA KURAOKA[4], KOJI MITA[5], MAYUMI KANEKO[6], KAZUHIRO SENTANI[2], NAOHIDE OUE[2] and JUN TEISHIMA[1]

[1]Department of Urology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan;
[2]Department of Molecular Pathology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan;
[3]Department of Urology, National Hospital Organization, Kure Medical Center and Chugoku Cancer Center, Kure, Japan;
[4]Department of Diagnostic Pathology, National Hospital Organization, Kure Medical Center and Chugoku Cancer Center, Kure, Japan;
[5]Department of Urology, Hiroshima City Asa Citizens Hospital, Hiroshima, Japan;
[6]Department of Diagnostic Pathology, Hiroshima City Asa Citizens Hospital, Hiroshima, Japan

Abstract. *Background/Aim: Sunitinib continues to be administered as a first-line therapeutic agent in metastatic renal cell carcinoma (mRCC). This study aimed to examine the role of CD44 in sunitinib resistance and as a predictive marker in mRCC. Materials and Methods: We analyzed the effect of CD44 knockdown on sunitinib resistance in RCC cell lines using WST-1 assays. CD44 expression in mRCC patients treated with first-line sunitinib was determined by immunohistochemistry. We validated the findings of this study by in silico analysis. Results: CD44 knockdown increased sensitivity to sunitinib. Immunohistochemical analysis revealed that 19 (34.5%) of 55 mRCC cases were positive for CD44. CD44-positive cases were associated with poor progression-free survival (PFS) after first-line sunitinib treatment. In the JAVELIN 101 study, high CD44 expression was significantly associated with poor PFS after sunitinib but not after avelumab + axitinib therapy. Conclusion: CD44 is involved in sunitinib resistance and may be a promising marker for sunitinib treatment in mRCC.*

Correspondence to: Yohei Sekino (ORCID ID: 0000-0003-1525-5463), MD, PhD, Department of Urology, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Tel: +81 822575146, Fax: +81 822575149, e-mail: akikosekino@gmail.com

Key Words: CD44, renal cell carcinoma, sunitinib, *in silico* analysis.

Renal cell carcinoma (RCC) represents approximately 90% of all renal tumors (1). Radical nephrectomy remains the standard treatment for patients with localized RCC (2), while systemic drug therapy is an established treatment for metastatic RCC (mRCC) (3). Sunitinib is a multi-targeted tyrosine kinase inhibitor (TKI) with anti-angiogenic effects through its blockade of vascular endothelial growth factor receptor and platelet-derived growth factor receptor (4). Although immune checkpoint inhibitors have been introduced, sunitinib treatment is still used as the first-line therapy in mRCC (5, 6). However, most patients treated with sunitinib become refractory due to sunitinib resistance (7). Furthermore, there are few reports regarding biomarkers to select suitable patients for sunitinib treatment. Therefore, identifying new molecular mechanisms underlying sunitinib resistance and a biomarker for sunitinib treatment will greatly improve outcomes for mRCC patients treated with this drug.

CD44 is a multi-structural and multi-functional transmembrane glycoprotein and a receptor for hyaluronan encoded by the *CD44* gene (8). CD44 is a cancer stem cell (CSC) marker that communicates with the microenvironment and regulates properties of stemness (9). A number of studies have reported that CD44 is involved in cancer initiation,

progression, and drug resistance in several cancers (10). Although some studies have shown that high CD44 expression was associated with poor progression-free survival (PFS) in mRCC treated with sunitinib (11, 12), the sample size was small. In this study, we examined the effect of CD44 knockdown on sunitinib resistance in RCC cell lines. We investigated the potential role of CD44 as a predictive marker for sunitinib treatment in mRCC using immunohistochemical analysis and *in silico* analysis. We also analyzed the involvement of CD44 in epithelial to mesenchymal transition (EMT).

Materials and Methods

Cell lines. The RCC cell lines 786-O, Caki-1, and ACHN were purchased from the American Type Culture Collection (Manassas, VA, USA). These cell lines were maintained as described previously (13). Sunitinib-resistant Caki-1 cells were established by culturing them with increasing concentrations of sunitinib (1-40 nM) for 6 months (14).

RNA interference. Silencer® Select (Ambion, Austin, TX, USA) against CD44 was used for RNA interference as described previously (15). Transfection was performed using Lipofectamine RNAiMAX (Invitrogen, CA, USA) according to the manufacturer's instructions. Cells were used 48 h after transfection.

Western blotting. For western blotting analysis, cells were lysed as described previously (16). The antibodiy against CD44 (Cell Signaling Technology, Inc., Danvers, MA, USA) was used at a 1:1000 dilution. β-actin (Sigma-Aldrich, St. Louis, MO, USA) was detected as a loading control.

Drug treatment. Sunitinib maleate was obtained from Funakoshi (Tokyo, Japan) and handled according to the manufacturer's recommendations. Cell lines were treated with vehicle (0.5% ethanol) or escalating doses of sunitinib. A WST-1 assay was performed to assess cell viability after exposure to sunitinib treatment for 48 h. Drug sensitivity curves and IC_{50} values were calculated using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA).

Tissue samples. We used 55 metastatic RCC tissue samples (Hiroshima cohort: Table I) for immunohistochemical analysis. The samples were collected from patients at Hiroshima University Hospital, Kure Medical Center, Chugoku Cancer Center, and Hiroshima City Asa Citizens Hospital under an institutional review board-approved protocol (IRB# E912: Hiroshima University, 2019-08: Kure Medical Center/Chugoku Cancer Center, 01-3-14: Hiroshima City Asa Citizens Hospital). Written comprehensive approvals for basic or clinical research were obtained from all patients whose samples were used. This study was conducted in accordance with the Ethical Guidance for Human Genome/Gene Research of the Japanese Government.

Immunohistochemistry. Immunohistochemistry was performed as described previously (17). Sections were incubated with an antibody against CD44 (1:100) (DAKO, Glostrup, Denmark) for 1 h at room temperature. CD44 expression in RCC was scored in all tumors as positive or negative. When more than 10% of tumor cells were stained, the specimen was considered positive for CD44 (according to the median cut-off values rounded to the nearest 10%). Using these definitions, two observers (KS and NO) without knowledge of the patients' clinical and pathologic parameters or outcomes independently reviewed immunoreactivity in each specimen.

Table I. *Clinicopathologic characteristics of 55 mRCC patients treated with sunitinib*.

Number of cases	55
Median age (years)	68 (40-89)
Gender	
Male	40
Female	15
Race	
Asian	55
Median follow-up periods (months)	4 (1-74)
Histology	
Clear cell	42
Papillary	4
Chromophobe	3
Unclassified	6
Metastasis sites	
Lung	43
Lymph node	20
Bone	17
Brain	5
Liver	7
Nephrectomy	
Yes	49
No	6
IMDC criteria	
Favorable	5
Intermediate	32
Poor	18
Tumor response	
Complete response	1
Partial response	6
Stable disease	27
Progression disease	21

mRCC: Metastatic renal cell carcinoma.

In silico analysis. The expression array data were downloaded from Gene Expression Omnibus (GEO) and Array Express under accession numbers GSE59264 (18), GSE64052 (19), GSE76088 (20), and E-MTAB-3267 (21). Gene expression data from 371 mRCC patients treated with sunitinib and 362 mRCC patients treated with avelumab+axitinib were downloaded from the JAVELIN RENAL 101 study by Motzer *et al*. (22). Gene expression data from 286 mRCC patients were downloaded from the checkmate 010-025 study (23). The information about sarcomatoid changes in the TCGA cohort was downloaded from the study by Bakouny *et al*. (24). The UCSC Xena web tool was used to determine the EMT signature (Table II) (25, 26).

Statistical analysis. All experiments were repeated at least three times with each sample in triplicate. The results are expressed as the mean±S.D. of the triplicate measurements. Statistical differences were evaluated using the Mann–Whitney *U*-test. A p-value of <0.05

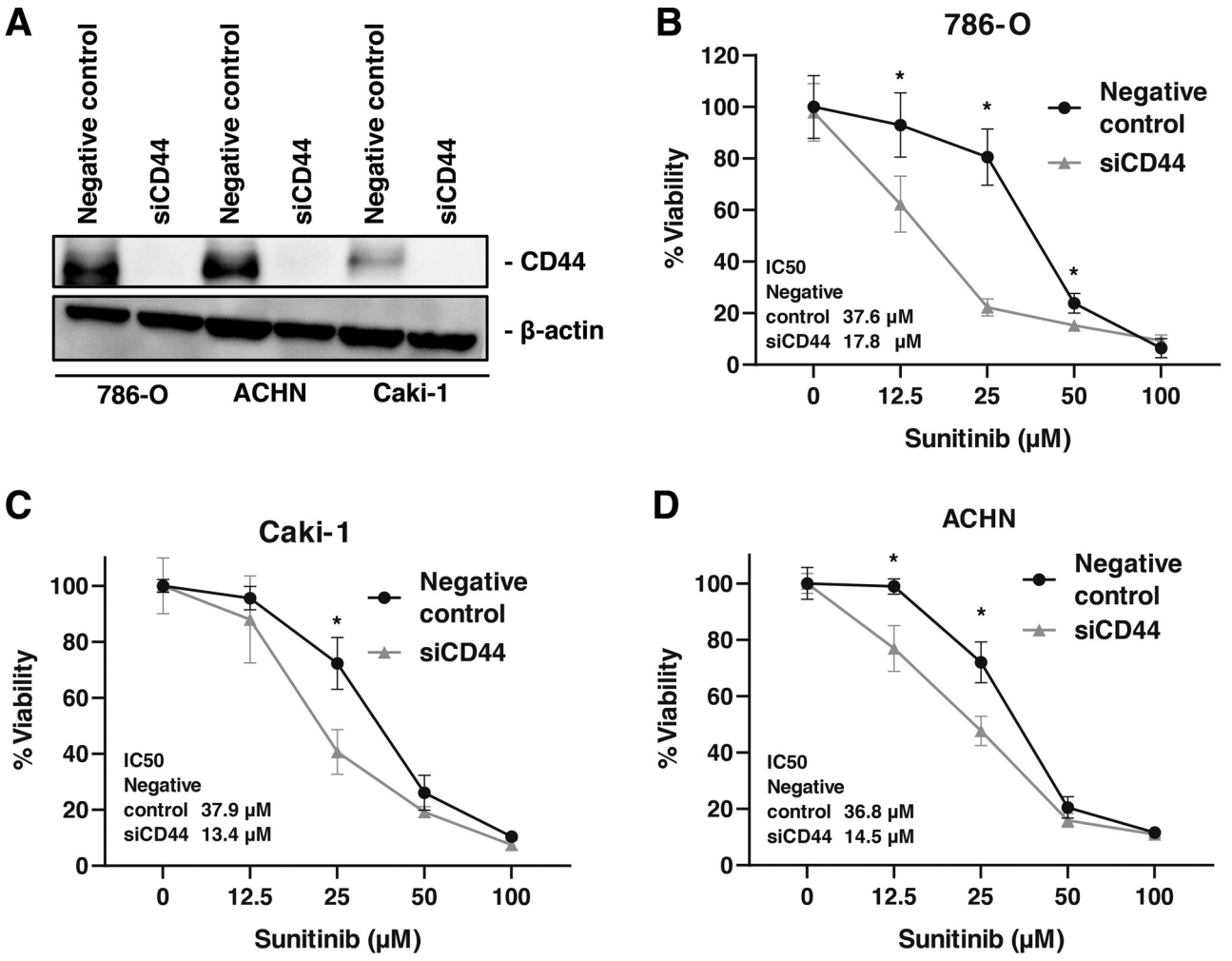

Figure 1. *CD44 knockdown increases sensitivity of renal cell carcinoma cell lines to sunitinib. (A) Western blotting of CD44 in 786-O, ACHN, and Caki-1 cells transfected with negative control or siRNA targeting CD44. β-actin was used as a loading control. (B-D) Dose-dependent effect of sunitinib on the viability of 786-O, Caki-1, and ACHN cells transfected with negative control or siRNA targeting CD44. The 50% inhibitory concentration (IC_{50}) values are indicated. *p<0.05.*

Table II. *Epithelial to mesenchymal transition signature.*

Gene	VIM+CDH2+FOXC2+SNAI1+SNAI2+TWIST1+GSC+FN1+ITBG6+MMP2+MMP3+MMP9+SOX10-CDH1-DSP-TJP1

was considered statistically significant. Kaplan–Meier analyses were performed, and the log-rank Mantel-Cox test was used to determine any statistical difference between the survival curves of the cohorts. Statistical analyses were conducted primarily using GraphPad Prism software (GraphPad Software Inc.).

Results

CD44 knockdown increased sensitivity to sunitinib in renal cell carcinoma. We examined CD44 expression and the efficacy of CD44 knockdown in RCC cell lines. Western blotting showed that CD44 is expressed in the 786-O, ACHN, and Caki-1 cells (Figure 1A). We used RNA interference targeting CD44 in the 786-O, ACHN, and Caki-1 cells and confirmed the efficiency of CD44 knockdown (Figure 1A). Then, we performed WST-1 assays to measure cell viability in these three cell lines with knockdown of CD44 under various concentrations of sunitinib. The WST-1 assays showed that down-regulation

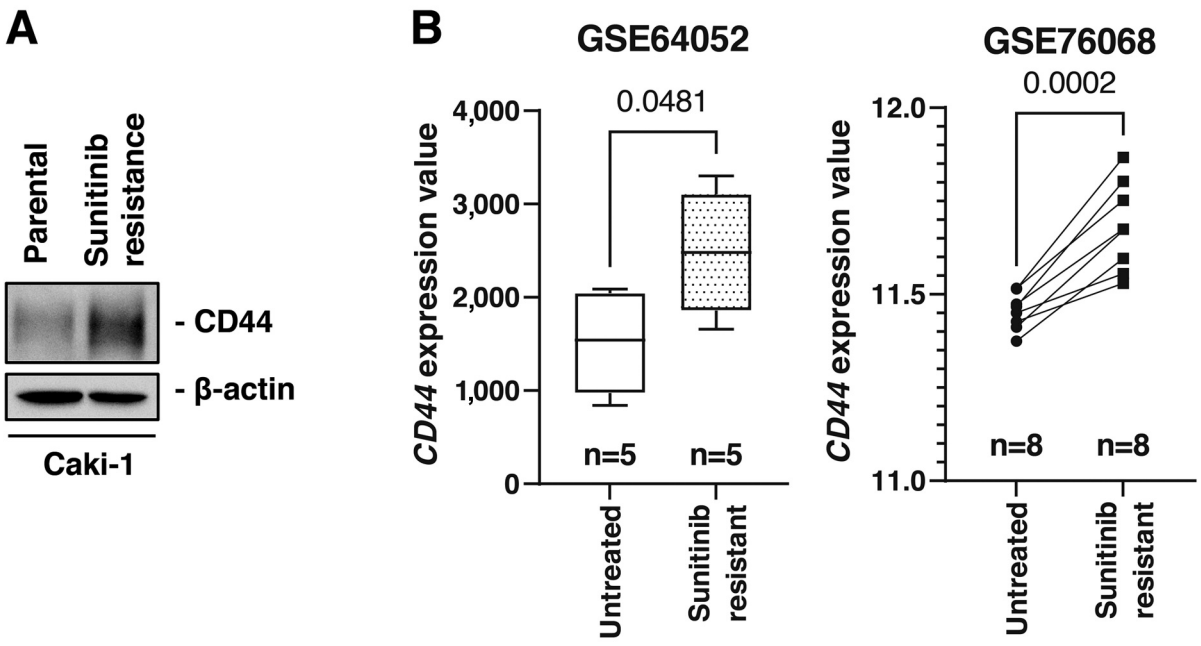

Figure 2. *CD44 is increased in sunitinib-resistant status. (A) Western blotting for CD44 in parental and sunitinib-resistant Caki-1 cells. β-actin was used as a loading control. (B) CD44 expression value in untreated and sunitinib-resistant samples from GSE64052 and GSE76088.*

of CD44 increased the sensitivity of the RCC cell lines to sunitinib (Figure 1B-D).

CD44 is over-expressed in sunitinib-resistant status. To verify whether CD44 is involved in sunitinib resistance, we investigated the expression of CD44 in parental and sunitinib-resistant Caki-1 cells. Western blotting showed that CD44 expression was increased in sunitinib-resistant Caki-1 cells compared with that in parental Caki-1 cells (Figure 2A). We also found that CD44 expression was increased in sunitinib-resistant samples compared to untreated samples from public databases (GSE64052 and GSE76088) (Figure 2B).

Clinical significance of CD44 in response to sunitinib treatment in mRCC. We performed immunohistochemistry for CD44 in 55 patients with mRCC (Hiroshima cohort) treated with sunitinib as first-line treatment to analyze the association between CD44 expression and therapeutic outcomes. Positive CD44 expression was found in 19 of the 55 (34.5%) patients (Figure 3A). The CD44-positive cases were associated with the unfavorable outcome: stable disease/ progression disease (p=0.011) (Table III). Kaplan–Meier analysis revealed that the CD44-positive cases treated with sunitinib as first-line treatment were associated with poor PFS (p=0.023) (Figure 3B). Although the p-value did not reach statistical significance, high *CD44* expression tended to be associated with poor PFS of patients with mRCC treated with first-line sunitinib in the public database (E-MTAB-3267) (p=0.128) (Figure 3C). In the JAVELIN101 study, the PFS of first-line avelumab+axitinib, and first-line sunitinib were examined in mRCC (22). High *CD44* expression was significantly associated with poor PFS in mRCC treated with first-line sunitinib (p=0.018) (Figure 3D). In contrast, high *CD44* expression was not associated with poor PFS in mRCC treated with first-line avelumab+axitinib (Figure 3E). In the CD44 high group, PFS was significantly longer in patients with mRCC treated with avelumab+axitinib than in those treated with sunitinib (Figure 3F).

CD44 is associated with epithelial to mesenchymal transition and increased in tissues with sarcomatoid changes. To clarify the involvement of CD44 in sunitinib resistance, we focused on epithelial to mesenchymal transition (EMT) because EMT is reported to be involved in TKI resistance in mRCC (27). In the JAVELIN 101 study, a moderate correlation between CD44 expression and EMT signature was found (p<0.001, R=0.43) (Figure 4A). Then, we analyzed the association between CD44 and sarcomatoid change in RCC. In the Hiroshima cohort, CD44 positive cases were associated with tissue sarcomatoid changes (p=0.050) (Table IV). In the public database (GSE59264), *CD44* expression was higher in sarcomatoid components than that in epithelial components (Figure 4B). Furthermore, in TCGA KIRC and the clinical study (Checkmate 010, 025), *CD44* expression was increased

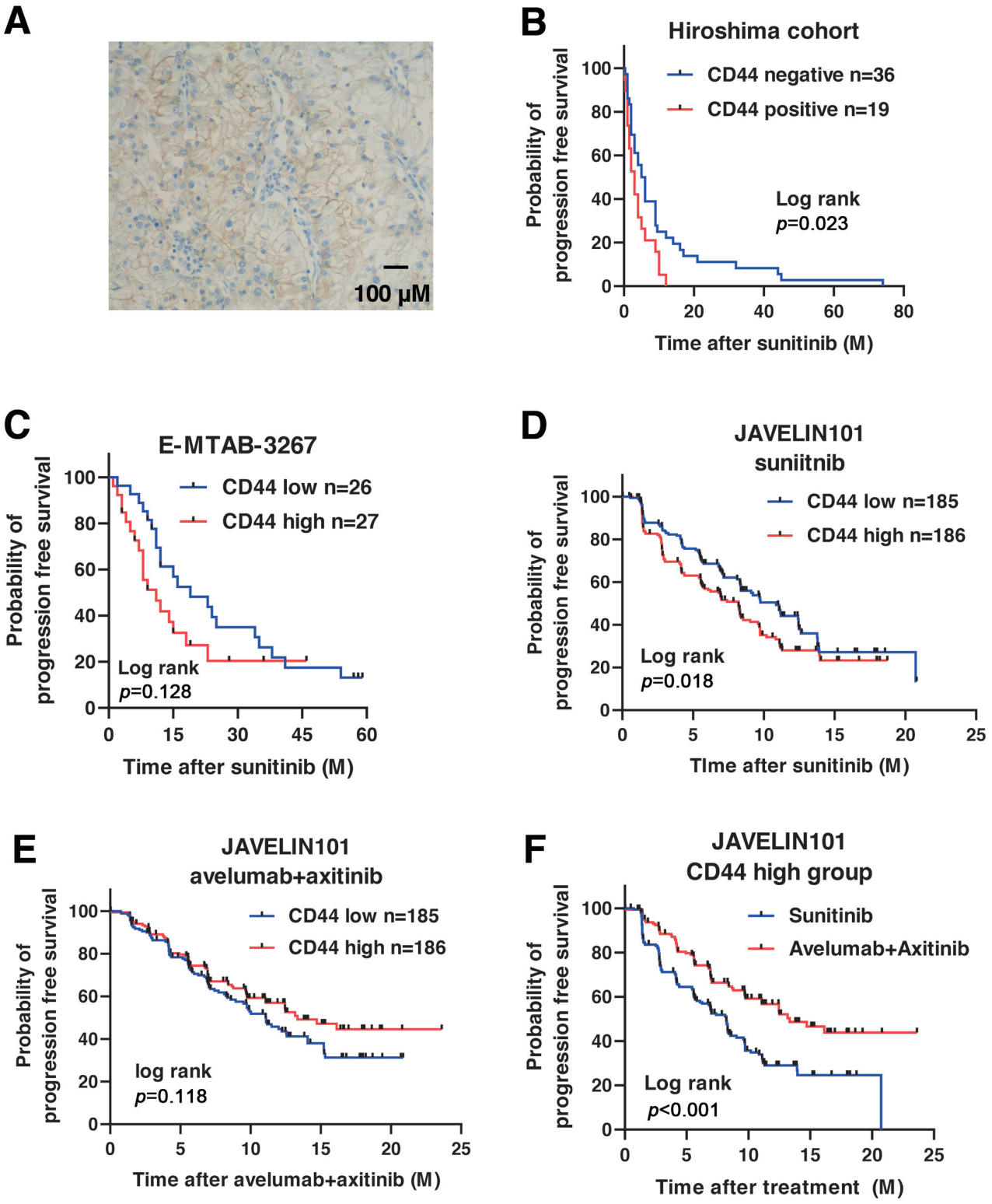

Figure 3. *Clinical significance of CD44 in response to sunitinib treatment in metastatic RCC (mRCC). (A) Immunohistochemical staining for CD44 in mRCC. Original magnification: 400×. (B-D) Kaplan–Meier plot of progression-free survival of mRCC patients treated with sunitinib according to CD44 expression from the Hiroshima cohort, E-MTAB-3267, and the JAVELIN 101 study. (E) Kaplan–Meier plot of progression-free survival of mRCC patients treated with avelumab+axitinib according to CD44 expression from the JAVELIN 101 study. (F) Kaplan–Meier plot of progression-free survival of mRCC patients treated with avelumab+axitinib and sunitinib in the CD44 high group from the JAVELIN 101 study.*

in the tissues with sarcomatoid changes (Figure 4C). In the Hiroshima cohort, the patients with sarcomatoid change were significantly associated with poor PFS in mRCC treated with first-line sunitinib (p=0.018) (Figure 4D). In the JAVELIN 101 study, a high EMT signature was significantly associated with poor PFS in mRCC treated with first-line sunitinib (p=0.007) (Figure 4E).

Discussion

Sunitinib resistance has been reported to be caused by multiple factors and molecular mechanisms (28, 29). Recent studies have reported that sunitinib targeted CSCs (30) and inhibited CSC-dependent tumor vasculogenesis in RCC (31). In breast cancer, dopamine has been shown to enhance the response to sunitinib partly through CSCs (32). Furthermore, CD133/CXCR4-expressing RCC cell lines, which are considered to be CSCs, showed decreased sensitivity to sunitinib (33). These findings indicate that CSCs play an essential role in sunitinib resistance. In the present study, we showed that CD44 knockdown increased the sensitivity to sunitinib in RCC cell lines. CD44 was upregulated in sunitinib-resistant Caki-1 cells, and this was also validated in public databases, indicating that CD44 is involved in sunitinib resistance in RCC. A recent review has shown that CD44 plays an essential role in EMT (10). In this study, CD44 expression was positively correlated with EMT signature in the public database JAVELIN 101. These findings may help to explain the mechanism through which CD44 is involved in sunitinib resistance. Although so far, the drug targeting CD44 has not been clinically utilized, a recent review reported that preclinical studies targeting CD44 are ongoing in various cancers (34). Collectively, these findings indicate that targeting CD44 may be a promising strategy to overcome sunitinib resistance in mRCC.

Although several biomarkers for sunitinib treatment have been reported so far (35), clinically relevant biomarkers to predict sunitinib response in mRCC are lacking. A recent study showed that high CD44 expression was associated with poor PFS after sunitinib treatment in RCC (12). In our study, CD44-positive cases were also associated with poor prognosis after sunitinib treatment. An ideal predictive biomarker is easily measured by using immunohistochemistry in a simple, inexpensive, and reliable way. Moreover, tumor tissue is routinely available for immunohistochemical analysis because patients with mRCC are usually treated by partial or radical nephrectomy. Of note, our findings were validated in the public database JAVELIN 101, which is a relatively large cohort. We also showed that high CD44 expression was not associated with PFS after avelumab + axitinib, but was significantly associated with poor PFS after sunitinib in the JAVELIN 101 study. A recent report has shown that ICI-based treatment improved efficacy *versus* sunitinib in patients with sarcomatoid changes (36). In our study, immunohistochemistry showed that CD44 positive cases were associated with patients with sarcomatoid changes, which was validated by *in silico* analysis. Collectively, these results suggest that CD44 may serve as a potential biomarker for drug selection.

There are some limitations in this study. First, we performed immunohistochemical analysis of CD44 expression using a relatively small sample size. Therefore, a study with a larger number of patients with mRCC will be necessary to verify the present findings. Second, CD44 has variant isoforms (37). In this study, we focused only on the standard form of CD44. In the future, we will analyze the involvement of variant forms of CD44 involved in sunitinib resistance.

In summary, our results showed that CD44 was involved in sunitinib resistance in RCC cell lines. The CD44-positive cases were associated with poor prognosis after sunitinib treatment, which was validated in public databases. CD44 expression was correlated with EMT signature and increased in patients with sarcomatoid changes. The data presented here highlight the potential of CD44 as a predictive marker and therapeutic target in patients with mRCC.

Table III. *Relationship between CD44 expression and tumor response in metastatic renal cell carcinoma treated with first line sunitinib.*

	CD44 expression		p-Value[a]
	Positive (n=19) (%)	Negative (n=36) (%)	
CR/PR (n=7)	0 (0%)	7 (100%)	
SD/PD (n=48)	19 (43%)	29 (57%)	0.011

CR: Complete response; PR: partial response; SD: stable disease; PD: progressive disease. [a]p-Values were calculated with Fisher's exact test.

Table IV. *Relationship between CD44 expression and sarcomatoid change in 55 metastatic renal cell carcinomas treated with first line sunitinib.*

	CD44 expression		p-Value[a]
	Positive (n=19) (%)	Negative (n=36) (%)	
Sarcomatoid change			
Negative n=43	12 (28%)	31 (72%)	
Positive n=12	7 (58%)	5 (42%)	0.050

[a]p-Values were calculated with Fisher's exact test.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

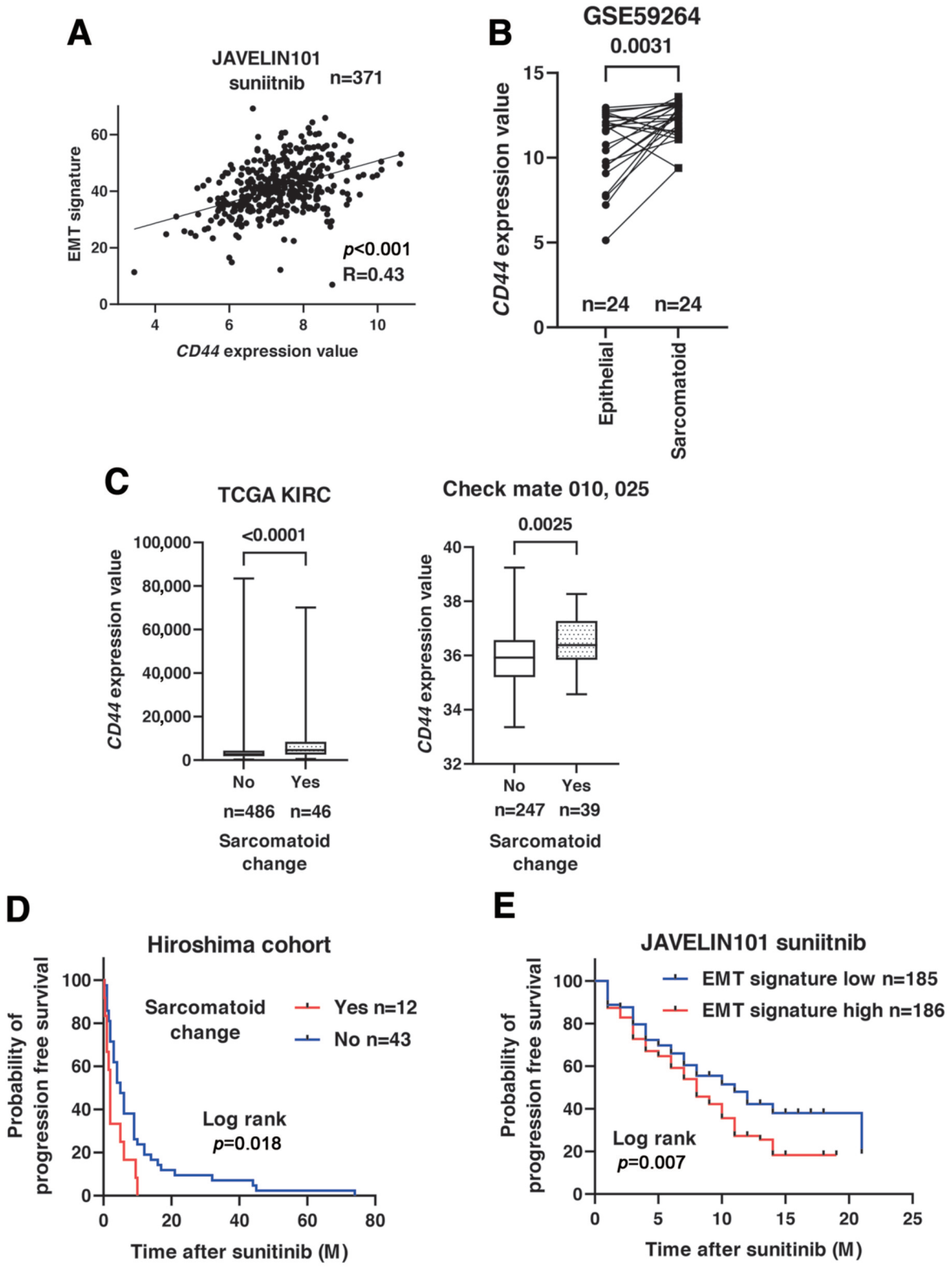

Figure 4. *CD44 is associated with epithelial to mesenchymal transition and increased in tissues with sarcomatoid changes. (A) The correlation between the expression of CD44 and epithelial-to-mesenchymal transition (EMT) signature in the JAVELIN 101 study. Spearman's correlation coefficients and p-Values are indicated. (B) CD44 expression value in epithelial and sarcomatoid samples from GSE59264. (C) CD44 expression value in untreated and sunitinib-resistant samples from GSE64052.*

Authors' Contributions

YS, and JT designed the study. TK, DM, SI, TH, DT, MS, KK, KM, and MK provided patients' clinical information. YS, TB, KK, HK, KI, and KG performed experiments and acquired data. KS and NO interpreted the results. YS drafted the manuscript. JT edited the article. All Authors approved the final content for journal submission and publication.

Acknowledgements

The Authors thank Mr. Shinichi Norimura for his excellent technical assistance. This work was carried out with the kind cooperation of the Research Center for Molecular Medicine of the Faculty of Medicine of Hiroshima University. The Authors would like to thank the Analysis Center of Life Science of Hiroshima University for the use of their facilities. This work was supported by the Japan Society for the Promotion of Science (19K18586).

References

1 Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, Heng DY, Larkin J and Ficarra V: Renal cell carcinoma. Nat Rev Dis Primers *3*: 17009, 2017. PMID: 28276433. DOI: 10.1038/nrdp.2017.9

2 Campbell SC, Clark PE, Chang SS, Karam JA, Souter L and Uzzo RG: Renal mass and localized renal cancer: evaluation, management, and follow-up: AUA guideline: Part I. J Urol *206(2)*: 199-208, 2021. PMID: 34115547. DOI: 10.1097/JU.0000000000001911

3 Hofmann F, Hwang EC, Lam TB, Bex A, Yuan Y, Marconi LS and Ljungberg B: Targeted therapy for metastatic renal cell carcinoma. Cochrane Database Syst Rev *10*: CD012796, 2020. PMID: 33058158. DOI: 10.1002/14651858.CD012796.pub2

4 Rini BI: Sunitinib. Expert Opin Pharmacother *8(14)*: 2359-2369, 2007. PMID: 17927489. DOI: 10.1517/14656566.8.14.2359

5 Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM and Figlin RA: Sunitinib *versus* interferon alfa in metastatic renal-cell carcinoma. N Engl J Med *356(2)*: 115-124, 2007. PMID: 17215529. DOI: 10.1056/NEJMoa065044

6 Quhal F, Mori K, Bruchbacher A, Resch I, Mostafaei H, Pradere B, Schuettfort VM, Laukhtina E, Egawa S, Fajkovic H, Remzi M, Shariat SF and Schmidinger M: First-line immunotherapy-based combinations for metastatic renal cell carcinoma: a systematic review and network meta-analysis. Eur Urol Oncol, 2021. PMID: 33757737. DOI: 10.1016/j.euo.2021.03.001

7 Morais C: Sunitinib resistance in renal cell carcinoma. J Kidney Cancer VHL *1(1)*: 1-11, 2014. PMID: 28326244. DOI: 10.15586/jkcvhl.2014.7

8 Tammi RH, Kultti A, Kosma VM, Pirinen R, Auvinen P and Tammi MI: Hyaluronan in human tumors: pathobiological and prognostic messages from cell-associated and stromal hyaluronan. Semin Cancer Biol *18(4)*: 288-295, 2008. PMID: 18468453. DOI: 10.1016/j.semcancer.2008.03.005

9 Skandalis SS, Karalis TT, Chatzopoulos A and Karamanos NK: Hyaluronan-CD44 axis orchestrates cancer stem cell functions. Cell Signal *63*: 109377, 2019. PMID: 31362044. DOI: 10.1016/j.cellsig.2019.109377

10 Chen C, Zhao S, Karnad A and Freeman JW: The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol *11(1)*: 64, 2018. PMID: 29747682. DOI: 10.1186/s13045-018-0605-5

11 Mikami S, Mizuno R, Kosaka T, Saya H, Oya M and Okada Y: Expression of TNF-α and CD44 is implicated in poor prognosis, cancer cell invasion, metastasis and resistance to the sunitinib treatment in clear cell renal cell carcinomas. Int J Cancer *136(7)*: 1504-1514, 2015. PMID: 25123505. DOI: 10.1002/ijc.29137

12 Ma X, Wang L, Li H, Zhang Y, Gao Y, Guo G, Liu K, Meng Q, Zhao C, Wang D, Song Z and Zhang X: Predictive immunohistochemical markers related to drug selection for patients treated with sunitinib or sorafenib for metastatic renal cell cancer. Sci Rep *6*: 30886, 2016. PMID: 27488093. DOI: 10.1038/srep30886

13 Sekino Y, Sakamoto N, Goto K, Honma R, Shigematsu Y, Quoc TP, Sentani K, Oue N, Teishima J, Kawakami F, Karam JA, Sircar K, Matsubara A and Yasui W: Uc.416+A promotes epithelial-to-mesenchymal transition through miR-153 in renal cell carcinoma. BMC Cancer *18(1)*: 952, 2018. PMID: 30286729. DOI: 10.1186/s12885-018-4863-y

14 Sekino Y, Hagura T, Han X, Babasaki T, Goto K, Inoue S, Hayashi T, Teishima J, Shigeta M, Taniyama D, Kuraoka K, Sentani K, Yasui W and Matsubara A: PTEN is involved in sunitinib and sorafenib resistance in renal cell carcinoma. Anticancer Res *40(4)*: 1943-1951, 2020. PMID: 32234883. DOI: 10.21873/anticanres.14149

15 Sekino Y, Sakamoto N, Goto K, Honma R, Shigematsu Y, Sentani K, Oue N, Teishima J, Matsubara A and Yasui W: Transcribed ultraconserved region Uc.63+ promotes resistance to docetaxel through regulation of androgen receptor signaling in prostate cancer. Oncotarget *8(55)*: 94259-94270, 2017. PMID: 29212226. DOI: 10.18632/oncotarget.21688

16 Sekino Y, Oue N, Shigematsu Y, Ishikawa A, Sakamoto N, Sentani K, Teishima J, Matsubara A and Yasui W: KIFC1 induces resistance to docetaxel and is associated with survival of patients with prostate cancer. Urol Oncol *35(1)*: 31.e13-31.e20, 2017. PMID: 27665358. DOI: 10.1016/j.urolonc.2016.08.007

17 Sekino Y, Oue N, Mukai S, Shigematsu Y, Goto K, Sakamoto N, Sentani K, Hayashi T, Teishima J, Matsubara A and Yasui W: Protocadherin B9 promotes resistance to bicalutamide and is associated with the survival of prostate cancer patients. Prostate *79(2)*: 234-242, 2019. PMID: 30324761. DOI: 10.1002/pros.23728

18 Sircar K, Yoo SY, Majewski T, Wani K, Patel LR, Voicu H, Torres-Garcia W, Verhaak RG, Tannir N, Karam JA, Jonasch E, Wood CG, Tamboli P, Baggerly KA, Aldape KD and Czerniak B: Biphasic components of sarcomatoid clear cell renal cell carcinomas are molecularly similar to each other, but distinct from, non-sarcomatoid renal carcinomas. J Pathol Clin Res *1(4)*: 212-224, 2015. PMID: 27499906. DOI: 10.1002/cjp2.23

19 Zhang L, Wang X, Bullock AJ, Callea M, Shah H, Song J, Moreno K, Visentin B, Deutschman D, Alsop DC, Atkins MB, Mier JW, Signoretti S, Bhasin M, Sabbadini RA and Bhatt RS: Anti-S1P antibody as a novel therapeutic strategy for VEGFR TKI-resistant renal cancer. Clin Cancer Res *21(8)*: 1925-1934, 2015. PMID: 25589614. DOI: 10.1158/1078-0432.CCR-14-2031

20 Ren NSX, Ji M, Tokar EJ, Busch EL, Xu X, Lewis D, Li X, Jin A, Zhang Y, Wu WKK, Huang W, Li L, Fargo DC, Keku TO, Sandler RS and Li X: Haploinsufficiency of SIRT1 enhances glutamine metabolism and promotes cancer development. Curr Biol *27(4)*: 483-494, 2017. PMID: 28162896. DOI: 10.1016/j.cub.2016.12.047

21 Beuselinck B, Job S, Becht E, Karadimou A, Verkarre V, Couchy G, Giraldo N, Rioux-Leclercq N, Molinié V, Sibony M, Elaidi R, Teghom C, Patard JJ, Méjean A, Fridman WH, Sautès-Fridman C, de Reyniès A, Oudard S and Zucman-Rossi J: Molecular subtypes of clear cell renal cell carcinoma are associated with sunitinib response in the metastatic setting. Clin Cancer Res *21(6)*: 1329-1339, 2015. PMID: 25583177. DOI: 10.1158/1078-0432.CCR-14-1128

22 Motzer RJ, Robbins PB, Powles T, Albiges L, Haanen JB, Larkin J, Mu XJ, Ching KA, Uemura M, Pal SK, Alekseev B, Gravis G, Campbell MT, Penkov K, Lee JL, Hariharan S, Wang X, Zhang W, Wang J, Chudnovsky A, di Pietro A, Donahue AC and Choueiri TK: Avelumab plus axitinib *versus* sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. Nat Med *26(11)*: 1733-1741, 2020. PMID: 32895571. DOI: 10.1038/s41591-020-1044-8

23 Braun DA, Hou Y, Bakouny Z, Ficial M, Sant' Angelo M, Forman J, Ross-Macdonald P, Berger AC, Jegede OA, Elagina L, Steinharter J, Sun M, Wind-Rotolo M, Pignon JC, Cherniack AD, Lichtenstein L, Neuberg D, Catalano P, Freeman GJ, Sharpe AH, McDermott DF, Van Allen EM, Signoretti S, Wu CJ, Shukla SA and Choueiri TK: Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. Nat Med *26(6)*: 909-918, 2020. PMID: 32472114. DOI: 10.1038/s41591-020-0839-y

24 Bakouny Z, Braun DA, Shukla SA, Pan W, Gao X, Hou Y, Flaifel A, Tang S, Bosma-Moody A, He MX, Vokes N, Nyman J, Xie W, Nassar AH, Abou Alaiwi S, Flippot R, Bouchard G, Steinharter JA, Nuzzo PV, Ficial M, Sant'Angelo M, Forman J, Berchuck JE, Dudani S, Bi K, Park J, Camp S, Sticco-Ivins M, Hirsch L, Baca SC, Wind-Rotolo M, Ross-Macdonald P, Sun M, Lee GM, Chang SL, Wei XX, McGregor BA, Harshman LC, Genovese G, Ellis L, Pomerantz M, Hirsch MS, Freedman ML, Atkins MB, Wu CJ, Ho TH, Linehan WM, McDermott DF, Heng DYC, Viswanathan SR, Signoretti S, Van Allen EM and Choueiri TK: Integrative molecular characterization of sarcomatoid and rhabdoid renal cell carcinoma. Nat Commun *12(1)*: 808, 2021. PMID: 33547292. DOI: 10.1038/s41467-021-21068-9

25 Chae YK, Chang S, Ko T, Anker J, Agte S, Iams W, Choi WM, Lee K and Cruz M: Epithelial-mesenchymal transition (EMT) signature is inversely associated with T-cell infiltration in non-small cell lung cancer (NSCLC). Sci Rep *8(1)*: 2918, 2018. PMID: 29440769. DOI: 10.1038/s41598-018-21061-1

26 Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN, Zhu J and Haussler D: Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol *38(6)*: 675-678, 2020. PMID: 32444850. DOI: 10.1038/s41587-020-0546-8

27 Hwang HS, Go H, Park JM, Yoon SY, Lee JL, Jeong SU and Cho YM: Epithelial-mesenchymal transition as a mechanism of resistance to tyrosine kinase inhibitors in clear cell renal cell carcinoma. Lab Invest *99(5)*: 659-670, 2019. PMID: 30683903. DOI: 10.1038/s41374-019-0188-y

28 Joosten SC, Hamming L, Soetekouw PM, Aarts MJ, Veeck J, van Engeland M and Tjan-Heijnen VC: Resistance to sunitinib in renal cell carcinoma: From molecular mechanisms to predictive markers and future perspectives. Biochim Biophys Acta *1855(1)*: 1-16, 2015. PMID: 25446042. DOI: 10.1016/j.bbcan.2014.11.002

29 Makhov P, Joshi S, Ghatalia P, Kutikov A, Uzzo RG and Kolenko VM: Resistance to systemic therapies in clear cell renal cell carcinoma: mechanisms and management strategies. Mol Cancer Ther *17(7)*: 1355-1364, 2018. PMID: 29967214. DOI: 10.1158/1535-7163.MCT-17-1299

30 Czarnecka AM, Solarek W, Kornakiewicz A and Szczylik C: Tyrosine kinase inhibitors target cancer stem cells in renal cell cancer. Oncol Rep *35(3)*: 1433-1442, 2016. PMID: 26708631. DOI: 10.3892/or.2015.4514

31 Brossa A, Grange C, Mancuso L, Annaratone L, Satolli MA, Mazzone M, Camussi G and Bussolati B: Sunitinib but not VEGF blockade inhibits cancer stem cell endothelial differentiation. Oncotarget *6(13)*: 11295-11309, 2015. PMID: 25948774. DOI: 10.18632/oncotarget.3123

32 Wang S, Mou Z, Ma Y, Li J, Li J, Ji X, Wu K, Li L, Lu W and Zhou T: Dopamine enhances the response of sunitinib in the treatment of drug-resistant breast cancer: Involvement of eradicating cancer stem-like cells. Biochem Pharmacol *95(2)*: 98-109, 2015. PMID: 25824241. DOI: 10.1016/j.bcp.2015.03.013

33 Varna M, Gapihan G, Feugeas JP, Ratajczak P, Tan S, Ferreira I, Leboeuf C, Setterblad N, Duval A, Verine J, Germain S, Mongiat-Artus P, Janin A and Bousquet G: Stem cells increase in numbers in perinecrotic areas in human renal cancer. Clin Cancer Res *21(4)*: 916-924, 2015. PMID: 25501128. DOI: 10.1158/1078-0432.CCR-14-0666

34 Xu H, Niu M, Yuan X, Wu K and Liu A: CD44 as a tumor biomarker and therapeutic target. Exp Hematol Oncol *9(1)*: 36, 2020. PMID: 33303029. DOI: 10.1186/s40164-020-00192-0

35 D'Aniello C, Berretta M, Cavaliere C, Rossetti S, Facchini BA, Iovane G, Mollo G, Capasso M, Pepa CD, Pesce L, D'Errico D, Buonerba C, Di Lorenzo G, Pisconti S, De Vita F and Facchini G: Biomarkers of prognosis and efficacy of anti-angiogenic therapy in metastatic clear cell renal cancer. Front Oncol *9*: 1400, 2019. PMID: 31921657. DOI: 10.3389/fonc.2019.01400

36 Choueiri TK, Larkin J, Pal S, Motzer RJ, Rini BI, Venugopal B, Alekseev B, Miyake H, Gravis G, Bilen MA, Hariharan S, Chudnovsky A, Ching KA, Mu XJ, Mariani M, Robbins PB, Huang B, di Pietro A and Albiges L: Efficacy and correlative analyses of avelumab plus axitinib *versus* sunitinib in sarcomatoid renal cell carcinoma: *post hoc* analysis of a randomized clinical trial. ESMO Open *6(3)*: 100101, 2021. PMID: 33901870. DOI: 10.1016/j.esmoop.2021.100101

37 Prochazka L, Tesarik R and Turanek J: Regulation of alternative splicing of CD44 in cancer. Cell Signal *26(10)*: 2234-2239, 2014. PMID: 25025570. DOI: 10.1016/j.cellsig.2014.07.011

Received August 2, 2021
Revised August 28, 2021
Accepted August 31, 2021