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PTEN Is Involved in Sunitinib and Sorafenib Resistance in Renal Cell Carcinoma

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Abstract. *Background/Aim: Targeted receptor tyrosine kinase inhibitor (TKI) is a standard treatment in advanced renal cell carcinoma (RCC). However, the role of PTEN in TKI resistance remains poorly understood. We aimed to determine the functional role of PTEN knockout and analyse the predictive significance of PTEN expression for TKI treatment in RCC. Materials and Methods: We developed PTEN knockout cells in RCC cell lines using the CRISPR-Cas9 system and analysed the effect of PTEN knockout on spheroid formation and resistance to sunitinib and sorafenib. Results: PTEN knockout promoted spheroid formation and decreased sunitinib/sorafenib sensitivity in RCC cell lines. PTEN immunohistochemistry in 74 metastatic RCCs treated with sunitinib and sorafenib revealed negative PTEN expression in 23% of samples. Kaplan–Meier analysis showed a significant association of negative PTEN expression with poor progression-free survival in metastatic RCC treated with sunitinib and sorafenib (p=0.024) or sunitinib alone (p=0.009). Conclusion: PTEN may be a biomarker and therapeutic target in patients with metastatic RCC.*

Renal cell carcinoma (RCC) accounts for around 90% of all renal tumours, and its incidence has been steadily increasing by 2-4% each year (1). More than 30% of patients diagnosed with RCC have metastatic disease (2). Patients with metastatic RCC have an approximately 13 months' median survival and a 5-year survival rate under 10% (3). Sunitinib is an oral multi-target tyrosine kinase inhibitor (TKI), which has potent anti-angiogenic effects and direct anti-tumour activities due to the inhibition of vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and stem cell growth factor receptor (4). Sunitinib treatment is used as a first-line therapy for metastatic RCC (5, 6) and significantly prolongs overall survival in metastatic RCC (7). Although approximately 70% of the patients show an initial response to sunitinib, eventually, sunitinib resistance and disease progression occur. Recent evidence has shown that the mechanisms of sunitinib resistance may be multifactorial (8-10); however, the mechanisms that underpin sunitinib resistance are not fully elucidated. Therefore, there is an urgent need to clarify the mechanisms of sunitinib resistance.

Phosphatase and Tensin Homolog Deleted from Chromosome 10 (PTEN) is a tumour-suppressive protein (11). The *PTEN* gene has both recurrent point mutations and focal deletions in RCC (12, 13). PTEN has been reported to antagonize the phosphoinositol-3-kinase (PI3K)/PTEN/AKT signalling pathway, which plays a crucial role in cell growth, differentiation, survival and drug resistance in RCC (14, 15). The Cancer Genome Atlas data showed that bi-allelic loss of PTEN is rare and associated with poor overall survival in RCC (16, 17). Although some studies have reported the prognostic significance of PTEN for TKI treatment (18, 19), the role of PTEN in sunitinib and sorafenib resistance in RCC has not been fully elucidated.

In this study, we aimed to analyse the effect of PTEN knockout on sunitinib and sorafenib resistance in RCC cell

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lines. We also analysed the expression of PTEN using immunohistochemistry, in specimens obtained from metastatic RCC patients treated with sunitinib and sorafenib.

Materials and Methods

Cell lines. Human RCC-derived cell lines Caki-1, ACHN, OSRC-2 and 786-O were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). All cell lines were maintained in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum (Whittaker, Walkersville, MD, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C.

Western blot analysis. For western blot analysis, cells were lysed as described previously (20). Primary antibodies against PTEN, Akt, and p-Akt (Cell Signaling Technology, Inc., Danvers, MA, USA) were used. β-Actin (Sigma-Aldrich, St. Louis, MO, USA) was used as a loading control. The IDs and dilutions of the primary and secondary antibodies used are summarized in Table I.

Generation of PTEN knockout cells. The CRISPR-Cas9 technology *via* the lentiviral transfection approach was used to knockout PTEN in ACHN and OSRC-2 cells. PTEN single-guide RNAs (PTEN-CRISPR vector) or scrambled single-guide RNAs (empty vector), and CRISPR/Cas9 All-in-One lentivector pLenti-U6-sgRNA-SFFV-Cas9-2A-Puro were purchased from ABM Inc. (Richmond, BC, Canada). The sgRNA sequence of the PTEN-CRISPR vector was TGGGAATAGTTACTCCC. Lentiviral particles were generated by co-transfection of HEK293T cells with Cas9-sgRNA constructs and packaging plasmids (GAG, VSVG, REV). After 48 h, the conditioned media containing lentiviral particles were harvested and used to infect Caki-1 cells using polybrene as the transfection agent. Stable PTEN knockout ACHN and OSRC-2 cells were selected by passaging in media containing 4 μg/ml puromycin.

Spheroid formation assay. For the generation of spheres, 2×10^3 cells (transfected with *TUBB3* siRNA, or negative control siRNA) were plated on 24-well ultra-low attachment plates (Corning, New York, NY, USA). Cells were grown in mTeSR medium (STEMCELL Technologies Inc., Cambridge, MA, USA). The plates were incubated at 37˚C in a 5% CO_2 incubator for 15 days. Sphere size was then determined using a microscope.

Sunitinib and sorafenib treatment. Sunitinib maleate and sorafenib tosylate were 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 and sorafenib. An MTT assay to assess cell viability was performed after the cells had been exposed to sunitinib and sorafenib 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) (21).

Tissue samples. We used 74 metastatic RCC tissue samples (Table II) for immunohistochemistry. The samples were collected from patients at the Hiroshima University Hospital, Kure Medical Center, and Chugoku Cancer Center under an institutional review board-approved protocol (IRB# E912, 2019-08). Written comprehensive approvals for basic or clinical research were obtained from all of the patients whose samples were used. This study was conducted in accordance with the Ethical Guidance for Human Genome/Gene Research of the Japanese Government.

Table I. *ID and dilution of primary and secondary antibody.*

Primary antibody	ID	Dilution
PTEN	138G6	1:500
Akt	9272	1:500
p-Akt	9271	1:500
β-actin	A5441	1:10000
Secondary antibody	**ID**	**Dilution**
Anti-IgG (H+L chain) (Mouse) pAb-HRP	330	1:500
Anti-IgG (H+L chain) (Rabbit) pAb-HRP	458	1:500

Table II. *Patient characteristics*.

Number of cases	74
Gender	
M	60 (81%)
F	14 (19%)
Median age (years)	65 (40-89)
Race	
Asian	74
Histology	
Clear cell	60 (81%)
Papillary	4 (5%)
Chromophobe	3 (4%)
Unclassified	7 (9%)
Metastasis sites	
Lung	57 (77%)
Lymph node	23 (31%)
Bone	25 (34%)
Brain	5 (7%)
Liver	11 (15%)
Nephrectomy	65 (88%)
IMDC criteria	
Favorable	11 (15%)
Intermediate	44 (59%)
poor	19 (26%)
1st line TKI	
Sunitinib	51 (69%)
Sorafenib	23 (31%)

RCC: Renal cell carcinoma; IMDC: International Metastatic RCC Database Consortium.

Immunohistochemistry. Immunohistochemistry was performed as described previously (22). Sections were incubated with a rabbit polyclonal anti-PTEN antibody (1:100) (Cell Signaling Technology, Inc.) for 1 h at room temperature. Cytoplasmic PTEN positivity and negativity in the tumour samples were recorded. Tumour samples with high PTEN intensity were considered positive, whereas no expression of the protein was considered

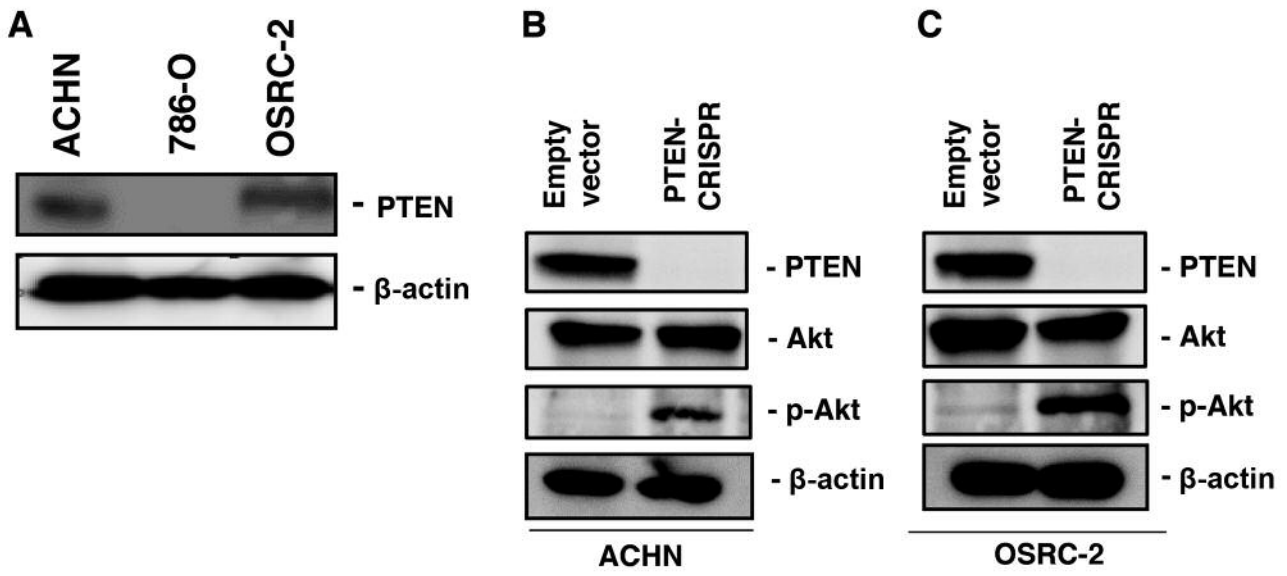

Figure 1. *Generation of PTEN knockout cells in RCC cell lines. (A) Western blot analysis of PTEN and β-actin in ACHN, 786-O, and OSRC-2 cells. β-actin was used as a loading control. (B, C) Western blot analysis of PTEN, Akt, p-Akt, and β-actin in ACHN and OSRC-2 cells transfected with PTEN-CRISPR vector or empty vector. β-actin was used as a loading control.*

negative (23). Two pathologists (DT and KS), without knowledge of the patients' clinical and pathologic parameters or their outcomes, independently reviewed immunoreactivity in each specimen.

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. Sample sizes for relevant experiments were determined by power analysis. Statistical differences were evaluated using the two-tailed Student *t*-test or Mann-Whitney *U*-test. A *p*-value of <0.05 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., CA, USA).

Results

Generation of PTEN knockout RCC cell lines. Previously, we generated PTEN knockout cells by using the PTEN-CRISPR vector in Caki-1 cells (24). Additionally, we generated PTEN knockout cells in ACHN and OSRC-2 cells, which are well established RCC cell lines (25, 26). An initial western blotting experiment performed before the knockout experiment showed that PTEN expression was detected in ACHN and OSRC-2 cells but not in 786-O cells (Figure 1A). Western blotting after transfection with the PTEN-CRISPR vector confirmed he loss of PTEN expression in ACHN and OSRC-2 cells, while the expression of phosphorylated AKT was upregulated (Figure 1B, C).

Knockout of PTEN promotes spheroid formation in RCC. Recent studies have shown that PTEN deletion induces spheroid formation in ovarian and colorectal cancer (27, 28). Therefore, we analysed the effect of PTEN knockout on spheroid formation in Caki-1, ACHN, and OSRC-2 cells. Caki-1 cells did not form spheroids. The size of the spheroids was larger in ACHN and OSRC-2 cells transfected with the PTEN-CRISPR vector than in ACHN and OSRC-2 cells transfected with the empty vector (Figure 2A, B).

Knockout of PTEN promoted sunitinib and sorafenib resistance in RCC. Some studies have shown that spheroid formation induces drug resistance (29, 30), and recent papers have indicated that sunitinib is more toxic to cells cultured in monolayers than in three-dimensional spheroids (31, 32). Therefore, to examine the effect of PTEN knockout on sunitinib and sorafenib resistance, we performed MTT assays to measure cell viability under various concentrations of sunitinib and sorafenib in Caki-1, ACHN, and OSRC-2 cells transfected with the empty vector or the PTEN-CRISPR vector. Previously, we have shown that knockout of PTEN promoted sunitinib resistance in Caki-1 cells (24). Knockout of PTEN decreased the sensitivity of ACHN and OSRC-2 cells to sunitinib (Figure 3A, B) and the sensitivity of all three cell types to sorafenib (Figure 3C-E).

Impact of PTEN on response of sunitinib and sorafenib treatment in metastatic RCC. We analysed the association between PTEN expression and therapeutic outcomes in 74

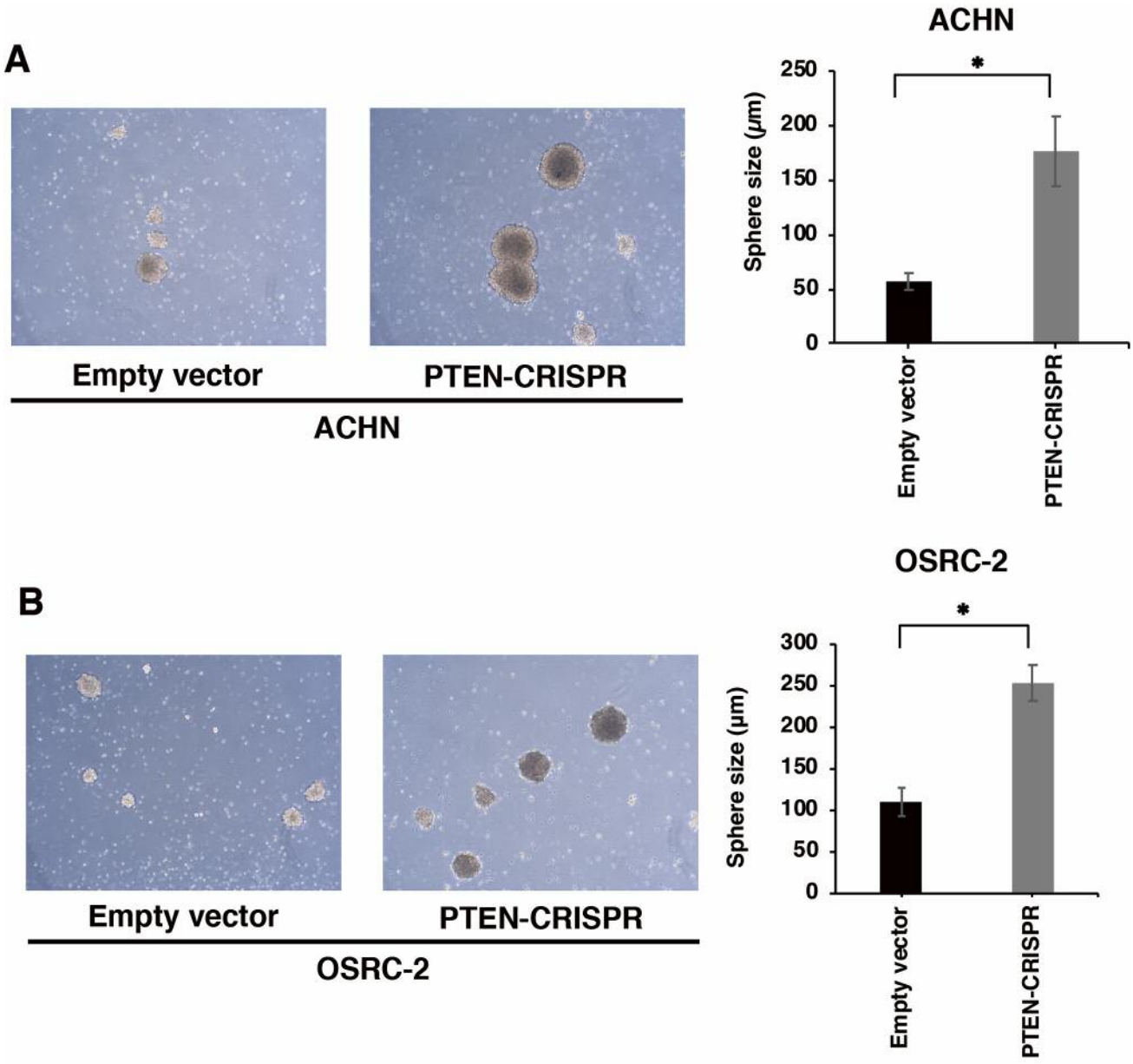

Figure 2. *PTEN knockout promoted spheroid formation in RCC cell lines. (A, B) The size of the spheres formed in ACHN and OSRC-2 cells transfected with PTEN-CRISPR vector or empty vector. Bars and error bars indicate the mean and S.D., respectively, of three independent experiments. Representative images of spheroid formation in ACHN and OSRC-2 cells. *p<0.01.*

metastatic RCCs treated with sunitinib and sorafenib as first-line treatment. Negative PTEN expression was found in 17 of 74 (23%) metastatic RCC patients (Figure 4A). Kaplan–Meier analysis revealed a significant association between negative PTEN expression and poor progression-free survival (PFS) in metastatic RCC treated with sunitinib and sorafenib as first-line treatment (p=0.024) (Figure 4B). We also analysed the PFS data in patients treated with sunitinib and sorafenib separately. In the sunitinib group, negative PTEN expression was significantly associated with poor PFS (p=0.009) (Figure 4C), whereas in the sorafenib group, negative PTEN expression was not significantly associated with poor PFS (p=0.744).

Discussion

PTEN has been reported to antagonize the PI3K/PTEN/AKT signalling pathway, which plays a crucial role in cell growth,

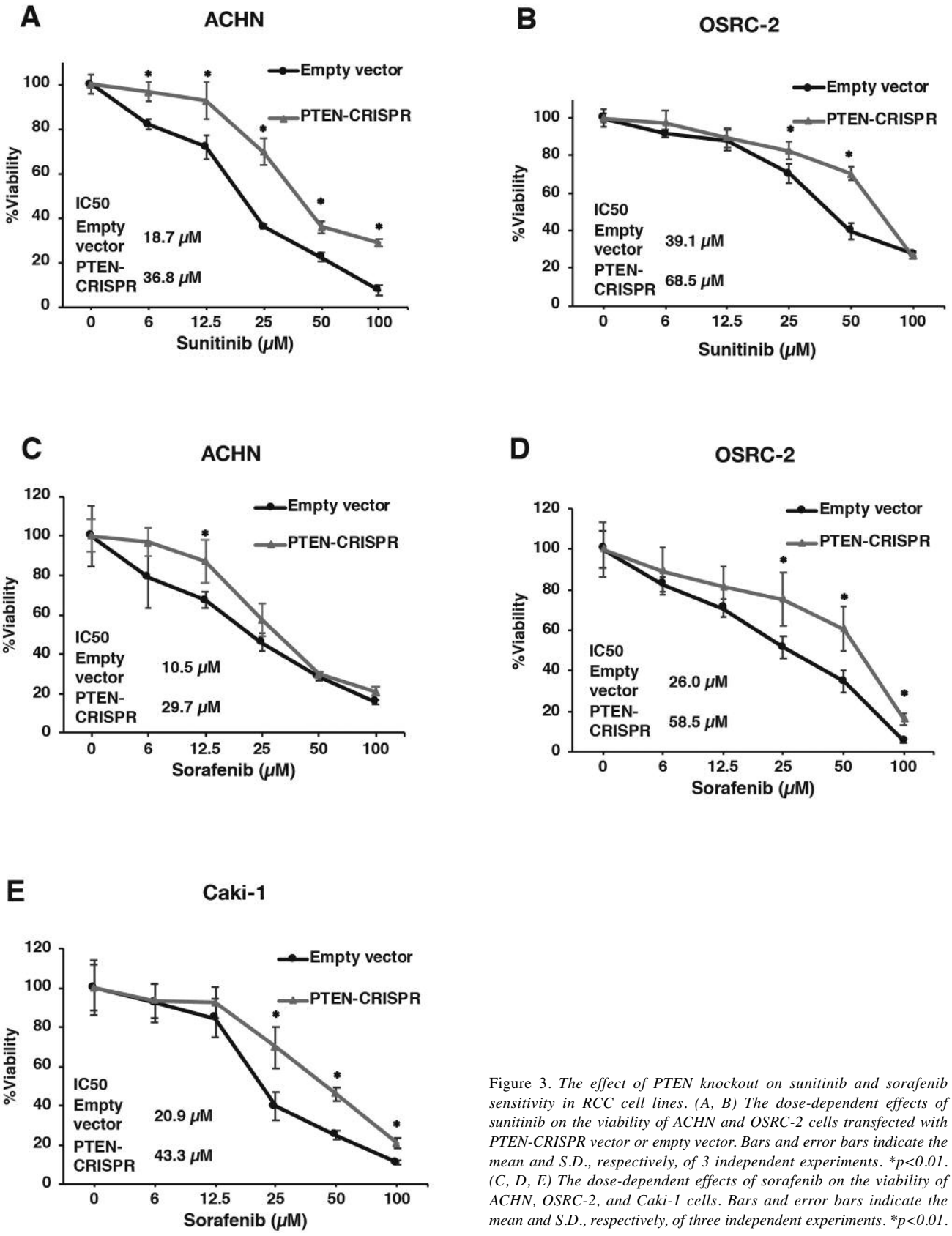

Figure 3. *The effect of PTEN knockout on sunitinib and sorafenib sensitivity in RCC cell lines. (A, B) The dose-dependent effects of sunitinib on the viability of ACHN and OSRC-2 cells transfected with PTEN-CRISPR vector or empty vector. Bars and error bars indicate the mean and S.D., respectively, of 3 independent experiments. *p<0.01. (C, D, E) The dose-dependent effects of sorafenib on the viability of ACHN, OSRC-2, and Caki-1 cells. Bars and error bars indicate the mean and S.D., respectively, of three independent experiments. *p<0.01.*

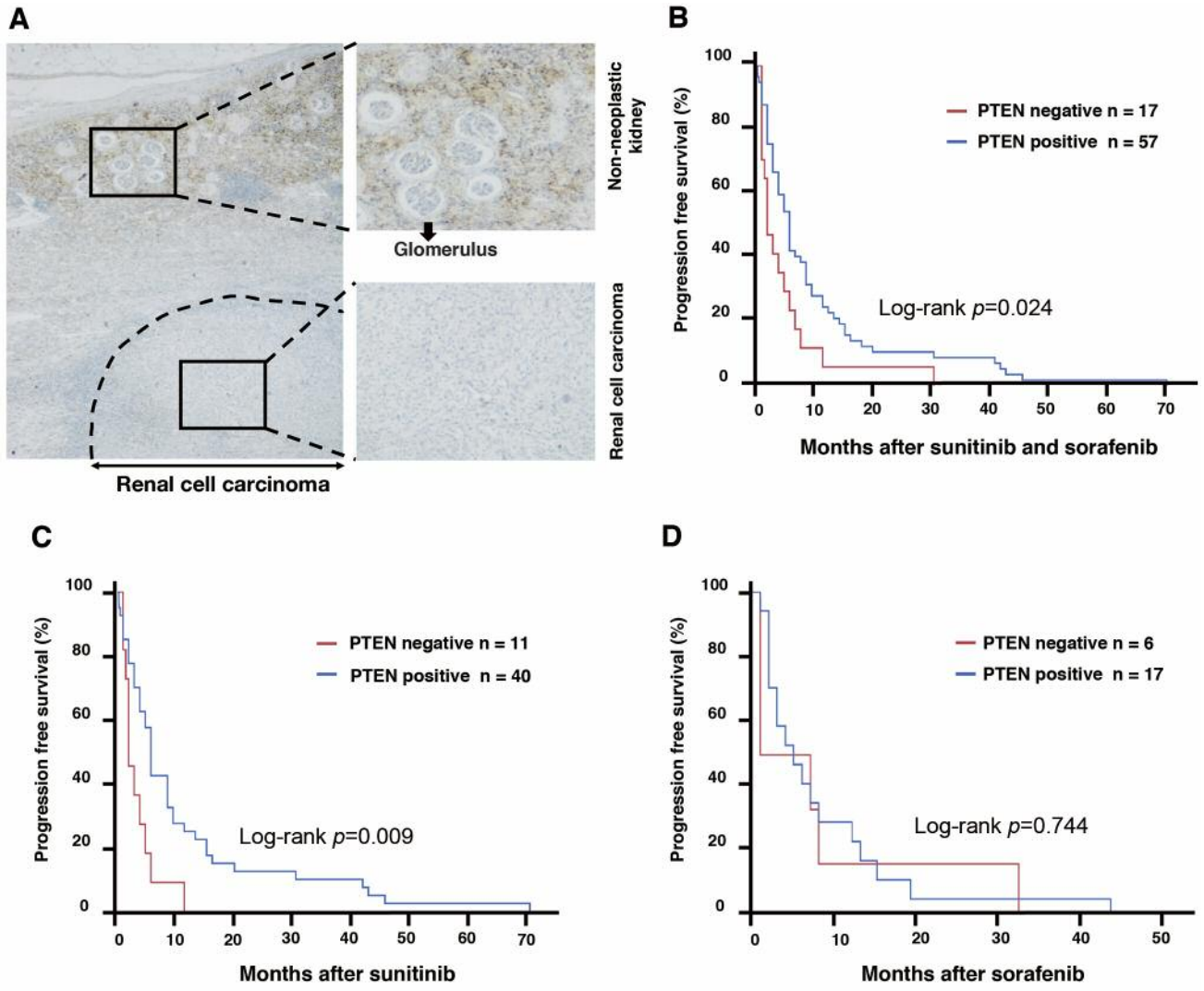

Figure 4. *The expression of PTEN in metastatic renal cell carcinoma (RCC) treated with sunitinib and sorafenib. Immunohistochemical staining of PTEN in the non-neoplastic kidney and RCC. Left panel: Original magnification, 100×. Right panel: Original magnification, 400×. (B) A Kaplan–Meier plot of progression-free survival (PFS) in metastatic RCC patients treated with sunitinib and sorafenib (C) A Kaplan–Meier plot of PFS in metastatic RCC patients treated with sunitinib (D) A Kaplan–Meier plot of PFS in metastatic RCC patients treated with sorafenib.*

differentiation, survival, and drug resistance in RCC (11, 33). Recent evidence has indicated that PTEN overexpression overcomes sunitinib resistance in RCC cell lines (34). We have previously shown that PTEN is involved in sunitinib resistance in Caki-1 cells (24). In the present study, loss of PTEN promoted sunitinib resistance in ACHN and OSRC-2 cells. Several reports have shown that PTEN is involved in sorafenib resistance in hepatocellular carcinoma (35, 36). However, the role of PTEN in sorafenib resistance in RCC is not well understood. In the present study, the loss of PTEN promoted sorafenib resistance in RCC cell lines. Collectively, these results indicated that PTEN may be involved in sunitinib and sorafenib resistance in RCC. Some preclinical studies have shown that PI3K inhibitors overcome sunitinib and sorafenib resistance in RCC (34, 37). Although further studies are necessary, the targeting of PTEN may be a promising strategy to overcome sunitinib and sorafenib resistance in RCC.

A number of reports have used sequencing and immunohistochemistry to identify the predictive biomarkers for TKI treatment (38-40). So far, however, no biomarker has been firmly established. A recent study has reported that negative expression of PTEN, determined by immunocytochemistry, tended to correlate with poor PFS of patients treated with sunitinib (18). In the present study, immunohistochemistry showed that negative PTEN expression was associated with poor PFS in metastatic RCC treated with sunitinib. Meanwhile,

a recent clinical trial (RECORD-3 trial) has shown that negative PTEN expression by immunohisto-chemistry was not associated with therapeutic outcomes sunitinib treatment (41). These results suggest that the predictive value of PTEN-regarding the response to sunitinib treatment is controversial. A recent study showed that the immunohistochemical evaluation of PTEN has not been sufficiently standardized (42). In our study, negative PTEN expression was observed in 23% of metastatic RCC patients. On the other hand, in the RECORD-3 clinical trial, negative PTEN expression was found in 53% of metastatic RCC patients. In addition, recent studies have shown that racial differences may contribute to the diversity of genomic aberrations including PTEN (43, 44). These findings help to explain why the predictive value of PTEN in sunitinib treatment is controversial. However, further studies are necessary to identify the predictive value of PTEN.

This study has some limitations. First, although we showed that negative PTEN expression was associated with sunitinib response in RCC, we used a relatively small sample size for immunohistochemical staining of PTEN. A prospective study with a larger number of patients with metastatic RCC will be necessary to verify the present data. Second, we used a CRISPR-Cas9 system to examine the effect of the loss of PTEN in RCC cell lines. To verify the current findings, we will need to analyse the overexpression of PTEN in 786-O cells.

In conclusion, the present study showed that knockout of PTEN induced spheroid formation and promoted sunitinib and sorafenib resistance in RCC cell lines. Immunohistochemistry revealed that negative PTEN expression was correlated with a poor survival outcome of sunitinib treatment. The data presented here emphasize the great potential of PTEN as a biomarker and therapeutic target in patients with metastatic RCC.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

YS, AM, and WY designed the study. JT, MS, DT, GK, and AM provided patients' clinical information. YS, HT, XH, and TB performed experiments and acquired data. SI, TH, JT, KS, and KK interpreted the results. YS drafted the manuscript. AM and WY edited the manuscript. All Authors approved the final content for journal submission and publication.

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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: 17009, 2017. PMID: 28276433. DOI: 10.1038/nrdp.2017.9

2 Vera-Badillo FE, Templeton AJ, Duran I, Ocana A, de Gouveia P, Aneja P, Knox JJ, Tannock IF, Escudier B and Amir E: Systemic therapy for non-clear cell renal cell carcinomas: A systematic review and meta-analysis. Eur Urol *67(4)*: 740-749, 2015. PMID: 24882670. DOI: 10.1016/j.eururo.2014.05.010

3 Janzen NK, Kim HL, Figlin RA and Belldegrun AS: Surveillance after radical or partial nephrectomy for localized renal cell carcinoma and management of recurrent disease. Urol Clin North Am *30(4)*: 843-852, 2003. PMID:14680319. DOI: 10.1016/s0094-0143(03)00056-9

4 Qu L, Ding J, Chen C, Wu ZJ, Liu B, Gao Y, Chen W, Liu F, Sun W, Li XF, Wang X, Wang Y, Xu ZY, Gao L, Yang Q, Xu B, Li YM, Fang ZY, Xu ZP, Bao Y, Wu DS, Miao X, Sun HY, Sun YH, Wang HY and Wang LH: Exosome-transmitted lncarsr promotes sunitinib resistance in renal cancer by acting as a competing endogenous rna. Cancer Cell *29(5)*: 653-668, 2016. PMID: 27117758. DOI: 10.1016/j.ccell.2016.03.004

5 Atkins MB and Tannir NM: Current and emerging therapies for first-line treatment of metastatic clear cell renal cell carcinoma. Cancer Treat Rev *70:* 127-137, 2018. PMID: 30173085. DOI: 10.1016/j.ctrv.2018.07.009

6 Vuorinen RL, Paunu N, Turpeenniemi-Hujanen T, Reunamo T, Jekunen A, Kataja V, Sintonen H, Purmonen T and Kellokumpu-Lehtinen PL: Sunitinib first-line treatment in metastatic renal cell carcinoma: Costs and effects. Anticancer Res *39(10)*: 5559-5564, 2019. PMID: 31570450. DOI: 10.21873/anticanres.13749

7 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

8 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

9 Rini BI and Atkins MB: Resistance to targeted therapy in renal-cell carcinoma. Lancet Oncol *10(10)*: 992-1000, 2009. PMID: 19796751. DOI: 10.1016/S1470-2045(09)70240-2

10 Yamaguchi N, Osaki M, Onuma K, Yumioka T, Iwamoto H, Sejima T, Kugoh H, Takenaka A and Okada F: Identification of micrornas involved in resistance to sunitinib in renal cell carcinoma cells. Anticancer Res *37(6)*: 2985-2992, 2017. PMID: 28551636. DOI: 10.21873/anticanres.11652

11 Lee YR, Chen M and Pandolfi PP: The functions and regulation of the pten tumour suppressor: New modes and prospects. Nat Rev Mol Cell Biol *19(9)*: 547-562, 2018. PMID: 29858604. DOI: 10.1038/s41580-018-0015-0

12 Mitchell TJ, Rossi SH, Klatte T and Stewart GD: Genomics and clinical correlates of renal cell carcinoma. World J Urol *36(12)*: 1899-1911, 2018. PMID: 30099580. DOI: 10.1007/s00345-018-2429-x

13 Alimov A, Li C, Gizatullin R, Fredriksson V, Sundelin B, Klein G, Zabarovsky E and Bergerheim U: Somatic mutation and homozygous deletion of pten/mmac1 gene of 10q23 in renal cell carcinoma. Anticancer Res *19(5B)*: 3841-3846, 1999. PMID: 10628321.

14 Fan C, Zhao C, Wang F, Li S and Wang J: Significance of pten mutation in cellular process, prognosis, and drug selection in clear cell renal cell carcinoma. Front Oncol *9:* 357, 2019. PMID: 31139560. DOI: 10.3389/fonc.2019.00357

15 Tang L, Li X, Gao Y, Chen L, Gu L, Chen J, Lyu X, Zhang Y and Zhang X: Phosphatase and tensin homolog (pten) expression on oncologic outcome in renal cell carcinoma: A systematic review and meta-analysis. PLoS One *12(7)*: e0179437, 2017. PMID: 28672019. DOI: 10.1371/journal.pone.0179437

16 Lee HJ, Lee HY, Lee JH, Lee H, Kang G, Song JS, Kang J and Kim JH: Prognostic significance of biallelic loss of pten in clear cell renal cell carcinoma. J Urol *192(3)*: 940-946, 2014. PMID: 24704020. DOI: 10.1016/j.juro.2014.03.097

17 Ricketts CJ, De Cubas AA, Fan H, Smith CC, Lang M, Reznik E, Bowlby R, Gibb EA, Akbani R, Beroukhim R, Bottaro DP, Choueiri TK, Gibbs RA, Godwin AK, Haake S, Hakimi AA, Henske EP, Hsieh JJ, Ho TH, Kanchi RS, Krishnan B, Kwiatkowski DJ, Lui W, Merino MJ, Mills GB, Myers J, Nickerson ML, Reuter VE, Schmidt LS, Shelley CS, Shen H, Shuch B, Signoretti S, Srinivasan R, Tamboli P, Thomas G, Vincent BG, Vocke CD, Wheeler DA, Yang L, Kim WY, Robertson AG, Cancer Genome Atlas Research N, Spellman PT, Rathmell WK and Linehan WM: The cancer genome atlas comprehensive molecular characterization of renal cell carcinoma. Cell Rep *23(1)*: 313-326 e315, 2018. PMID: 29617669. DOI: 10.1016/j.celrep.2018.03.075

18 Muriel Lopez C, Esteban E, Berros JP, Pardo P, Astudillo A, Izquierdo M, Crespo G, Sanmamed M, Fonseca PJ and Martinez-Camblor P: Prognostic factors in patients with advanced renal cell carcinoma. Clin Genitourin Cancer *10(4)*: 262-270, 2012. PMID: 22959659. DOI: 10.1016/j.clgc.2012.06.005

19 Park I, Cho YM, Lee JL, Ahn JH and Lee DH: Prognostic tissue biomarker exploration for patients with metastatic renal cell carcinoma receiving vascular endothelial growth factor receptor tyrosine kinase inhibitors. Tumour Biol *37(4)*: 4919-4927, 2016. PMID: 26526582. DOI: 10.1007/s13277-015-4339-5

20 Oue N, Hamai Y, Mitani Y, Matsumura S, Oshimo Y, Aung PP, Kuraoka K, Nakayama H and Yasui W: Gene expression profile of gastric carcinoma: Identification of genes and tags potentially involved in invasion, metastasis, and carcinogenesis by serial analysis of gene expression. Cancer Res *64(7)*: 2397-2405, 2004. PMID: 15059891. DOI: 10.1158/0008-5472.can-03-3514

21 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

22 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, 2018. PMID: 27665358. DOI: 10.1016/j.urolonc.2016.08.007

23 Hager M, Haufe H, Kemmerling R, Mikuz G, Kolbitsch C and Moser PL: Pten expression in renal cell carcinoma and oncocytoma and prognosis. Pathology *39(5)*: 482-485, 2007. PMID: 17886097. DOI: 10.1080/00313020701570012

24 Sekino Y, Sakamoto N, Sentani K, Oue N, Teishima J, Matsubara A and Yasui W: Mir-130b promotes sunitinib resistance through regulation of pten in renal cell carcinoma. Oncology *97(3)*: 164-172, 2019. PMID: 31195398. DOI: 10.1159/000500605

25 Brodaczewska KK, Szczylik C, Fiedorowicz M, Porta C and Czarnecka AM: Choosing the right cell line for renal cell cancer research. Mol Cancer *15(1)*: 83, 2016. PMID: 27993170. DOI: 10.1186/s12943-016-0565-8

26 Kinouchi T, Kotake T, Mori Y and Abe T: Human renal cell carcinoma: Establishment and characterization of a new cell line (os-rc-2). In Vitro Cell Dev Biol *21(4)*: 195-199, 1985. PMID: 4008433. DOI: 10.1007/bf02620929

27 Dean M, Jin V, Bergsten TM, Austin JR, Lantvit DD, Russo A and Burdette JE: Loss of pten in fallopian tube epithelium results in multicellular tumor spheroid formation and metastasis to the ovary. Cancers (Basel) *11(6)*, 2019. PMID: 31242614. DOI: 10.3390/cancers11060884

28 Chandrasekaran S, Deng H and Fang Y: Pten deletion potentiates invasion of colorectal cancer spheroidal cells through 3d matrigel. Integr Biol (Camb) *7(3)*: 324-334, 2015. PMID: 25625883. DOI: 10.1039/c4ib00298a

29 Langhans SA: Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. Front Pharmacol *9:* 6, 2018. PMID: 29410625. DOI: 10.3389/fphar.2018.00006

30 Khawar IA, Park JK, Jung ES, Lee MA, Chang S and Kuh HJ: Three dimensional mixed-cell spheroids mimic stroma-mediated chemoresistance and invasive migration in hepatocellular carcinoma. Neoplasia *20(8)*: 800-812, 2018. PMID: 2998150. DOI: 10.1016/j.neo.2018.05.008

31 Lichner Z, Saleeb R, Butz H, Ding Q, Nofech-Mozes R, Riad S, Farag M, Varkouhi AK, Dos Santos CC, Kapus A and Yousef GM: Sunitinib induces early histomolecular changes in a subset of renal cancer cells that contribute to resistance. FASEB J *33(1)*: 1347-1359, 2019. PMID: 30148679. DOI: 10.1096/fj.201800596R

32 Brodaczewska KK, Bielecka ZF, Maliszewska-Olejniczak K, Szczylik C, Porta C, Bartnik E and Czarnecka AM: Metastatic renal cell carcinoma cells growing in 3d on polydlysine or laminin present a stemlike phenotype and drug resistance. Oncol Rep *42(5)*: 1878-1892, 2019. PMID: 31545459. DOI: 10.3892/or.2019.7321

33 Hafsi S, Pezzino FM, Candido S, Ligresti G, Spandidos DA, Soua Z, McCubrey JA, Travali S and Libra M: Gene alterations in the pi3k/pten/akt pathway as a mechanism of drug-resistance (review). Int J Oncol *40(3)*: 639-644, 2012. PMID: 22200790. DOI: 10.3892/ijo.2011.1312

34 Makhov PB, Golovine K, Kutikov A, Teper E, Canter DJ, Simhan J, Uzzo RG and Kolenko VM: Modulation of akt/mtor signaling overcomes sunitinib resistance in renal and prostate cancer cells. Mol Cancer Ther *11(7)*: 1510-1517, 2012. PMID: 22532600. DOI: 10.1158/1535-7163.MCT-11-0907

35 Ruan ZP, Xu R, Lv Y, Tian T, Wang WJ, Guo H and Nan KJ: Pten enhances the sensitivity of human hepatocellular carcinoma cells to sorafenib. Oncol Res *20(2-3)*: 113-121, 2012. PMID: 23193917. DOI: 10.3727/096504012x13477145152995

36 Liu K, Liu S, Zhang W, Jia B, Tan L, Jin Z and Liu Y: Mir-494 promotes cell proliferation, migration and invasion, and increased sorafenib resistance in hepatocellular carcinoma by targeting pten. Oncol Rep *34(2)*: 1003-1010, 2015. PMID: 26045065. DOI: 10.3892/or.2015.4030

37 Roulin D, Waselle L, Dormond-Meuwly A, Dufour M, Demartines N and Dormond O: Targeting renal cell carcinoma with nvp-bez235, a dual pi3k/mtor inhibitor, in combination with sorafenib. Mol Cancer *10:* 90, 2011. PMID: 21791089. DOI: 10.1186/1476-4598-10-90

38 Voss MH, Reising A, Cheng Y, Patel P, Marker M, Kuo F, Chan TA, Choueiri TK, Hsieh JJ, Hakimi AA and Motzer RJ: Genomically annotated risk model for advanced renal-cell carcinoma: A retrospective cohort study. Lancet Oncol *19(12)*: 1688-1698, 2018. PMID: 30416077. DOI: 10.1016/S1470-2045(18)30648-X

39 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

40 Stubbs C, Bardoli AD, Afshar M, Pirrie S, Miscoria M, Wheeley I and Porfiri E: A study of angiogenesis markers in patients with renal cell carcinoma undergoing therapy with sunitinib. Anticancer Res *37(1)*: 253-259, 2017. PMID: 28011500. DOI: 10.21873/anticanres.11315

41 Voss MH, Chen D, Reising A, Marker M, Shi J, Xu J, Ostrovnaya I, Seshan VE, Redzematovic A, Chen YB, Patel P, Han X, Hsieh JJ, Hakimi AA and Motzer RJ: Pten expression, not mutation status in tsc1, tsc2, or mtor, correlates with the outcome on everolimus in patients with renal cell carcinoma treated on the randomized record-3 trial. Clin Cancer Res *25(2)*: 506-514, 2019. PMID: 30327302. DOI: 10.1158/1078-0432.CCR-18-1833

42 Agoston EI, Micsik T, Acs B, Fekete K, Hahn O, Baranyai Z, Dede K, Bodoky G, Bursics A, Kulka J, Krenacs T, Gyorffy B, Harsanyi L and Szasz AM: In depth evaluation of the prognostic and predictive utility of pten immunohistochemistry in colorectal carcinomas: Performance of three antibodies with emphasis on intracellular and intratumoral heterogeneity. Diagn Pathol *11(1)*: 61, 2016. PMID: 27392434. DOI: 10.1186/s13000-016-0508-0

43 Heath EI, Lynce F, Xiu J, Ellerbrock A, Reddy SK, Obeid E, Liu SV, Bollig-Fischer A, Separovic D and Vanderwalde A: Racial disparities in the molecular landscape of cancer. Anticancer Res *38(4)*: 2235-2240, 2018. PMID: 29599344. DOI: 10.21873/anticanres.12466

44 Spratt DE, Chan T, Waldron L, Speers C, Feng FY, Ogunwobi OO and Osborne JR: Racial/ethnic disparities in genomic sequencing. JAMA Oncol *2(8)*: 1070-1074, 2016. PMID: 27366979. DOI: 10.1001/jamaoncol.2016.1854

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