ANTICANCER RESEARCH *41*: 4287-4294 (2021)
doi:10.21873/anticanres.15233

P53 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 examined the potential role of p53 in sunitinib resistance and as a predictive marker in mRCC. Materials and Methods: We analysed the effects of p53 knockout on sunitinib resistance. p53 expression in 53 mRCC patients receiving first-line sunitinib was determined immunohistochemically. We performed in silico analysis to examine the predictive value of p53 in mRCC. Results: WST-1 assays showed that p53 knockout decreased sensitivity to sunitinib. Sunitinib and nutlin-3 together suppressed cell growth. Immunohistochemistry revealed 11 p53-positive cases among 53 patients with mRCC. Kaplan–Meier analysis showed that p53-positive cases tended to be associated with poor progression-free survival (PFS) after first-line sunitinib treatment. In the JAVELIN 101 study, TP53 mutation was significantly associated with poor PFS after sunitinib treatment. Conclusion: p53 may be involved in sunitinib resistance and represent a valuable marker for sunitinib treatment in mRCC.*

Correspondence to: Yohei Sekino (ORCID ID: 0000-0003-1525-5463), MD, Ph.D., 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: p53, renal cell carcinoma, sunitinib, in silico analysis.

Renal cell carcinoma (RCC) accounts for approximately 90% of all renal tumours (1), and more than 30% of patients are diagnosed with metastatic RCC (mRCC) (2). Morbidity and mortality rates are high with the survival rate for mRCC ranging from 0-20% (3). Sunitinib is a small molecule multi-targeted tyrosine kinase inhibitor (TKI) that exerts its potent anti-angiogenic effects by blocking vascular endothelial growth factor receptor and platelet-derived growth factor receptor (4). Although immune checkpoint inhibitor (ICI)-based therapies are being increasingly used in the management of mRCC, sunitinib treatment continues to be administered as a first-line therapy for mRCC (5). Further, the combination of ICI and TKI was recently reported to improve overall survival in mRCC (6). Therefore, clarifying the molecular mechanisms underlying sunitinib resistance will significantly improve outcomes for patients treated with sunitinib.

Tumour protein p53, encoded by *TP53*, has a tumour suppressor role. p53 controls cell proliferation, DNA repair, senescence, and apoptosis through the induction of target genes such as *p21* and *BAX* (7). A recent review showed that *TP53* mutations contribute to the resistance of chemotherapy in cancer (8), and a recent report showed that a complex interaction between p53 and von Hippel-Lindau tumour

suppressor (VHL) influences the sensitivity to sunitinib in RCC cell lines (9). A recent sequence study found that the rate of *TP53* genomic alterations in mRCC increased after first-line treatment, including that with TKI, compared to the rate before first-line treatment (10). Another study reported that *TP53* mutations had independent prognostic value in mRCC treated with first-line TKIs including sunitinib (11). The present study aimed to examine the involvement of p53 in sunitinib resistance and the predictive value of p53 in mRCC treated with first-line sunitinib. We generated p53 knockout RCC cell lines by CRISPR-Cas9 and examined the effect of p53 knockout on sunitinib treatment. The effect of the combination of sunitinib with nutlin-3 was examined in RCC cell lines. We also immunohistochemically analysed p53 in mRCC treated with first-line sunitinib and performed in silico analysis to analyse the predictive value of p53 in mRCC.

Materials and Methods

Cell lines. Two RCC cell lines (Caki-1 and ACHN) were purchased from the American Type Culture Collection (Manassas, VA, USA). These cell lines were maintained as described previously (12). Sunitinib-resistant Caki-1 cells were established by culturing them with increasing concentrations of sunitinib (1-40 nM) for 6 months (13).

Generation of p53 knockout cells. To knock out p53 in Caki-1 and ACHN cells, we used the CRISPR-Cas9 technology, which was performed as described previously (14). p53 single-guide RNAs (sgRNAs; CRISPR-P53 vector) and scrambled sgRNAs (empty vector) were purchased from ABM Inc. (Richmond, BC, Canada). The sgRNA sequence of the CRISPR-P53 vector was GACGGAA ACCGTAGCTGCCC. Lentiviral particles were generated by cotransfection of HEK 293T cells with Cas9-sgRNA constructs and packaging plasmids (GAG, VSVG, and REV). After 48 h, the conditioned media containing lentiviral particles were harvested and used to infect cells using Polybrene (Funakoshi, Tokyo, Japan) as the transfection agent. Stable p53 knockout cells were selected by passaging in media containing 4 μg/ml puromycin.

Quantitative RT-PCR analysis. Quantitative RT-PCR analysis was performed as described previously (15). Total RNA was isolated from frozen samples or cancer cell lines using Isogen (Nippon Gene, Tokyo, Japan), and 1 g of total RNA was converted to cDNA with a First Strand cDNA Synthesis Kit (Amersham Biosciences Corp., Piscataway, NJ, USA). qPCR was performed with SYBR Select Master Mix (Applied Biosystems, Austin, TX, USA). The TP53 primer sequences were: Forward: GAGCTGAATGAGGCCTTGGA and Reverse: CTGAGTCAGGCCCTTCTGTCTT. Real-time detection of the emission intensity of SYBR green bound to double-stranded DNA was performed with a CFX Connect Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA). Actin Beta (ACTB)-specific PCR products, which were amplified from the same RNA samples, served as internal controls.

Western blotting analysis. Western blotting analysis was performed as described previously (16). Antibodies against p53 (DO-1), phosphorylated p53, p21, and cleaved PARP (Cell Signaling Technology, Inc., Danvers, MA, USA) were used at a 1:1,000 dilution. β-actin (Sigma-Aldrich, St. Louis, MO, USA) was detected as a loading control.

Table I. *Clinicopathologic characteristics of 53 mRCC patients treated with sunitinib*.

Number of cases	53
Median age (years)	68 (40-89)
Gender	
Male	40
Female	13
Race	
Asian	55
Median follow-up periods (months)	4 (1-74)
Histology	
Clear cell	42
Papillary	4
Chromophobe	3
Unclassified	4
Metastasis sites	
Lung	43
Lymph node	20
Bone	17
Brain	5
Liver	7
Nephrectomy	
Yes	48
No	5
IMDC criteria	
Favorable	5
Intermediate	30
Poor	18
Tumor response	
Complete response	0
Partial response	5
Stable disease	27
Progression disease	15
Not evaluable	6

mRCC: Metastatic renal cell carcinoma.

Sunitinib treatment. Sunitinib maleate and nutlin-3 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. A WST-1 assay was performed to assess cell viability after the cells had been exposed to drug (sunitinib and nutlin-3) treatment for 48 h. Drug sensitivity curves and 50% inhibitory concentration (IC_{50}) values were calculated using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA).

Tissue samples. We used 53 mRCC tissue samples obtained from the 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 (Hiroshima University, IRB# E912; Kure Medical Center/Chugoku Cancer Center: 2019-08; Hiroshima City Asa Citizens Hospital: 01-3-14). 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 anti-p53 antibody (1:100) (DAKO, Glostrup, Denmark) for 1 h at room temperature. p53 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 p53 (according to the median cut-off values rounded off to the nearest 10%). Using this definition, two observers (KS and NO) without knowledge of the patients' clinical and pathologic parameters or outcomes independently reviewed immunoreactivity in each specimen.

In silico analysis. Expression array data were downloaded from the Gene Expression Omnibus (GEO) and Array Express under accession numbers GSE64052 (18), GSE76088 (19), and E-MTAB-3267 (20). Gene expression data from 370 mRCC patients treated with sunitinib and 352 mRCC patients treated with avelumab+axitinib were downloaded from the JAVELIN RENAL 101 study performed by Motzer *et al*. (21).

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 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

p53 knockout decreases sensitivity to sunitinib in RCC. We examined p53 expression and the efficacy of p53 knockout by CRISPR-Cas9 in Caki-1 and ACHN cells. Western blotting showed that p53 expression was not detected in the Caki-1 and ACHN cells transfected with p53-CRISPR vector at mRNA and protein levels (Figure 1A and B). Then, we performed a WST-1 assay to measure cell viability in these two cell lines with knockout of p53 under various concentrations of sunitinib. The WST-1 assay showed that p53 knockout decreased the sensitivity to sunitinib (Figure 1C and D).

Phosphorylated p53 is overexpressed in sunitinib-resistant Caki-1 cells. To verify whether p53 is involved in sunitinib resistance, we investigated its expression in two public databases (GSE64052 and GSE76068). The expression of *TP53* did not change significantly in either the untreated or sunitinib-resistant statuses (Figure 2A). Although p53 expression was not changed in parental and sunitinib-resistant Caki-1 cells, phosphorylated p53 expression was suppressed in sunitinib-resistant Caki-1 cells under sunitinib treatment (Figure 2B).

Anti-proliferative effects of sunitinib and nutlin-3 in RCC cell lines. The above findings indicate that p53 is involved in sunitinib resistance. Nutlin-3 activates the p53 pathway by preventing the interaction of MDM2 with p53 (22). Therefore, we evaluated the efficacy of the combination of nutlin-3 and sunitinib in RCC cell lines. We measured cell viability following treatment with sunitinib alone or its combination with nutlin-3 in Caki-1 and ACHN cells. The combination of sunitinib and nutlin-3 significantly reduced cell viability compared to sunitinib alone in the Caki-1 and ACHN cells (Figure 3A). p21 expression was induced by nutlin-3 treatment, indicating that nutlin-3 activated the p53 pathway. We also analysed cleaved PARP, which is a marker of apoptosis. Western blotting showed that cleaved PARP was increased with the combined treatment of sunitinib and nutlin-3 compared to that with sunitinib alone (Figure 3B).

Clinical significance of p53 in response to sunitinib treatment in mRCC. We performed immunohistochemistry of p53 to analyse the association between p53 expression and therapeutic outcome in 53 patients with mRCC (Hiroshima cohort) treated with sunitinib as first-line treatment. Missense mutations of *TP53* led to prolonged half-life of the protein, which is immunohistochemically detected as nuclear accumulation (23). Positive p53 expression was found in 11 of the 53 (20.7%) patients (Figure 4A) but was not associated with objective response rate (complete response and partial response) (p=0.941) (Table II). Although not statistically significant, the results of Kaplan–Meier analysis revealed that the p53-positive cases of mRCC treated with first-line sunitinib tended to be associated with poor progression-free survival (PFS) (p=0.113) (Figure 4B). Low *TP53* expression was associated with poor PFS in mRCC treated with first-line sunitinib in the public database (E-MTAB-3267) (p=0.038) (Figure 4C). A recent large clinical study (JAVELIN101) examined PFS of patients with mRCC treated with first-line avelumab+axitinib or first-line sunitinib (21). Mutation of *TP53* was significantly associated with poor PFS in mRCC patients treated with first-line sunitinib (p=0.047) (Figure 4D) but not in those treated with first-line avelumab+axitinib (p=0.53) (Figure 4E).

Discussion

Considering that combination therapy with an ICI and TKI improves outcomes in mRCC, overcoming TKI resistance remains an essential goal. Recent evidence has led to the proposal that the mechanisms of sunitinib resistance might be multifactorial (24). In the present study, we showed that p53 knockout decreased sensitivity to sunitinib in RCC cell lines. What is more, phosphorylated p53 expression was suppressed in sunitinib-resistant RCC cell lines. A recent report showed that p53 knockdown suppressed the sensitivity to sunitinib through interaction with VHL (9). These results suggest that the p53 pathway is involved in sunitinib resistance in RCC cell lines. In

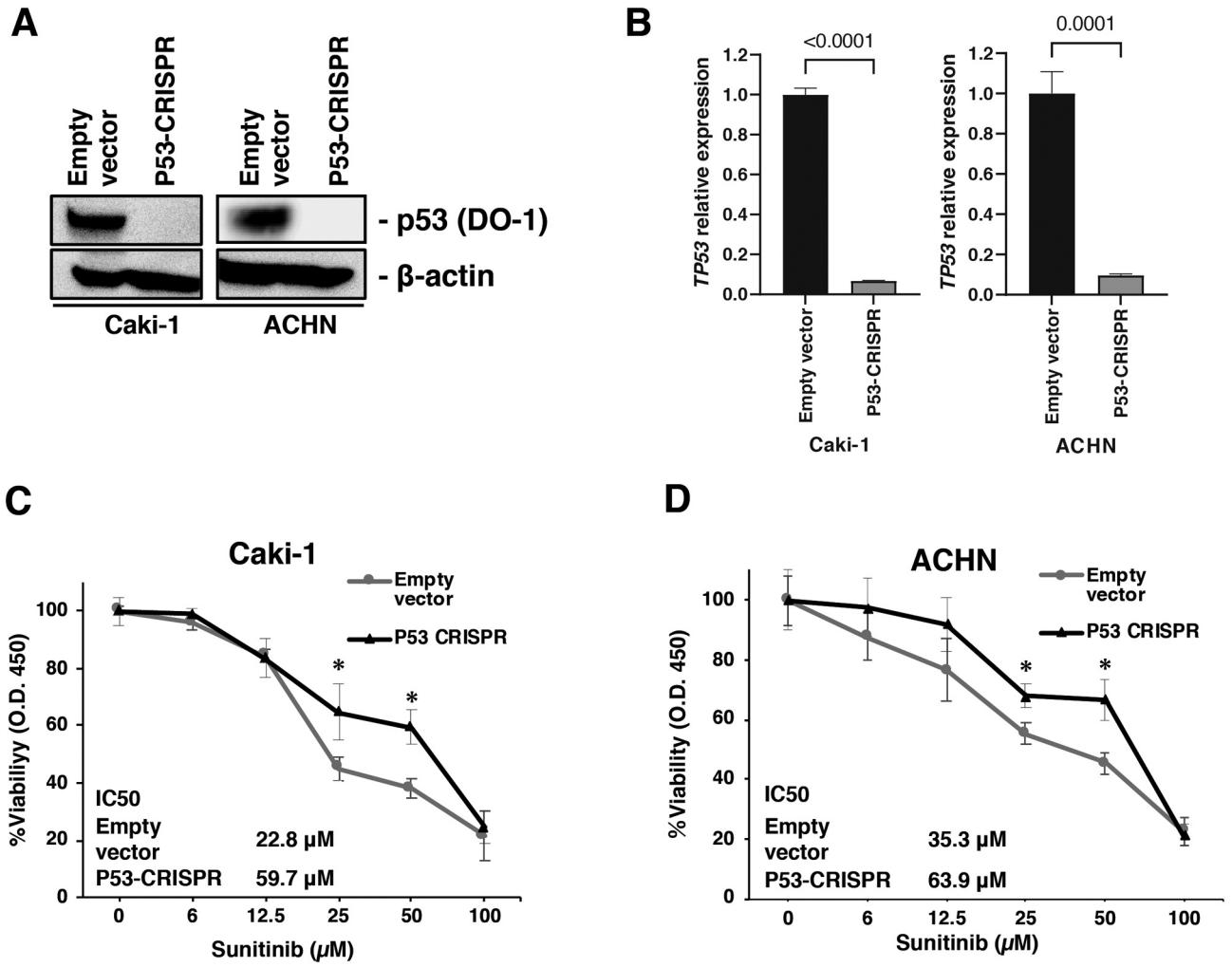

Figure 1. *p53 decreased sensitivity to sunitinib in RCC cell lines. (A) Western blotting of p53 in Caki-1 and ACHN cells transfected with empty vector and P53-CRISPR vector. β-actin was used as a loading control. (B) qRT-PCR of p53 in Caki-1 and ACHN cells transfected with empty vector and P53-CRISPR vector. The results are expressed as the mean±S.D. of triplicate measurements. (C-D) Dose-dependent effect of sunitinib on the viability of Caki-1 and ACHN cells transfected with empty vector and P53-CRISPR vector. The 50% inhibitory concentration (IC_{50}) values are indicated. *p<0.05.*

the present study, the combination of sunitinib with nutlin-3 significantly reduced cell growth and increased cleaved PARP expression. One study showed that nutlin-3 enhances the efficacy of another TKI, sorafenib, in RCC (25). Although a recent review showed that seven p53-MDM2 inhibitors are promising in cancer treatment, they have not been clinically utilized (26). Although further studies are needed, the p53 pathway may be a promising therapeutic target.

Although a recent paper reviewed biomarkers predictive of TKI efficacy, including sunitinib treatment, in mRCC (27), clinically relevant biomarkers are lacking. In the present study, immunohistochemical analysis showed that p53-positive cases tended to be associated with poor prognosis after first-line sunitinib treatment. Tumor tissue is easily available for immunohistochemical analysis because patients with mRCC are usually treated by partial or radical nephrectomy. Of note, we showed that mutation of *TP53* was not associated with poor PFS after treatment with avelumab+axitinib but was significantly associated with poor PFS after sunitinib treatment. A recent clinical trial study (IMmotion151) also showed mutation of *TP53* to be associated with poor PFS in mRCC treated with first-line sunitinib (p=0.001). On the contrary, mutation of *TP53* was not associated with atezolizumab+bevacizumab (p=0.161) (28). Collectively, these results suggest that p53 may serve as a potential biomarker for drug selection.

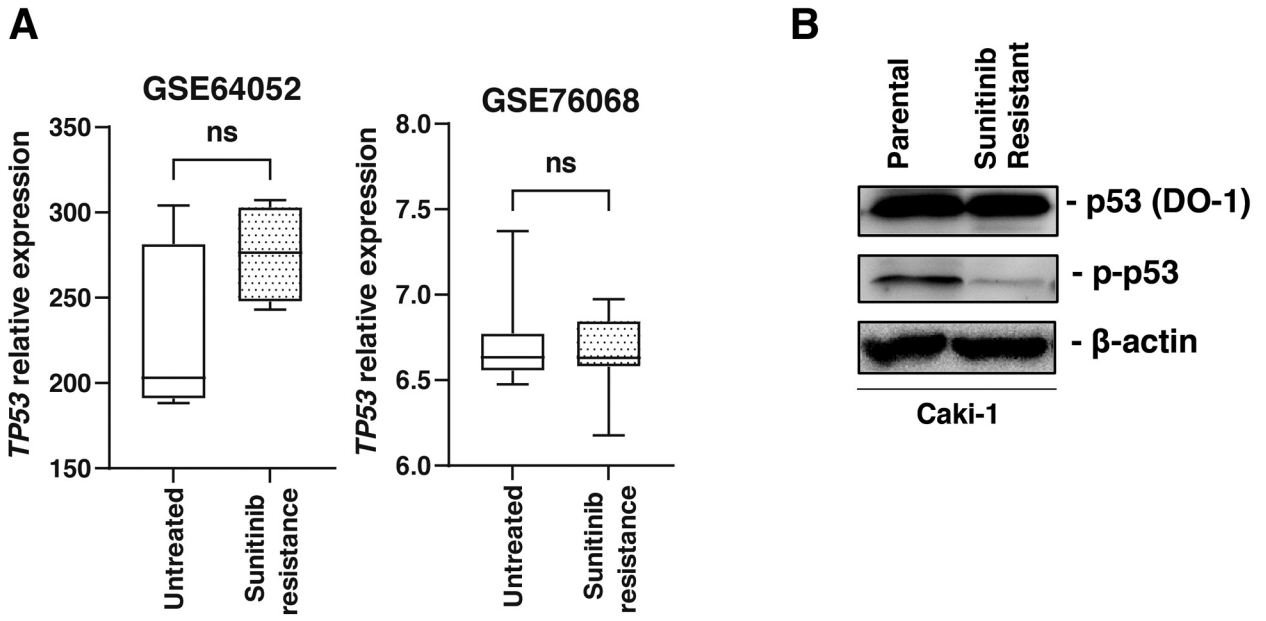

Figure 2. *The expression of p53 in sunitinib-resistant status. (A) TP53 expression value in untreated and sunitinib-resistant samples from GSE64052 and GSE76088. (B) Western blotting of p53 and phosphorylated p53 in parental and sunitinib-resistant Caki-1 cells upon treatment with 10 nM sunitinib. β-actin was used as a loading control. p-p53: Phosphorylated p53.*

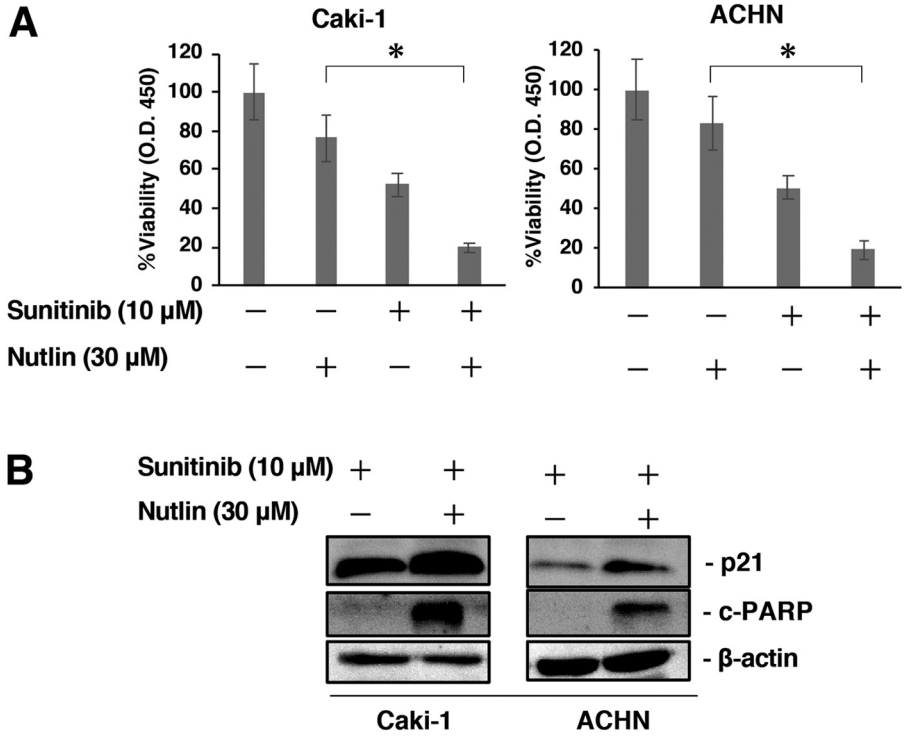

Figure 3. *Anti-proliferative effects of sunitinib and nutlin-3 in RCC cell lines. (A) The effect of combination therapy with sunitinib (10 μM) and nutlin-3 (30 μM) on the viability of Caki-1 and ACHN cells. The results are expressed as the mean±SD of triplicate measurements. *p<0.01. (B) Western blotting of p21 and c-PARP in Caki-1 and ACHN cells treated with either sunitinib (10 μM) alone or with sunitinib (10 μM) and nutlin-3 (30 μM). β-actin was used as a loading control. c-PARP: Cleaved PARP.*

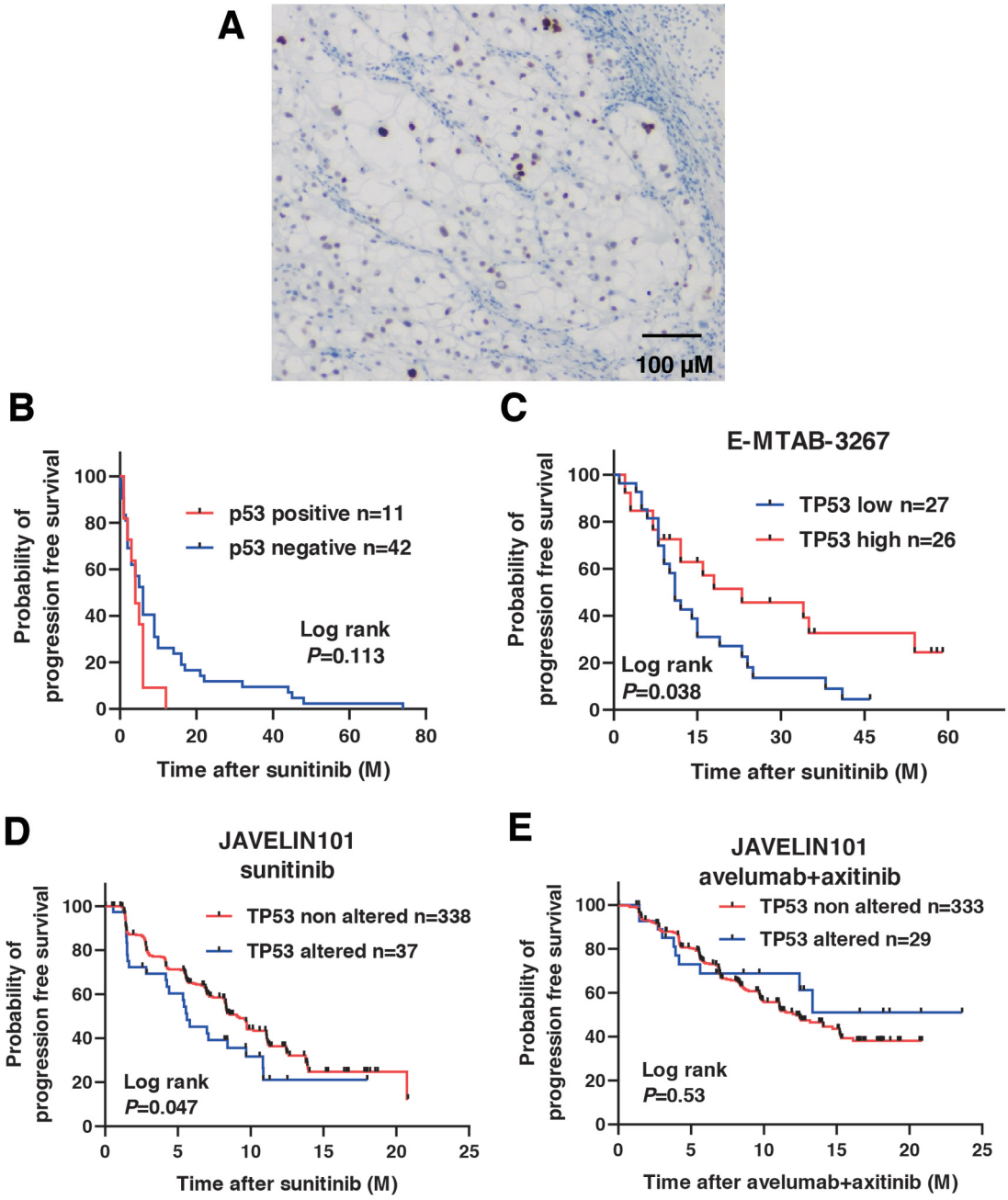

Figure 4. *Clinical significance of p53 in response to sunitinib treatment in metastatic RCC (mRCC). (A) Immunohistochemical staining of p53 in mRCC. Original magnification: 400×. (B) Kaplan–Meier plot of progression-free survival (PFS) of mRCC patients treated with sunitinib according to p53 expression from the Hiroshima cohort. (C, D) Kaplan–Meier plot of PFS of mRCC patients treated with sunitinib according to TP53 expression from E-MTAB-3267 and the JAVELIN 101 study. (E) Kaplan–Meier plot of PFS of mRCC patients treated with avelumab+axitinib according to TP53 expression from the JAVELIN 101 study.*

There are some limitations in this study. We performed immunohistochemistry of p53 in 53 patients with mRCC treated with first-line sunitinib. A prospective study with a larger number of patients with mRCC will be necessary to verify the present data. Although we showed that p53 was involved in sunitinib resistance, the detailed mechanism was not fully clarified.

In summary, our results showed that p53 was involved in sunitinib resistance in RCC cell lines. The combination of sunitinib with nutlin-3 reduced cell growth compared to

Table II. *Relationship between p53 expression and tumor response in 47 metastatic renal cell carcinomas treated with first line sunitinib.*

	P53 expression		*p*-Value[a]
	Positive (n=10) (%)	Negative (n=37) (%)	
CR/PR (n=5)	1 (20%)	4 (80%)	
SD/PD (n=42)	9 (21%)	33 (79%)	0.941

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

sunitinib alone in RCC cell lines. The p53-positive cases tended to be associated with poor prognosis after sunitinib treatment. In silico analysis showed that mutation of *TP53* was significantly associated with poor PFS after sunitinib therapy. The data presented here highlight the potential of p53 as a predictive marker and therapeutic target in patients with mRCC.

Conflicts of Interest

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

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 it. 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 also 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 Rini BI, Campbell SC and Escudier B: Renal cell carcinoma. Lancet *373(9669)*: 1119-1132, 2009. PMID: 19269025. DOI: 10.1016/S0140-6736(09)60229-4

3 Mekhail TM, Abou-Jawde RM, Boumerhi G, Malhi S, Wood L, Elson P and Bukowski R: Validation and extension of the Memorial Sloan-Kettering prognostic factors model for survival in patients with previously untreated metastatic renal cell carcinoma. J Clin Oncol *23(4)*: 832-841, 2005. PMID: 15681528. DOI: 10.1200/JCO.2005.05.179

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 Bedke J, Albiges L, Capitanio U, Giles RH, Hora M, Lam TB, Ljungberg B, Marconi L, Klatte T, Volpe A, Abu-Ghanem Y, Dabestani S, Pello SF, Hofmann F, Kuusk T, Tahbaz R, Powles T and Bex A: The 2021 updated European Association of Urology guidelines on renal cell carcinoma: Immune checkpoint inhibitor-based combination therapies for treatment-naive metastatic clear-cell renal cell carcinoma are standard of care. Eur Urol, 2021. PMID: 34074559. DOI: 10.1016/j.eururo.2021.04.042

7 Murray-Zmijewski F, Slee EA and Lu X: A complex barcode underlies the heterogeneous response of p53 to stress. Nat Rev Mol Cell Biol *9(9)*: 702-712, 2008. PMID: 18719709. DOI: 10.1038/nrm2451

8 He C, Li L, Guan X, Xiong L and Miao X: Mutant p53 gain of function and chemoresistance: The role of mutant p53 in response to clinical chemotherapy. Chemotherapy *62(1)*: 43-53, 2017. PMID: 27322648. DOI: 10.1159/000446361

9 Zhao Z, Chen C, Lin J, Zeng W, Zhao J, Liang Y, Tan Q, Yang C and Li H: Synergy between von Hippel-Lindau and P53 contributes to chemosensitivity of clear cell renal cell carcinoma. Mol Med Rep *14(3)*: 2785-2790, 2016. PMID: 27485825. DOI: 10.3892/mmr.2016.5561

10 Pal SK, Sonpavde G, Agarwal N, Vogelzang NJ, Srinivas S, Haas NB, Signoretti S, McGregor BA, Jones J, Lanman RB, Banks KC and Choueiri TK: Evolution of circulating tumor DNA profile from first-line to subsequent therapy in metastatic renal cell carcinoma. Eur Urol *72(4)*: 557-564, 2017. PMID: 28413127. DOI: 10.1016/j.eururo.2017.03.046

11 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

12 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

13 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

14 Sekino Y, Han X, Babasaki T, Miyamoto S, Kitano H, Kobayashi G, Goto K, Inoue S, Hayashi T, Teishima J,

Sakamoto N, Sentani K, Oue N, Yasui W and Matsubara A: TUBB3 is associated with high-grade histology, poor prognosis, p53 expression, and cancer stem cell markers in clear cell renal cell carcinoma. Oncology *98(10)*: 689-698, 2020. PMID: 32585672. DOI: 10.1159/000506775

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 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

19 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

20 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

21 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

22 Wang S, Zhao Y, Aguilar A, Bernard D and Yang CY: Targeting the MDM2-p53 protein-protein interaction for new cancer therapy: Progress and challenges. Cold Spring Harb Perspect Med *7(5)*: a026245, 2017. PMID: 28270530. DOI: 10.1101/cshperspect.a026245

23 Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M and Levine AJ: Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. Mol Cell Biol *8(2)*: 531-539, 1988. PMID: 2832726. DOI: 10.1128/mcb.8.2.531-539.1988

24 Sharma R, Kadife E, Myers M, Kannourakis G, Prithviraj P and Ahmed N: Determinants of resistance to VEGF-TKI and immune checkpoint inhibitors in metastatic renal cell carcinoma. J Exp Clin Cancer Res *40(1)*: 186, 2021. PMID: 34099013. DOI: 10.1186/s13046-021-01961-3

25 Vatsyayan R, Singhal J, Nagaprashantha LD, Awasthi S and Singhal SS: Nutlin-3 enhances sorafenib efficacy in renal cell carcinoma. Mol Carcinog *52(1)*: 39-48, 2013. PMID: 22006587. DOI: 10.1002/mc.20875

26 Nayak SK, Khatik GL, Narang R, Monga V and Chopra HK: p53-Mdm2 interaction inhibitors as novel nongenotoxic anticancer agents. Curr Cancer Drug Targets *18(8)*: 749-772, 2018. PMID: 28669344. DOI: 10.2174/1568009617666170623111953

27 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

28 Motzer RJ, Banchereau R, Hamidi H, Powles T, McDermott D, Atkins MB, Escudier B, Liu LF, Leng N, Abbas AR, Fan J, Koeppen H, Lin J, Carroll S, Hashimoto K, Mariathasan S, Green M, Tayama D, Hegde PS, Schiff C, Huseni MA and Rini B: Molecular subsets in renal cancer determine outcome to checkpoint and angiogenesis blockade. Cancer Cell *38(6)*: 803-817.e4, 2020. PMID: 33157048. DOI: 10.1016/j.ccell.2020.10.011

Received July 2, 2021
Revised July 22, 2021
Accepted July 23, 2021