Exceptional Response to Temsirolimus in a Metastatic Clear Cell Renal Cell Carcinoma With an Early Novel *MTOR*-Activating Mutation

Juan Francisco Rodríguez-Moreno, MD[a,b,*]; María Apellaniz-Ruiz, PhD[c,*]; Juan María Roldan-Romero, MS[c]; Ignacio Durán, MD[d]; Luis Beltrán, MD[e]; Cristina Montero-Conde, PhD[c]; Alberto Cascón, PhD[c,f]; Mercedes Robledo, PhD[c,f]; Jesus García-Donas, MD, PhD[a,b,†]; and Cristina Rodríguez-Antona, PhD[c,f,†]

Abstract

mTOR pathway inhibitors are important drugs for the treatment of advanced renal cell carcinoma (RCC). However, no valid predictive markers have been identified to guide treatment selection and identify patients who are sensitive to these drugs. Mutations activating the mTOR pathway have been suggested to predict response; however, their predictive value is still unclear. Here, we present the genomic and functional characterization of a patient with metastatic clear cell RCC (ccRCC) who experienced a partial response to temsirolimus after a poor response to 2 previous lines of treatment. At the time of publication, the patient was disease-free 8 years after temsirolimus treatment. Multiregion whole-exome sequencing (WES) on 3 regions of the primary tumor, 1 metastasis, and blood revealed tumor mutations in driver genes in ccRCC: a missense mutation in *VHL* (p.W88L), a loss-of-function mutation in *BAP1* (p.E454Rfs*15), and a novel missense mutation in *MTOR* (p.Y1974H). The *MTOR* mutation was present in all tumor regions, with similar allele frequency as the *VHL* mutation, and in vitro functional assessment of the *MTOR* variant demonstrated that it increased mTORC1 activity. Consistently, immunohistochemistry in the tumor samples demonstrated increased levels of phospho-S6. In conclusion, multiregion WES identified a novel *MTOR* mutation acquired early during tumor development as the event leading to a high sensitivity to temsirolimus treatment. This study supports tumor multiregion sequencing to detect truncal mutations in the mTOR pathway to identify patients sensitive to mTOR inhibitors.

J Natl Compr Canc Netw 2017;15(11):1310–1315
doi: 10.6004/jnccn.2017.7018

Treatment of advanced renal cell carcinoma (RCC) has changed drastically in the past decade. The mTORC1 inhibitors, everolimus and temsirolimus (also known as *rapalogs*), have been shown to be key drugs for use in first-line treatment[1] and pretreated patients.[2] Although recent evidence showed they had inferior global efficacy compared with modern immunotherapy[3] or new targeted agents,[4] approximately 20% of all patients with RCC respond to rapalogs. Furthermore, mTOR inhibitors combined with novel antiangiogenic agents have become a standard of care in pretreated patients,[5,6] and ongoing trials are exploring the value of these combinations in first-line treatment (ClinicalTrials.gov identifier: NCT02811861).

From [a]Oncology Unit, Clara Campal Comprehensive Cancer Center, Madrid, Spain; [b]Spanish Oncology GenitoUrinary Group (SOGUG), Madrid, Spain; [c]Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain; [d]Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain; [e]Department of Surgery, Whipps Cross Hospital, London, United Kingdom; and [f]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

*These authors contributed equally.

†These authors acted as senior authors.

Submitted March 15, 2017; accepted for publication August 2, 2017.

The authors have disclosed that they have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors.

This study was supported by Projects SAF2015-70820-ERC and SAF2015-64850-R (Spanish Ministry of Economy, Industry and Competitiveness MEIC, co-funded by the European Regional Development Fund ERDF) and by the Instituto de Salud Carlos III (Acción Estratégica en Salud grant number PI13/00622).

Correspondence: Cristina Rodríguez-Antona, PhD, Spanish National Cancer Research Centre (CNIO), Hereditary Endocrine Cancer Group, Melchor Fernández Almagro 3, 28029 Madrid, Spain.
E-mail: crodriguez@cnio.es;

Jesus García-Donas, MD, Clara Campal Comprehensive Cancer Center, Oncology Unit, Oña Street 10, 28050 Madrid, Spain.
E-mail: jgarciadonas@gmail.com

Extraordinary responses to mTOR inhibitors have been described in few patients with mutations in *TSC1*, *TSC2*, or *MTOR*.[7–9] However, a recent study in RCC showed that not all patients with mTOR-activating mutations responded to hh inhibitors, whereas some without mutations did.[10] Additionally, Lim et al[11] explored *MTOR*, *TSC1*, *TSC2*, *NF1*, and *PIK3CA* mutations in a cohort of 22 patients with different tumors with significant response to everolimus, identifying candidate mutations in only 50%. These studies suggest that additional mechanisms, such as clonal heterogeneity,[12,13] modulate response. Thus, understanding of the underlying mechanisms leading to mTOR inhibitor tumor sensitivity is currently incomplete, and additional investigations and cases demonstrating exquisite responses are needed.

This study describes a patient with metastatic clear cell RCC (ccRCC) refractory to multiple lines of anti–vascular endothelial growth factor (VEGF) therapy that, on temsirolimus treatment, exhibited an exceptional clinical response. Multiregional whole-exome sequencing (WES), in vitro functional assessment, and immunohistochemistry (IHC) of the tumor samples identified a novel *MTOR* mutation acquired early during tumor development as being responsible for the drug sensitivity. The molecular characterization of patients experiencing long responses to rapalogs could help define a subset who would benefit from these drugs.

Case Report

A 57-year-old Caucasian woman with an unremarkable past medical history presented with lumbar pain. Physical examination revealed a mass on the right flank. An FDG-PET scan revealed a renal mass highly suggestive of malignancy, signs of liver spread, and pelvic and lumbar spinal cord bone metastases (Figure 1A). Tumor staging at initial diagnosis was pT1bN0M1 (stage IV). In April 2007, an open right radical nephrectomy was performed. The histopathologic report revealed Fuhrman grade 4 advanced ccRCC. According to the Memorial Sloan Kettering Cancer Center (MSKCC) prognostic model,[14] the patient was classified as being in the intermediate-risk group based on 2 risk factors for the prognostic score: Karnofsky performance status of 70% and a serum lactic dehydrogenase level >1.5 times the upper limit of normal. She received palliative radiotherapy for a painful right iliac metastasis and started sunitinib treatment at 50 mg/d on a 4 weeks on, 2 weeks off schedule (4/2). After 2 cycles, all tumoral lesions had progressed and treatment was switched to sorafenib, 400 mg twice daily. New response assessment after 2 cycles revealed disease progression with new liver and bone lesions and clinical deterio-

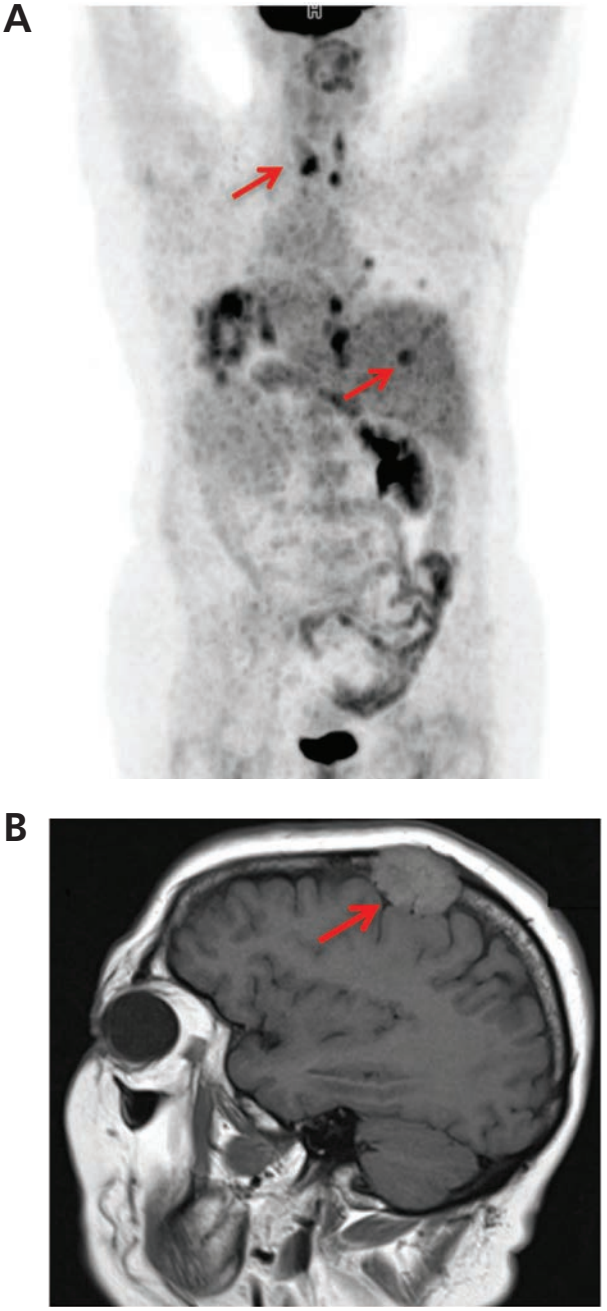

Figure 1. (A) Initial tumor dissemination detected by PET/CT (arrows). (B) MRI showing skull metastasis (arrow), which was surgically removed in 2015.

ration. In March 2008, the patient started temsirolimus at 25 mg weekly and experienced a remarkable clinical benefit. She had a partial response according to RECIST criteria, with a time to best response after starting temsirolimus of 6 weeks (temsirolimus start: March 3, 2008; response at first reevaluation: April 16, 2008), and experienced overall good tolerance; she continued temsirolimus for >7 years. Main toxicity consisted of 3 different pneumonitis episodes (grade 1–3) that required dose interruptions or reductions. In August 2015, a new solitary bone metastasis arose in the skull, whereas others remained with no change (Figure 1B). A complete resection of the lesion was performed followed by local radiotherapy. At 21 months after the metastasectomy, the patient continues on treatment with biweekly temsirolimus in combination with denosumab, with no tumor progression.

We performed WES in 3 different regions of the primary tumor obtained before treatment, in the skull metastasis resected 7 years after temsirolimus treatment initiation, and in the germline DNA obtained from the patient's blood. The mean depth of coverage was >80x for the 3 regions of the primary tumors, 136x for the metastasis, and 94x for the blood (see supplemental eAppendix 1 for details regarding methodology, available online with this article at JNCCN.org). The number of tumor mutations (single nucleotide variants and indels) leading to nonsynonymous coding or loss-of-function (LOF) variants in the primary tumor was 160. Tumor mutations refer to those detected in the tumor but absent in the blood. In total, 116, 76, and 65 mutations were detected in each of the 3 tumor regions and from these, 41 were shared (Figure 2; supplemental eTable 1). Among the shared tumor mutations, 39 were missense variants and 2 were LOF variants caused by frameshifts. The variants affecting genes frequently mutated in ccRCC were detected in all tumor samples analyzed: *VHL* (c.263G>T; p.W88L), *BAP1* (c.1359dup; p.E454Rfs*15), and *MTOR* (c.5920T>C; p.Y1974H), and were validated by Sanger sequencing. In ccRCC the primary driver event is *VHL* inactivation, and thus *VHL* mutations are early alterations present in all tumor cells.[15] The *MTOR* mutation frequencies detected in the different tumor samples sequenced were similar to those found for *VHL*, indicating that the *MTOR* mutation was also a truncal event in this patient (average ratio

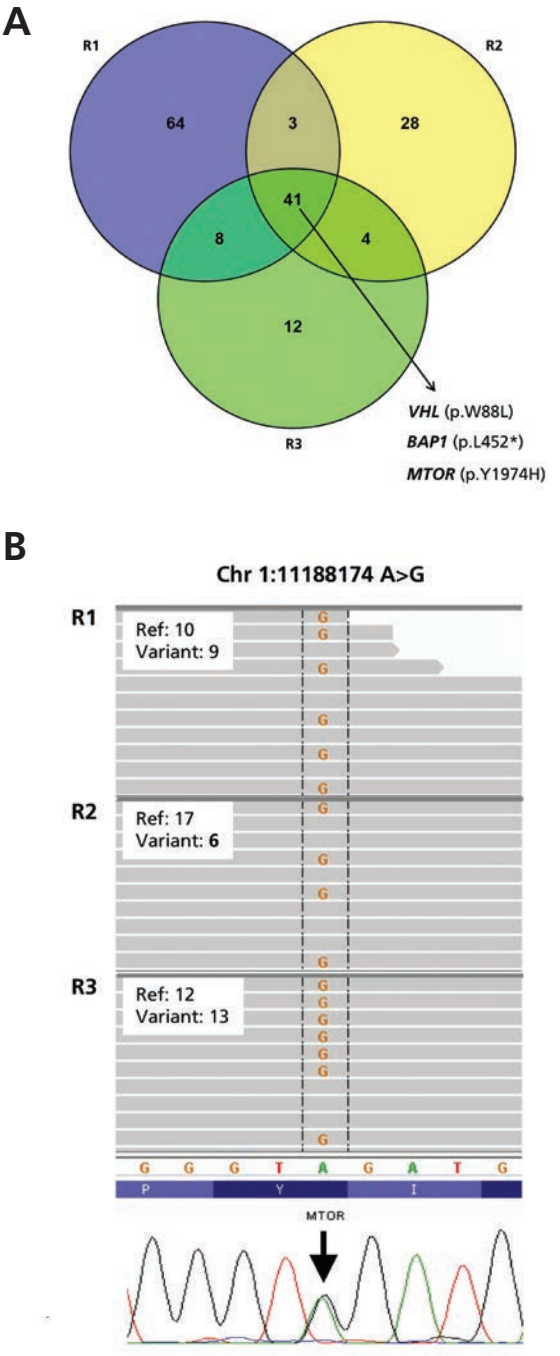

Figure 2. Mutations revealed by whole-exome sequencing (WES). (A) Venn diagram indicating the number of tumor (absent in blood) loss-of-function and nonsynonymous coding variants identified through WES in 3 regions (R1–R3) of the primary tumor. Shared variants affecting relevant clear cell renal cell carcinoma genes are indicated below the arrow. (B) Representative genome images from the Integrative Genomics Viewer (Broad Institute), along with the number of reads for the reference (ref) and variant allele of the *MTOR* Y1974H mutation, and Sanger sequencing chromatogram.

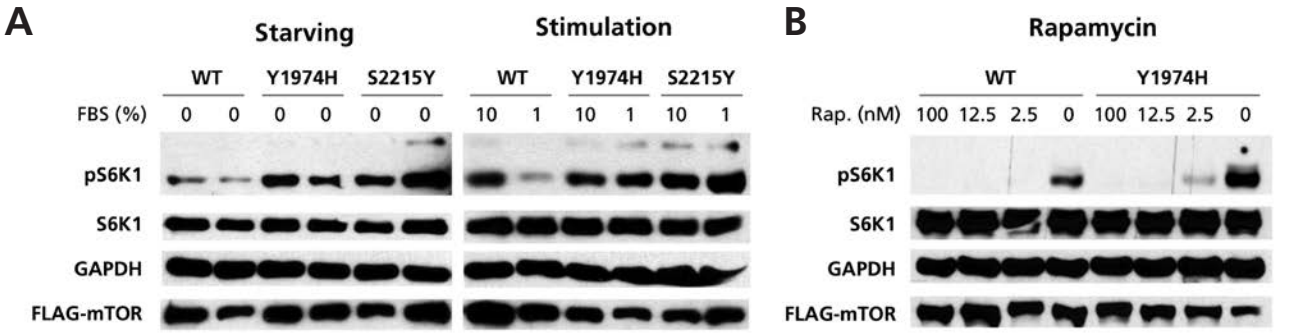

Figure 3. Functional assessment of *MTOR* p.Y1974H variant. Constructs expressing wild-type (WT) mTOR, mTOR Y1974H, and mTOR S2215Y (a positive control for mTOR activation) were transfected in HEK293 cells and the effect of phosphorylation of the downstream target S6K1 (pS6K1) was tested with Western blot. (A) Western blot analysis 24 hours after transfection cells were serum-starved for 24 hours (Starving) and then stimulated with 1% or 10% fetal bovine serum for 2 hours (Stimulation). In the Starving assay, lanes 1–2, 3–4, and 5–6 are duplicates from different experiments. (B) Cells transfected with *mTOR* constructs were treated with 2.5, 12.5, and 100 nM of rapamycin (rap) for 6 hours.
Abbreviation: GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

of 1.1; supplemental eTable 1). For *BAP1* mutation, the average ratio was 0.66. Other genes implicated in cancer with mutations included *BCL11B*, *CIC*, *EML4*, *KMT2C*, *NOTCH1*, and *RANBP2* (see supplemental eTable 1).

To assess the effect of the *MTOR* novel variant p.Y1974H, HEK293 cells were transfected with constructs expressing the wild-type mTOR protein or the Y1974H variant protein. The mTOR-activating mutation S2215Y was used as control for the experiments. The Thr389 phospho-S6K1 (pS6K1) levels, which represent TORC1 activity, were augmented for the Y1974H variant compared with mTOR wild-type, and similar to those obtained for S2215Y (Figure 3A), indicating that mTOR Y1974H activates mTORC1. In addition, we observed that mTOR Y1974H was sensitive to rapamycin (Figure 3B). mTOR Y1974H also led to an increased number of cells in S phase with a concomitant decrease in G0/G1, and an alteration in forward scatter (data related to an alteration in cell size) compared with mTOR wild-type and similar to mTOR S2215Y (supplemental eFigure 1).[16] Immunohistologic analysis of the tumor samples revealed activation of the mTOR pathway through a positive immunostaining of phospho-S6, a downstream target of mTOR (Figure 4A–C), with the metastasis staining being more intense than that in the primary tumor. The staining of p-ERK (Figure4 D–F) was weak in the primary tumor, whereas the metastasis exhibited a nuclear and cytoplasmatic intense and extensive staining.

WES of the metastasis that arose 7 years after starting temsirolimus treatment revealed 80 tumor nonsynonymous coding or LOF variants (71 missense, 2 in-frame deletions, and 7 LOF) present in the tumor and absent in the blood. Of these, 51 were shared with the primary tumor and 29 were exclusively present in the metastasis (supplemental eTable 1). Among the genes exclusively mutated in the metastasis, only 3 (*CRTC3*, *KAT6B*, *PBRM1*) were in the Cancer Gene Census (CGC) but none were directly related to the *MTOR* pathway, and the variants have not been previously described in tumors, according to the COSMIC database.

The full region of the *MTOR* FKBP-rapamycin binding domain (FRB), including intronic regions, was sequenced by Sanger. However, no metastasis-specific variants were detected.

Discussion

Rapalogs are drugs approved for metastatic ccRCC in first-line treatment[1] and pretreated patients.[2] Although recent studies have shown less efficacy of rapalogs compared with nivolumab[3] and cabozantinib,[4] combination of everolimus and lenvatinib has become a second-line standard,[5,6] and ongoing trials are assessing combinations in first-line treatment. Interestingly, monotherapy with mTOR inhibitors is very useful in a considerable subgroup of patients, with long-term responses, regardless of the line of treatment.[10,12] Mutations affecting mTOR pathway genes have been explored as possible biomarkers of activity for these drugs, and despite initial descriptions of highly sensitive patients with mutations in *MTOR*, *TSC1*, and *TSC2*,[7–9] more recent studies show only

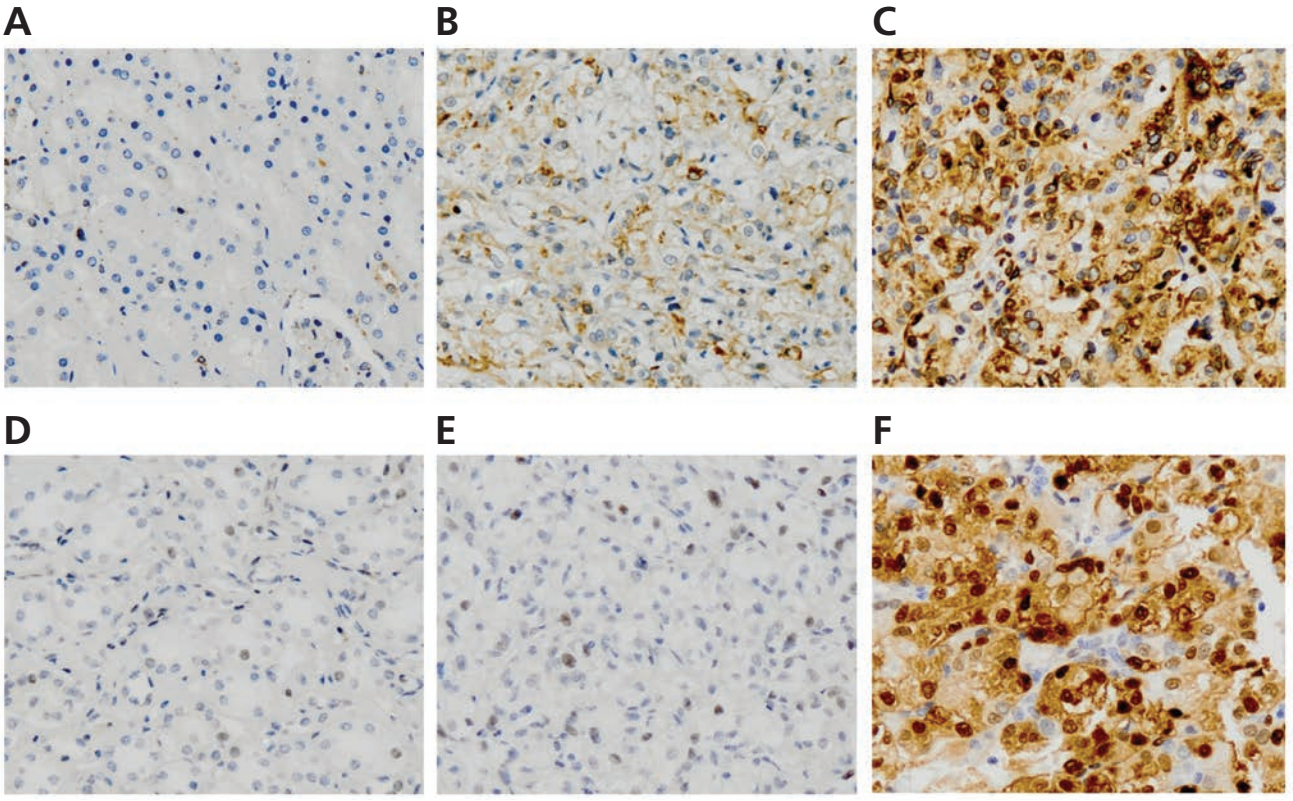

Figure 4. Immunohistochemical (IHC) study of *TSC2* and phospho-S6. Representative pictures of phospho-S6 IHC staining (original magnification x20) in (A) nontumoral kidney, (B) primary tumor, and (C) bone metastasis from the patient. Phospho-ERK staining (original magnification x20) in (D) nontumoral kidney, (E) primary tumor, and (F) bone metastasis.

partial correspondence between mTOR pathway mutations and response to mTOR inhibitors.[10] Thus, understanding of the molecular mechanisms conferring sensitivity to mTOR drugs is incomplete, and it is crucial to identify biomarkers that would allow tailored treatment of advanced RCC.

This report presents a patient with metastatic ccRCC who experienced an unusual prolonged response to temsirolimus. Multiregion sequencing of the tumor, functional studies on a candidate variant, and immunohistologic characterization of the tumors allowed the identification of an *MTOR* mutation (p.Y1974H) as causative for the response. This mutation is located in the FAT (FRAP-ATM-TTRAP) domain of mTOR, a region that has a prominent cluster of hyperactivating mutations in RCC that lead to an increase in mTORC1 and mTORC2 activities.[17,18] These mutations may destabilize the structure of the FAT domain, directly deregulating mTOR kinase activity and affecting the binding of mTOR complex proteins.[18] As expected for ccRCC, a *VHL* mutation (p.W88L) was found in all regions of the primary tumor and in the metastasis, in agreement with an early event.[15,19] Additional alterations in genes frequently mutated in clear cell histology were present in *BAP1* and *MTOR*.[20] The *MTOR* mutation (p.Y1974H) was detected in the 3 regions of the primary tumor and in the metastasis with similar frequency to *VHL* mutation, the major ccRCC driver event (supplemental eTable 1), and functional in vitro assays confirmed it was an activating mutation. ccRCC intratumor heterogeneity has been shown to affect the mTOR pathway,[15] and the moment during clonal evolution in which the mutations occur has been suggested to impact drug response.[12,13] Our results are consistent with a truncal *MTOR* mutation, which would render the tumor ubiquitously addicted to mTORC1 hyperactivity. In addition, the *BAP1* mutation found in the patient suggested a poor outcome, because these mutations are associated with poor RCC prognosis.[21,22] The remarkable response obtained with third-line temsirolimus highlights the high sensitivity to this drug.

Interestingly, the patient developed a bone metastasis while receiving temsirolimus. To date, there is one report identifying a mechanism for acquired mTOR inhibitor resistance, which consisted of a secondary mutation (p.F2108L) in mTOR FRB domain.[9] In the present patient, the only *MTOR* mutation found in the metastasis was p.Y1974H. Sequencing of the intronic region of *MTOR* FRB domain ruled out mutations that may lead to alternative splicing events altering this region. Regarding other genes, WES revealed mutations exclusive of the metastasis; however, none of the mutated genes was directly related to the mTOR pathway. IHC of the tumors revealed more intense staining for phospho-S6 in the metastasis than in the primary tumor, suggesting that the metastasis had acquired an additional alternative mechanism hyperactivating mTORC1. Phospho-ERK–strong IHC staining in the metastasis (Figure 4F) suggested the mechanism could be connected with the Ras-ERK pathway; however, none of the metastasis mutations were directly linked with this pathway. At any rate, the resistance mechanism may involve alterations not detectable by DNA sequencing (eg, epigenetic changes).

Conclusions

Although patients with extraordinary responses to mTOR inhibitors have been reported, the mechanisms underlying these responses are still not well understood. In this study, multiregional WES directly linked a truncal novel activating *MTOR* mutation with the exceptional response to temsirolimus in a patient with ccRCC. Our results support the sequencing of multiple tumor areas to identify early mutations affecting the mTOR pathway, to ultimately reveal predictive markers able to personalize RCC treatments.

Acknowledgments

The authors would like to thank the National Centre for Genomic Analysis personnel, and especially Sergi Beltran and Sophia Derdak, for their excellent support in whole-exome sequencing. They also want to thank the CNIO Flow Cytometry Core Unit and Dolores Martinez for their excellent support and assistance.

References

1. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356:2271–2281.
2. Motzer RJ, Escudier B, Oudard S, et al. Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. Cancer 2010;116:4256–4265.
3. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1803–1813.
4. Choueiri TK, Escudier B, Powles T, et al. Cabozantinib versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1814–1823.
5. Motzer RJ, Hutson TE, Glen H, et al. Lenvatinib, everolimus, and the combination in patients with metastatic renal cell carcinoma: a randomised, phase 2, open-label, multicentre trial. Lancet Oncol 2015;16:1473–1482.
6. Motzer RJ, Hutson TE, Ren M, et al. Independent assessment of lenvatinib plus everolimus in patients with metastatic renal cell carcinoma. Lancet Oncol 2016;17:e4–5.
7. Iyer G, Hanrahan AJ, Milowsky MI, et al. Genome sequencing identifies a basis for everolimus sensitivity. Science 2012;338:221.
8. Wagle N, Grabiner BC, Van Allen EM, et al. Activating *MTOR* mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. Cancer Discov 2014;4:546–553.
9. Wagle N, Grabiner BC, Van Allen EM, et al. Response and acquired resistance to everolimus in anaplastic thyroid cancer. N Engl J Med 2014;371:1426–1433.
10. Kwiatkowski DJ, Choueiri TK, Fay AP, et al. Mutations in TSC1, TSC2, and *MTOR* are associated with response to rapalogs in patients with metastatic renal cell carcinoma. Clin Cancer Res 2016;22:2445–2452.
11. Lim SM, Park HS, Kim S, et al. Next-generation sequencing reveals somatic mutations that confer exceptional response to everolimus. Oncotarget 2016;7:10547–10556.
12. Voss MH, Hakimi AA, Pham CG, et al. Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from *MTOR* inhibitor therapy. Clin Cancer Res 2014;20:1955–1964.
13. Voss MH, Hsieh JJ. Therapeutic guide for mTOuRing through the braided kidney cancer genomic river. Clin Cancer Res 2016;22:2320–2322.
14. Motzer RJ, Bacik J, Mazumdar M. Prognostic factors for survival of patients with stage IV renal cell carcinoma: Memorial Sloan-Kettering Cancer Center experience. Clin Cancer Res 2004;10:6302S–6303S.
15. Gerlinger M, Horswell S, Larkin J, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 2014;46:225–233.
16. Sato T, Nakashima A, Guo L, et al. Single amino-acid changes that confer constitutive activation of *MTOR* are discovered in human cancer. Oncogene 2010;29:2746–2752.
17. Grabiner BC, Nardi V, Birsoy K, et al. A diverse array of cancer-associated *MTOR* mutations are hyperactivating and can predict rapamycin sensitivity. Cancer Discov 2014;4:554–563.
18. Ghosh AP, Marshall CB, Coric T, et al. Point mutations of the *MTOR*-RHEB pathway in renal cell carcinoma. Oncotarget 2015;6:17895–17910.
19. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883–892.
20. TCGA. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 2013;499:43–49.
21. Pena-Llopis S, Vega-Rubin-de-Celis S, Liao A, et al. BAP1 loss defines a new class of renal cell carcinoma. Nat Genet 2012;44:751–759.
22. Kapur P, Pena-Llopis S, Christie A, et al. Effects on survival of BAP1 and PBRM1 mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. Lancet Oncol 2013;14:159–167.

See JNCCN.org for supplemental online content.