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CLINICAL RESEARCH

mTORC1 Inhibition Is an Effective Treatment for Sporadic Renal Angiomyolipoma

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Introduction: Renal angiomyolipoma (AML) is the most common benign renal tumor. Despite a generally benign histology, AML can result in significant morbidity, from intra-abdominal hemorrhage and reduction in kidney function. While classically associated with the autosomal dominant disorder tuberous sclerosis complex (TSC) or with pulmonary lymphangioleiomyomatosis, most AMLs are sporadic. Mammalian target of rapamycin complex 1 (mTORC1) inhibitors (e.g., sirolimus) have been found to be effective in treating TSC- or lymphangioleiomyomatosis-associated AML, but to date it is unknown whether this strategy is effective for sporadic AML.

Methods: We stained tumor specimens of sporadic AML patients for pS6 to assess for mTORC1 activation.

Results: We detected strong activation of the mTORC1 pathway, similar to TSC-associated AML. Consequently, we showed that *in vitro* treatment with sirolimus results in significant growth inhibition of the human sporadic AML cell line SV7Tert, similar to the effect seen when the same treatment is applied to the human TSC-associated AML cell line UMBSV-tel. To further investigate the potential of mTORC1 inhibition for treating sporadic AML and assess whether the *in vitro* results are clinically relevant, we identified a patient with sporadic, bilateral AMLs, showing continued tumor growth following a partial nephrectomy. Using immunostaining, we detected strong mTORC1 activation in the patient's AML tissue. Accordingly, upon treatment with sirolimus, we noted significant reduction in the patient's tumor volume and resolution of hydronephrosis, without any significant side effects.

Conclusion: We propose mTORC1 inhibition as an effective treatment option for patients with sporadic AML, which represents the vast majority of patients with this tumor.

Kidney Int Rep (2018) **3,** 155–159; http://dx.doi.org/10.1016/j.ekir.2017.07.016

Angiomyolipoma (AML), the most common benign kidney tumor, is characterized by a unique histology, consisting of blood vessels, adipose tissue, and smooth muscle in varying proportions.[1,2] Despite its benign histology, AML can result in severe hemorrhage or renal failure.[3] Furthermore, an aggressive variant, termed "epithelioid" AML, has been described and shown to possess metastatic potential.[4] AML is strongly associated with tuberous sclerosis complex (TSC), an autosomal dominant syndrome characterized by the emergence of benign tumors in various organs, including the kidneys, brain, and skin. TSC has been shown to result from mutations in *TSC1* or *TSC2*, which code for hamartin or tuberin, respectively. The latter act as a tumor suppressor complex, inhibiting the activity of mammalian target of rapamycin complex 1 (mTORC1). mTORC1 is a key regulator of various cellular functions that acts to promote cell growth, protein synthesis, and vasculogenesis via phosphorylation of 2 main downstream effectors, S6K and 4EBP1. TSC1/2 loss of function leads to constitutive mTORC1 activation and aberrant cell growth. Despite the strong association with TSC, only 20% of AML patients have underlying TSC, and most cases arise sporadically. Several randomized controlled trials have demonstrated that mTORC1 inhibitors reduce the size of AML tumors in patients with TSC. Nonetheless, there are currently no available data regarding the efficacy of mTORC1 inhibition in treating sporadic AML, which represents the majority of cases. Interestingly, sporadic AMLs have been shown to harbor somatic *TSC2* mutations and to exhibit mTORC1 activation.[5] In this study, we were interested in asking whether mTORC1 inhibition

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Received 24 June 2017; revised 26 July 2017; accepted 31 July 2017; published online 11 August 2017

could be effective in treating not only TSC-associated AML, but also sporadic AML as well. First, we demonstrated that sporadic AML tumors indeed show activation of the mTORC1 pathway. Next, we treated a human sporadic AML cell line with rapamycin and showed that the treatment resulted in significant growth inhibition, to a similar extent to that seen when a human TSC-associated AML cell line is treated with rapamycin. Finally, to assess whether these results could be translated for use in the clinic, we detected a patient with large bilateral sporadic AMLs, which exhibited continued tumor growth following partial nephrectomy. Following the demonstration of mTORC1 activation in the patient's AML tissue, using pS6 staining, we treated her with rapamycin and followed tumor growth using magnetic resonance imaging. We detected significant and continued tumor shrinkage over several years, while exhibiting minimal side effects. In summary, we demonstrate that mTORC1 inhibitors may well represent an effective treatment for patients with sporadic AML, representing the majority of AML patients.

MATERIALS AND METHODS

See Supplementary Data.

RESULTS

Sporadic AML Exhibits Activation of the mTORC1 Pathway

So as to validate that sporadic AML tumors show mTORC1 activation, which could serve as the basis for targeting this pathway in this group of patients, we first carried out immunohistochemical staining for pS6, a marker of mTORC1 activation in normal human adult kidneys (hAK), sporadic AML tumors, and TSC-related AML (Figure 1). hAK demonstrated varying levels of pS6 expression, mostly in distal tubules (DT) and collecting ducts (CD) (Figure 1). Within the tumor tissue of sporadic AML specimens we detected a strong pS6 expression, whereas the normal kidney borders exhibited an expression pattern similar to that of hAK, involving mostly DT and CD. As expected, TSC-related AML demonstrated a strong pS6 expression in both tumor tissue and within normal kidney tissue, reflecting the germline mutation in TSC1/2 leading to widespread mTORC1 activation (Figure 1). Taken together, these results indicate that the tumor tissue of sporadic AML exhibits strong activation of the mTORC1 pathway.

mTORC1 Inhibition Halts the *In Vitro* Growth of Human Sporadic AML Cells

Having shown enhanced mTORC1 activity in sporadic AML tumors, we next asked whether mTORC1 blockade would inhibit the growth of sporadic AML cells *in vitro*. For this purpose, we treated SV7Tert cells, representing a human sporadic AML cell line,[6,7] with rapamycin for 96 hours. Treated SV7Tert cells demonstrated significant growth inhibition compared to vehicle-treated SV7Tert cells (Figure 2a). In order to quantify this effect, we tested the viability of rapamycin- and vehicle-treated SV7Tert cells using the MTS assay. UMBSV-tel cells, representing a cell line derived from TSC-associated AML, served as control. We detected a significant reduction in cell viability in rapamycin-treated compared to vehicle-treated SV7Tert cells. Importantly, UMBSV-tel cells demonstrated a similarly significant reduction in proliferation following rapamycin treatment (Figure 2b). Taken together, these results indicate that sporadic AML cells, similarly to TSC-related AML cells, are sensitive to mTORC1 inhibition.

A Sporadic AML Patient Showing Continued Tumor Growth Despite Surgical Treatment

Next, we were interested in assessing the clinical potential of our findings and testing whether mTORC1 inhibition could be effective in treating sporadic AML. For this purpose, we detected a sporadic AML patient, who demonstrated continued tumor growth following surgical treatment. The patient, a 35-year-old female, born to healthy, nonconsanguineous parents with an unremarkable family history, presented with abdominal pain at age 23 years. Her history and physical examination were unremarkable. Computed tomography (CT) revealed a 37-mm left lateral kidney mass consistent with AML. Two years later, CT demonstrated continued AML growth, now reaching 50 mm, alongside multiple 5- to 9-mm AMLs in the lower left kidney pole. The patient underwent renal angiography, which demonstrated a hypovascular mass, precluding embolization. Hence, left partial nephrectomy was performed, with excision of the large mass alongside small, 2- to 3-mm masses. Histological analysis revealed fat-predominant AML that was positive for human melanoma black (HMB)-45 and α—smooth muscle actin. One year later, CT revealed a new, 16-mm lateral left kidney AML, which progressed over the next 6 years, when abdominal magnetic resonance imaging (MRI) demonstrated in the left renal pelvis, a fatty 22 × 49-mm mass (Figure 2c), leading to mild hydronephrosis. Several additional AMLs of up to 6 mm were seen in the left cortex. The right kidney demonstrated another 3-mm AML. Notably, 2 years following the nephrectomy, the patient became pregnant via spontaneous conception, and gave birth to a healthy daughter. Aside from this transient hyperestrogenic state induced by pregnancy, the patient was not

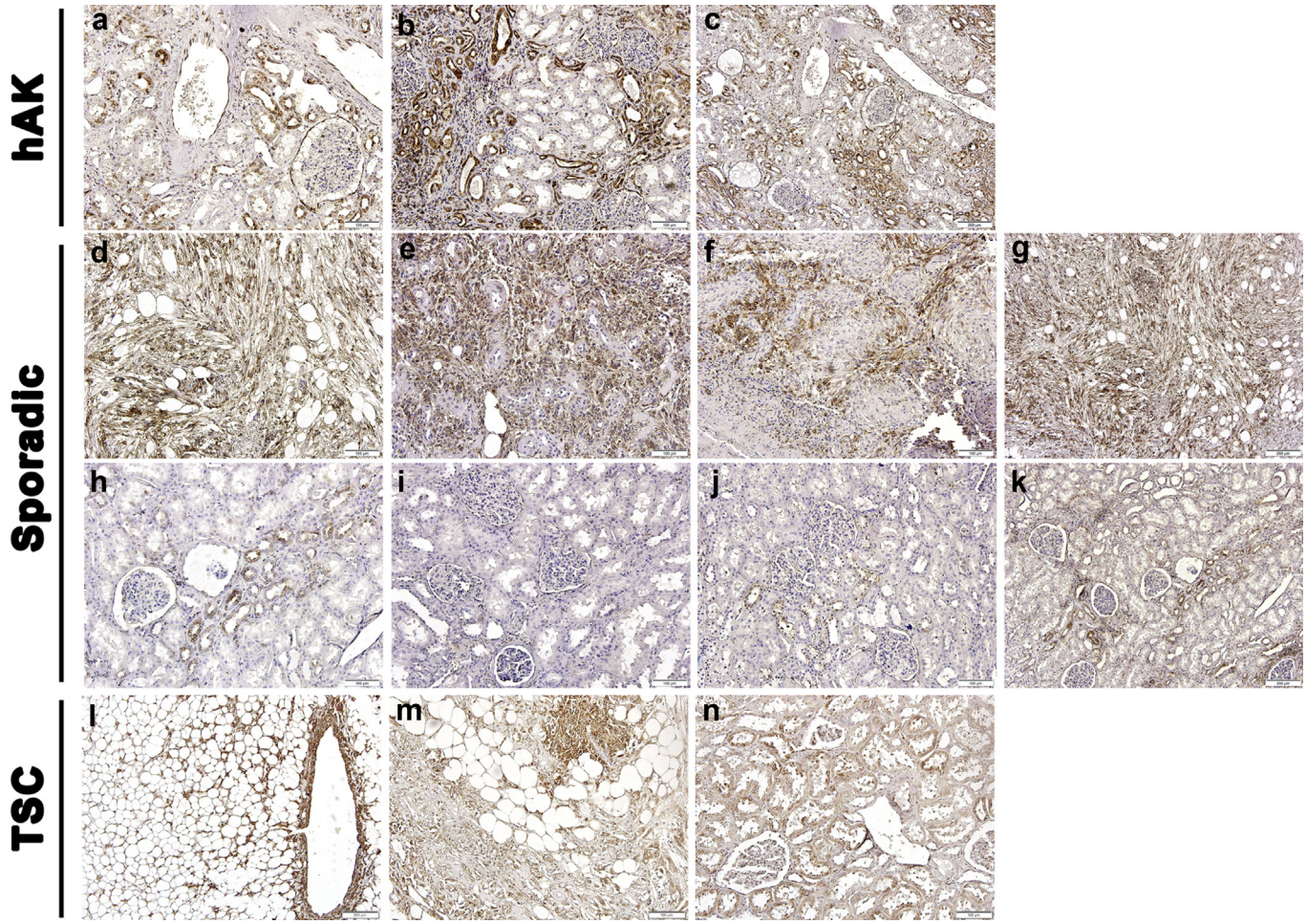

Figure 1. pS6 staining of normal human adult kidney (hAK) and AML tumors. (a–c) hAK demonstrates varying pS6 expression, seen mostly in distal tubules (DT) and collecting ducts (CD). (d–g) Sporadic AMLs demonstrate abundant pS6 expression. (h–k) Normal kidney borders of sporadic AML demonstrate varying pS6 expression in DT and CD, similarly to hAK. (l–n) TSC-related AML demonstrates a strong pS6 expression in both tumor (l,m) and normal kidney tissue (n). AML, renal angiomyolipoma; TSC, tuberous sclerosis complex.

exposed to excessive estrogen levels (e.g., oral contraceptives), known to promote AML growth. At this point, a workup for TSC was commenced. Brain MRI, chest CT, dermatological examination, and ophthalmological evaluation were unremarkable. Sanger sequencing of *TSC1/2*, encompassing the coding regions and their flanking regions (~50 bp from each side) yielded no mutation or polymorphism in *TSC1* and no mutation in *TSC2*, where several known polymorphisms (Supplementary Data) were found. Multiplex ligation-dependent probe amplification excluded any deletion or duplication in *TSC1/2*. The patient was thus diagnosed with sporadic AML. MRI performed 1 year later identified continued tumor growth, now reaching 30 × 50 mm.

The Patient's Tumor Demonstrates mTORC1 Activation

In order to assess whether the patient could benefit from mTORC1 inhibitors, we were interested in determining whether the tumors could result from mTORC1 overactivation. We therefore stained the patient's tumor for pS6 (Figure 2d). We detected robust pS6 expression in all tumor compartments, similar to the results obtained in the staining of sporadic AML tumors of other patients (Figure 1).

Rapamycin Treatment Results in Significant Reduction in the Patient's AML Volume

Having demonstrated strong mTORC1 activation in the patient's AML, we decided to treat her with rapamycin at a dose of 1 mg/d, obtaining an average trough level of 3.7 ng/ml. MRI revealed significant and consistent reduction in tumor volume. Within 5 and 13 months from commencement of treatment, the tumor exhibited a 22.2% and 42.3% volume reduction, respectively, compared to baseline, measured 6 months prior to the beginning of treatment (Figure 2c). Moreover, the hydronephrosis was resolved. The treatment resulted in transient mild stomatitis, hypercholesterolemia, and

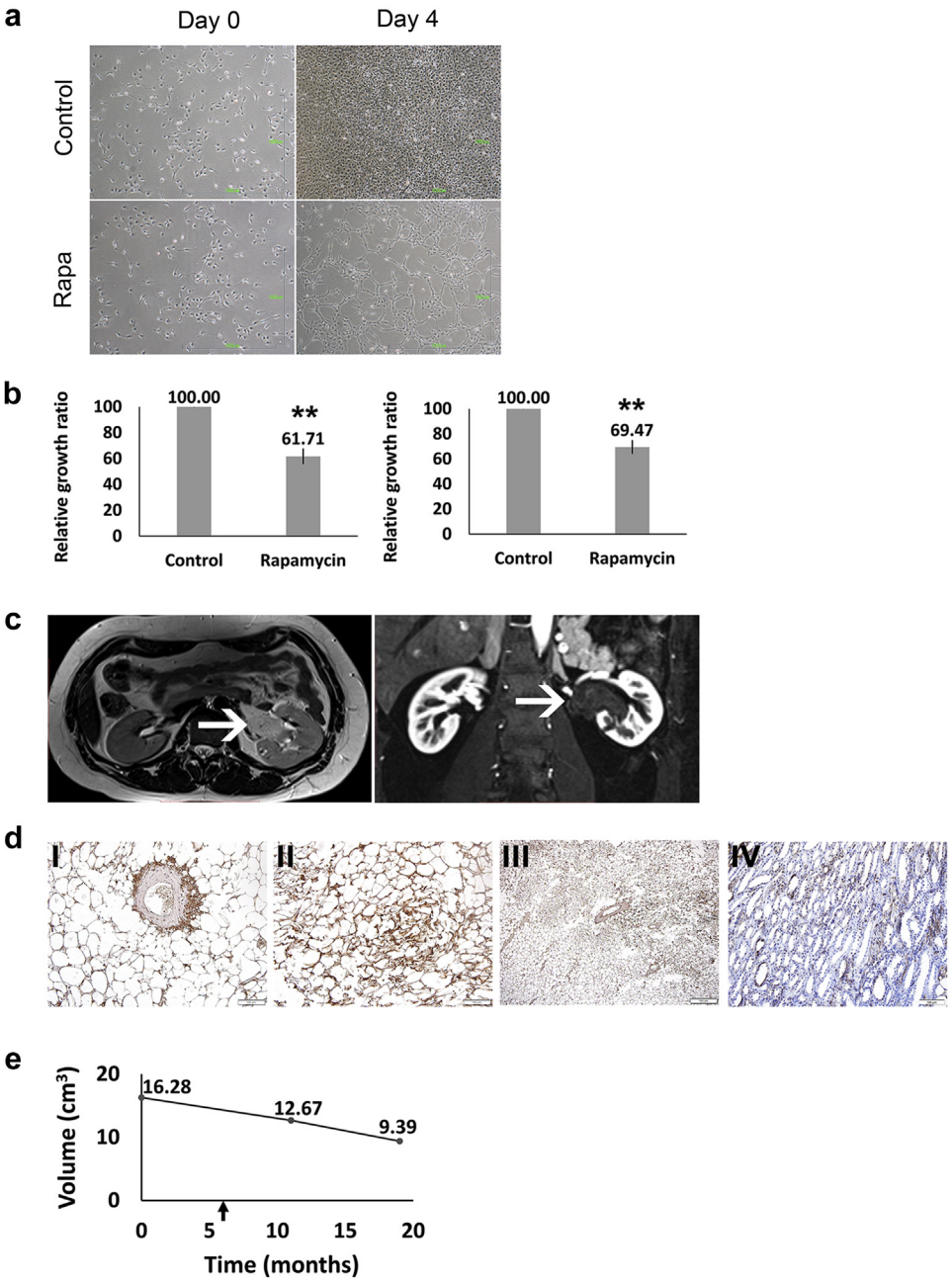

Figure 2. Rapamycin suppresses the growth of sporadic AML cells, both *in vitro* and *in vivo*. (a) SV7Tert cells, representing a sporadic AML cell line, were treated with 20 nM rapamycin or vehicle for 4 days. At day 4, rapamycin-treated cells demonstrated a significantly lower cell number, compared to control, vehicle-treated cells. (b) SV7Tert cells and UMBSV-tel cells, representing a cell line derived from TSC-associated AML, were treated for 4 days with rapamycin or vehicle, and then assessed for cell viability using the MTS assay. Both SV7Tert (left panel) and UMBSV-tel cells (right panel) exhibit a significant and similar reduction in cell viability following rapamycin treatment, compared to control cells. (c) A large, mostly lipoid, angiomyolipoma is seen in the patient's left kidney (arrows). (d) The patient's AML tumor demonstrates pS6 expression in the tumor tissue (I–III). Normal areas of the patient's kidney (IV) demonstrate relatively low pS6 expression, seen mostly in DT and CD, similarly to other sporadic AMLs. (e) The patient's tumor volume following rapamycin treatment. Shown is the tumor volume before treatment commencement and at 2 time points during the treatment. The arrow marks the beginning of the treatment. Significant gradual decrease in tumor volume is seen. AML, renal angiomyolipoma; CD, collecting ducts, DT, distal tubules.

acne. Altogether, these results indicate that rapamycin is effective and safe in treating sporadic AML.

DISCUSSION

The understanding of AML pathogenesis has advanced significantly in recent years, mainly with the identification of mTORC1 as a key driving factor and integration of mTORC1 inhibitors into clinical use for TSC-associated AML.[8,9] Nonetheless, because approximately 80% of cases are sporadic,[10] most AML patients have no pharmacological therapeutic option. A recent systematic analysis[10] found that among these patients,

92.7% require intervention, most commonly partial nephrectomy (37%) or embolization (29%). Moreover, although more recent data support a conservative approach in most patients,[11] tumor embolization results in re-intervention within 3 years in 36.5% of cases.[9] Furthermore, this strategy is obviously difficult to implement in non-TSC patients exhibiting multiple tumors. These issues underscore the need for noninvasive therapies for AML. Herein, we evaluated the potential of mTORC1 inhibition as such a method. Consistent with previous work,[5] we found that our patient's AML exhibited mTORC1 activation. Furthermore, we demonstrated that rapamycin effectively inhibits the growth of sporadic AML cells *in vitro* with efficacy similar to that seen in TSC-related AML cells. Importantly, rapamycin resulted in significant reduction in tumor volume and disappearance of hydronephrosis, and was well tolerated. Notably, although Kenerson *et al*. found mTORC1 activation in 15 of 15 sporadic AMLs,[5] TSC2 loss of heterozygosity could be detected only in 4 of 8 sporadic AMLs.[12] Thus, although empirical rapamycin treatment could be considered in light of the relatively safe side effect profile, acquisition of tumor sample and assessment of mTORC1 activity (e.g., via fine-needle aspiration[13]) would allow a more definite indication for treatment. In summary, we demonstrate that sporadic AML can be effectively treated using rapamycin. Although this concept needs to be proved in larger cohorts, we propose that the strong mTORC1 activity in sporadic AML and antiproliferative effect *in vitro* indicate that rapalogs should be considered for sporadic AML.

ACKNOWLEDGMENTS

BD is supported by the Ziering Foundation, The Israel Cancer Fund (grant number PG-11-3072), the Israel Cancer Association (grant number 20150916), and the ICRF project grant (grant number PG-14-112). We wish to express our gratitude to Prof. Iris Barshack of the Department of Pathology, Sheba Medical Center, Tel Hashomer, Israel, for providing angiomyolipoma histology specimens. We wish to thank Prof. John Bissler for the interpretation of the imaging studies and Prof. Jack Arbiser for supplying the AML cell lines. This work is dedicated in loving memory to Dr. Lia, Mircea, and Florin Pleniceanu.

SUPPLEMENTARY MATERIAL

Supplementary Data. Description of the cells and cell culturing procedures used in this paper, as well as the methods for immunohistochemical staining, assessment of cell viability, statistical methods, imaging, supplemental results, and supplemental references are described in the supplementary data section.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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