

Cooperative benefit for the combination of rapamycin and imatinib in tuberous sclerosis complex neoplasia

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Abstract

Tuberous sclerosis (TS) is a common autosomal-dominant disorder characterized by tumors of the skin, lung, brain, and kidneys. Monotherapy with rapamycin however resulted in partial regression of tumors, implying the involvement of additional pathways. We have previously implicated platelet-derived growth factor-BB in TS-related tumorigenesis, thus providing a rationale for a combination of mTOR/PDGF blockade using rapamycin and imatinib. Here, we test this combination using a well-established preclinical model of cutaneous tumorigenesis in TS, *tsc2ang1* cells derived from a skin tumor from a mouse heterozygous for *tsc2*. Treatment of *tsc2ang1* cells with a combination of rapamycin and imatinib led to an inhibition of proliferation compared with either vehicle treatment or treatment with rapamycin or imatinib monotherapy. Combination therapy also led to a decrease in Akt activation. Potent *in vivo* activity in animal experiments by combination therapy was noted, without toxicity to the animals. Our findings provide a rationale for the combined use of rapamycin and imatinib, both FDA approved drugs, for the treatment of TS.

Introduction

TS is a common autosomal-dominant disorder characterized by the development of tumors of the brain, kidney, skin and lung. The disorder is characterized by mutations or deletions in one of two large genes, hamartin (*TSC1*) and tuberin (*TSC2*). In addition, neuro-developmental complications of TS, including seizures and autism, lead to significant morbidity. While no strict genotype-phenotype relationship has been established, the disease is more severe in patients with *tsc2* mutations. Loss of heterozygosity (LOH), which deletes the unaffected allele, is required for the development of some neoplastic features of TS, including renal angiomyolipomas, lymphangiomyomatosis, and skin lesions, while LOH is not observed in brain tubers which often cause epileptic foci [1]. Apart from deletions, there

are other mechanisms causing inactivation of *tsc1* and *tsc2*.

The signaling pathways implicated in TS are complex. A hotspot for mutations in *tsc2* involves regions implicated in controlling rheb, although multiple other signaling pathways have also been linked to TS-related neoplasia, including mTORC1, notch, p42/44 MAP kinase, NFκB, and Akt [2-11]. Tuberin (*TSC2*) was weak or absent in angiomyolipomas, but present in healthy kidney, whereas, phosphorylated p70 S6 kinase and pS6 were present only in angiomyolipomas. Activation of a mammalian target of rapamycin metabolic pathway in tuberous sclerosis lesions, which contributes to their growth [2]. The upregulation of mTORC1 observed in neoplasms of patients with TS has served as a rationale for recent use of mTOR inhibitor rapamycin (sirolimus) to treat TS.

Rapamycin treatment was shown to result in partial regression of kidney, lung and skin lesions but not complete disappearance of tumors. In addition, tumor re-growth was observed upon cessation of therapy, consistent with the known cytostatic rather than cytotoxic effects of rapamycin. The use of mTOR inhibitors is becoming increasingly accepted, especially for the treatment in TSC [2, 12, 13]. These clinical findings suggest that additional signaling pathways are active in TS-related tumors [14].

We have previously demonstrated that platelet-derived growth factor β receptor (PDGFR β) is present and active in human and murine TS lesions [15-17]. Other groups have demonstrated an inverse relationship between mTOR activation and PDGFR β levels in TS-derived cells [18]. Therefore, we reasoned that mTOR blockade might be compensated for by PDGF activation *in vitro* and *in vivo*, and combined blockade might be more efficacious than rapamycin monotherapy. To test this idea, we used the FDA-approved drug imatinib (gleevec) to treat mouse tumors formed by implanted *tsc2ang1* cells, isolated from a cutaneous

sarcoma that arose in a *tsc2* heterozygous mouse and a well-validated model of TS-related neoplasia. Imatinib functions as a specific inhibitor of a number of tyrosine kinase enzymes leading to downregulation of MMP-2 [19]. Currently, imatinib is approved for chronic myelogenous leukemia carrying the *bcr-abl* translocation, as well as hypereosinophilic syndrome and eosinophilic leukemia with the FIP1L1-PDGFR α fusion kinase. Solid tumors for which imatinib is approved include c-kit positive gastrointestinal stromal tumor and inoperable dermatofibrosarcoma protuberans (<http://www.gleevec.com>). We demonstrate that addition of imatinib to rapamycin decreases the levels and phosphorylation of PDGFR β . Consistent with this, combination treatment resulted in a greatly decreased phosphorylation of Akt, and Akt is a major determinant of tumorigenesis *in vivo*. Finally, we show that the combination of rapamycin and imatinib has a greater antitumor effect compared to vehicle alone than either rapamycin or imatinib compared to vehicle *in vivo*. These findings provide a rationale for combination therapy with rapamycin and imatinib in TS.

Results

Rapamycin and imatinib treatment inhibits TSC2 *ang1* cell proliferation

To assess if rapamycin and imatinib combination was superior to either agent alone, we assessed their effects on the proliferation of *Tsc2 ang1* cells. Treatment of cells for 72 h with rapamycin (5nM) or imatinib (10 μ M) alone reduced the level of

proliferation significantly compared with control ($p = 0.0006$ for imatinib vs control, $p = 0.0076$ for rapamycin vs control). However, combined treatment with both agents led to significantly better inhibition of proliferation (Figure 1a), compared with control ($p = 0.0002$). Combination of drugs was significantly better than rapamycin alone ($p = 0.0243$), while imatinib vs combination did not reach significance at p less than 0.05. Experiments were done in triplicates for reproducibility.

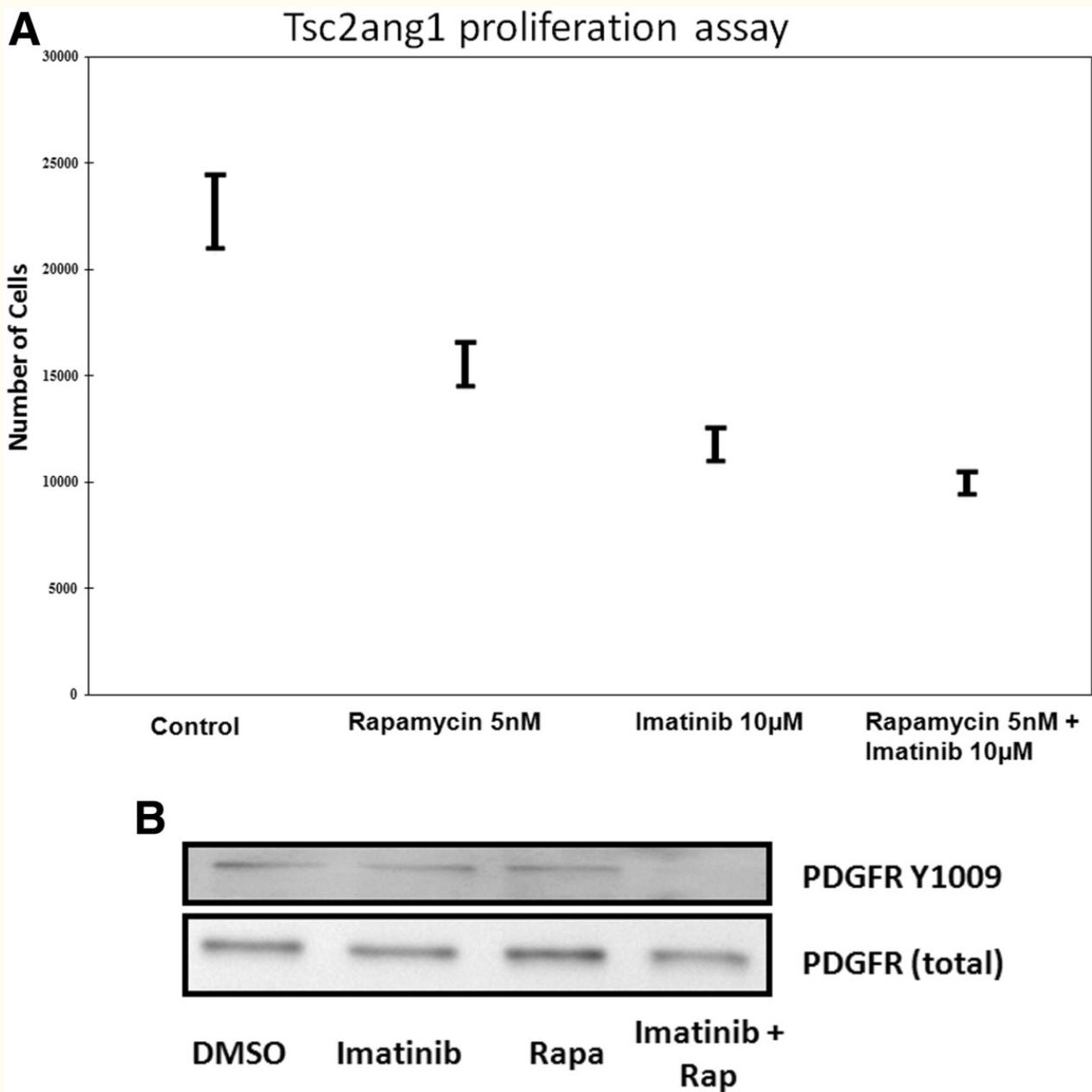


Figure 1

Figure 1 caption

(A) Effect of rapamycin, imatinib or combination of imatinib and rapamycin on tsc2ang1 cell line proliferation. Cells were treated with vehicle control (DMSO), rapamycin (5nM), imatinib (10 µM) or imatinib (10 µM) + rapamycin (5nM) (shown along X-axis) for 24 h and counted using a Coulter counter. Treatment at each concentration was performed in triplicate. The Y-axis represents cell number. (B) Downregulation of Phospho-PDGFR-β by combinational treatment; Tsc2ang1 cells were treated with DMSO, rapamycin5nM, imatinib10uM or rapamycin5nM + imatinib10uM for 24 hours prior to harvest. Combination rapamycin + imatinib treatment inhibits the levels of phospho-PDGFR-β (Tyr 1021).

Combination of rapamycin and imatinib inhibits PDGFRβ activation inTsc2 ang1

cells

In view of the effects of rapamycin and imatinib

combination on Tsc2Ang1 cell proliferation together with previous findings that mTOR inhibitor treatment of TS is associated with increased PDGFR β levels, we examined the effects of imatinib and rapamycin or a combination of these on PDGFR β expression. Experiments were done in triplicates for reproducibility. We tested imatinib (10 μ M), rapamycin (5nM) or their combination on the levels of phosphorylated PDGFR β (at Tyr-1009) in Tsc2ang1cells. Combined treatment led to downregulation of PDGFR β phosphorylation, (Figure 1b).

Treatment with combination of rapamycin and imatinib down regulates phosphoAkt473 protein levels in Tsc2ang1 cells

To verify the effect of imatinib and rapamycin, as well as combination therapy on the activation of

Akt, we treated Tsc2ang1 cells with imatinib (10 μ M), rapamycin (5 nM) or their combination and the levels of Akt and p Akt were measured by densitometry scanning of the signals on western blot membrane. Total levels of Akt were unchanged by treatment and served as loading controls. We found first that overall, treatment with drug led to significantly decreased phospho Akt compared to vehicle controls ($p = 0.0002$, general linear modeling). In addition, we found that treatment with rapamycin and imatinib reduced the levels of pAkt (Figure 2) and a significant reduction in the levels of pAkt ($p < 0.0002$) in imatinib + rapamycin treated cells compared to vehicle control. The mean of combination treatment versus imatinib alone or rapamycin alone also decreased. However, significance was only observed when the combination treatment was compared to rapamycin alone ($p < 0.0243$) and not when compared to imatinib alone ($p < 0.4273$).

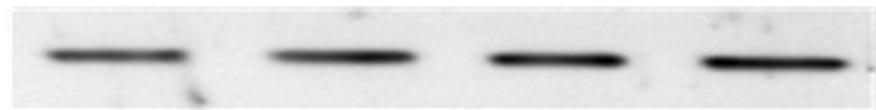
pAktSer473



Akt



GAPDH



Control

+ - - -

Imatinib

- + - +

Rapamycin

- - + +

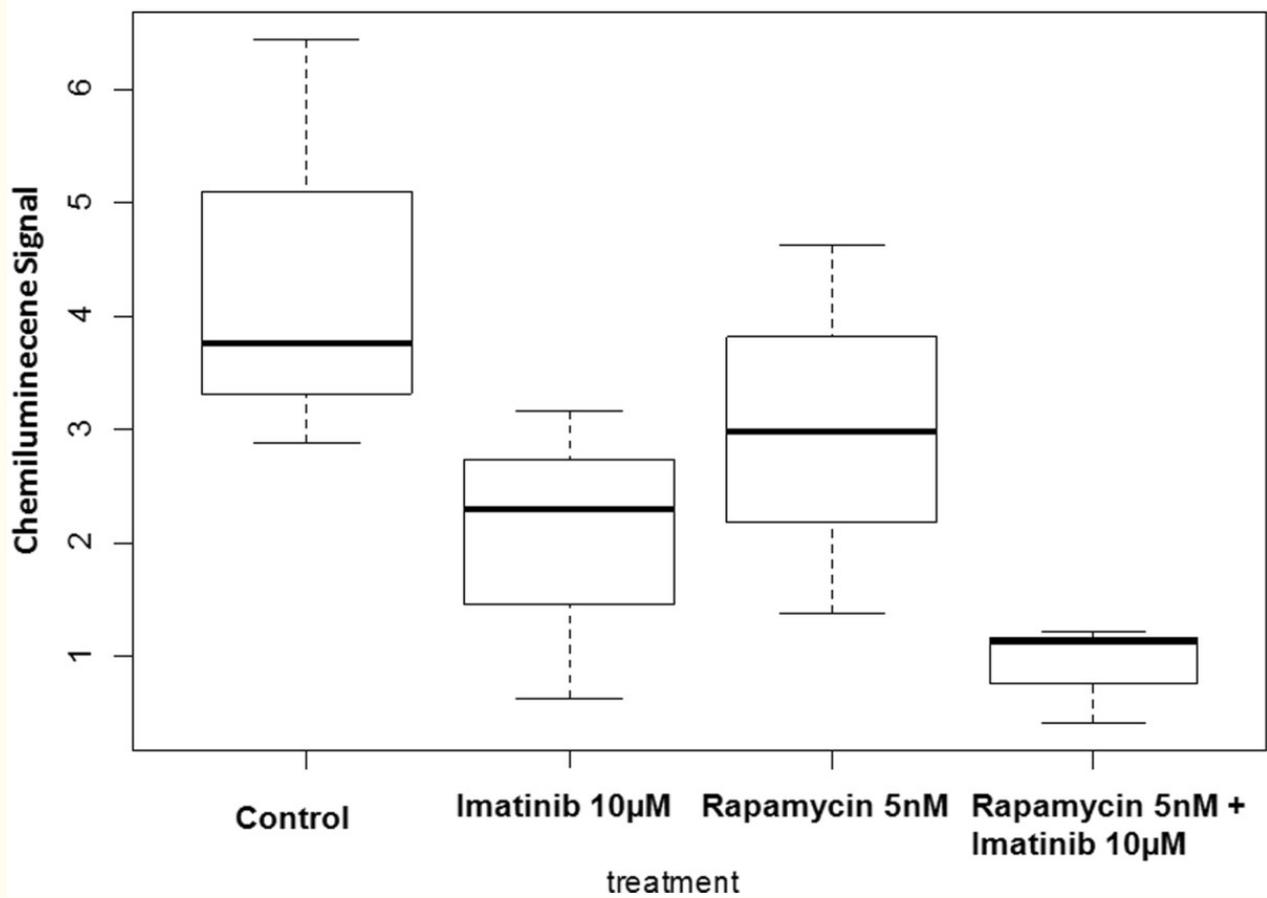


Figure 2

Figure 2 caption

blot analysis using Akt and pAkt were conducted on Tsc2 ang1 cells treated with treated with DMSO, rapamycin5nM, imatinib10uM or rapamycin5nM + imatinib10uM for 24 hours. Cells were lysed and analyzed by using antibodies specific for the total Akt and pAkt473. Rapamycin + imatinib treatment downregulates pAkt levels ($p < 0.0002$). GAPDH was used as the loading control by using monoclonal anti-GAPDH antibody. All treatment groups were significant when compared to the vehicle control, Imatinib 10nM ($p < 0.0006$); Rapamycin 5nM ($p < 0.0076$); Rapamycin 5nM + Imatinib 10nM ($p < 0.0002$). However, the combinational therapy only show significance against Rapamycin at 5nM ($p < 0.0243$) and not Imatinib at 10nM ($p < 0.4273$).

Matrix metalloproteinase analysis

Cell migration and invasion are fundamental components of tumor cell invasion and neovascularization. Matrix metalloproteinases (MMPs) are essential for successful cell migration through extracellular matrix. In order to determine the levels of MMPs in our samples, conditioned media from equivalent numbers of imatinib- or rapamycin-treated cells along with that of control cells were collected and quantitatively analyzed using monospecific ELISAs. MMP-2 levels in conditioned media from tsc2ang1 cells treated with either imatinib ($p = .0243$), rapamycin or combination treatment with rapamycin and imatinib

($p = .0092$) were significantly decreased compared to that of controls (Figure 3). However, MMP-2 levels in the conditioned media of cells treated with rapamycin ($p = .0744$) were not significantly decreased compared to that of controls consistent with previous reports [20]. Differences between rapamycin alone and combination therapy trended toward significance ($p = 0.0744$), and between imatinib alone and combination ($p = 0.329$), indicating that imatinib has a more potent effect on MMP-2 activity than rapamycin. Moreover, no significant difference was observed in MMP-9 levels between the different treatment groups and controls (data not shown).

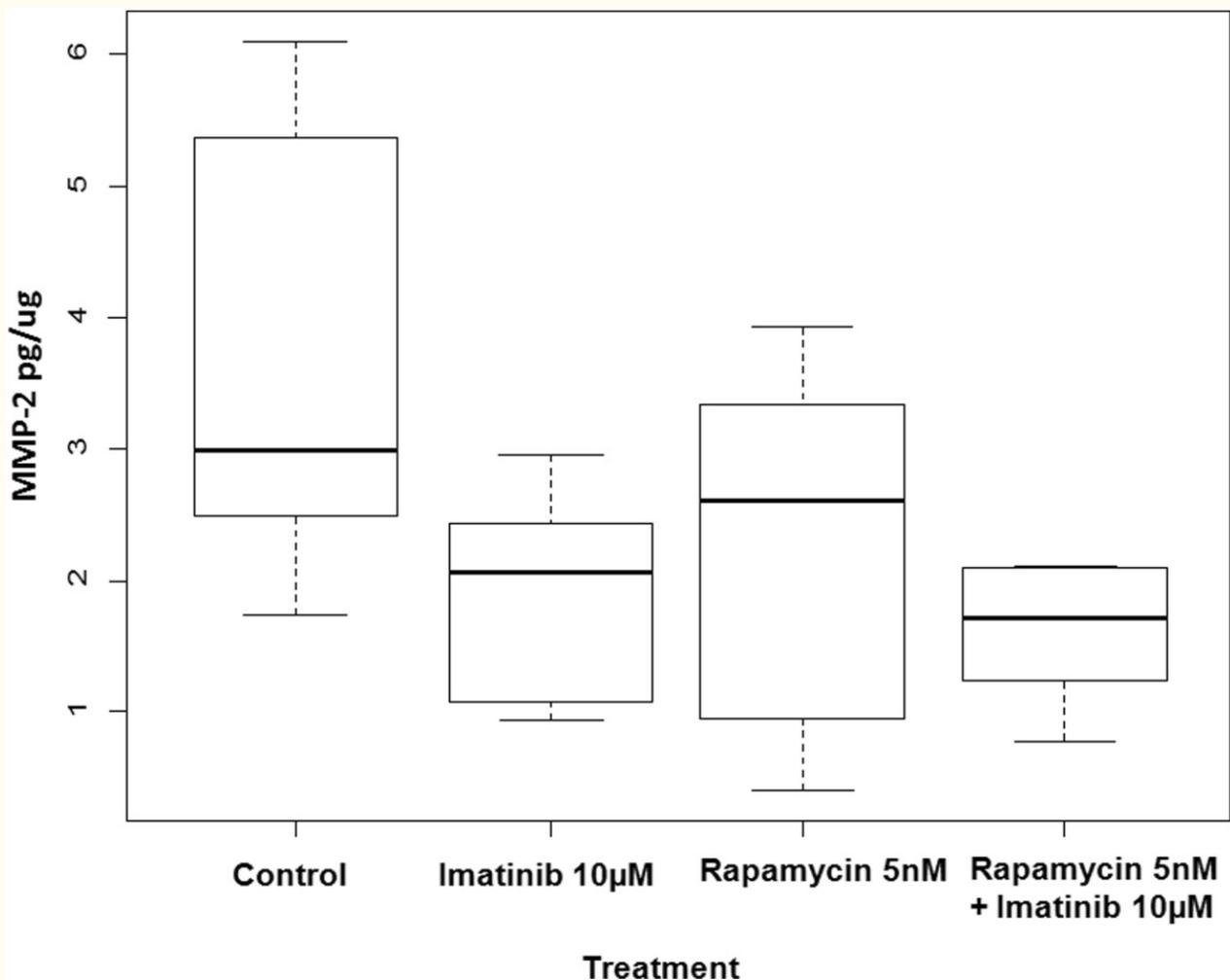


Figure 3

Figure 3 caption

MMP-2 ELISA analysis of conditioned media from *tsc2ang1* cells treated with either rapamycin, imatinib or the combination for 24 hours resulted in a decrease in the amount of MMP-2 produced in comparison to controls. MMP-2 levels in the *tsc2ang1* cells treated with imatinib only and imatinib + rapamycin were significantly decreased compared to that of controls.

In vivo tumorigenesis

Given the effects of combined treatment with imatinib and rapamycin on cell proliferation as well as the ability of this combination to reduce the levels of phospho-PDGFRβ and phosphor-Akt, we assessed the effects of this combination on *tsc2ang1* tumorigenesis *in vivo*. While each drug individually reduced the size of tumors formed compared to vehicle control, combined treatment with imatinib and rapamycin resulted in a nearly complete abrogation of tumor growth (97% decrease in tumor volume compared with control, $p < 0.0001$) (Figure 4). Tumor volume was

significantly different in comparison between control vs rapamycin/imatinib, rapamycin vs rapamycin/imatinib, and imatinib vs rapamycin/imatinib ($p < 0.05$). There was no significant difference between rapamycin alone and imatinib alone. Tumor volume comparing imatinib alone versus combination was significantly different ($p = 0.0209$), while tumor volume between rapamycin alone and combination did not reach significance ($p = 0.19$). Neither local nor systemic toxicity was observed in any of the treatment groups indicating that combined effective inhibition of tumor growth is feasible with imatinib and rapamycin with minimal toxicity.

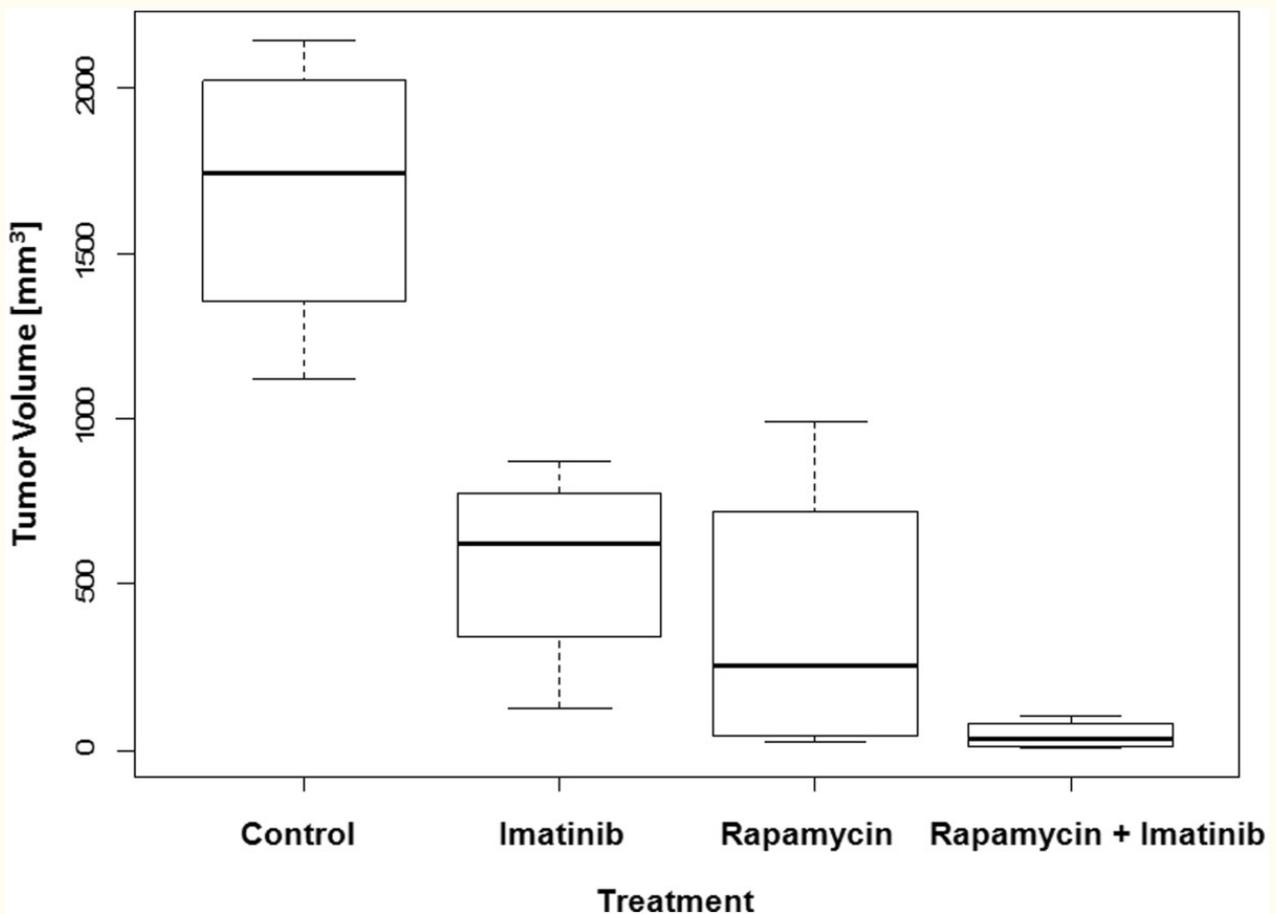


Figure 4

Figure 4 caption

Effect of rapamycin and imatinib on growth of *tsc2ang1* xenografts in nude mice. The y axis represents tumor volume.

Discussion

TS is a multisystem disorder characterized by benign or malignant neoplasia, as well as autism and seizures. No highly effective and long lasting therapy exists for TS neoplasia. Renal lesions such as angiomyolipomas may cause massive bleeding and compromise renal function, often requiring a kidney transplant. Lymphangiomyomatosis, a neoplastic complication most commonly seen in young women, can only be cured by lung transplantation. Deforming skin lesions cause significant psychological distress [21]. Finally, brain tumors such as subependymal giant cell astrocytomas, can cause morbidity and mortality, and multiple tubers results in refractory seizures and exacerbation of mental retardation. Thus, urgent therapy is required for TS. Here, we demonstrate in a validated preclinical model a

nearly complete tumor inhibition with a combination of two FDA-approved drugs (rapamycin and imatinib) targeting two distinct signaling pathways (mTORC1 and PDGFR β , respectively) implicated in TS-associated neoplasia.

The lack of total blockade by rapamycin is consistent with the incomplete clinical response of tumors to systemic rapamycin, implying that either additional pathways are already present in these tumors, or are induced by rapamycin monotherapy. High among the candidates are the PDGFR β mediated signal pathways, especially since mTORC1 activation and PDGFR β signaling have been shown to have an inverse relationship [18].

Treatment with combination of rapamycin and imatinib led to downregulation of PDGFR β in *tsc2ang1* cells, in a c-cbl-independent manner (data

not shown). *In vitro* proliferation assays demonstrated an additive effect of rapamycin and imatinib on tsc2ang1 cells.

Rapamycin has been used as monotherapy in patients with TS, resulting in benefit only as long as the patients are exposed to the drug [12]. Our findings that rapamycin therapy alone does not address PDGFR β signaling, as well as modest efficacy against Akt activation are potential reasons for the failure of rapamycin as monotherapy in solid tumors. In highly malignant tumors, mTORC1 inhibition has shown to cause a paradoxical activation of Akt, in part through activation of mTORC2, which is an Akt kinase [22, 23]. We demonstrate that the combination of rapamycin and imatinib blocks Akt activation compared with

monotherapy, and likely accounts for the decrease in tumor volume. Our findings demonstrate the following. Combination therapy of TS relevant cells with rapamycin and imatinib results in downregulation of PDGFR β and Akt signaling. This decrease is independent of c-cbl-mediated degradation. Finally, the combination of rapamycin and imatinib has at least additive activity against the tsc2ang1 model of TS *in vivo*. Given that the combination of these two approved drugs is well tolerated *in vivo*, and that rapamycin alone does not cause long-lasting remissions, this study provides a rationale for the combination of these two drugs in humans.

Materials and methods

Generation of murine model of TS

Tsc2ang1 (ATCC CRL 2620) is a murine cell line derived from a cutaneous sarcoma that arose in the extremity of a mouse heterozygous for tsc2; these mice develop cutaneous sarcomas at a frequency of 10 to 15%. The cells were cultured in complete DMEM medium supplemented with 10% FBS (Sigma Aldrich, St Louis, MO).

In vitro proliferation assay

10,000 tsc2ang1 cells per well were plated in 24-well dishes in triplicate. The next day, fresh medium containing the compounds or vehicle controls was added. Cells were incubated at 37° C for 24 h, and cell number was determined using a Coulter Counter (Hiialeah, FL).

Western blot analysis

Lysates of Tsc2ang1 cells treated with vehicle or indicated drugs were prepared in NP-40 lysis buffer (1% NP-40, 150 mmol/L NaCl, 10% glycerol, 20 mmol/L HEPES, 1 mmol/L phenylmethylsulfonyl fluoride, 2.5 mmol/L EDTA, 100 μ mol/L Na₃VO₄, and 1% aprotinin). Protein concentration in cleared lysates was determined using an Eppendorf BioPhotometer. Samples were resolved using SDS-PAGE (National Diagnostics) and transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in 10 mmol/L Tris/0.1% Tween 20/100 mmol/L NaCl and incubated with primary antibodies followed by horseradish peroxidase-conjugated secondary antibody. The immunoreactive bands were visualized by enhanced chemiluminescence (Amersham Biosciences). The antibodies used were: Phospho-PDGFR- β antibody (Tyr 1021) (Cell signaling Laboratories); Akt antibody (9272) (Cell signaling Laboratories), pAKT (4058) antibody (Cell signaling Laboratories) monoclonal anti-GAPDH antibody (Santa Cruz L-18S-48167) was used as a loading control.

Matrix metalloproteinase analysis

The presence of MMPs in conditioned media samples was determined using MMP ELISA Quantikine Kits (R&D Systems, Inc.). Specimens, standards and reagents were prepared according to manufacturer's instructions. Protein concentration was determined via the Bradford method using bovine serum albumin as the standard as described previously [24].

In vivo tumor growth

To test the *in vivo* activity of compounds that inhibit tsc2ang1 growth *in vitro*, groups of four nude mice per compound (or control). We injected 1 million Tsc2ang1 cells s.c. into 4 nude mice in each group. I.p. treatment with imatinib, rapamycin and imatinib and rapamycin were conducted for 30 days. Rapamycin and imatinib were obtained from LC Laboratories (Woburn, MA). Beginning 2 days later, the mice received daily i.p. injections of vehicle (control), rapamycin (12 mg/kg/day), imatinib (120 mg/kg/day) or imatinib plus rapamycin. The compounds were suspended in 0.1 ml of ethanol and 0.9 ml of Intralipid solution (Fresenius Kabi, Uppsala, Sweden) [25]. No local or systemic toxicity was observed in any of the animals. Injections were given over a period of 4 weeks, after which the mice were sacrificed due to overwhelming tumor burden in the control group. Tumor volume was calculated using the equation ($w^2 \times L$)0.52, where w(width) represents the shortest diameter of the tumor.

Statistics

One way ANOVA, and non parametric test were performed for the tumor volume statistics. We did parametric analysis when the conditions for ANOVA were satisfied and in case where conditions are not satisfied and if the variables are not normally distributed, we conducted corresponding non parametric test and opted to present results for Wilcoxon test.

Ethical approval

Animals studies were performed in compliance with the Emory IACUC.

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References

1. Boer K, Jansen F, Nellist M, Redeker S, van den Ouweland AM, Spliet WG, van Nieuwenhuizen O, Troost D, Crino PB, Aronica E. Inflammatory processes in cortical tubers and subependymal giant cell tumors of tuberous sclerosis complex. *Epilepsy Res.* 2008;78:7-21.
2. El-Hashemite N, Zhang H, Henske EP, Kwiatkowski DJ. Mutation in TSC2 and activation of mammalian target of rapamycin signalling pathway in renal angiomyolipoma. *Lancet.* 2003;361:1348-1349.
3. Finlay GA, Thannickal VJ, Fanburg BL, Kwiatkowski DJ. Platelet-derived growth factor-induced p42/44 mitogen-activated protein kinase activation and cellular growth is mediated by reactive oxygen species in the absence of TSC2/tuberin. *Cancer Res.* 2005;65:10881-10890.
4. Goncharova EA, Goncharov DA, Eszterhas A, Hunter DS, Glassberg MK, Yeung RS, Walker CL, Noonan D, Kwiatkowski DJ, Chou MM, Panettieri RA, Krymskaya VP. Tuberin regulates p70 S6 kinase activation and ribosomal protein S6 phosphorylation. A role for the TSC2 tumor suppressor gene in pulmonary lymphangioleiomyomatosis (LAM). *J Biol Chem.* 2002;277:30958-30967.
5. Karbowniczek M, Zitserman D, Khabibullin D, Hartman T, Yu J, Morrison T, Nicolas E, Squillace R, Roegiers F, Henske EP. The evolutionarily conserved TSC/Rheb pathway activates Notch in tuberous sclerosis complex and *Drosophila* external sensory organ development. *J Clin Invest.* 2010;120:93-102.
6. Kwiatkowski DJ. Rhebbing up mTOR: new insights on TSC1 and TSC2, and the pathogenesis of tuberous sclerosis. *Cancer Biol Ther.* 2003;2:471-476.
7. Li Y, Inoki K, Vikis H, Guan KL. Measurements of TSC2 GAP Activity Toward Rheb. *Methods Enzymol.* 2005;407:46-54.
8. Li Y, Inoki K, Yeung R, Guan KL. Regulation of TSC2 by 14-3-3 binding. *J Biol Chem.* 2002;277:44593-44596.
9. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell.* 2005;121:179-193.
10. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. *Nat Cell Biol.* 2002;4:658-665.
11. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol.* 2003;5:578-581.
12. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmithorst VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med.* 2008;358:140-151.
13. Franz DN, Leonard J, Tudor C, Chuck G, Care M,

- Sethuraman G, Dinopoulos A, Thomas G, Crone KR. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Ann Neurol.* 2006;59:490-498.
14. Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin WG. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell.* 2003;4:147-158.
 15. Arbiser JL, Govindarajan B, Bai X, Onda H, Kazlauskas A, Lim SD, Amin MB, Claesson-Welsh L. Functional tyrosine kinase inhibitor profiling: a generally applicable method points to a novel role of platelet-derived growth factor receptor-beta in tuberous sclerosis. *Am J Pathol.* 2002;161:781-786.
 16. Govindarajan B, Brat DJ, Csete M, Martin WD, Murad E, Litani K, Cohen C, Cerimele F, Nunnolley M, Lefkove B, Yamamoto T, Lee C, Arbiser JL. Transgenic expression of dominant negative tuberin through a strong constitutive promoter results in a tissue-specific tuberous sclerosis phenotype in the skin and brain. *J Biol Chem.* 2005;280:5870-5874.
 17. Govindarajan B, Shah A, Cohen C, Arnold RS, Schechner J, Chung J, Mercurio AM, Alani R, Ryu B, Fan CY, Cuezva JM, Martinez M, Arbiser JL. Malignant transformation of human cells by constitutive expression of platelet-derived growth factor-BB. *J Biol Chem.* 2005;280:13936-13943.
 18. Zhang H, Bajraszewski N, Wu E, Wang H, Moseman AP, Dabora SL, Griffin JD, Kwiatkowski DJ. PDGFRs are critical for PI3K/Akt activation and negatively regulated by mTOR. *J Clin Invest.* 2007.
 19. Schultz JD, Rotunno S, Erben P, Sommer JU, Anders C, Stern-Straeter J, Hofheinz RD, Hormann K, Sauter A. Down-regulation of MMP-2 expression due to inhibition of receptor tyrosine kinases by imatinib and carboplatin in HNSCC. *Oncol Rep.* 2011;25:1145-1151.
 20. Lee PS, Tsang SW, Moses MA, Traves-Gibson Z, Hsiao LL, Jensen R, Squillace R, Kwiatkowski DJ. Rapamycin-insensitive up-regulation of MMP2 and other genes in tuberous sclerosis complex 2-deficient lymphangiomyomatosis-like cells. *Am J Respir Cell Mol Biol.* 2010;42:227-234.
 21. Li S, Takeuchi F, Wang JA, Fuller C, Pacheco-Rodriguez G, Moss J, Darling TN. MCP-1 overexpressed in tuberous sclerosis lesions acts as a paracrine factor for tumor development. *J Exp Med.* 2005;202:617-624.
 22. Frias MA, Thoreen CC, Jaffe JD, SCHRODER W, Sculley T, Carr SA, Sabatini DM. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol.* 2006;16:1865-1870.
 23. Sun SY, Rosenberg LM, Wang X, Zhou Z, Yue P, Fu H, Khuri FR. Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res.* 2005;65:7052-7058.
 24. Smith ER, Zurakowski D, Saad A, Scott RM, Moses MA. Urinary biomarkers predict brain tumor presence and response to therapy. *Clin Cancer Res.* 2008;14:2378-2386.
 25. Bhandarkar SS, Bromberg J, Carrillo C, Selvakumar P, Sharma RK, Perry BN, Govindarajan B, Fried L, Sohn A, Reddy K, Arbiser JL. Tris (dibenzylideneacetone) dipalladium, a N-myristoyltransferase-1 inhibitor, is effective against melanoma growth in vitro and in vivo. *Clin Cancer Res.* 2008;14:5743-5748.

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