

Effects of Combining Rapamycin and Resveratrol on Apoptosis and Growth of TSC2-Deficient Xenograft Tumors

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Abstract

Lymphangiomyomatosis (LAM) is a rare neoplastic metastatic disease affecting women of childbearing age. LAM is caused by hyperactivation of the mechanistic target of rapamycin complex 1 (mTORC1) as a consequence of tuberous sclerosis complex (TSC) 1/2 inactivation. Clinically, LAM results in cystic lung destruction. mTORC1 inhibition using rapamycin analogs (rapalogs) is partially effective in reducing disease progression and improving lung function. However, cessation of treatment results in continued progression of the disease. In the present study, we investigated the effectiveness of the combination of rapamycin treatment with resveratrol, an autophagy inhibitor, in the TSC2-null xenograft tumor model. We determined that this combination inhibits phosphatidylinositol-4,5-bisphosphate 3-kinase PI3K/Akt/mTORC1 signaling and activates apoptosis. Therefore, the combination of rapamycin and resveratrol may be an effective clinical strategy for treatment of LAM and other diseases with mTORC1 hyperactivation.

Keywords: rapamycin; resveratrol; LAM; TSC2; mTORC1

Clinical Relevance

Rapamycin is an effective treatment for lymphangiomyomatosis (LAM), but the disease progresses in some patients and in patients who discontinue treatment for various reasons. The combination of resveratrol and rapamycin may achieve the goal of eliminating TSC2-deficient cells and allow the patients to stop rapamycin without further disease progression. Going forward, it would be interesting to proceed to a clinical trial of the use of resveratrol in combination with rapamycin compared with rapamycin monotherapy in patients with LAM. Because resveratrol has a low toxicity profile, it may be an attractive option.

Lymphangiomyomatosis (LAM) is a rare, slowly progressing neoplastic disorder primarily affecting women of childbearing age (1, 2). It is characterized by the presence of metastatic, smooth-muscle-like cells in the lungs that destroy pulmonary connective tissue and form multiple cysts (3, 4). LAM is caused by

inactivating mutations in the tumor suppressor proteins tuberous sclerosis complex (TSC) (5, 6). The TSC1/TSC2 heterodimer is a negative regulator of the mechanistic target of rapamycin complex 1 (mTORC1). TSC1/2 acts as a GTPase-activating protein for the small GTPase Rheb (7, 8), which directly binds to mTOR

and activates it in a GTP-dependent manner (9). Therefore, the phosphorylation and negative regulation of TSC2 leads to the activation of mTORC1. mTOR, a serine/threonine kinase that belongs to the phosphatidylinositol 3-kinase-related kinase family, was discovered as a target of a naturally occurring molecule called

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rapamycin (10). mTOR is a master sensor of nutrient availability in the cell that regulates proliferation, cellular metabolism, protein and lipid synthesis, and autophagy in response to extracellular signals such as nutrient availability and growth factors (11). mTOR is a key component of two distinct complexes: mTORC1 and mTORC2. These complexes differ in their protein composition, downstream targets, and sensitivity to rapamycin. mTORC1 is acutely rapamycin sensitive, and mTORC2 is rapamycin insensitive (11). The growth factor input to mTORC1 via the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt signaling cascade is mediated through the inhibition of TSC2 protein (12–14), and several growth factor-stimulated signaling pathways converge on TSC2. Akt, acting downstream of activated PI3K, phosphorylates TSC2 at S939 and T1462 (15), whereas Ras-activated extracellular signal-regulated protein kinase (ERK)1/2 phosphorylates TSC2 at residue S664, also leading to inactivation of TSC2 (16). Finally, ribosomal S6 kinase, a downstream effector of ERK, has also been shown to directly phosphorylate TSC2 on S1798 (17).

In LAM, loss of TSC1/2 leads to uncontrolled activation of mTORC1 and unrestricted cell growth. LAM can occur sporadically or is associated with TSC syndrome, an inherited, autosomal-dominant tumor suppressor disorder that is characterized by benign tumors of the brain, heart, and kidneys as well as seizures, cognitive impairment, and skin lesions (18). Whereas LAM in women with TSC is caused by mutations in either the *TSC1* or *TSC2* genes, sporadic LAM is almost exclusively caused by mutations in *TSC2*. Because of the hyperactivation of mTORC1 in LAM, mTORC1 inhibitors are an effective therapeutic tool for blocking LAM cell proliferation. Clinical trials with rapamycin and its analog (rapalog) everolimus reduced disease-related symptoms and stabilized pulmonary function in patients; however, treatment was not able to reverse the preexisting damage, and pulmonary function decline resumed after cessation of treatment (19, 20). Several factors likely contribute to the partial effectiveness of rapalogs. First, inhibition of mTORC1 relieves the negative feedback loop to PI3K/Akt, promoting Akt activation, an oncogene that suppresses apoptosis and promotes cell survival. Thus,

rapamycin is cytostatic rather than cytotoxic. Second, increased mTORC1 activity in LAM cells negatively regulates autophagy (21), rendering LAM-derived cells acutely dependent on autophagy for survival. Thus, rapamycin causes reactivation of autophagy, which paradoxically allows LAM cells to survive. Therefore, the use of autophagy inhibitors in addition to rapamycin treatment could cause LAM cells to undergo apoptosis (22, 23).

We have previously demonstrated that the autophagy inhibitor resveratrol synergized with rapamycin in suppressing PI3K/mTORC1 activity, preventing rapamycin-induced autophagy and promoting death of TSC2-deficient cells *in vitro* (23). In this study we investigated whether the combination of rapamycin and resveratrol is effective in reducing TSC2-deficient tumor burden *in vivo*, thereby increasing the clinical usefulness of rapamycin therapy.

Materials and Methods

Wound Healing Assay

Cells were seeded in 12-well plates in complete DMEM and grown to confluency in monolayer overnight. Diagonal wound/scratch was created using a 200- μ l pipette tip. Cell debris was removed by washing once with PBS, followed by the addition of fresh media containing 20 nM rapamycin and/or 100 μ M resveratrol for 17 hours. Vehicle alone served as control. For each condition, three different areas were imaged, and three measurements were taken per image. Images were collected, and migration distance was measured using an EVOS FL Auto microscope and software (Life Technologies, Grand Island, NY) at 20 \times magnification.

Boyden Chamber Assay

Cells were cultured in media without serum in the presence of rapamycin and/or resveratrol. Nineteen hours later, 1×10^5 cells per well were plated on tissue culture inserts with 8.0- μ m pores (BD Biosciences, San Jose, CA). The inserts were incubated with serum-free media containing 20 nM rapamycin and/or 100 μ M resveratrol. Complete media were added to the lower chamber and allowed to migrate for 6 hours. After 6 hours, cells remaining on the upper side of the membrane were

scraped off, and the cells that had migrated to the lower side of the membrane were fixed in 4% paraformaldehyde. The insert membranes were removed, stained, and mounted on coverslips using DAPI Fluoromount (Southern Biotech, Birmingham, AL). Images were collected at 10 \times magnification using an EVOS FL Auto microscope. Nuclei were counted manually, and data were analyzed using two-tailed Student's *t* test.

Animal Studies

All animal work was performed in accordance with protocols approved by the Institutional Animal Care and Use Committee-Children's Hospital Boston (IACUC-CHB). Intact female CB17-SCID mice, 6 to 8 weeks of age, were purchased from Taconic (Hudson, NY). Eker rat uterine leiomyoma-derived TSC2-deficient (ELT3) cells were developed by Howe and colleagues (24, 25). ELT3 cells were transduced with Luciferase tag for bioluminescent imaging. For xenograft tumor establishment, 2.5×10^6 cells were inoculated bilaterally into the posterior back region of mice. Five weeks after cell inoculation, mice bearing subcutaneous tumors were randomized into four groups: vehicle control ($n = 5$; 10% ethanol in 10% 2-hydroxypropyl- β -cyclodextrin, 200 μ l/d, orally), rapamycin ($n = 4$; 3 mg/kg/d, intraperitoneally), resveratrol ($n = 5$; 200 mg/kg/d in 10% 2-hydroxypropyl- β -cyclodextrin, orally), and rapamycin plus resveratrol ($n = 5$; 3 mg/kg/d, intraperitoneally and 200 mg/kg/d, orally). The concentrations were selected based on effective doses as previously determined (23). Animal toxicity was analyzed using body weight as previously described (21). Drug treatment was initiated 5 weeks after cell inoculation. Body weight was measured every week. Tumor volume $[(\text{length} \times \text{width})^2/2]$ was measured weekly using calipers.

Bioluminescent Reporter Imaging

Ten minutes before imaging, animals were injected with luciferin (Xenogen, Alameda, CA) (120 mg/kg, intraperitoneally). Bioluminescent signals were recorded using the Xenogen IVIS System. Total photon flux of tumors was analyzed (26).

Statistical Analysis

For animal experiments, all data are presented as means \pm SEM. Measurements at single time points were analyzed by ANOVA and, if demonstrated significance,

were further analyzed by a two-tailed Student's *t* test. Time courses were analyzed by a repeated measures (mixed model) ANOVA with Bonferroni post *t* tests. Statistical tests and graphs were performed with GraphPad Prism 6.0 (GraphPad Software, San Diego, CA). $P < 0.05$ indicates statistical significance.

For *in vitro* experiments, statistical analysis was performed using a two-tailed Student's *t* test and graphed using Excel (Microsoft, Redmond, WA).

Further details are provided in the online supplement.

Results

The Combination of Rapamycin and Resveratrol Suppresses PI3K/mTORC1 Signaling, Promotes Apoptosis, and Reduces Motility of TSC2-Null LAM Cells

We first confirmed the ability of the rapamycin and resveratrol combination to inhibit PI3K/mTORC1 activation in human LAM-derived cells, which were obtained from biopsies of lung lesions from patients with LAM (5). Due to TSC2 deficiency and hyperactivation of mTORC1, these cells have high basal levels of p-S6K1 and p-S6 (Figure 1A). Combination treatment with rapamycin and resveratrol was able to inhibit S6K1 signaling, as evidenced by the reduction of S6K1 phosphorylation at T389 and S6 at S240/244. We also observed that rapamycin-induced phosphorylation of Akt on S473 was suppressed by the addition of resveratrol, and these results are in agreement with our previous findings (23). Combination therapy also induced stabilization of the autophagy protein p62/SQSTM1, corresponding to suppression of autophagy induction, and we observed reduced levels of survivin (Figure 1B). Similar results were seen with the use of combination therapy in ELT3 uterine leiomyoma cells (ELT3) derived from Eker rat (24). As shown in Figure 1C, combination or rapamycin and resveratrol inhibited TORC1/S6K1 signaling as indicated by the suppression of S6K1 phosphorylation at T389, eIF4B at S422, and S6 at S240/244, as well as stabilization of PDCD4 protein levels. mTORC1 signaling to 4EBP1 was also suppressed, as evidenced by dephosphorylation of 4EBP1 at S65. Additionally, the rapamycin and resveratrol combination was able to inhibit

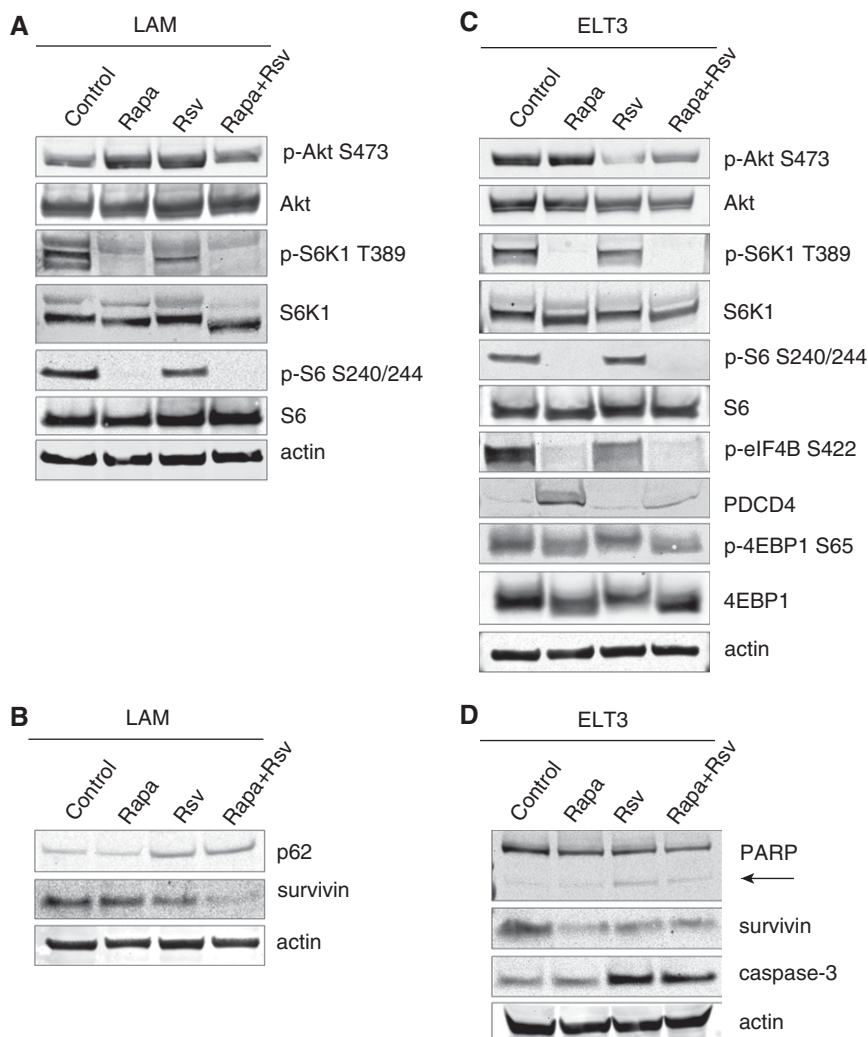


Figure 1. Combination of rapamycin (Rapa) and resveratrol (Rsv) inhibits phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt and mechanistic target of rapamycin (mTOR) signaling pathways in lymphangioliomyomatosis (LAM) and Eker rat uterine leiomyoma-derived TSC2-deficient (ELT3) cells. (A) LAM cells were treated with 20 nM rapamycin and/or 100 μ M resveratrol for 24 hours. Cells were lysed, and indicated proteins were detected by immunoblot. (B) LAM cells were treated as described in (A). (C and D) ELT3 cells were treated as described in (A). Each experiment was performed at least three times to ensure reproducibility. PARP, poly(ADP-ribose) polymerase-1.

rapamycin-induced activation of Akt by decreasing p-Akt levels compared with that of vehicle-treated controls. Combination therapy with rapamycin and resveratrol also dramatically down-regulated survivin levels and induced the apoptotic markers cleaved poly(ADP-ribose) polymerase-1 (PARP) and caspase-3 (Figure 1D).

To test whether combination therapy with rapamycin and resveratrol affects cell motility, migration of ELT3 and LAM cells was measured by means of the wound-healing assay (Figure 2). Although rapamycin treatment alone resulted in

complete wound closure 17 hours after treatment (Figures 2A and 2C), the newly populated area showed lower cell density than the untreated controls, indicating that rapamycin treatment delays cell migration (27). Additionally, as previously reported, resveratrol treatment alone impaired cell migration by approximately 25% compared with controls (28). Significantly, the combination treatment produced a synergistic effect on wound healing: only approximately 50% of the scratched area was repopulated. Similar effects on wound healing were seen with ELT3 cells (Figures

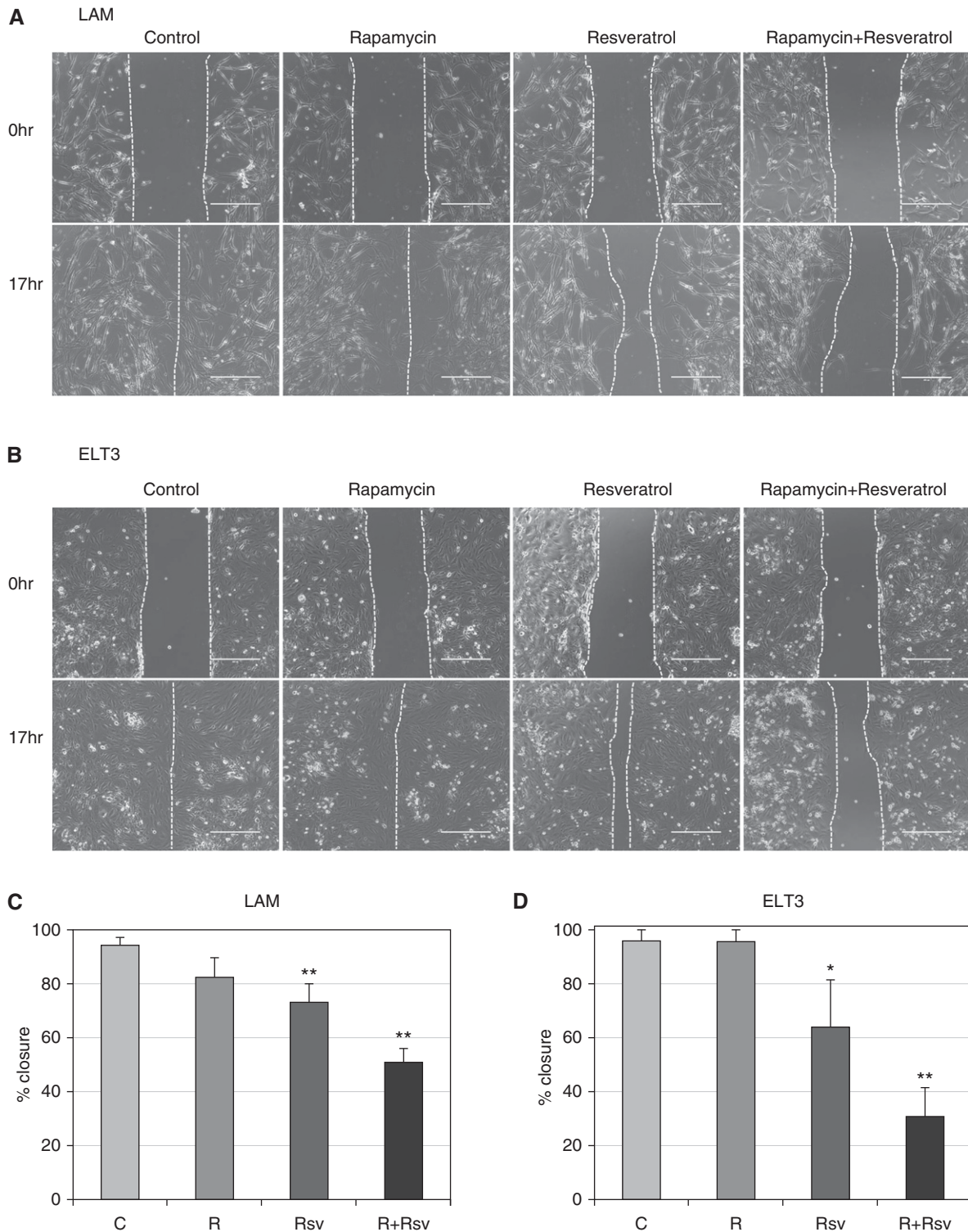


Figure 2. The combination of rapamycin and resveratrol reduces cell migration of LAM and ELT3 cells. (A) LAM cells grown to confluent monolayer were scratched and treated with 20 nM rapamycin and/or 100 μ M resveratrol for 17 hours. Representative images at the 0- and 17-hour time points are shown. (B) ELT3 cells were treated as described in (A). (C) Measurement and quantification of LAM cell migration was performed using EVOL FL Auto and Excel software. Statistical analysis was performed using two-tailed Student's *t* test. (D) Measurement and quantification of the ELT3 cell migration was performed as described in (C). Scale bars, 400 μ m. **P* < 0.05; ***P* < 0.01. C, control; R, rapamycin.

2B and 2D), where rapamycin treatment alone did not prevent complete wound closure, resveratrol treatment alone produced approximately 65% repopulation, and the combination of rapamycin and resveratrol resulted in approximately 30% repopulation. Additionally, transwell cell migration assay confirmed that the combination of rapamycin and resveratrol reduced cell migration of TSC2-null ELT3 cells by over 90% (Figure 3). Thus, our data indicate that the combination of rapamycin and resveratrol is effective in inhibiting PI3K/mTORC1 signaling and reducing cell motility of TSC2-null cells.

Combined Administration of Rapamycin and Resveratrol Has Low Toxicity in Mice and Causes a Significant Reduction of TSC2-Deficient Xenograft Tumor Size and Growth

To evaluate the role of rapamycin and resveratrol inhibition in cells with mTORC1 hyperactivation *in vivo*, we used an ELT3 cell xenograft model (26). Mice bearing ELT3-luciferase-expressing xenograft tumors were treated with rapamycin and resveratrol as single-agent treatments or in combination. Drug toxicity was evaluated by body weight changes, and no significant effect was observed after the drug treatments (Figure 4A). It is important

to note that tumors from control and resveratrol groups grew faster than the ones from the rapamycin alone or the rapamycin with resveratrol groups; thus, ulcers developed in the skin of tumor areas. We therefore had to kill these two groups at Week 3. We next assessed the possible benefits of combination treatment on tumor development by analysis of the xenograft tumor size. As previously published (21), rapamycin treatment alone showed a slight but significant reduction in tumor size in just 3 weeks of treatment. Strikingly, the combination of rapamycin and resveratrol caused a statistically significant reduction in tumor size as compared with rapamycin treatment alone by as early as Week 2 of treatment, and this reduction in tumor size was maintained at 4 weeks after treatment (Figure 4B). Subsequently, in comparison with the vehicle control, single-agent treatment of rapamycin or the combination of rapamycin and resveratrol for 3 weeks reduced tumor growth by 0.54-fold as measured by bioluminescence intensity (Figures 4C and 4D). More importantly, unlike single-agent rapamycin treatment, the combination of rapamycin and resveratrol suppressed xenograft tumor progression at 4 weeks of treatment (Figures 4C and 4D). Our results indicate

that combination therapy of rapamycin with resveratrol is significantly more effective than rapamycin treatment alone in reducing tumor size and tumor growth while maintaining low toxicity.

The Combination of Rapamycin and Resveratrol Suppresses PI3K/Akt/mTORC1 Activation in the Tumor

We next examined the effect of the rapamycin and resveratrol combination on the activity of the PI3K/Akt/mTORC1 signaling pathways in tumor samples. The combination of rapamycin and resveratrol resulted in severe reduction of S6 phosphorylation and approximately 40% inhibition of 4EBP1 phosphorylation (Figures 5A and 5B). Although rapamycin treatment alone caused a slight inhibition of S6 phosphorylation, it was not statistically significant. This could be due to compensatory S6 phosphorylation by ribosomal S6 kinase (29). Moreover, the combination of rapamycin and resveratrol caused a 45% inhibition of Akt phosphorylation at S473. This is important because inhibition of mTORC1 signaling leads to suppression of the negative feedback loop to Akt, causing reactivation of Akt (30). The combination treatment was also able to stabilize PDCD4 protein levels. Finally, rapamycin and resveratrol, alone or in combination, did not alter p-ERK levels,

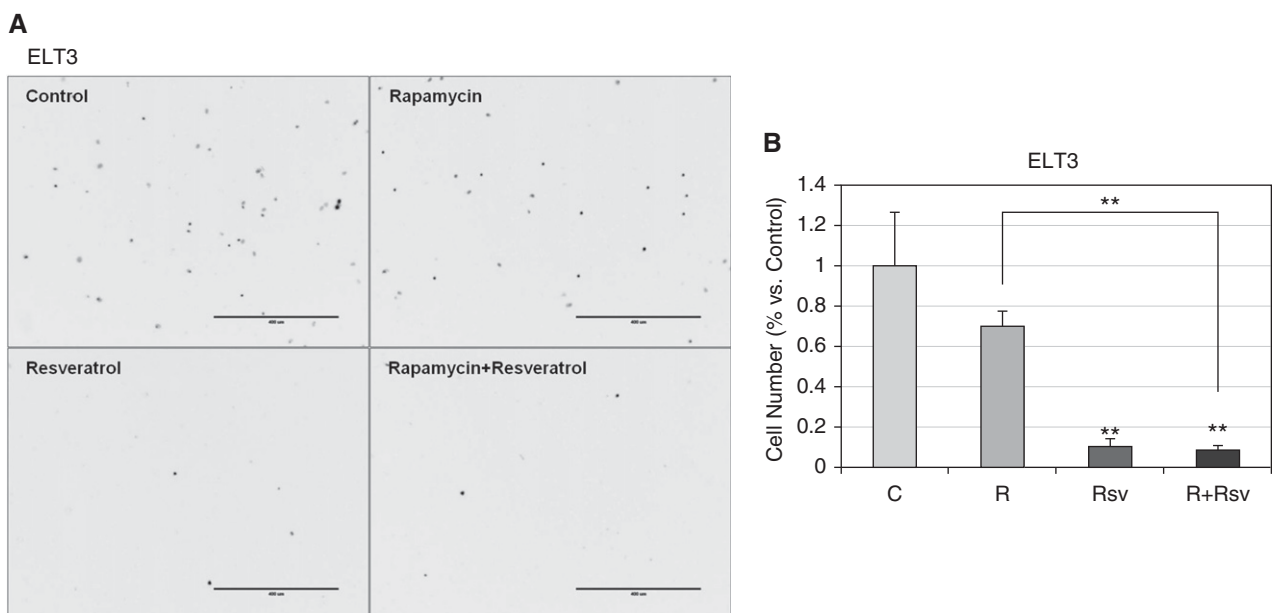


Figure 3. The combination of rapamycin and resveratrol prevents cell migration. Cell migration/Boyden chamber assay was performed as described in MATERIALS AND METHODS. (A) Representative images of ELT3 cells stained with 4',6-diamidino-2-phenylindole after a 6-hour migration assay. (B) Histogram representing the number of cells migrated relative to untreated control. Scale bars, 400 μ m. $**P < 0.01$.

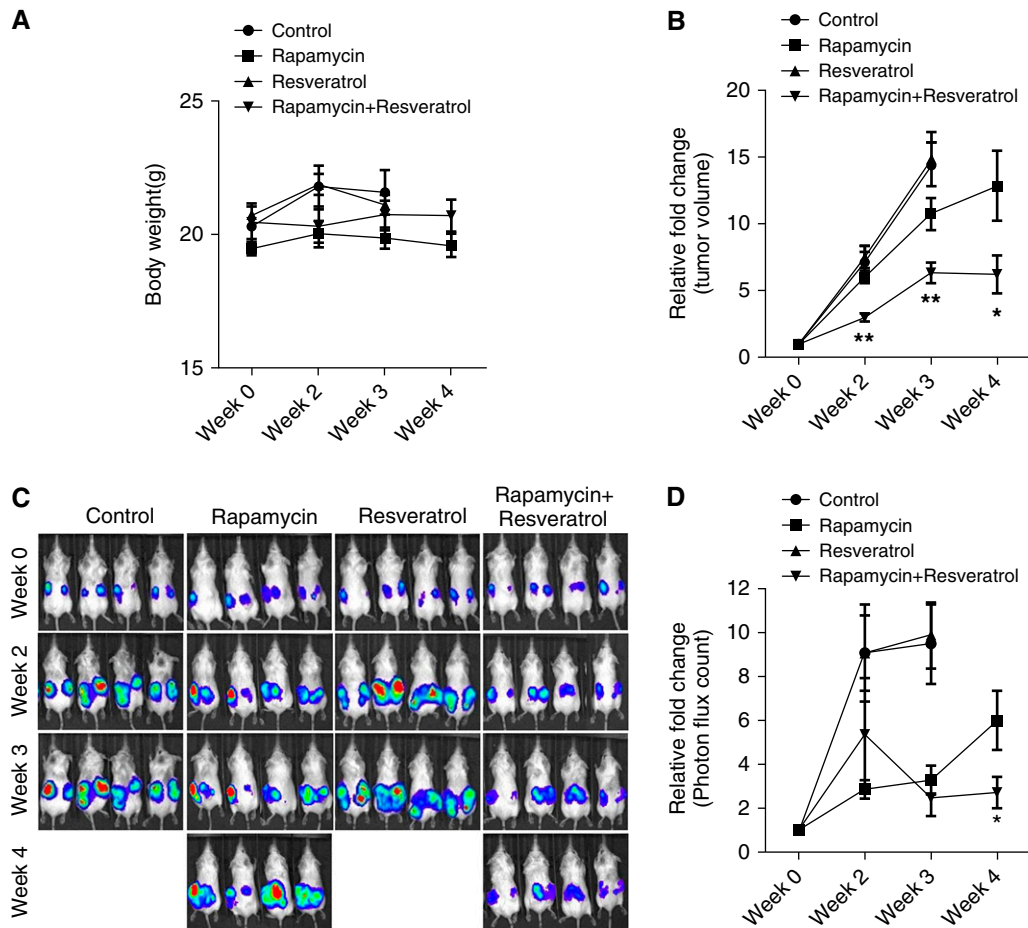


Figure 4. Combination treatment with rapamycin and resveratrol reduces tumor size and inhibits growth. Female CB17-scid mice were inoculated with ELT3-luciferase cells subcutaneously. Mice were treated with vehicle, rapamycin, resveratrol, or a combination of rapamycin and resveratrol for 4 weeks. (A) Body weight was measured every week. (B) Tumor volume was measured weekly using a digital caliper. The y axis indicates the relative fold growth of tumor size versus the baseline measurement before drug treatment. (C and D) Bioluminescent intensity in xenograft tumors was recorded and quantified weekly. The y axis indicates the relative tumor growth versus the baseline quantification before drug treatment. * $P < 0.05$; ** $P < 0.01$. Vehicle- and resveratrol-treated tumors progressed faster than the tumors in rapamycin or rapamycin-resveratrol groups, and ulcers developed in the skin of tumor areas; thus, those animals had to be killed at Week 3.

indicating the specificity of this treatment in inhibiting Akt/mTORC1 signaling, as we have previously observed (31).

Combined Administration of Rapamycin and Resveratrol Reduces Proliferation and Induces Apoptosis within the Tumor

To examine the cause of reduced tumor size as observed within the combination rapamycin and resveratrol treatment group, we first examined tumors for expression of Ki67, a marker of proliferative index. Single-agent or combination treatment reduced the number of proliferating cells by 50% compared with untreated tumors (Figure 6). We subsequently examined the levels of cleaved caspase-3, a marker of apoptosis. Rapamycin and resveratrol combination treatment caused

a 2-fold up-regulation of cleaved caspase-3 (Figures 7A and 7B). In addition, analysis of TUNEL staining of tumor sections showed that combination treatment increased the levels of apoptotic TUNEL-positive cells by 3-fold, whereas single-agent treatment with either rapamycin or resveratrol had no effect on apoptosis (Figures 7C–7G). Taken together, these data indicate that a 4-week administration of the combination treatment of rapamycin and resveratrol inhibits mTORC1 signaling, inhibits Akt activation, induces apoptosis, and inhibits cells motility.

Discussion

Because loss of TSC function leads to an enhancement of signaling through

mTORC1, rapamycin has always been an attractive therapy for LAM. Clinical trials for sirolimus resulted in significant improvement of symptoms during the treatment period. However after treatment was discontinued, symptoms returned, often to levels comparable with the placebo group (19, 20, 32–34). The cytostatic response of LAM cells to sirolimus may be due to activation of autophagy as a result of the inhibition of mTORC1 activity, causing cell survival (1).

Although it would be expected that a loss of TSC function would lead to an enhancement of cell growth due to increased mTORC1 signaling, this is not observed in LAM cells. mTORC1 activity negatively regulates autophagy, and it has been suggested that the mTORC1-induced

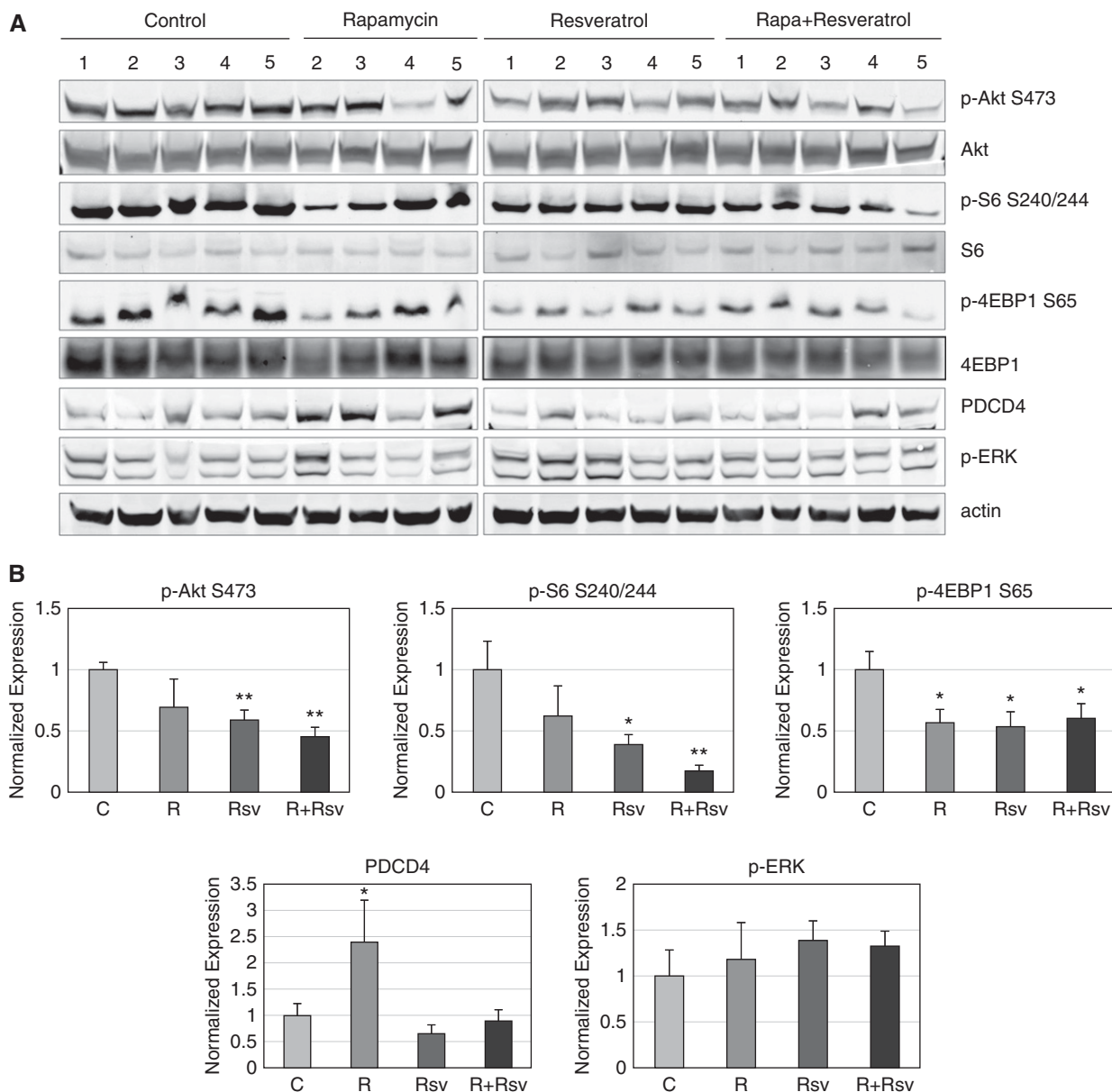


Figure 5. The combination of rapamycin and resveratrol inhibits mechanistic target of rapamycin complex 1/Akt signaling in xenograft tumors. (A) Tumors were lysed as described in MATERIALS AND METHODS, and indicated proteins were detected by immunoblot. Numbers represent different mice within the treatment group. (B) Quantification of immunoblots was performed using Image Studio 4.0 program and Excel. * $P < 0.05$; ** $P < 0.01$.

inhibition of autophagy maintains the LAM cells in a stagnant metabolic state, preventing cell growth and proliferation that would have otherwise been stimulated by mTORC1 activity (21, 35). In addition, LAM cells seem to be dependent on even the low levels of autophagy because further inhibition of autophagy induces cell death (21).

Our previous studies showed that combination therapy of rapamycin with resveratrol was able to block autophagy and

induce apoptosis in TSC2-null cells and in cells with mTORC1 pathway hyperactivation (22, 23). In this study, we observed that, in TSC2-null cells, the combination therapy prevented rapamycin-induced up-regulation of Akt while maintaining inhibition of S6K1 signaling, indicating that this combination therapy can counter hyperactivation of mTORC1 signaling caused by the loss of TSC2. Additionally, resveratrol treatment was able to prevent rapamycin-induced up-

regulation of autophagy, as evidenced by stabilization of p62, in accord with our previous findings (23). Interestingly, expression of survivin, an inhibitor of apoptosis in TSC2-null cells, was found to correlate with resistance to chemotherapeutic agents (36). We found that survivin expression is down-regulated by the combination treatment, an important consequence for TSC2-null/LAM cells because rapamycin treatment alone does not suppress survivin expression

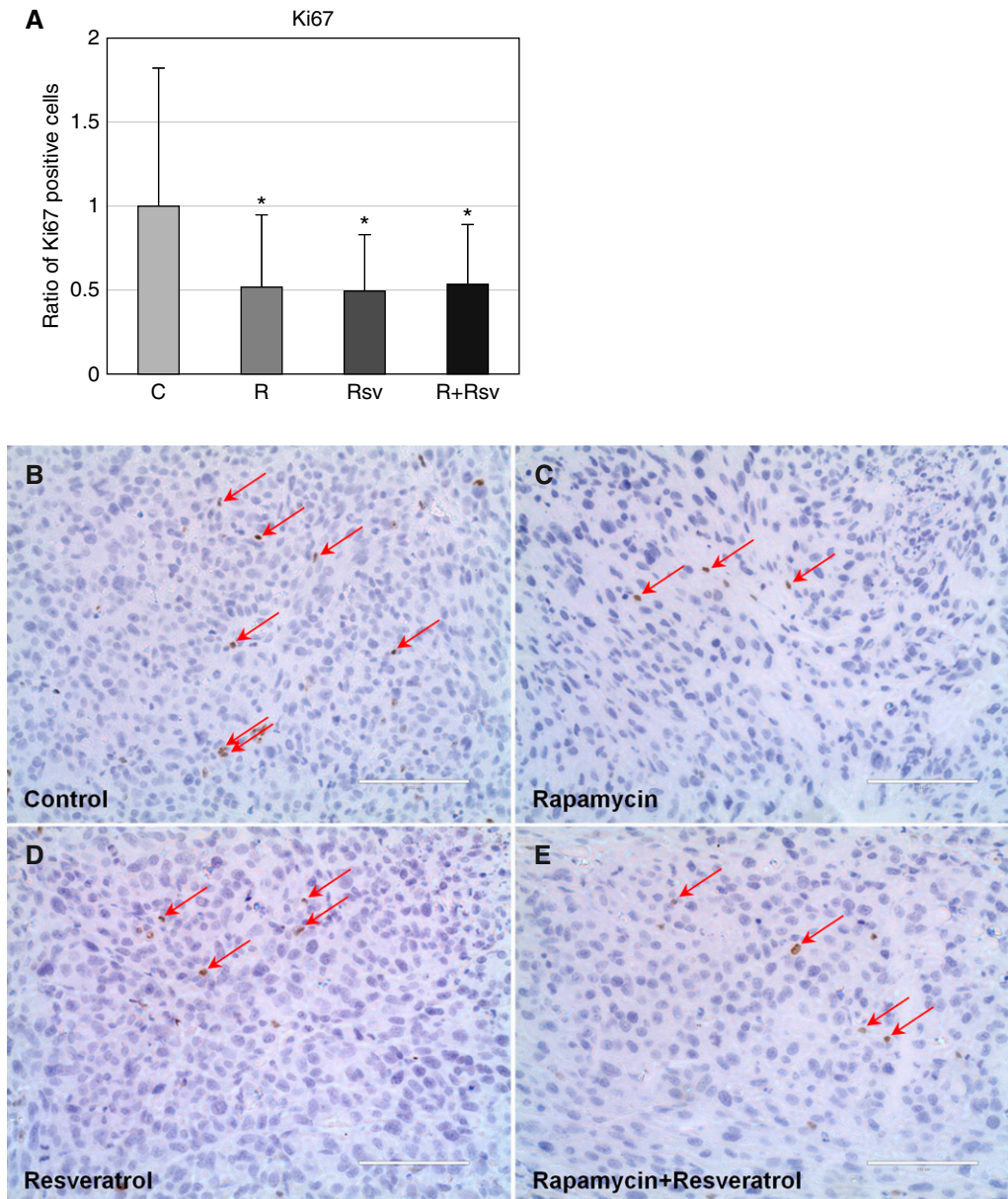


Figure 6. The combination of rapamycin and resveratrol reduces proliferation in xenograft tumors. (A) Quantification of Ki67-positive cells was performed as described in MATERIALS AND METHODS. * $P < 0.05$. After immunohistochemistry, representative images for control (B), rapamycin (C), resveratrol (D), or the combination of rapamycin and resveratrol (E) were created using an EVOS FI Auto microscope (original magnification: $\times 40$). Arrows indicate Ki67-positive cells. Scale bars, 100 μm .

and could be one of the mechanisms for therapy resistance. In addition, combination treatment significantly induced apoptosis in cell culture, as measured by the increase in cleavage of apoptotic markers caspase-3 and PARP. Rapamycin and resveratrol also synergized in inhibiting cell motility, in accord with reduced lung colonization *in vivo* we have previously observed (22).

Because LAM is caused by the loss of TSC2, we examined the effectiveness of this

combination therapy in the TSC2-null mouse xenograft model. Although resveratrol treatment alone had no effect on the TSC2-deficient xenograft tumor size grown *in vivo*, the combination of rapamycin and resveratrol had a statistically significant effect on reducing the tumor size by Week 2 of treatment as compared with rapamycin treatment alone, and this effect was maintained throughout the treatment. Additionally, analysis of tumor progression revealed that although both single-agent

rapamycin and the combination therapy reduced xenograft tumor growth after 3 weeks of treatment, combination therapy was able to suppress tumor growth, whereas rapamycin delayed tumor growth, as seen by the increase in photon flux from Week 3 to Week 4 of treatment. Signaling data from treated tumor tissue consistently depicted strong suppression of Akt/mTORC1 signaling but had no effect on MAPK signaling. Combination therapy also significantly increased apoptosis in the

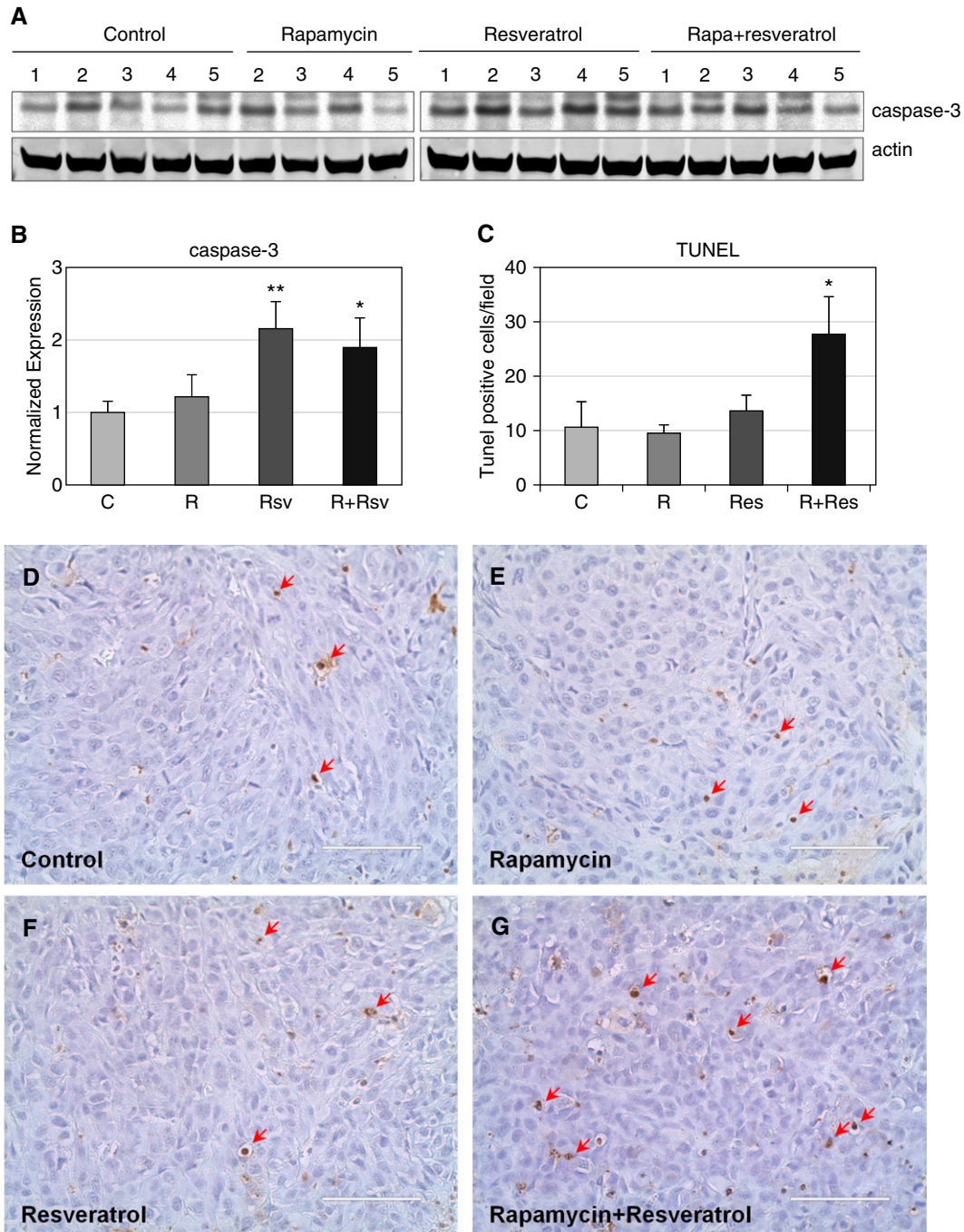


Figure 7. The combination of rapamycin and resveratrol induces apoptosis in xenograft tumors. (A) Tumors were lysed as described in MATERIALS AND METHODS, and caspase-3 and actin were detected by immunoblot. (B) Quantification of caspase-3 was performed using Image Studio 4.0 program and Excel. * $P < 0.05$; ** $P < 0.01$. (C) Quantification of TUNEL-positive cells was performed as described in MATERIALS AND METHODS. * $P < 0.05$. After immunohistochemistry, representative images for control (D), rapamycin (E), resveratrol (F), or the combination of rapamycin and resveratrol (G) were created using an EVOS FI Auto microscope (original magnification: $\times 40$). Arrows indicate TUNEL-positive cells. Scale bars, 100 μm . TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

TSC2-deficient xenograft tumors and inhibited proliferation. Although both single treatments and the combination treatment reduced the proliferative index, only combination therapy induced apoptosis. This further supports the model

whereby the synergistic interaction of rapamycin and resveratrol results in reduction of xenograft tumors. Our findings are very exciting because rapamycin and resveratrol combination therapy could benefit patients with LAM

not only by arresting tumor growth but also by eliminating lesions, possibly through induction of apoptosis.

Combination therapy with rapamycin and an autophagy inhibitor is an attractive strategy for the treatment of LAM because

it may result in better long-term clinical outcomes than rapamycin alone. Based on our previous *in vitro* work, resveratrol was able to suppress autophagy in TSC2-deficient cells even in the presence of rapamycin. Based on the current *in vivo* study,

rapamycin administered in combination with resveratrol is a potent inhibitor of TSC2-deficient lesion growth partly via induction of apoptosis. Based on these data, resveratrol is a good candidate for a rapamycin cotherapy in patients with LAM. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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