

Topical application of an mTOR inhibitor suppresses mast cells in a murine skin: A novel option for cutaneous mastocytosis

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Abstract

Mastocytosis is classified into three major types: cutaneous mastocytosis (CM), systemic mastocytosis (SM), and mast cell sarcoma. SM can present with cutaneous lesions while CM is characterized by the cutaneous proliferation of mast cells. Systemically administered mTOR inhibitors are effective against SM, but there are, as of yet, no reports of effectiveness of topical agent for CM and in vivo experiments using a topical formulation. First, the effect of rapamycin, an mTOR inhibitor, on the cell growth and cycle of human mast cell lines (HMC-1.1 and HMC-1.2) was assessed via cell growth, cell cycle apoptosis assays and evaluating phosphorylation of the mTOR pathway signal. Next, the therapeutic efficacy of a topical formulation of rapamycin was tested in a subcutaneous HMC-1 xenograft model. Human mast cells (HMC-1.2) were subcutaneously injected into the dorsum of mice with severe combined immunodeficiency (SCID) before topical rapamycin application. In vitro analysis demonstrated that rapamycin had not induced apoptosis in the human mast cell line but had significantly suppressed cell proliferation by inducing cell cycle arrest. In a murine severe combined immunodeficiency model of HMC-1 xenograft, by day 35, tumor growth was markedly suppressed in the rapamycin group than in a control group. Immunohistochemistry and western blotting of tumor tissue demonstrated that rapamycin had suppressed mTOR signaling in the tumors, thereby inhibiting their growth. Topical rapamycin suppressed mTOR signaling and the growth of D816V-mutated c-Kit mast cells in a HMC-1 xenograft model.

Introductions

Mastocytosis is a heterogeneous disease characterized by the abnormal growth and accumulation of clonal mast cells in one or more organs. Cutaneous mastocytosis (CM) was described as “brown lesions that wheal after scratching” and were subsequently named urticaria pigmentosa (UP) [1]. The World Health Organization (WHO) classified the disease as a systematic mastocytosis, cutaneous mastocytosis and mast cell sarcoma by major criteria. The skin lesions of CM reportedly resolve spontaneously in about 50% of the patients by adulthood but persist into adulthood in 15–30% of cases [2]. The skin lesions have a significantly adverse impact on the quality of life of the patients [2, 3].

In addition to causing cosmetic concerns [4], particularly in children and young adults, cutaneous lesions may also serve as a trigger for mast cell activation and increase the risk of severe systemic reactions, including anaphylaxis [5, 6]. Current treatment options for mastocytosis include systemic corticosteroids, biologic agents, and oral mTOR inhibitors [7–9]. However, these therapies are associated with potential adverse effects, and biologic agents and targeted therapies may impose a substantial financial burden on patients. Therefore, the development of a low-risk and effective topical treatment capable of controlling local mast cells is of considerable clinical importance [10, 11].

Previous studies have examined the efficacy of mammalian target of rapamycin (mTOR) inhibitors as a potential treatment for CM. Rapamycin inhibits the activation of mTOR complex 1, a regulatory kinase important for the suppression of cell activation and proliferation and antibody production [12]. The

mTOR pathway regulates mast cell growth and survival and mTOR inhibitors suppress the growth and survival of neoplastic mast cells [13, 14]. Rapamycin is known to inhibit the growth and survival of mutated c-kit mast cells [15].

Consequently, the present study used *in vitro* mastocytosis cell lines (human mast cell [HMC]1.1 and 1.2) and a HMC-1 xenograft model to investigate the efficacy of topical rapamycin as a novel treatment option for CM.

Methods

Cell cultures

HMC-1 is a human mast cell line derived from a patient with mast cell leukemia. HMC-1 comprises two sublines, HMC-1.1 (RRID:CVCL_H206) and HMC-1.2 (RRID:CVCL_H205). Both cell lines used in this study were clones derived from an original population. HMC-1.1 contains only the V560G mutation of *c-kit* gene and HMC-1.2 contains D816V and V560G, both of which induce substitution of amino acids in juxtamembrane and catalytic domain in KIT tyrosine kinase. Both sublines were cultured in IMDM medium (Nacalai tesque, Kyoto, Japan, Cat# 11506-05) supplemented with 10% FBS, l-glutamine 2 mM, penicillin 100 units/ml, and streptomycin 100 mg/ ml.

Mice

Female, 8-week-old mice with severe combined immunodeficiency (SCID) (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, J; Cat# 005557, RRID: IMSR_JAX:005557) were purchased from The Jackson Laboratory Japan, Inc. (Kanagawa, JAPAN). The maintenance and experimental procedures were approved by the Animal Care and Use Committee of Tokyo Medical University School of Medicine (Tokyo Medical University ethics approval number: R5-090: 2023.3.24).

mTOR inhibitor

Rapamycin (Cat# S1039) was purchased from Selleck Chemicals (Houston, U.S.). Sirolimus gel (Rapalimus® Gel) purchased from Nobelpharma Co., Ltd. (Osaka, Japan) was impregnated with a 0.2% concentration of rapamycin. Rapamycin gel demonstrated the highest bioavailability compared with other vehicles.

Cell growth

Cell growth of HMC-1.1 and HMC-1.2 was assessed over time in culture medium supplemented with rapamycin at concentrations ranging from 0.05 to 500 nM. The cells were cultured in 96-well plates at a density of 1×10^4 /well. At hours 24 and 72 and day 7 of the culture, the number of viable cells was

estimated using an MTT assay and cell count reagent SF (Nacalai tesque, Cat#07553-15). Then, MTT solution 10 μ l (stock concentration, 5.0 mg/ml in PBS) was added to each well and incubated for two hours under the same conditions. The absorbance values were read using an ELISA plate reader (Bio-Rad Laboratories, CA) at 450 nm with a reference wavelength of 620 nm.

Cell cycle and apoptosis assay

The HMC-1.1 and HMC-1.2 cells (0.5×10^6) were incubated for 24, 48, and 72 hours in the 5ml medium alone or in a medium containing various concentrations of rapamycin (50, 500 nM and 5 μ M). The cell cycle and apoptosis were then analyzed by flow cytometry (Facs Calibur; Becton Dickinson, San Jose, CA) according to manufacturer's protocol. In brief, the cells were incubated for 10 minutes at 4°C with FITC-conjugated annexin V and 7-AAD (Immuno Immunotech - Beckman Coulter, Marseille, France), and fluorescence intensity was analyzed by flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). Cells were incubated with Bromodeoxyuridine (BrdU) for 3 hours to evaluate the cell cycle in detail. BrdU is used to detect cellular proliferation during the transition from the G1 to the S phase.

Subcutaneous HMC-1 xenograft model

A total of 2.0×10^6 HMC-1.1 or HMC-1.2 were suspended in 40 μ l of culture medium before subcutaneous injection into the bilateral dorsal skin of SCID mice (day - 2). The four quadrants surrounding the injection site were marked with indocyanine green (ICG). The mice were placed in a gauge singly. Beginning 3 days after injection (day 1), sirolimus gel was applied daily to the right dorsum while vaseline was applied to the left dorsum as a vehicle control. The topical treatments were continuously applied to the dorsum until the mice were sacrificed on day 35 after the initiation of topical application.

Evaluation of tumor size

Tumor size was assessed weekly by measuring the short and long diameters of the visible lesions after shaving the fur. The tumor area was calculated using the formula for an ellipse: Area = $(\pi/4) \times$ long diameter $\times$ short diameter (mm^2). After the mice were sacrificed, the skin was reflected to expose the subcutaneous tumors. Data represent the results of three independent experiments.

Human cutaneous mastocytosis

Human cutaneous mastocytosis skin samples from biopsies were used for diagnosis and screening for the c-kit gene mutation. Written informed consent was obtained for the use of these samples, including for genetic analysis. This research was approved by the Tokyo Medical University Medical Ethics Committee (approval number: T2019-0160).

Histopathological analysis, immunohistochemistry, and evaluation of mTOR signaling

For histopathological analysis, the tumor samples obtained from the HMC-1 xenograft model and human samples were fixed with a 20% formalin neutral buffer solution and stained with hematoxylin and eosin (New Hematoxylin Solution; 30141, MUTO Chemicals Tokyo, JAPAN). Anti-p70S6kinase (Cat# 9234, RRID:AB_2269803), anti-phospho-p70S6kinase (threonine [Thr]389)(Cat# 34475, RRID:AB_2943679), anti-4E-binding protein 1 (4E-BP1) (Cat# 9644, RRID:AB_2097841), anti-phospho-4E-BP1 (Thr37/46) (Cat# 2855, RRID:AB_560835), and anti-mTOR (Cat# 2983, RRID:AB_2105622), anti-phospho-mTOR (serine [Ser]2448) (Cat# 5536, RRID:AB_10691552) rabbit monoclonal antibodies (Cell Signaling Technology, Beverly, MA) were used, followed with matched secondary antibodies: Histofine Simple Stain mouse MAX-PO (R) (Nichirei Biosciences, Cat# 414341F, RRID:AB_2819094). Immune complexes were visualized with Simple Stain AEC solution (Nichirei Biosciences). Images were obtained using a Nanozoomer virtual slide scanner (Hamamatsu photonics).

Immunofluorescence Staining

For immunofluorescence staining, formalin fixed paraffin embedded human skin sections were deparaffinized. After heat-induced antigen retrieval with an edetic acid solution (pH 9.0). The sections were then incubated with primary antibodies against Ki67 (Abcam, Cat# ab15580, RRID:AB_443209) and Mast Cell Tryptase (G3) (Santa Cruz Biotechnology, Cat# sc-33676, RRID:AB_628406) at 6 hr of incubation at room temperature, followed by 30 min of incubation at room temperature with matched secondary antibodies: Alexa fluor 488 goat anti-rabbit and Alexa fluor 594 goat anti-mouse (Thermo Fisher Scientific). Images were obtained using a Nikon AX (Nikon) confocal microscope after DAPI staining.

Western blot

The cells were lysed with RIPA lysis buffer (Cell Signaling Technology, Cat# 9806). The concentration of total protein was measured using a Qubit Protein Assay Kit (Invitrogen, Waltham, MA). Cell lysates were loaded on 4%–12% Nupage Bis-Tris Gels and transferred to PVDF membranes using Trans Blot Turbo Transfer Pack (Bio-Rad, Hercules, CA), then probed for immunoreactive proteins with Western blot analysis using the following protein-specific antibodies: b-actin (Cat# 4967, RRID:AB_330288), anti-p70S6kinase (Cat# 9234, RRID:AB_2269803), anti-phospho-p70S6kinase (threonine [Thr]389)(Cat# 34475, RRID:AB_2943679), anti-4E-BP1 (Cat# 9644, RRID:AB_2097841), anti-phospho-4E-BP1 (Thr37/46) (Cat# 2855, RRID:AB_560835), and anti-mTOR (Cat# 2983, RRID:AB_2105622), anti-phospho-mTOR (serine [Ser]2448) (Cat# 5536, RRID:AB_10691552) rabbit monoclonal antibodies (all from Cell Signaling Technology, Beverly, MA). The proteins were visualized using WesternBreeze Chemiluminescent Kit (Invitrogen, Waltham, MA), and signals were detected using ChemiDoc XRS system (Bio-Rad, Hercules, CA).

Statistics

All statistical analyses were performed using SPSS (International Business Machines Corporation, Armonk, NY). The two-tailed paired Student's t-test was used. All the data were expressed as the mean $\pm$ standard error of the mean. $P < 0.05$ was considered to indicate statistical significance.

Results

Rapamycin inhibited mastocytosis cell lines proliferation

Rapamycin is known to suppress the proliferation of HMC-1 cells [15]. In our experiments, various concentrations of rapamycin slowed HMC-1.1 and HMC-1.2 cell proliferation (Fig. 1a). As absorbance is proportional to the number of cells, no difference was observed between rapamycin-treated and untreated cells on day 1 after the start of the culture. However, by day 3, absorbance in the rapamycin-treated cells were significantly lower than in the untreated controls. By day 7, absorbance had decreased to a significantly greater degree in the rapamycin-treated cells compared with controls.

Growth inhibition of mastocytosis cell lines by rapamycin was induced by G1 cell cycle arrest

Although rapamycin reportedly induces apoptosis in HMC-1 cells [15, 16], in the present study, 72 hours treatment of this agent did not increase the proportion of apoptotic cells of both cell lines at concentrations higher than 500 nM (Fig. 1b). Based on these results, it was hypothesized that rapamycin suppresses cell cycle progression rather than inducing apoptosis. BrdU was therefore added to evaluate the cell cycle. As a result, the rapamycin treatment was found to have increased the proportion of cells in the G0/G1 phase (HMC-1.1: 66.8% vs 83.6%; HMC-1.2: 60.5% vs 81.4%) and decreased the proportion of cells in the S phase (HMC-1.1: 26.4% vs 11.0%; HMC-1.2: 19.8% vs 9.2%) (Fig. c, d). These results indicated that rapamycin suppressed cell proliferation by inducing G1 cell cycle arrest in the HMC-1.1 and HMC-1.2.

Rapamycin inhibited mTOR signaling in the HMC-1.1 and HMC-1.2 cells by suppressing p70S6K phosphorylation

Western blot analysis was used to determine the mechanism of rapamycin activity on HMC-1.1 and HMC-1.2 cells (Fig. 2a). p70S6K and 4EBP-1 are major downstream targets of mTORC1 [17], and the phosphorylation of these molecule indicates the activation of mTOR signaling. In the both cell lines, p70S6K and 4EBP-1 were constitutively phosphorylated, suggesting that mTOR signaling was activated, with this phosphorylation being more pronounced in the HMC-1.2 cells. The phosphorylation of mTOR was not inhibited in the HMC-1.1 cells following the rapamycin treatment but was significantly suppressed in the HMC-1.2 cells (Fig. 2b). In contrast, the phosphorylation of p70S6K was completely inhibited by rapamycin in both cell lines (Fig. 2c). The phosphorylation of 4EBP-1 was significantly inhibited only in the HMC-1.2 cells (Fig. 2d). These data suggested that rapamycin may be more effective in HMC-1.2 cells than in HMC-1.1 cells.

Topical rapamycin gel suppressed HMC-1 tumor growth

To date, no murine CM model has been established. Therefore, we established a HMC-1 xenograft model by subcutaneously inoculating a human mast cell line. HMC-1 cells were subcutaneously injected into SCID mice and resulted in the gradual growth of tumors. Approximately three weeks after the cell injection, tumor mass had become macroscopically detectable. The topical treatment of rapamycin gel (Rapa) was begun three days after the cell injection (Fig. 3a). On day 22, the HMC-1.1 cells in the tumors of the rapamycin group were significantly smaller than those of the control group (Fig. 3b). However, the tumors of the rapamycin group continued to increase in size. Although rapamycin gel delayed tumor growth, it did not completely halt tumor progression. A clear macroscopic difference in HMC-1.1 tumor size between the two groups was observed on day 36 (Fig. 3c).

On day 36, the HMC-1.2 cells in the tumors of the rapamycin group were significantly smaller than those of the control group (Fig. 3d). The tumors treated with vaseline were not macroscopically visible. After the mice were sacrificed, a macroscopic examination of the inverted skin revealed that the tumors treated with vaseline had markedly enlarged (Fig. 3e). Although the growth rate of these tumors varied substantially, all the tumors treated with topical rapamycin gel were macroscopically undetectable. Histopathological examination of serial sections of tumors from the rapamycin group demonstrated the presence of only a small number of residual HMC-1.2 cells (Fig. 3f), suggesting that the topical rapamycin gel had suppressed tumor growth not by inducing the apoptosis of the HMC-1.2 cells, but rather by arresting the cell cycle, thereby inhibiting tumor expansion. Although rapamycin significantly inhibited the proliferation of HMC-1.1 cells, residual cell proliferation remained, whereas proliferation of HMC-1.2 cells was almost completely suppressed.

Topical rapamycin gel inhibited 4EBP-1 phosphorylation and suppressed the HMC-1.2 cell cycle progression

To assess the effect of rapamycin gel immunohistochemically, tissue sample of the HMC-1.2 tumors were collected three days after topical application. Immunohistochemical analysis revealed strong staining for phosphorylated 4EBP-1 (p-4EBP-1) in the control group but no staining in the rapamycin group. In addition, Ki-67 staining demonstrated a decrease in the number of positive cells following the topical rapamycin treatment, indicating cell cycle arrest (Fig. 4a). In contrast, no difference was observed in the staining pattern of phosphorylated mTOR (p-mTOR) or phosphorylated p70S6K (p-p70S6K). Similar histopathological findings were consistently observed in all four mice.

Next, to evaluate the suppression of mTOR signaling quantitatively, Western blot analysis was performed (Fig. 4b). Tumor cells were collected after three days of rapamycin gel application, once the injected tumors had become macroscopically detectable. Phosphorylation of 4EBP-1 was markedly suppressed by the topical rapamycin treatment (Fig. 4c), whereas the phosphorylation level of mTOR and p70S6K did not differ significantly (Fig. 4d), indicating that topical rapamycin gel inhibited mTOR signaling and suppressed tumor growth.

Mast cells in CM exhibited activation of the mTOR signaling pathway and proliferative activity

Most of the mast cells in CM are generally considered indolent [18]; however, whether the mTOR pathway is activated in these cells remains unclear. To clarify the degree of mTOR signaling activation in CM, human skin biopsy specimens were analyzed immunohistochemically. The immunohistochemical staining for mTOR and p-mTOR demonstrated a distinct pattern (Fig. 5a). To assess proliferative activity, Ki-67 immunofluorescence staining was performed with CM cells harboring the human KIT D816V mutation and those without the KIT D816V mutation. Some mast cells in both groups were positive for Ki-67 (Fig. 5b). Therefore, cells harboring the KIT D816V mutation had higher p-mTOR and Ki-67-positive expression. Similar histopathological findings were consistently observed in all four Japanese pediatric patients.

Discussion

The prevalence of mastocytosis, including CM, in Sweden was reportedly 23.9 per 100,000 (95% confidence interval: 22.8–25.0) [19], and an epidemiological study in Denmark and the United States reported a prevalence of approximately 1 per 20000 persons and 4 per 100,000 persons, respectively, in both children and adults [20, 21]. As of yet, no cases of SM have been reported in Japan. Possibly due to differences in genetic background between Japanese and Western populations, fewer cases of mastocytosis, with only a small number of CM cases without SM are reported annually. In fact, the present study center has, to date, treated only about 25 patients with CM.

CM resolves or improves spontaneously in 90% of pediatric patients but skin lesions persisted into adulthood in 15–30% of cases [22–24]. In the authors' own, decade-long experience of managing the disease, none of the appropriately treated skin lesions resolved. Thus, the prognosis of CM varies considerably, and given the relative unlikelihood of the disease improving spontaneously during childhood, a safe and effective topical medication is needed. The present study investigated the potential utility of an mTOR inhibitor, given the role of mTOR in mast cell regulation, as a topical treatment for CM. Rapamycin is known to inhibit the growth and survival of mutated c-kit mast cells [15] and has been used to treat angiofibroma in TSC patients [25]. Given that topical rapalimus gel is already available for clinical use, the favorable results observed in our study may facilitate its future clinical application and potential indication expansion for patients with CM.

The present studies found that rapamycin suppressed the HMC-1.1 cells; furthermore, both HMC-1.1 and HMC-1.2 cells demonstrated an equally slow rate of proliferation in the presence of rapamycin 500nM. The next analysis was that different concentrations of rapamycin were stimulated to HMC-1.1 and HMC-1.2 cells to assess whether rapamycin induces apoptosis or necrosis in HMC-1 cells. The resulting percentage of apoptosis and necrosis indicated that rapamycin 500nM, a relatively high concentration, had not damaged the cells on fluorescence-activated cell-sorting (FACS) and that the HMC-1 cells had reached a steady state in the presence of the drug (Fig. 1c, d). Rapamycin has also reportedly induced

G1 cell-cycle arrest and apoptosis in HMC-1 cells [15, 19]. Our study revealed that, as in some of the previous reports [26, 27], the cell cycle of the HMC-1 cells had been arrested but that apoptosis had not occurred.

After treatment of HMC-1.1 and HMC-1.2 cells with rapamycin, inhibition of mTOR signaling was assessed by Western blotting. However, assessing mTOR signaling solely by mTOR phosphorylation is challenging, it was necessary to assess downstream targets, including p70S6K and 4EBP-1. Rapamycin does not primarily inhibit phosphorylation of mTOR itself; instead, the FKBP12–rapamycin complex binds to the FRB domain of mTORC1 and suppresses its kinase activity by restricting substrate access. Consequently, phosphorylation of downstream targets such as S6K and 4E-BP1 is reduced even when p-mTOR levels remain unchanged. As a result, rapamycin was found to be more effective in HMC-1.2 cells than in HMC-1.1 cells, in line with the findings of a previous report [15].

In vitro research has demonstrated that mast cells harboring a mutation in the D816V c-kit domain tended to respond to rapamycin, suggesting that topical rapamycin may be effective against CM with the c-kit D816V mutation (Fig. 2a-d). To assess this hypothesis, at first, we planned to examine the effect of topical rapamycin on a murine CM model as in a previous study [28], in which membrane-bound / soluble stem cell factor (SCF) was found to be overexpressed on murine keratinocytes. However, this model was unable to be used in the present study because most of the mast cell had proliferated only in juvenile mice. Moreover, the number of mast cells tended to decrease as the transgenic mice aged.

Therefore, the present study employed subcutaneous injections of HMC-1.2 cells into SCID mice to induce tumor formation. After these cells were injected into the dorsal skin, rapamycin gel was applied every day for 35 days. As a result, no tumors developed in the rapamycin group unlike in the control group, indicating the efficacy of the rapamycin gel application. This effect was confirmed by immunohistochemistry and Western blotting. The rapamycin treatment group demonstrated a decrease in the quantity of the Ki-67 (Fig. 4a), and Western blotting confirmed mTOR, 4EBP-1, and p70S6K phosphorylation (Fig. 4b-d). However, the *in vitro* data showed that phosphorylation of p70S6K and 4EBP-1 was inhibited, the reasons for the discrepancy between these data and the *in vitro* data remains unclear. One possible reason is that the tissue had been harvested only three days after injecting the HMC-1.2 cells into the mice, raising the possibility that the short treatment duration was insufficient to produce measurable changes in the phosphorylation of mTOR and p70S6K in HMC-1.2 cells. Previous study demonstrated that rapamycin rapidly suppresses phosphorylation of 4E-BP1, with detectable effects observed within six hours after its exposure [15].

Finally, the expression and phosphorylation of mTOR and Ki-67 were examined in skin samples from patients with CM with and without the D816V mutation. Our data demonstrated mTOR positivity in mast cells infiltrating the dermis regardless of their KIT D816V mutation status. The proportion of Ki-67-positivity was higher in the cells harboring the KIT D816V mutation. In contrast to neoplastic mast cells, normal mast cells are typically negative for Ki-67 immunostaining [19]. The data in the present study suggested that mast cells harboring the KIT D816V mutation had higher proliferative activity than those

which did not harbor the mutation. The mast cells harboring the mutation were thus considered likely to respond to rapamycin. While the HMC-1.1 cell group differed significantly from the control group (unlike the HMC-1.2 cells), rapamycin did not completely suppress cell proliferation in the HMC-1.1 cells. The cells with no KITD816V mutation responded more weakly to rapamycin gel than those harboring the KITD816V mutation, but this finding nonetheless suggested that the drug may be effective even in patients without the KIT D816V mutation.

In conclusion, the topical application of rapamycin, an mTOR inhibitor, suppressed mTOR signaling and lesion size in a subcutaneous HMC-1 xenograft model of CM possessing D816V-mutated c-Kit mast cells. The findings of this study may support the repurposing of topical rapamycin as a potential treatment option for CM.

Abbreviations

4E-BP1
4E-binding protein 1
BrdU
Bromodeoxyuridine
CM
cutaneous mastocytosis
HMC
human mast cell
ICG
indocyanine green
mTOR
mammalian target of rapamycin
MDS
myelodysplastic syndromes
SCID
severe combined immunodeficiency
Ser
serine
SCF
stem cell factor
SM
systemic mastocytosis
Thr
threonine
TSC
tuberous sclerosis complex
UP

urticaria pigmentosa

WHO

World Health Organization.

Declarations

Conflicts of interest

The authors have no conflicts of interest to declare.

Competing Interests Statement

The authors did not receive support from any organization for the submitted work.

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Conflict of interest

The authors declare no competing interests.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. This study had the approval of the Animal Care and Use Committee, Faculty of Medicine, Tokyo Medical University on 24th March 2023, with approval number R5-090.

Consent for publication

All authors have read the final version of the manuscript and declared their consent for publication.

Consent to participate

Written informed consent was obtained from the parents.

Consent to publish

The participant has consented to the submission of the case report to the journal.

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Author Contribution

KH and TI contributed to the study conception and design. Material preparation, data collection and analysis were performed by CE and TM. The first draft of the manuscript was written by AI and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

All datasets will be provided upon the reasonable request.

References

1. Sanger A (1878) An anomalous mottled rash, accompanied by pruritus, factious urticaria and pigmentation, urticaria pigmentosa. *Clin Soc Lond* 11:161–163
2. Hartmann K, Escribano L, Grattan C et al (2016) Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. *J Allergy Clin Immunol* 137:35–45.
<https://doi.org/10.1016/j.jaci.2015.08.034>
3. Jennings S, Russell N, Jennings B et al (2014) The Mastocytosis Society survey on mast cell disorders: patient experiences and perceptions. *J Allergy Clin Immunol Pract* 2:70–76.
<https://doi.org/10.1016/j.jaip.2013.09.004>
4. Meni C, Bruneau J, Georgin-Lavialle S et al (2015) Paediatric mastocytosis: a systematic review of 1747 cases. *Br J Dermatol* 172:642–651. <https://doi.org/10.1111/bjd.13567>

5. Brockow K, Jofer C, Behrendt H, Ring J (2008) Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *J Allergy Clin Immunol* 121:1517–1521. <https://doi.org/10.1111/j.1398-9995.2007.01569.x>
6. Alvarez-Twose I, Zanotti R, González-de-Olano D et al (2014) Nonaggressive systemic mastocytosis (SM) without skin lesions associated with insect-induced anaphylaxis shows unique features versus other indolent SM. *J Allergy Clin Immunol* 133:520–528. <https://doi.org/10.1016/j.jaci.2013.06.020>
7. Valent P, Akin C, Hartmann K et al (2021) Updated diagnostic criteria and classification of mast cell disorders: a consensus proposal. *HemaSphere* 5:e646. <https://doi.org/10.1097/hs9.0000000000000646>
8. Castells M, Butterfield J (2019) Mast cell activation syndrome and mastocytosis: initial treatment options and long-term management. *J Allergy Clin Immunol Pract* 7:1097–1106. <https://doi.org/10.1016/j.jaip.2019.02.002>
9. Akin C, Arock M, Carter MC, George TI, Valent P (2025) Mastocytosis. *Nat Rev Dis Primers* 11:30. <https://doi.org/10.1038/s41572-025-00611-8>
10. Heide R, Zuidema E, Beishuizen A et al (2009) Clinical aspects of diffuse cutaneous mastocytosis in children: two variants. *Dermatology* 219:309–315. <https://doi.org/10.1159/000243808>
11. Hartmann K, Metcalfe DD (2000) Pediatric mastocytosis. *Hematol Oncol Clin North Am* 14:625–640. [https://doi.org/10.1016/s0889-8588\(05\)70299-9](https://doi.org/10.1016/s0889-8588(05)70299-9)
12. Yang F, Tanaka M, Wataya-Kaneda M et al (2014) Topical application of rapamycin ointment ameliorates *Dermatophagoides farinae* body extract-induced atopic dermatitis in NC/Nga mice. *Exp Dermatol* 23:568–572. <https://doi.org/10.1111/exd.12463>
13. Kim MS, Kuehn HS, Metcalfe DD, Gilfillan AM (2008) Activation and function of the mTORC1 pathway in mast cells. *J Immunol* 180:4586–4595. <https://doi.org/10.4049/jimmunol.180.7.4586>
14. Shin J, Pan H, Zhong XP (2012) Regulation of mast cell survival and function by tuberous sclerosis complex 1. *Blood* 119:3306–3314. <https://doi.org/10.1182/blood-2011-05-353342>
15. Gabillot-Carré M, Lepelletier Y, Humbert M, de Sepuvelde P, Hamouda NB, Zappulla JP et al (2006) Rapamycin inhibits growth and survival of D816V-mutated c-kit mast cells. *Blood* 108:1065–1072. <https://doi.org/10.1182/blood-2005-06-2433>
16. Chan IJ, Kasproicz S, Tharp MD (2013) Distinct signalling pathways for mutated KIT(V560G) and KIT(D816V) in mastocytosis. *Clin Exp Dermatol* 38:538–544. <https://doi.org/10.1111/ced.12000>
17. Smrz D, Kim MS, Zhang S, Mock BA, Smrzová S, DuBois W et al (2011) mTORC1 and mTORC2 differentially regulate homeostasis of neoplastic and non-neoplastic human mast cells. *Blood* 118:6803–6813. <https://doi.org/10.1182/blood-2011-06-359984>
18. Hartmann K, Hermes B, Rappersberger K, Sepp N, Mekori YA, Henz BM (2003) Evidence for altered mast cell proliferation and apoptosis in cutaneous mastocytosis. *Br J Dermatol* 149:554–559. <https://doi.org/10.1046/j.1365-2133.2003.05598.x>
19. Bergström A, Häggglund H, Berglund A, Nilsson G, Lambe M (2024) Epidemiology of mastocytosis: a population-based study (Sweden). *Acta Oncol* 63:44–50. <https://doi.org/10.2340/1651->

20. Cohen SS, Skovbo S, Vestergaard H et al (2014) Epidemiology of systemic mastocytosis in Denmark. *Br J Haematol* 166:521–528. <https://doi.org/10.1111/bjh.12916>
21. Carter MC, Metcalfe DD, Komarow HD (2014) Mastocytosis. *Immunol Allergy Clin North Am* 34:181–196. <https://doi.org/10.1016/j.iac.2013.09.001>
22. Akin C, Broesby-Olsen S, Valent P (2020) Mastocytosis: Overview of Diagnosis and Classification. In: Akin C (ed) *Mastocytosis*. Springer, Cham, pp 23–24. https://doi.org/10.1007/978-3-030-27820-5_2
23. Søndergaard J, Asboe-Hansen G (1987) Mastocytosis in Childhood. In: Happle R, Grosshans E (eds) *Pediatric Dermatology*. Springer, Berlin, Heidelberg, pp 148–154. https://doi.org/10.1007/978-3-642-71524-2_20
24. Carter MC, Lange M, Alvarez-Twose I et al (2026) Management of Mastocytosis and Mast Cell Activation in Children. *J Allergy Clin Immunol Pract* 14:30–42. <https://doi.org/10.1016/j.jaip.2025.11.016>
25. Wataya-Kaneda M, Tanaka M, Nakamura A, Matsumoto S, Katayama I (2011) A topical combination of rapamycin and tacrolimus for the treatment of angiofibroma due to tuberous sclerosis complex (TSC): a pilot study of nine Japanese patients with TSC of different disease severity. *Br J Dermatol* 165:912–916. <https://doi.org/10.1111/j.1365-2133.2011.10471.x>
26. Metcalfe SM, Canman CE, Milner J, Morris RE, Goldman S, Kastan MB (1997) Rapamycin and p53 act on different pathways to induce G1 arrest in mammalian cells. *Oncogene* 15:1635–1642. <https://doi.org/10.1038/sj.onc.1201341>
27. Chatterjee A, Mukhopadhyay S, Tung K, Patel D, Foster DA (2015) Rapamycin-induced G1 cell cycle arrest employs both TGF- β and Rb pathways. *Cancer Lett* 360:134–140. <https://doi.org/10.1016/j.canlet.2015.01.043>
28. Kunisada T, Lu SZ, Yoshida H et al (1998) Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. *J Exp Med* 187:1565–1573. <https://doi.org/10.1084/jem.187.10.1565>

Figures

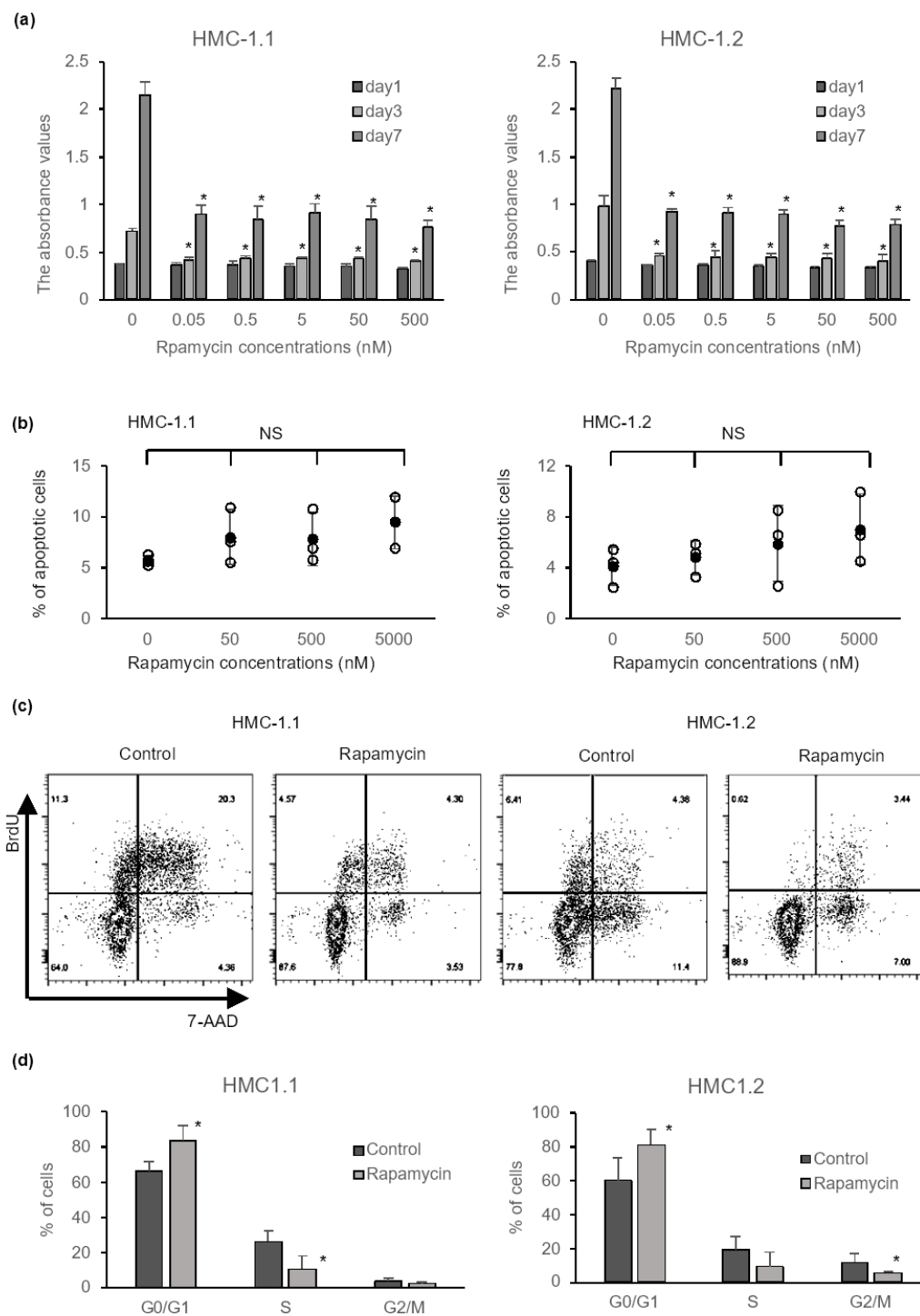


Figure 1

Rapamycin inhibited HMC-1.1 (V560G) and HMC-1.2 (V560G and D816V) proliferation.

(a) Inhibitory effect of various concentrations of rapamycin on HMC-1 cell proliferation. Rapamycin activity in the proliferation of HMC-1.1 was assessed and HMC-1.2 was assessed. The cells were

cultured with various concentrations of rapamycin (0.05 – 500 nM) or without rapamycin for 1 - 7 days. Rapamycin inhibited HMC-1 cell proliferation even at low concentrations. * $p < .001$.

(b) Apoptotic cells were analyzed by flow cytometry using annexin V. Rapamycin’s effect on the cell cycle of HMC-1 cells. The cells were incubated in the presence of rapamycin 500 nM for 24 hours.

(c) The cell cycle was analyzed by flow cytometry with FITC-conjugated BrdU and 7-AAD. BrdU was incorporated into DNA during the S phase, allowing quantitative assessment of cell cycle progression and cell proliferation. The percentages indicate the number of cells at each cell cycle stage (G0-G1, S, and G2-M). The lower left quadrant of the histogram (BrdU⁻/7-AAD⁻), upper quadrant (BrdU⁺), and lower right quadrant (BrdU⁻/7-AAD⁺) represents the cells in the G0–G, S, and G2–M phase, respectively. A representative experiment (of three) is shown.

(d) The proportion of cells in the G0–G1, S, and G2–M phases was compared between the groups with and without rapamycin. All the experiments were performed at least three times and yielded similar results.

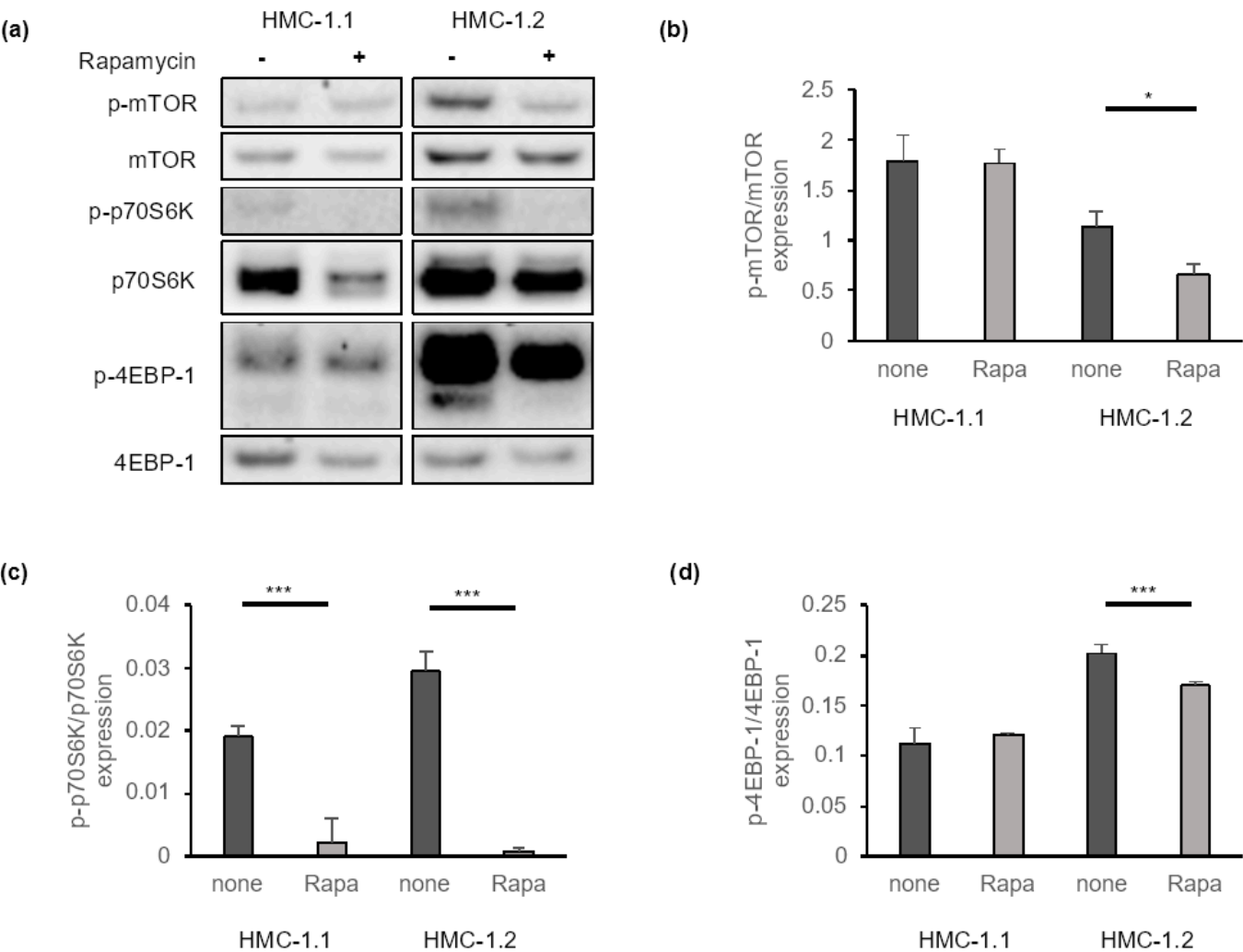


Figure 2

Effect of rapamycin on the phosphorylation of p70S6kinase and 4E-BP1 in the HMC-1 cells.

(a) Western blot analysis of the HMC-1.1 and HMC-1.2 cells with or without exposure to rapamycin 500 nM for 24 hours is shown. P indicates phosphorylation.

The data on phosphorylated mTOR expression were normalized to mTOR expression. $*p < .05$, $**p < .01$, $***p < .001$.

(b) The data on phosphorylated p70S6K expression were normalized to p70S6K expression. $*p < .05$, $**p < .01$, $***p < .001$.

(c) The data on phosphorylated 4EBP-1 expression were normalized to 4EBP-1 expression. $*p < .05$, $**p < .01$, $***p < .001$.

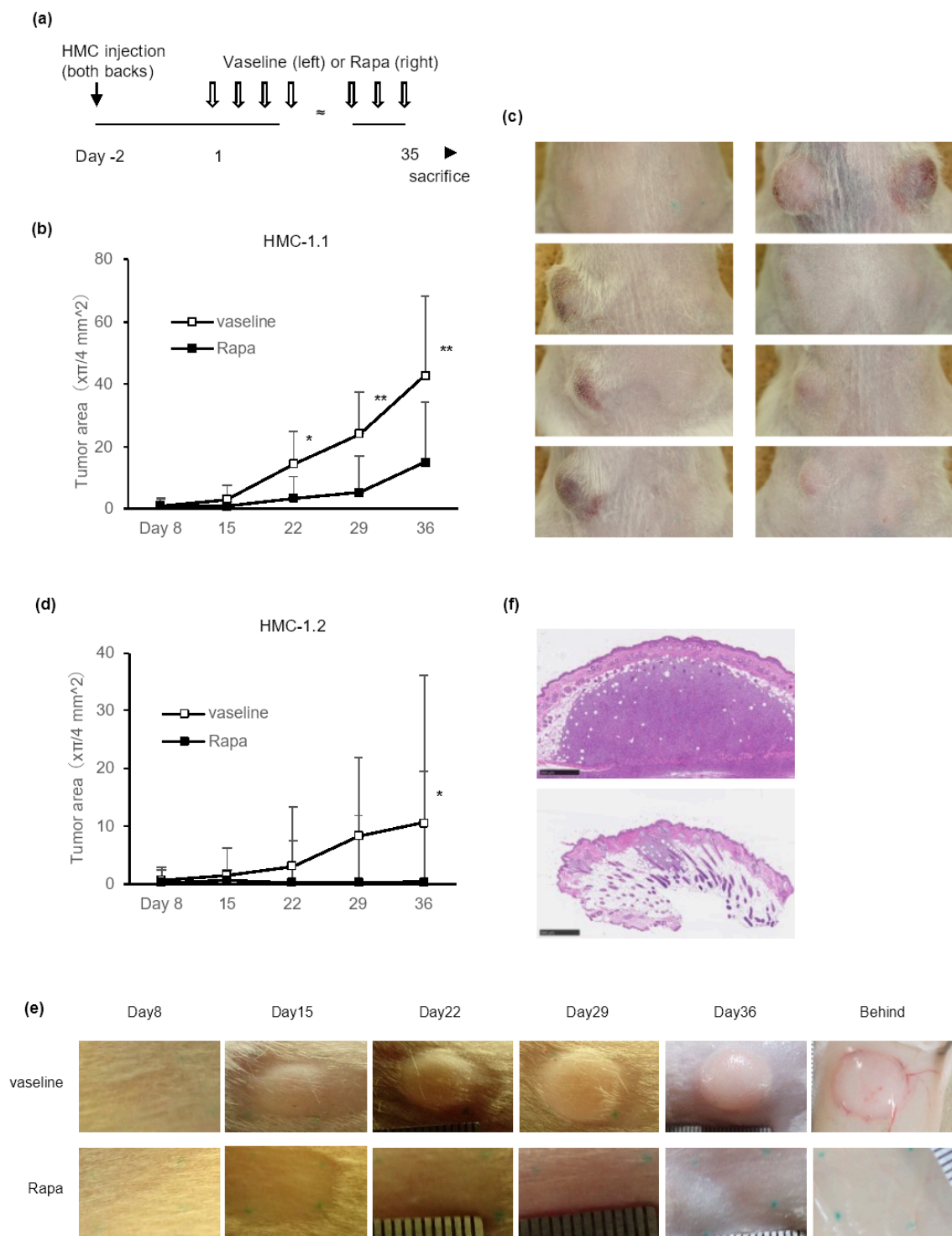


Figure 3

Topical rapamycin gel suppressed the growth of HMC-1 cells subcutaneously implanted in SCID mice.

(a) Schema of the animal model. The HMC-1 cells were dissolved in 40 μ l of a culture medium and subcutaneously injected into the bilateral dorsum of SCID mice (day -2). After 3 days, rapamycin gel was

applied to the right dorsum, and vaseline was applied to the left dorsum daily (day 1). The topical treatments were continued until the mice were sacrificed on day 35.

(b) Growth curve of HMC-1.1 tumor area showed in the Rapa group and the control group. Topical rapalimus gel suppresses the growth of subcutaneously implanted HMC-1.1 cells in SCID mice (n=8). * $p < .05$, ** $p < .01$, *** $p < .001$.

(c) Macroscopic images of the back of a set of eight mice on day 35. Rapamycin gel was continuously applied to the right back, while vaseline was applied to the left back. Tumors on the left side were larger than those on the right side in most mice, with the exception of one mouse (upper right), in which tumor sizes were comparable between both sides.

(d) Growth curve of HMC-1.2 tumor area showed in the Rapa group and the control group. Topical rapalimus gel suppresses the growth of subcutaneously implanted HMC-1.2 cells in SCID mice (n=8). * $p < .05$, ** $p < .01$.

(e) Representative macroscopic images showing the growth of subcutaneous HMC-1.2 tumors in SCID mouse treated with vaseline or rapamycin gel. The rightmost images show the inverted skin after incision.

(f) Histopathological findings of dorsal HMC-1.2 tumors on day 35. The upper panel shows the control group, and the lower panel shows the rapamycin group. (hematoxylin and eosin staining; scale bar, 500 μm). The grossly visible subcutaneous tumor was bisected through its center to obtain the histological section. As no grossly visible tumor was identified, the tissue was sectioned through the center of the marked injection site.

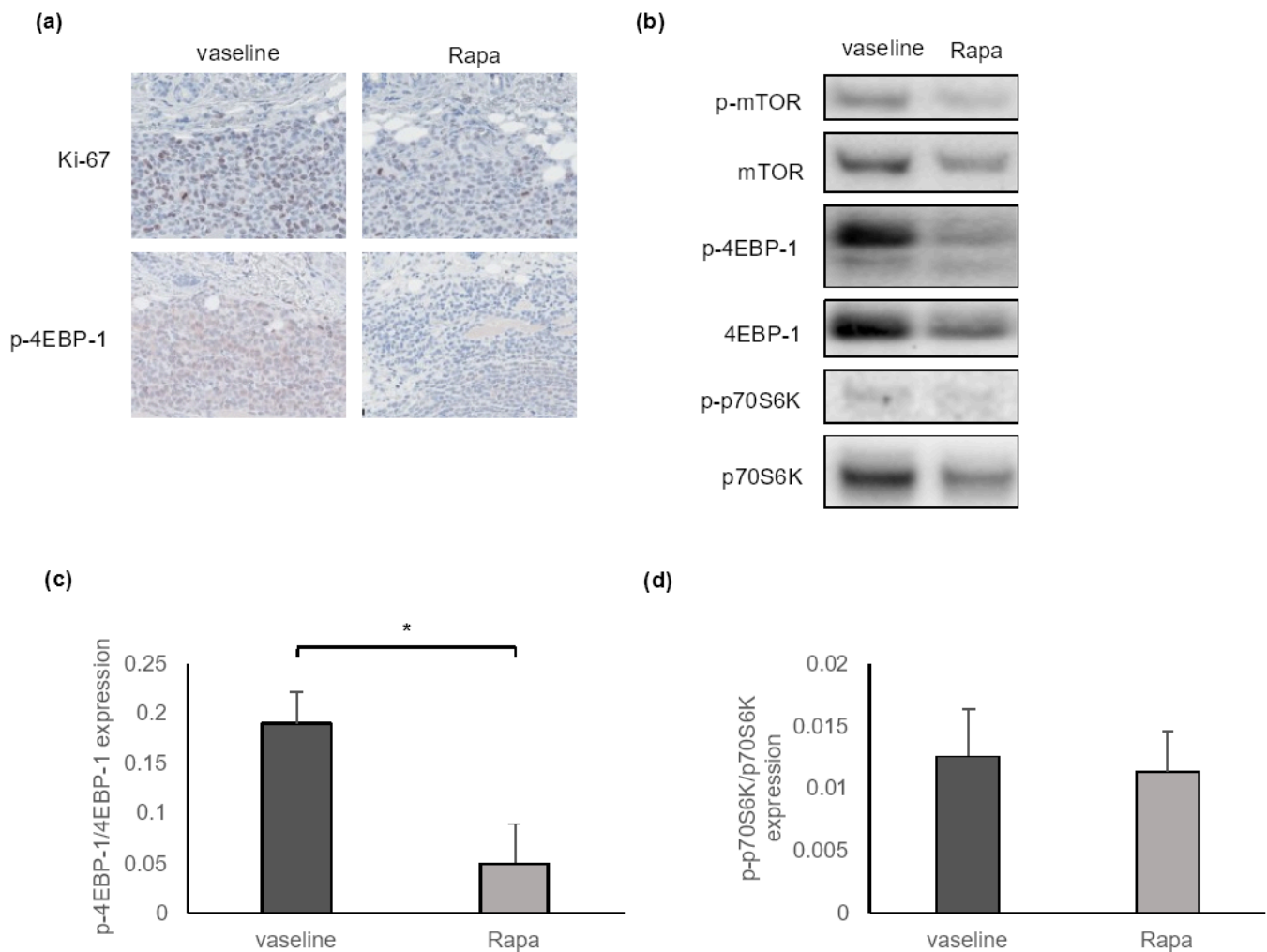


Figure 4

Effect of rapamycin gel on the mTOR pathway.

(a) Immunohistochemical staining of the HMC-1.2 tumors in the control group and rapamycin group on day 3. The panels show Ki-67 staining (the upper panels) and 4EBP-1 phosphorylation staining (the lower panels).

(b) Western blot analysis of the HMC-1.2 tumors after three days of treatment. P indicates phosphorylation.

(c) The data on phosphorylated 4EBP-1 expression were normalized to 4EBP-1 expression (n=4). * $p < .05$, ** $p < .01$.

(d) The data on phosphorylated p70S6K expression were normalized to p70S6K expression (n=4).

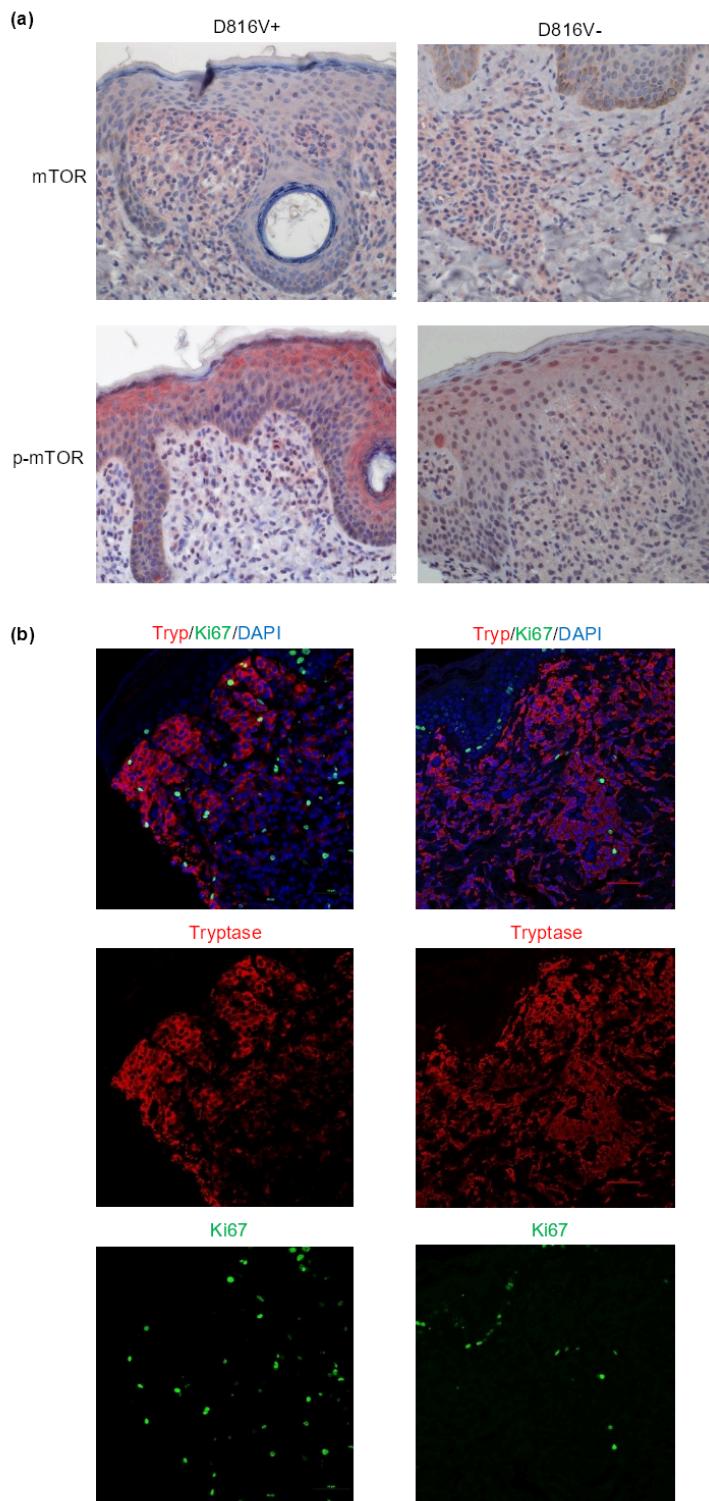


Figure 5

Characterization of mTOR pathway activation in human tissue specimens by immunostaining.

(a) The left panels show skin specimens from a patient with KIT D816V mutation–positive human CM, and the right panels show specimens from KIT D816V–negative human CM. The upper panels show mTOR staining, and the lower panels show phosphorylated mTOR (p-mTOR) staining.

(b) Co-immunostaining for tryptase (Tryp) and Ki-67 demonstrated low Ki-67 expression in D816V mutation-positive and negative CM.