Clinical and Histologic Analysis of the Efficacy of Topical Rapamycin Therapy Against Hypomelanotic Macules in Tuberous Sclerosis Complex

Mari Wataya-Kaneda, MD, PhD; Mari Tanaka, MD, PhD; Lingli Yang, MD, PhD; Fei Yang, BS; Daisuke Tsuruta, MD, PhD; Ayumi Nakamura, BPharm; Shoji Matsumoto, PhD; Toshimitsu Hamasaki, PhD; Atushi Tanemura, MD, PhD; Ichiro Katayama, MD, PhD

IMPORTANCE Tuberous sclerosis complex (TSC) is an autosomal dominant disorder leading to the aberrant activation of the mammalian target of rapamycin complex 1. Although the efficacy of mammalian target of rapamycin complex 1 inhibitors against tumors in patients with TSC, including facial angiofibroma, has been well investigated, their efficacy against hypomelanotic macules in patients with TSC is unknown.

OBJECTIVES To evaluate objectively the efficacy of topical rapamycin treatment of hypomelanotic macules in patients with TSC and to elucidate the mechanisms of how rapamycin improves the macules.

DESIGN, SETTING, AND PARTICIPANTS We performed a prospective, baseline-controlled trial of 6 patients with TSC and hypomelanotic macules in non–sun-exposed and sun-exposed skin at the Department of Dermatology, Osaka University, from August 4, 2011, through September 27, 2012. Rapamycin gel, 0.2%, was applied to the lesions twice a day for 12 weeks. Histologic examinations and blood tests were conducted at the start and completion of treatment. Blood rapamycin levels were analyzed at completion.

EXPOSURES Topical rapamycin treatment for hypomelanotic macules.

MAIN OUTCOMES AND MEASURES Objective evaluation of rapamycin treatment of hypomelanotic macules in TSC with δ-L (L indicates the brightness of the color) levels on spectrophotometry at the start and completion (12 weeks) of treatment and at 4 and 12 weeks after discontinuation of treatment (16 and 24 weeks, respectively).

RESULTS Improvement of hypomelanotic macules (in δ-L values) was significant at 12 weeks (mean [SD], 2.501 [1.694]; P < .05), 16 weeks (1.956 [1.567]; P < .01), and 24 weeks (1.836 [1.638]; P < .001). Although efficacy tended to be prominent in sun-exposed skin, we did not observe significant differences (in δ-L values) between sun-exposed and non–sun-exposed skin at 12 weeks (mean [SD], 1.859 [0.629] and 3.142 [2.221], respectively), 16 weeks (1.372 [0.660] and 2.539 [2.037], respectively), and 24 weeks (1.201 [0.821] and 2.471 [2.064], respectively). No adverse events were observed, and rapamycin was not detected in the blood of any patient. Electron microscopic analysis of hypomelanotic macules revealed that topical rapamycin treatment significantly improved the uniformity of the melanosome numbers in the TSC melanocytes (pretreatment macules: mean [SD], 25.71 [21.90] [range, 5-63]; posttreatment macules: 42.43 [3.60] [range, 38-49]; P < .001). Moreover, rapamycin treatment induced the recovery of melanosomes in TSC–knocked-down melanocytes from depleted amounts (mean [SD], 16.43 [11.84]) to normal levels (42.83 [14.39]; P < .001).

CONCLUSIONS AND RELEVANCE Topical rapamycin treatment was effective and safe against hypomelanotic macules arising from TSC. This efficacy of rapamycin was corroborated as stemming from the improvement of impaired melanogenesis in TSC melanocytes.

Published online February 18, 2015.

Copyright 2015 American Medical Association. All rights reserved.
Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that causes multiple hamartomas, epilepsy, autism, and hypopigmented macules. Tuberous sclerosis complex is caused by mutations in the TSC1 gene (OMIM 605284) or the TSC2 gene (OMIM 191092); the genes encode hamartin and tuberin, respectively. The hamartin-tuberin complex downregulates the mammalian target of rapamycin complex 1 (mTORC1). The constitutive activation of mTORC1 that results from the abnormality of the TSC1 or TSC2 gene is associated with abnormal cellular proliferation, which causes TSC-related hamartomas. Recent reports suggest that mTORC1 inhibitors, such as rapamycin, may be effective for the treatment of TSC-related tumorigenesis, including facial angiofibromas. The efficacy of topical rapamycin treatment for facial angiofibroma in patients with TSC also has been reported. With regard to hypomelanotic macules, which are resistant to effective treatment, the mechanism by which mTORC1 affects melanogenesis is also still unknown, although hypomelanotic macules are one of the major diagnostic criteria for TSC. Hypomelanotic macules in TSC are very important because they arise at birth or in early infancy, resulting in the early diagnosis of TSC. In addition, hypomelanotic macules appearing on the face are cosmetically displeasing and require treatment. Ohguchi et al and Hah et al reported that rapamycin upregulated microphthalmia-associated transcription factor, a master regulator of melanogenesis, in B16 and MNT-1 melanoma cells. Jóźwiak and Galus reported the inhibition of microphthalmia-associated transcription factor by mTOR in TSC. Ho et al reported that TORC1 is related to melanogenesis through the activation of the melanogenic enzyme and the formation of mature melanosomes. In addition, Murase et al reported that autophagy, which is regulated by mTORC1, is involved in the degradation of melanosomes in keratinocytes. Recently, Wataya-Kaneda et al reported 2 cases of hypomelanotic macules in patients with TSC who recovered after the topical administration of rapamycin. The mTORC1 inhibitors, such as rapamycin, might be effective not only for tumors but also for hypomelanotic macules in patients with TSC.

The aims of this study were to evaluate objectively and precisely the efficacy of topical rapamycin treatment and to investigate the histologic and cytologic effects of topical rapamycin on TSC-related hypomelanotic macules. To assess the findings objectively, we evaluated the efficacy of rapamycin with the use of a spectrophotometer (CM-700d; Konica Minolta). In addition, the specimens of hypomelanotic macules before and after rapamycin treatment were examined by electron microscopy. To corroborate the effect of rapamycin on the melanogenesis that was observed in the rapamycin-treated hypomelanotic macules in TSC, the effect of rapamycin on TSC2-knockdown melanocytes (TSC-model melanocytes) was examined. We also compared the efficacy of topical rapamycin treatment on hypomelanotic macules in sun-exposed and non–sun-exposed skin in patients with TSC to investigate the effect of sun exposure.

### Methods

#### Ethical Consideration

This study was approved by the ethics committee of the Osaka University Faculty of Medicine (approval 10339) and was disclosed to the University Hospital Medical Information Network (report number UMIN000006108). All the patients volunteered for this trial and signed written informed consent agreements. On behalf of the children, written informed consent was obtained from each legal representative.

#### Study Design

This study was a prospective, baseline-controlled trial. Six patients with definitive TSC and hypomelanotic macules in non–sun-exposed and sun-exposed skin who wanted to participate in this trial were enrolled among 250 outpatients at the Department of Dermatology, Osaka University.

Rapamycin gel, 0.2%, was applied to hypomelanotic macules on non–sun-exposed and sun-exposed (on the face in 5 patients and above the knee in 1 patient) skin on each patient twice a day for 12 weeks. Assessment of each patient was performed once a month during the 12 weeks of rapamycin treatment and at 4 and 12 weeks after discontinuation of the treatment (16 and 24 weeks, respectively). Serum rapamycin levels were analyzed at the end of treatment using liquid chromatography–electrospray ionization mass spectrometry (detection limit, 0.6 ng/mL).

Powder prepared from 2-mg rapamycin tablets (sirolimus [Rapamune]) was mixed with a 100-mg gel to a concentration of 0.2% rapamycin. We enrolled 6 patients aged 3 to 33 (mean age, 11.7) years who were diagnosed as having definitive TSC according to the diagnostic criteria update. Four of the 6 patients were male; 2 were female. All non–sun-exposed skin areas were on the trunk except for 1 thigh, and all the sun-exposed skin areas were on the face except for 1 knee (Table).

To determine the treatment efficacy objectively, assessment was performed using a spectrophotometer to measure the δ-L value of each hypomelanotic macule before and after treatment. Decreased δ-L values reflected improved hypomelanotic macules.

#### Evaluation of Rapamycin Efficacy

**Immunohistochemical Examinations**

Skin specimens of hypomelanotic macules, obtained before treatment from 6 participants and after treatment from 1 participant, were fixed in buffered 10% formalin and embedded in paraffin. The paraffin-embedded tissue slides were stained with hematoxylin-eosin and Fontana-Masson. After the antigen was retrieved by boiling each specimen in an oil bath for 15 minutes in a 10 mM TRIS/1 mM EDTA buffer (pH 9.0), the slides were also incubated with a goat anti–melan A monoclonal antibody (1:50) (Dako) at 4°C overnight and with a biotinylated-link universal antibody, conjugated streptavidin–alkaline phosphatase, and a fuchsin substrate-chromogen buffer. These slides were observed under a microscope.
Electron Microscopic Examination of the Skin Tissue

Punched skin samples (1 mm each) from the hypomelanotic macules in patients with TSC before and after treatment were fixed in 2.5% glutaraldehyde in a 0.1M phosphate buffer (pH, 7.4) at 4°C. Postfixation took place in 1% osmium tetroxide in a 0.1M phosphate buffer (pH, 7.4) for 1 hour, after which the samples were dehydrated in an ethanol dilution series and embedded in epoxy resin (Quetol-812; Nisshin EM). Semithin sections (0.5 μm) were stained with toluidine blue O, and ultrathin sections (0.1 μm) were stained with saturated uranyl acetate and lead citrate. Sections on copper mesh were examined using a commercially available electron microscope (JEM-1200EX; Jeol).

Melanin Counts in Melanocytes

Seven melanocytes were randomly selected in each sample for counting. The number of melanosomes in each melanocyte was counted directly at a magnification ×8000 and then summarized in a graph.

RNA Interference

For the short interfering RNA (siRNA) experiments, double-stranded RNA duplexes composed of 21-nucleotide sense and antisense oligonucleotides (Cosmo Bio Ltd) were synthesized. The RNA oligonucleotides used for targeting human TSC2 in this study were 5′-CGAACGAGGUGGUGUCCUATT-3′ for TSC2 sense and 5′-UAGGACACCACCUCGUUCGTT-3′ for TSC-2 antisense.

Transfection of Short Interfering SC2 and Rapamycin Stimulation With siRNA-Transfected Melanocytes

Human neonatal epidermal melanocytes from a moderately pigmented donor (HEMn-MP) were purchased from Invitrogen and cultured in medium 254 (M-254-500; Gibco) with the addition of human melanocyte growth supplement (Invitrogen) at 37°C in an atmosphere of 5% carbon dioxide. The melanocytes were used in passages 6 through 8. We seeded HEMn-MP cells onto 6-well plates at a density of 5 × 10⁴ cells/well 12 hours before transfection. Transfection reagent (Lipofectamine 2000; Invitrogen) was used with 30nM siRNA according to the manufacturer’s instructions. The efficacy of the transfection was verified with reverse transcription-polymerase chain reaction at 48 hours after siRNA transfection; HEMn-MP cells were stimulated with 30nM rapamycin (Calbiochem) for 72 hours.

Electron Microscopic Examination of Cultured Cells

After being washed 3 times with phosphate-buffered solution, cultured cells were fixed with 0.5% glutaraldehyde in a 0.1M phosphate buffer (pH, 7.4) at 4°C for 15 minutes at room temperature. After being washed 3 times with phosphate-buffered solution, cells were fixed with 1% osmium tetroxide in phosphate-buffered solution for 3 hours at 4°C. After they were rinsed with water to remove the osmium tetroxide, the cells were dehydrated in a sequential ethanol dilution series. After infiltration in a 1:1 mixture of epoxy resin and ethanol, the cells were embedded in epoxy resin, drained for 60 minutes by inverting the culture dishes on a paper towel, and polymerized overnight at 60°C in a vacuum oven. Ultrathin sections prepared in the same manner as described for the tissue sample were examined with an electron microscope, and the number of melanosomes in each randomly selected melanocyte was counted directly.

Statistical Analysis

We used a nonparametric approach because the data were not normally distributed. To compare the homogeneity and the mean number of melanosomes before and after rapamycin treatments, we used a Levene test and Wilcoxon rank sum test, respectively. To compare the improvements in the hypomelanotic macules before and after the topical rapamycin treatment, we used simulation-based multiple comparisons based on generalized estimating equations. P < .05 was considered significant.

Results

Clinical Improvement of Hypomelanotic Macules

The patient information, treated lesions, and histologic characteristics are summarized in the Table. We have included representative photographs of treatment in sun-exposed and non-sun-exposed skin before and after treatment. The photographs demonstrate that topical rapamycin treatment improved the hypomelanotic macules and that the sun-
exposed skin improved more than the non–sun-exposed areas (Figure 1). In this trial, blood tests were performed on all participants before and after treatment to examine the systemic influence. Blood rapamycin concentration was also analyzed. No adverse effects were observed in any participant, and the rapamycin concentration in the blood was lower than the detection limit (0.6 ng/mL).

Improvement of δ-L Spectrophotometry Values
Five of 6 patients demonstrated lower δ-L values at the end of treatment than at initiation, with no significant difference in non–sun-exposed skin at the end of treatment (mean [SD] at 12 weeks, 3.142 [2.221]). However, at 4 weeks after discontinuation of treatment (16 weeks), δ-L values for the non–sun-exposed skin decreased significantly (mean [SD], 2.539 [2.037]; P = .046). The significant decline in δ-L values continued for 12 weeks after discontinuation of treatment (mean [SD] at 24 weeks, 2.471 [2.064]; P < .01) (Figure 2A). Unlike the non–sun-exposed skin, the δ-L values for the sun-exposed skin indicated significant differences at the completion of treatment (mean [SD], 1.859 [0.629]; P = .046) and 4 and 12 weeks after discontinuation of treatment (mean [SD], 1.372 [0.660] and 1.201 [0.821], respectively; P < .01 for both) (Figure 2B).

To assess the effect of sun exposure on rapamycin treatment, we compared the mean δ-L values for the 6 patients between the non–sun-exposed and sun-exposed skin. Although the mean δ-L values in the sun-exposed skin were lower than those for the non–sun-exposed skin at all points, including at treatment initiation (mean [SD] at initiation, 3.049 [1.912] vs 3.589 [2.580]; at 12 weeks, 1.859 [0.629] vs 3.142 [2.221]; at 16 weeks, 1.372 [0.661] vs 2.539 [2.037]; and at 24 weeks, 1.201 [0.822] vs 2.471 [2.064]), and although both graphs were approximately parallel, we found no significant difference between the δ-L values for the non–sun-exposed and sun-exposed skin (Figure 2C). This result indicated that sun exposure did not affect the efficacy of the topical rapamycin therapy.

Histochemical Examinations of Hypomelanotic Macules
To corroborate the efficacy of rapamycin treatment, we undertook histologic investigations of the TSC-related hypomelanotic macules before and after treatment. Specimens of hypomelanotic macules before treatment were obtained from all 6 participants voluntarily, but an after-treatment specimen was obtained from only patient 6 because the remaining 5 patients refused biopsy of their healed lesions. Six pretreatment skin specimens, 1 posttreatment specimen, and 1 control specimen were stained with Fontana-Masson and anti-melan A antibody. The pretreatment and posttreatment specimens of hypomelanotic macules in patients with
TSC demonstrated melan A–positive cells, as did the control skin specimen (eFigure 1A, C, and E in the Supplement). However, in the pretreatment TSC specimens, we observed dispersed faint Fontana-Masson staining. By contrast, as with the control samples, higher and darker Fontana-Masson–stained granules were detected in the posttreatment TSC specimens than in pretreatment specimens (eFigure 1B, D, and F in the Supplement). These results indicated that melanocytes were present but melanin granule levels were decreased in the TSC-related hypomelanotic macules, and topical rapamycin therapy increased the volume of melanin granules observed by microscope in the basal layers.

Electron Microscopic Examinations of Hypomelanotic Macules
Electron microscopic examinations suggested that in TSC hypomelanotic macules, abnormal melanogenesis might occur. To examine the precise abnormality of the melanogenesis, we assessed the numbers and morphologic features of the melanocytic melanosomes in the TSC hypomelanotic macules using an electron microscope. We examined 5 pretreatment samples and 1 posttreatment sample. Before rapamycin treatment, all the melanosomes observed in the samples were well matured at stage III or stage IV. The number of stages III and IV melanosomes varied between melanocytes. Even within the same hypomelanotic lesion from the same patient, some areas had extreme contrasts of few and many melanosomes (Figure 3A and B). By contrast, in posttreatment TSC hypomelanotic macules, the numbers of melanosomes in the melanocytes became uniform and were equal to those in the control melanocytes (Figure 3C).

To confirm these findings, 7 melanocytes were selected at random from each sample, and we counted the numbers of pretreatment and posttreatment melanosomes in each melanocyte from patient 6 in microscopic images at a magnification of ×8000. The numbers of melanosomes in the melanocytes in the pretreatment TSC hypomelanotic macules ranged from 5 to 63. By contrast, the numbers of melanosomes in the posttreatment melanocytes ranged from 38 to 49. The melanosomes in the normal control skin sample ranged from 29 to 58. The range of melanosomes in the melanocytes from the pretreatment TSC hypomelanotic macules was significantly broader than the range from the control specimen (P = .02). By contrast, the range of melanosomes in the melanocytes from the posttreatment TSC hypomelanotic macules was narrower than that for the pretreatment macules (P = .049) (Figure 3D). The numbers of melanosomes in each melanocyte from the pretreatment hypomelanotic macules obtained from the remaining 4 participants were also examined. Three of the 5 patients’ pretreatment specimens exhibited significant variation in the numbers of melanosomes. One specimen (patient 2) that did not show a difference in variability reflected the significantly small number of melanosomes in each melanocyte (range, 1-65; P = .17) (Table and eFigure 2 in the Supplement). Given that the keratinocytes had abnormalities in the number and morphologic features of melanosomes similar to those of their adjacent melanocytes (Figure 3A), we believe that melanin had been transferred to the adjacent keratinocytes.

These results indicate that in hypomelanotic macules in patients with TSC, melanocytes existed, melanin transfer from melanocyte to keratinocyte was normal, and melanogenesis in melanocytes was impaired. As a result, maturation of the
Electron Microscopic Examination of Cultured TSC–Knocked-Down Melanocytes

To corroborate the characteristics of TSC melanocytes and the efficacy of rapamycin, TSC2–knocked-down melanocytes (ie, TSC-model melanocytes) were prepared using short interfering TSC2, and the effects of rapamycin were examined. Because the effectiveness rate of the TSC2-model melanocytes was approximately 70%, we found verification of the variability to be difficult. However, many melanocytes with only a small number of melanosomes were observed in the TSC-model melanocytes. After rapamycin treatment, the number of melanosomes in the TSC-model melanocytes increased (Figure 4A and B), although no change occurred in the control melanocytes (Figure 4C and D). To confirm the effect of the rapamycin, the numbers of melanosomes in the TSC-model and control melanocytes were counted. The TSC-model melanocytes had significantly fewer melanosomes (mean [SD], 16.43 [11.84]) than did the control melanocytes (mean [SD], 42.5 [20.1]; P < .001). However, after rapamycin treatment, the number of melanosomes in the TSC-model melanocytes significantly increased (mean [SD], 42.83 [14.39]; P < .001) and approached the numbers in the control melanocytes (Figure 4E). These results corroborated that abnormal melanogenesis had occurred in the TSC-model melanocytes and that rapamycin reduced the abnormalities without affecting normal cells.

Discussion

Tuberous sclerosis complex is an autosomal dominant disorder caused by the inactivation of 1 of 2 tumor suppressor genes, TSC1 or TSC2, leading to the aberrant activation of mTORC1.3-4 Thus, mTORC1 inhibitors, such as rapamycin, have been used to treat patients with TSC. The mechanism of tumorigenesis via mTORC1 is well investigated, and the efficacy of rapamycin for treating tumors in TSC has been already reported.5,28-30 By contrast, only 1 study of hypomelanotic macules has been reported,22 and the mechanisms and therapeutic approaches to hypomelanotic macules in TSC remain unknown, although these macules are a major feature of TSC.15,16 In this report, we treated hypomelanotic macules in 6 patients with definitive TSC and objectively verified the efficacy of a topical rapamycin treatment using a spectrophotometer.

At the completion of 12 weeks of treatment, hypomelanotic macules on non–sun-exposed and sun-exposed skin improved clinically (Figure 1), but significant differences in their conditions at the initiation of treatment were demonstrated only.
on sun-exposed skin. However, after the discontinuation of treatment, the hypomelanotic macules were more improved than they had been at the completion of treatment, and significant differences were observed at 4 weeks after discontinuation of treatment for non–sun-exposed and sun-exposed skin ($P < .05$ and $P < .01$, respectively) and 12 weeks ($P < .01$) after discontinuation of the treatment on both areas (Figure 2). These results suggest that topical rapamycin therapy was as effective for the hypomelanotic macules in TSC as it is for TSC angiofibromas. However, recovery of the skin color took longer than recovery after angiofibromas, and once skin color was recovered, the effect lasted for several weeks after the discontinuation of treatment. Although topical rapamycin was effective in hypomelanotic macules and angiofibromas, the mechanisms by which the rapamycin improved the hypopigmented macules compared with the angiofibromas might be different.

To examine how rapamycin was involved in the melanogenesis, histochemical and electron microscopic examinations of the hypomelanotic macules in patients with TSC were conducted. Skin color is related to the number of melanin granules in keratinocytes that are transferred from melanocytes. Our observations revealed that the melanosomal transfer from melanocytes to keratinocytes was normal (Figure 3A). Therefore, hypomelanotic macules in patients with TSC could be attributed to abnormal melanization in melanocytes but not to the abnormal transfer of melanosomes from melanocytes to keratinocytes or to the increased degradation of melanosomes in keratinocytes, as reported by Murase et al. We demonstrated that maturation of melanosomes was observed in the melanocytes of hypomelanotic macules in the patients with TSC (Figure 3A), but the numbers of matured melanosomes in the melanocytes varied (range, 5-63) between melanocytes...
Hypomelanotic Macules in Tuberous Sclerosis Complex

ORIGINAL INVESTIGATION
Research

(All figures in the Supplement). Jimbow reported that TSC-related hypomelanotic macules are associated with a decrease in melanization and melanosome size and an increase in aggregated melanosomes in keratinocytes. Our observations confirmed those findings. The abnormal melanization that resulted in variable numbers of melanosomes between melanocytes might be characteristic of the hypomelanotic macules in TSC. Rapamycin treatment reduced these abnormalities significantly, and the numbers of matured melanosomes in melanocytes after rapamycin treatment became uniform (range 38-49) (Fig 3D).

To corroborate the effects of rapamycin on the melanocytes in the patients with TSC, TSC-model melanocytes before and after rapamycin treatment were observed with an electron microscope (Fig 4). The TSC-model melanocytes had significantly small numbers of melanosomes. The clinical findings in the pretreatment biopsy samples showed a variable number of melanosomes. However, TSC-model melanocytes showed reduced numbers of melanosomes. We attribute this discrepancy to influence on melanocytes in vivo by many other surrounding cells and the intricate effect of the microenvironment, in contrast to cultured cells. In addition, TSC2 may be inactivated by a mutation or deletion, and these modes may not be functionally identical. Some abnormality might induce the variability and another might induce the decrease of melanosomes. In patient 2, TSC2 abnormality may have induced the decrease of melanosomes rather than the variability (Fig 2 in the Supplement). Rapamycin treatment significantly increased the numbers of melanosomes in the TSC-model melanocytes, although no influence appeared in the control melanocytes (Fig 4). These results were consistent with the clinical symptoms after topical rapamycin treatment; namely, rapamycin treatment affected only the hypomelanotic macule but did not affect normal skin.

Although clinical improvement of hypomelanotic macules in sun-exposed skin was remarkable compared with the non-sun-exposed skin throughout the study period, including at initiation (Fig 1), the difference in δ-L values between the sun-exposed skin and the non-sun-exposed skin was not statistically significant (Fig 2C). Recently, Kalie et al reported that ULK1 regulates melanin levels in MNT-1 melanoma cells independently of mTORC1. Sun exposure might improve melanogenesis in an mTOR-independent manner.

In this trial, no adverse effects were observed, and the rapamycin blood concentrations were lower than the detection limit (0.6 ng/mL) in all participants. Because mTORC1 is a conserved and ubiquitous protein, adverse effects are a concern with the systemic administration of rapamycin. Topical rapamycin treatment is recommended not only for facial angiofibromas but also for hypomelanotic macules attributable to TSC.

Conclusions

In this report, we clarified that topical rapamycin therapy was effective and safe for treating TSC-related hypomelanotic macules. We also revealed that the small or varied numbers of melanosomes in melanocytes might be characteristic of TSC-related hypomelanotic macules and that rapamycin improved the abnormalities. Sun exposure might improve melanogenesis in an mTOR-independent manner. Further studies with more samples are expected.

ARTICLE INFORMATION

Accepted for Publication: October 5, 2014.

Author Contributions: Dr Wataya-Kaneda had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Wataya-Kaneda, Tanaka, L. Yang, F. Yang, Tanemura.

Acquisition, analysis, or interpretation of data: Wataya-Kaneda, Tanaka, L. Yang, F. Yang, Tsuruta, Nakamura, Matsumoto, Hamasaki, Katayama.

Drafting of the manuscript: Wataya-Kaneda, Tanaka, Hamasaki, Tanemura.

Critical revision of the manuscript for important intellectual content: Wataya-Kaneda, L. Yang, F. Yang, Tsuruta, Nakamura, Matsumoto, Katayama.

Statistical analysis: Tanaka, L. Yang, F. Yang, Hamasaki.

Obtained funding: Wataya-Kaneda.

Administrative, technical, or material support: Wataya-Kaneda, L. Yang, F. Yang, Tsuruta, Nakamura, Matsumoto, Tanemura.

Study supervision: Wataya-Kaneda, Tanemura, Katayama.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a grant from the Ministry of Health, Labor and Welfare of Japan.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES


