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Letter to the Editor

Topical delivery of mTOR inhibitor halts scarring

Dear Editor,

Scar management is considered a major medical need affecting patients with large wounds, extensive burns, and enhanced genetic susceptibility for scar formation. Scarring impacts the human's well-being interfering with aesthetics, skin functions, and physical restriction of the body movement [1]. Scarring is not limited to the integumentary system (e.g., skin), it also affects multiple organs like the respiratory and digestive (e.g., lungs and salivary glands) systems, among others. Moreover, surgical interventions can also lead to undesired scarring and fibrosis, especially in the skin [2]. The development of novel pharmacological strategies targeting fibrosis and scarring constitute an urgent need to improve the lives of affected individuals. The biology of the scar formation is unique as it develops later after wound closure and during tissue maturation phase. Such a long course of scar formation provides a unique window of opportunity for pharmacological intervention.

Current therapies offer limited results and mainly rely on enhanced skin hydration, moderate relieve of tissue tension, and protection against ultraviolet (UV). Conventional treatment includes a plethora of surgical and non-surgical strategies as pressure therapy, silicone gel, dermabrasion, chemical peels, as well as corticosteroid injections, and even the use of radiation and chemotherapy (e.g., 5-fluorouracil or bleomycin). Most of the over-the-counter formulations contain ingredients like onion extract and vitamins A and E; however, the literature lacks well-designed studies to test their efficacy [3].

From a signaling standpoint, the activation of the mTOR pathway has been associated with wound healing and scar formation [4–6]. Recently mTOR inhibitors, particularly rapamycin, has been shown to play a major role in many physiological processes including prolonging lifespan in mammals [7]. These studies also include outcomes as the prevention of age-related effects and diseases like cancer. Rapamycin and its derivatives are FDA approved drugs, also known in the clinic as Rapamune (Sirolimus), Everolimus (Afinitor), Temsirolimus, and Deforolimus (Ridaforolimus). They are used to treat diseases like cancer, diabetes, neurodegeneration, and transplantation rejection [8].

We previously showed that rapamycin reduces skin proliferative lesions and hamartomas, even suggesting it as a treatment to block fibroproliferative lesions [9]. We next asked whether rapamycin could be beneficial for scars treatment. Here we used a well-established in vivo scar model to generate pre-clinical data that supports the use of rapamycin to prevent scarring [10]. Our model shows that scars (Fig. 1A, Suppl. Fig. S1A) were characterized by the absence of hair follicles, continuous epithelial coverage, and increased mesenchymal cellular density (n = 7 mice per group). Furthermore, the scars displayed the activation of AKT (p-AKT) and downregulation of PTEN (compare to healthy skin - Suppl. Fig. S1B), indicating the activation of PI3K/AKT signaling in scars (Fig. 1B).

Next, we tested whether topical rapamycin gel treatment could affect scarring in vivo (Fig. 1C). We found that the scar size treated with 0.2% rapamycin gel (n = 8 mice) is smaller than the scars of the mice receiving vehicle alone (n = 8 mice) (Fig. 1D and Suppl. Fig S1A). We further analyzed the scars using four parameters: scar area (Fig. 1E) and length, determined by scar edges and the distance between the adipose tissues (Fig. 1F), muscle (Fig. 1G), and hair follicles (Fig. 1H). We found that rapamycin treatment resulted in statistically significant reductions of all analyzed parameters ($^{*}p < 0.05$ and $^{**}p < 0.01$), which indicates a reduction in scarring (Fig. 1D–H).

Mechanistically, rapamycin forms an FKBP12-rapamycin-mTOR complex that inhibits the ribosomal protein pS6. Thus, pS6 is a molecular biomarker for rapamycin efficacy [8]. In our study, the results showed that rapamycin application reduced scarring and efficiently inhibit pS6 in vivo (Fig. 2A and Suppl. Fig. S1C). Furthermore, collagen is a crucial skin component. Changes on the levels of collagen type I and III are evident during scarring [11]. Indeed, the scars from the vehicle-treated group had thin and elongated green fibers positioned in parallel, indicating the presence of collagen type III. Remarkably, the administration of rapamycin resulted in smaller scars comprised of thick and short yellow/orange fibers, suggesting the presence of collagen type I. The reduced scars close resembled the uninjured skin, which had predominantly short, thick and intertwined yellow/orange fibers (collagen type I) (Fig. 2B). We also investigated the changes in the collagen organization and found significant differences in fiber's orientations. Notably, scars from the rapamycin-treated group had collagen fiber orientation similar to the control mice (Suppl. Figs. S1D and S2A).

During tissue repair, the scarring is associated with angiogenesis. Here we used a well-established endothelial cell marker CD31 (Suppl. Fig. S2B) to identify and quantify the endothelial vessels within scar tissues. We showed that microvessels were predominantly on the scars and that rapamycin administration statistically reduced the number of these blood vessels (Fig. 2D, $^{***}p < 0.001$). Also, we previously showed that Periostin (OSF-2; *Postn* gene) requires the activation of the mTOR signaling to enhance migration and proliferation [12]. Considering that Periostin is associated with high tissue tension and the knockout mice displayed adverse effects in healing and excess of collagen deposition [12,13], we asked whether Periostin would be involved in the rapamycin-dependent reduction of scars. Our results showed that Periostin is upregulated in the scars (Fig. 2E_arrows and Suppl. Fig S2C); and conversely, rapamycin prevented the augmentation of Periostin (Fig. 2E).

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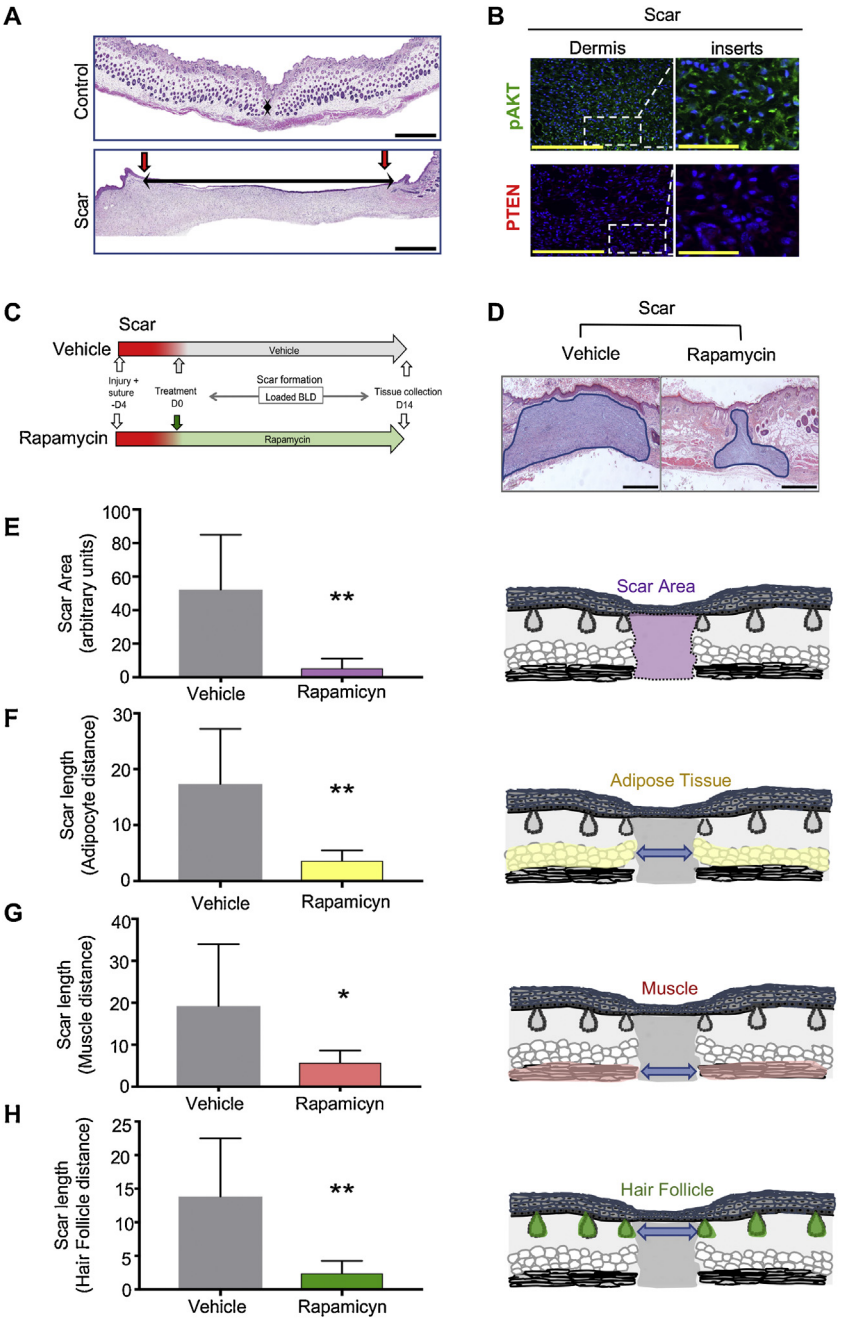

Fig. 1. mTOR inhibitor rapamycin reduces scarring. A) Representative tissue samples stained for hematoxylin and eosin (H&E) show defined scar in the experimental group (n = 7 mice). To induce scarring, the skin was injured (incision plus suture/surgical glue closing of the wound) and biomechanical loading devices (BLD) were placed and activated at 4 days postinjury (see details in material and methods). Resting BLDs served as controls (n = 7 mice). Note that the scar displays classic histological features such as increased cellularity within the scar and loss of rete pegs, adnexae, and hair follicles. Red arrows indicate the scar borders or limits, and black arrows indicate the extension of the scar in the experimental group (scale bar: 3 mm). B) The scars have increased AKT activation indicated by the pAKT (in green), while the tumor suppressor PTEN inactive (scale bar: 250 μm; inserts - scale bar: 80 μm). C) Next, the scars were treated with rapamycin (n = 8 mice) or vehicle (n = 8mice). The schematic representation shows the time course of the experiment, which included the administration of rapamycin gel or vehicle on the scars. D) Striking differences were found in the scars treated with mTOR inhibitor rapamycin. The rapamycin-treated scars were significantly smaller (scale bar: 100 μm). E–H) The quantitative cross-sections measurements confirm that the mTOR inhibitor treatment resulted in a significant reduction of scar's dimensions (E) and length (F–H). The schematic representations indicate the length or distance (blue arrows) among the anatomical landmarks or tissues like fat or adipocytes (F), muscle (G), and hair follicles (H) that demarcate the scars. (Data are presented as mean ± SEM; $^{*}p < 0.05$ and $^{**}p < 0.01$).

Here, we demonstrated that topical application of rapamycin is effective in preventing scarring. Nevertheless, it is important to notice that an adverse effect of mTOR inhibitors is the increased risk for delayed wound healing. According to the prescription information, mTOR inhibitors, (Rapamune (sirolimus) and Afinitor (everolimus), delayed wound healing in <1% of individuals who had the risk increased if their body mass index was higher than 30 kg/m2. Consequently, one should consider the use of mTOR inhibitors during the remodeling phase of the healing (after wound closure) to avoid possible delay in healing and effectively target scarring.

In conclusion, we found that topical administration of 0.2% rapamycin gel effectively reduced scarring. Along with reduced scar size, rapamycin differentially regulated the content of collagen

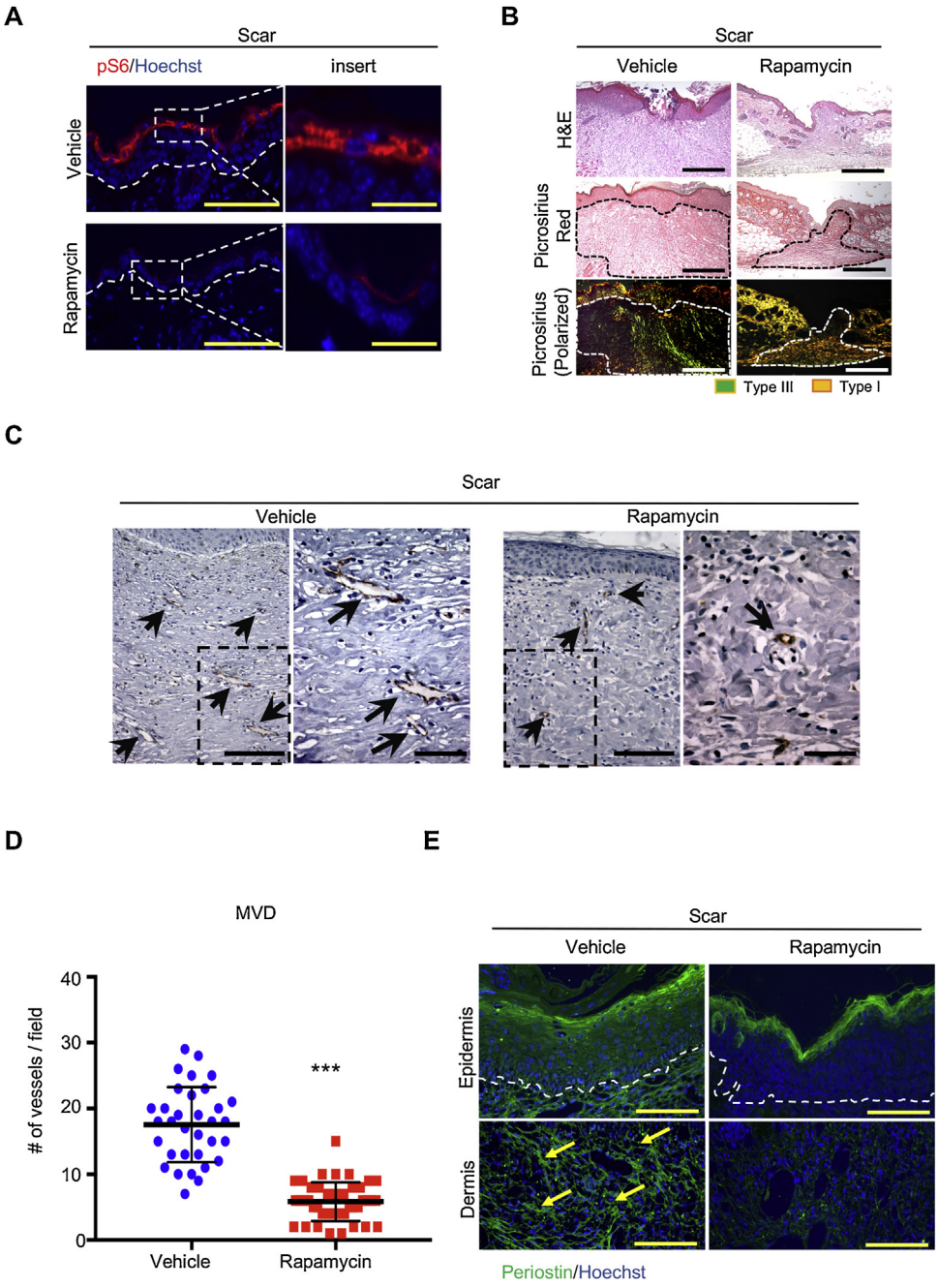

Fig. 2. Effective delivery and action of rapamycin gel affect scar composition. **A)** Skin treated with rapamycin gel shows mTOR inhibition confirmed by the loss of the biomarker pS6, compared to the vehicle. PS6 is in red (Insert) (scale bar: 250 μm; and inserts- scale bar: 80 μm). B) Hematoxylin and eosin (H&E) and Picrosirius Red stains of representative samples of scars tissues (treated with vehicle or rapamycin). The polarization of picrosirius stains demonstrates different content on collagen I and collagen III composition between the groups. Note that rapamycin treated scars have a notable presence of yellow collagen fiber (collagen I). In contrast, large amounts of collagen III (green) are seen in the scar (+ vehicle). (Scale bar: 100 μm). C) Representative images of CD31+ blood vessels with the scar (20x and 40x magnifications; scale bars: 250 μm and 50 μm). Arrows show the blood vessels stained in brown. Note an increasing number of blood vessels in the scars (+vehicle) compared to rapamycin-treated scars. D) The graphic depicts the increased microvessel density (MVD) on scar tissues that is abrogated by rapamycin gel treatment (mean ± SEM; *** $p < 0.001$). E) The microphotographs show rapamycin application in the scar reduces Periostin production. Yellow arrows point at Periostin stained in green (scale bar: 250 μm) (see Supplemental information for methods).

type I and III within the lesions and decreased abnormal vascularization observed within scars. Altogether, our findings are encouraging as rapamycin efficiently reduces scarring.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.06.008.

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Liana P. Webber, Brian Yip, Carlos H.V. do Nascimento Filho, Ha B. Park
Laboratory of Epithelial Biology, Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, 48109-1078, USA

Rogerio M. Castilho[a,b], Cristiane H. Squarize[a,b,*]
[a]Laboratory of Epithelial Biology, Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, 48109-1078, USA, [b]The Michigan Medicine Rogel Cancer Center, University of Michigan, Ann Arbor, MI, 48109, USA

* Corresponding author at: Laboratory of Epithelial Biology, Department of Periodontics and Oral Medicine, University of Michigan, 1011 N. University Ave, office 3210, Ann Arbor, MI 48109-1078, USA.
E-mail address: csquariz@umich.edu (C. Squarize).

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