Periostin modulates extracellular matrix behavior in tendons

Kevin I. Rolnicka, Joshua A. Choea, Ellen M. Leifermana, Jaclyn Kondratko-Mittnachtb, Anna E. B. Clementsa, Geoffrey S. Baera, Peng Jiangb,c, Ray Vanderbya and Connie S. Chamberlaina

a - Department of Orthopedics and Rehabilitation, University of Wisconsin, Madison, WI 53705, USA
b - Center for Gene Regulation in Health and Disease (GRHD) and Department of Biological, Geological and Environmental Sciences, Cleveland State University, Cleveland, OH 44115, USA
c - Center for RNA Science and Therapeutics, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA

Correspondence to Connie S. Chamberlain: Dept. of Orthopedics and Rehabilitation, 1111 Highland Ave., WIMR Room 5057, University of Wisconsin, Madison, WI 53705, USA. Chamberlain@ortho.wisc.edu (C.S. Chamberlain)

Abstract

Periostin, originally named osteoblast-specific factor 2 (OSF-2) has been identified primarily in collagen rich, biomechanically active tissues where its role has been implicated in mechanisms to maintain the extracellular matrix (ECM), including collagen fibrillogenesis and crosslinking. It is well documented that periostin plays a role in wound healing and scar formation after injury, in part, by promoting cell proliferation, myofibroblast differentiation, and/or collagen fibrillogenesis. Given the significance of periostin in other scar forming models, we hypothesized that periostin will influence Achilles tendon healing by modulating ECM production. Therefore, the objective of this study was to elucidate the effects of periostin during Achilles tendon healing using periostin homozygous (Postn−/−) and heterozygous (Postn+/−) mouse models. A second experiment was included to further examine the influence of periostin on collagen composition and function using intact dorsal tail tendons. Overall, Postn−/− and Postn+/− Achilles tendons exhibited impaired healing as demonstrated by delayed wound closure, increased type III collagen production, decreased cell proliferation, and reduced tensile strength. Periostin ablation also reduced tensile strength and stiffness, and altered collagen fibril distribution in the intact dorsal tail tendons. Achilles tendon outcomes support our hypothesis that periostin influences healing, while tail tendon results indicate that periostin also affects ECM morphology and behavior in mouse tendons.

© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Rupture of the Achilles tendon is a common injury in North America with a mean incidence of 8.3 per 100,000 people.[1] The healing process can protract years, producing a fibrotic tissue with impaired mechanical properties. Despite new surgical techniques and other therapeutic advancements designed to improve tendon healing, none eliminate scar formation, thereby making them prone to further re-rupture.[2,3] A mechanism to accelerate wound healing and/or reduce scar formation could reduce the likelihood of re-injury and time spent recovering.

Periostin, originally named osteoblast-specific factor 2 (OSF-2) is a nonstructural ECM matricellular protein able to modify cell behavior and biomechanical properties of collagenous-based tissues. It is induced and secreted by activated fibroblasts and myofibroblasts in areas...
of tissue injury where it accumulates within the ECM to facilitate type I collagen maturation and crosslinking. Periostin also plays a role in maintaining ECM homeostasis through interactions with proteins including tenasin-C and fibronectin.

It is well documented that periostin plays a role in wound healing and scar formation after injury, in part, by promoting cell proliferation, myofibroblast differentiation, and/or collagen fibrillogenesis. [3–10] Within skin wounds, periostin is upregulated after injury and peaks 7 days later. [11,12] Similarly, periostin is increased after myocardial infarction and is required for proper scar formation. Previously, we reported the upregulation of periostin within the medial collateral ligament (MCL) and Achilles tendon during early healing. [13,14] The increase in periostin production coincided with upregulated type III collagen, thereby supporting a role in scar formation. [14]

Studies using Postn null (Postn−/−) mice have further supported its role in healing and scar formation. After injury, Postn−/− mice had impaired skin wound closures and a reduced population of myofibroblasts typically present at the wound borders. [4] Exogenous delivery of periostin to the Postn−/− mice, returned myofibroblast presence within the granulation tissue. [11] Postn−/− mice also exhibited impaired healing after acute myocardial infarction which resulted from cardiac rupture as a consequence of reduced myocardial stiffness, fewer myofibroblasts, and impaired collagen fibril formation. [5]

Given the significance of periostin in other scar forming models, we hypothesized that periostin will influence Achilles tendon healing by modulating ECM production. Therefore, the objective of the present study was to elucidate the effects of periostin during Achilles tendon healing using periostin homozygous knockout (Postn−/−) and heterozygous (Postn+/−) mouse models. A second experiment was included to further examine the influence of periostin on collagen composition and function using intact mouse dorsal tail tendons.

Results

Achilles tendon results

Achilles Tendon Mechanical Testing. To determine if periostin affects tendon function, the intact, day 14+, and day 28-post-injured wild type (WT), Postn+/−, and Postn−/− tendons were mechanically tested. Intact tendons failed primarily at the calcaneal insertion (93%) and to a lesser extent the musculo-tendinous junction (MTJ; 7%). Failure of the injured tendons occurred at the midsubstance (40.7%), calcaneal insertion (55.9%) and MTJ (3.4%). Mechanical results indicated that the ultimate load of intact tendons was significantly lower in Postn+/− tendons compared to the intact WT samples; no differences were noted by the intact Postn−/− tendons (Fig. 1A, G). Within the healing tendon, ultimate load at day 28 post-injury was lower in the Postn−/− tendons compared to the injured WT tendons. No other differences in ultimate load were noted (Fig. 1A, G). Ultimate stress was significantly lower in intact Postn+/− tendons compared to Postn−/− tendons. No differences were noted in ultimate stress after tendon injury (Fig. 1B, G). In contrast, ultimate strain was significantly reduced within the Postn−/− and Postn+/− tendons 28 days post-injury compared to WT samples (Fig. 1C, G). No other significant genotype differences were noted in modulus, stiffness, or cross sectional area (Fig. 1C-G). As expected, a day effect was noted in all metrics tested, except strain, regardless of genotype; day 28 and/or day 14 tendons were significantly weaker than the intact tendons (Fig. 1F).

Achilles Tendon Immunohistochemistry. IHC was performed to examine the effects of periostin on tendon cell number and ECM production. To first confirm that Postn−/− was abrogated within the Postn−/− tissue, tendons were stained for periostin. Very little/no periostin was evident in intact tendons, regardless of genotype (Supp. Fig. 1). Injured tendons collected at days 7, 14, and 28 were positive for periostin within both the WT and Postn−/− tendons. In contrast, very low/no periostin was detected in the injured Postn−/− tendons. Ki67, a marker of proliferating cells, was significantly decreased over time from day 7 to day 28 post-injury, regardless of genotype (Fig. 2A-B). Proliferating cells were also significantly different within the injured tendon based on genotype; Postn−/− tendons collected at 7 days post-injury contained fewer proliferating cells compared to the WT tendons. In contrast, more cells were present within the day 14 Postn−/− tendons compared to the WT tendons (Fig. 2C). No other differences were noted. Myofibroblasts, a marker of scar formation, were reduced over time within the injured tendon, regardless of genotype (Fig. 2D-E). Further analysis indicated that myofibroblasts were significantly higher within the Postn+/− tendons compared to the Postn−/− tendons at 7 days post-injury (Fig. 2F). To note, the number of myofibroblasts remained similar across time within the Postn−/− tendons but were significantly reduced over time within the day 14 Postn+/− and day 28 wt samples. Type I collagen, a marker for native tendon, was reduced at 7 days post-injury compared to days 14 and 28, regardless of genotype (Fig. 2G-H). No other changes were noted in type I collagen (Fig. 2I). Type III collagen, another marker of scar formation, was significantly increased from day 7 to day 28 during healing, regardless of genotype (Fig. 2J-K). Differences were also noted between genotypes. Type III
collagen was elevated at days 7, 14, and 28 post-injury within Postn+/− tendons compared to the WT and/or Postn+/− tendons. While type III collagen localization within Postn+/− tendons was similar to WT tendons at days 7 and 14, levels were significantly lower at day 28 post-injury. (Fig. 2J-L). Another method to monitor tendon scar formation is via calculating the type I:type III collagen ratio. Regardless of genotype, the type I:type III collagen ratio was reduced over the course of healing from day 7 to day 28 (Fig. 2M). The ratio was also different between genotypes. Specifically, type I:III collagen was decreased within the Postn+/− and Postn+/− tendons compared to the WT samples at 7 days post-injury (Fig. 2N). While no changes were noted at day 14, Postn+/− tendons remained higher in type I:III collagen compared to the WT and Postn+/− at day 28 post-injury. Lastly, CD31, a marker to identify endothelial cells, was significantly decreased from day 7 to day 14 post-injury, regardless of genotype. (Fig. 2O). No other changes were noted in endothelial cells (Fig. 2P-Q).

Achilles Tendon Histology. To examine the effects of periostin on wound size, tendons were stained with H&E, imaged, and wound size was measured. Injury to the Achilles tendon in Postn+/− and Postn+/− mice, resulted in a larger wound size at days 7 and 14 post-injury compared to the WT tendons. (Fig. 3A-B). By day 28, wound size was similar between genotypes.

Tail Tendon Results:

Tail Tendon Mechanical Testing. To further explore the effects of periostin on mechanical properties, the mouse dorsal tail tendons were examined between the intact Postn+/−, Postn+/−, and WT animals. Intact tail tendons from Postn+/− and Postn+/− mice exhibited a significant reduction in ultimate load, stiffness, stress, and Young’s modulus, compared to WT tendons (Fig. 4A-D, F). No differences were noted between the Postn+/− and Postn+/− groups. Cross sectional area of the tendons were also not significantly different between groups (Fig. 4E-F).

Tail Tendon TEM. To elucidate the compositional differences between the Postn+/− and WT tail tendon fibrils, samples were subjected to TEM. As no differences were noted between the Postn+/− and Postn+/− groups within the mechanical testing results, the Postn+/− group was omitted from analysis. The overall average diameter of fibrils was not significantly different between the two genotypes (Fig. 5A, F). However, when fibrils were grouped according to size, a difference in diameter was noted in large fibrils ranging from 300 to 400 nm. Postn+/− fibrils were...
significantly larger compared to the WT tendons (Fig. 5B-C, F). Fibril volume fraction analysis indicated Postn/C0/C0 tendons contained fewer fibrils (and more matrix) compared to the WT fibrils (Fig. 5D-F). Overall, these results indicate that Postn/C0/C0 tendons contain fewer but larger sized fibrils within a given area compared to the WT tendons, possibly accounting for the reduced mechanical and structural behaviors observed.

Discussion

The goal of this study was to elucidate the role of periostin on tendon behavior using Postn+/− and Postn/C0/C0 mouse models. To our knowledge, this is the first study to report impaired healing by the Postn−/− Achilles tendon as demonstrated by delayed wound closure, increased type III collagen production, decreased type I:type III collagen, altered cell proliferation, and reduced tensile strength. Tendon healing from Postn+/− tendons was also impacted but with less severity. Intact Postn/C0/C0 and Postn+/− dorsal tail tendons showed reduced tensile strength and stiffness. Postn−/− tail tendons also demonstrated abnormal collagen fibril distribution. The localization of periostin has been previously reported in various musculoskeletal systems. Our own studies reported periostin localization within WT Achilles tendons and medial collateral ligaments.[13,14]
Within the intact structures, periostin levels are typically low. Injury to these tissues significantly upregulates periostin such that levels are similar to the increases noted with type III collagen. Other labs have reported that fibrocartilage development within the post-natal anterior cruciate ligament (ACL) insertion, is delayed in Postn−/−/− mice.[15] In muscle injury models, Postn−/−/− animals also exhibited a loss of muscle fibers.[16] Combined, these results suggest that periostin plays a significant role in ECM homeostasis and regulation of cell phenotype within the musculoskeletal tissues.

The association of periostin and type I collagen has been well documented in skin and cardiac models.[6,7,10,17,18] Fewer studies have reported correlations between periostin and type I collagen within the tendon. [8,19] In a previous study, we found no association between type I collagen and periostin during normal Achilles tendon healing.[14] However, a concomitant increase in periostin and type III collagen, as well as a decrease in tendon tensile strength was noted.[14] Within the current study, no significant changes in type I collagen were observed within the Postn−/−/− or Postn+/−/− tendons. Unexpectedly, type III collagen was increased within the Postn−/−/− and to a lesser extent, the Postn+/−/− Achilles tendons, which led to a decrease in the type I:type III collagen ratio at day 7. The rationale for the changes in collagen production is unclear but suggests that periostin in part, contributes to controlling the amount of scar that forms. Few studies have reported the influence of periostin on type III collagen behavior during healing. [9,10,20] One study reported that, periostin levels were upregulated within the forming scar of the remodeling tendon, typically dominated with type III collagen.[20] Another study reported that periostin-releasing mesenchymal stromal cells (MSCs) ectopically implanted into a mouse, mediated formation of tendon-like tissue and enhanced type III collagen production.[9] Similarly, periostin levels were positively correlated with type III collagen levels but not type I collagen during chronic cardiac remodeling.[10] Altogether, these results support the concept that periostin influences type III collagen output, although the type of collagen most influenced by periostin may be tissue specific and healing specific.

Periostin modulates cell behavior by inducing cell proliferation and myofibroblast differentiation.[11,12,21] Within the infarcted heart, periostin has been shown to control migration and proliferation of fibroblasts via α[3] which leads to collagen production.[5,17] Periostin is also expressed by fibroblasts and myofibroblasts, cells responsible for production of contractile proteins (such as α-SMA)
and extracellular matrix proteins (including collagen).\[^{22}\] In our study, cell proliferation was reduced, and wound repair was delayed by the healing \(\text{Postn}^{-/-}\) Achilles tendons, supporting a beneficial role of periostin in the repair response. While the role of periostin on myofibroblast differentiation has been significantly documented in other healing models, we found only minor temporal differences in myofibroblasts between the WT and \(\text{Postn}^{-/-}\) Achilles tendons. This subtle outcome may be due to the low number of myofibroblasts detected within the healing tendon at the times tested regardless of phenotype. Overall, the reduction in cell populations within the healing \(\text{Postn}^{-/-}\) tendons suggests decreased/delayed wound closure during the early stages of healing.

Aside from modulating cell behavior, periostin may also play a role in collagen fibrillogenesis. Indeed a number of reports have demonstrated that collagen fibrillogenesis is altered within the \(\text{Postn}^{-/-}\) tissues, as a reduction in collagen fibril diameter and/or decreased collagen fibril cross-linking was reported.\[^{[5,8]}\] Our TEM results detected no statistical difference in overall collagen fibril diameter between genotypes. Moreover, fibrils ranging in size from 300 to 400 nm were larger in periostin homozygous animals. While no significant reductions in collagen diameter were detected, there was a significant decrease in tensile strength by the \(\text{Postn}^{-/-}\) tendons. As a decrease in collagen cross-linking can be expected to reduce tensile strength or stiffness, this may partly account for the reduction in tensile strength by the intact \(\text{Postn}^{-/-}\) tail tendons.\[^{[6]}\] Our findings also suggest periostin may control collagen fibrillogenesis by altering the amount of type I:type III collagen normally found within the tendon.

While some similarities in tendon properties were noted between \(\text{Postn}\) heterozygous and homozygous animals, significant discrepancies...
Experimental procedures

Animal model

The study was approved by the University of Wisconsin Institutional Animal Use and Care Committee. A total of 165 male and female 8–10 week mice were randomly included in the study. Mouse breeding pairs for B6; 129-Postn<sup>tm1jouer</sup>/J (strain 009067) were obtained from Jackson Laboratories (Sacramento, CA). Postn<sup>+/+</sup> females were bred with Postn<sup>+/−</sup> males at the University of Wisconsin, Biotron Core. Genotyping of offspring to identify Postn<sup>+/−</sup> mice was performed via Transnetxty (Cordova, TN). C57Bl/6J mice (strain 000664; Jackson Laboratories, Sacramento, CA) were used as the wild type (WT) controls.

To examine the effects of periostin on Achilles tendon healing, a unilateral surgically transected Achilles tendon was used as an experimental model to create a uniform defect for healing as previously described. A total of 55 Postn<sup>+/−</sup>, 55 Postn<sup>+/+</sup>, and 55 wt mice were included in the study.

Mice were anesthetized using isoflurane. A small, 1 cm skin incision was made over the posterior aspect of the hind limb, distal to the gastrocnemius muscle and proximal to the calcaneus. Underlying fascia was dissected to expose the Achilles tendon. The superficial digital flexor (SDF) tendon was dissected from the Achilles tendon and surgically removed. The Achilles was transected in the midsubstance of the tendon (half way between the calcaneal insertion and the musculotendinous junction) and the tendon ends were repaired using 10–0 suture. The muscular, subcutaneous, and subdermal tissue layers were each closed with 4–0 Dexon suture. The hindlimb was then immobilized using a wire cerclage. All animals were allowed unrestricted cage movement immediately after surgery. Tendons were then collected at 7, 14, or 28 days post-injury and used for immunohistochemistry (IHC; 5 mice/genotype/day) or mechanical testing (10 mice/genotype/day).

Tendons designated for IHC were carefully dissected, measured, weighed, and immediately snap-frozen in optimal cutting temperature (OCT) media. Mice used for mechanical testing were stored at −80°C until use. A day 7 mechanical group was not included as the tendon is too structurally compromised for meaningful mechanical data. Intact Achilles tendons were also included in the study and used for histology/IHC (n = 10 mice/genotype) and mechanical testing (n = 10 mice/genotype).

Immunohistochemistry/Histology

Immunohistochemistry (IHC) was performed as previously described. Briefly, cryosectioned tendons were fixed with acetone, exposed to 3 %
hydrogen peroxide to eliminate endogenous peroxidase activity, and blocked using Background Buster (Innovex Biosciences, Richmond, CA). Sections were incubated in rabbit polyclonal primary antibodies Ki67 (1:750, Ab155580), CD31 (1:50, Ab28364), alpha-SMA (α-SMA; 1:300, Ab5694), collagen type I (1:800; Ab34710), and collagen type III (1:150; Ab7778), to identify proliferating cells, endothelial cells, myofibroblasts, collagen type I and collagen type III, respectively (all from Abcam, Cambridge, MA). To confirm that that periostin protein was absent in Postn−/− samples, a rabbit polyclonal primary antibody for periostin (1:100, PAB16942, Abnova, Walnut, CA) was included. After primary antibody exposure, sections were treated with biotin and streptavidin conjugated to horseradish peroxidase using the StatQ staining kit (Innovex Biosciences). The bound antibody complex was then visualized using diaminobenzidine (DAB). Stained sections were dehydrated with ethanol, cleared, cover-slipped, and viewed under light microscopy. Negative controls omitting the primary antibody were included with each experiment. H&E staining was also performed to measure size of wound region.

Cell counting

Following IHC and H&E staining, images were collected using an Olympus camera-assisted microscope (Nikon Eclipse microscope, model E6000, Melville, NY; Olympus model DP79 Center Valley, PA). Three blocked random pictures were obtained from each sample. Images of IHC markers were captured within the healing region. H&E-stained sections were imaged to include the entire healing region, including the borders. Three sections were counted per animal resulting in fifteen sections per genotype per time point. Each captured image was then quantified with ImageJ [29]. For wound healing measurements, wound borders were outlined and the area was quantified via Image J.

Achilles tendon mechanical testing

Mechanical testing was performed to measure the functional effect of periostin on both the intact and healing tendons. Achilles tendons were dissected and the surrounding tissue was excised with care to keep the calcaneal insertion site and the musculotendinous junction (MTJ) intact. Sutures were not removed from the injured tendons based on our previous tests indicating that sutures carried no significant load, and to avoid disruption of the injured region. Tendons remained hydrated via phosphate buffered saline (PBS). Tendon length, width, and thickness were repeatedly measured using digital calipers and the cross-sectional area (assumed to be an ellipse) was estimated. Tendons were tested in a custom-designed load frame, which gripped and loaded the tendons along their longitudinal axis. The calcaneus was trimmed and press-fit into a custom bone grip. The soft tissue end was fixed to strips of Tyvek with a cyanoacrylate adhesive, which were held in a soft-tissue grip. Dimensional measurements for the tendons were recorded at pre-load. Mechanical testing was performed at room temperature. A low preload of 0.1 N was applied in order to obtain a uniform zero point prior to preconditioning (20 cycles at 0.5 Hz) to 0.5 %. Pull-to failure testing was performed on tendons at a rate of 3.33 mm/sec. Force and displacement data from the test system were recorded at 10 Hz. Ultimate load was the highest load prior to a complete rupture of the tendon and stress was calculated by dividing the ultimate load by the initial cross-sectional area. Stiffness was calculated as the slope of the linear portion of the load–displacement curve. Young’s modulus was calculated as the slope of the linear portion of the stress–strain curve. Ultimate strain was calculated by dividing tendon deformation by original tendon length.

Tail tendon mechanical testing

A second experiment was performed to examine the effects of periostin on mouse tail tendons. Dorsal tail tendons were obtained from Postn−/−, Postn+/−, and WT mice (n = 14 tendons/genotype) initially used for the Achilles tendon healing study. Mechanical testing was completed to compare the functional differences between the Postn−/−, Postn+/−, and WT tail tendons. To obtain the tail tendons, the skin was removed to expose the dorsal side tendon. Fascia was carefully cut and tendons were collected. A total of 14 tendons from each genotype (n = 42 total) were tested. Tendon length was measured using a 0–150 mm (0.01 mm resolution) digital caliper. Tendon width and thickness were measured optically using ImageJ (measurements taken at three locations along the tendon length and averaged) and the cross-sectional area (assumed to be an ellipse) was calculated. Tendons were loaded into a mechanical testing frame (Mark-10 Corporation, Copiague, NY), and pulled to failure at 15 mm/min. Load and deformation were recorded, while stiffness, stress, strain, and Young’s modulus were calculated as reported above.

Tail tendon transmission electron microscopy (TEM)

Tail dorsal tendons were obtained from Postn−/− and WT mice (n = 3 tendons/genotype) initially used for the Achilles tendon healing study. Dorsal tail tendons were fixed in glutaraldehyde and paraformaldehyde. Areas containing collagen bundles were dissected, post-fixed in osmium tetroxide, post-stained with uranyl acetate and lead citrate, and viewed with a Zeiss JEM 1400 transmission electron microscope at 75 kv.
tetroxide, dehydrated using a series of graded ethanols, and rinsed in propylene oxide. Samples were then embedded in Spurr’s resin. Embedded tendons were cross-sectioned at 70 nm using an ultramicrotome, mounted on 200-mesh copper grids and stained with uranyl acetate and lead citrate. Images were collected from non-overlapping regions of the tendon via Philips CM200 electron microscope (Philips Medical System, Andover, MA) equipped with SIS MegaView 3 digital camera. A total of 9 regions per tendon were obtained. Fibril diameter and distribution were calculated via ImageJ.[29] Fibril volume fraction was calculated according to the American Society of Testing and Materials (ASTM) methods E562-11 for biphasic materials.[30] The ASTM method estimates the volume fraction of an identifiable phase from sections through the microstructure by means of a point grid quantification.

Statistical analysis
A two-way analysis of variance (ANOVA) was used to examine differences between genotypes across time for all Achilles tendon immunohistochemistry and mechanical testing results. IHC staining differences were examined within the healing region. Three replicates per tendon sample (n = 5 samples/day/genotype) were collected and averaged. For each stain, WT and Postn−/+ samples from days 7, 14, and 28 were analyzed for differences in genotype, time, or genotype and time interaction. If the overall p-value was significant, post-hoc comparisons were performed using Tukey’s post-hoc test. Tail tendon mechanical results as well as TEM outcomes were examined using one-way ANOVA. P ≤ 0.05 was used as the criterion for statistical significance. All analyses were performed using KaleidaGraph, version 4.03 (Synergy Software, Inc., Reading, PA).

Funding
Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number AR059916 and by the AO Foundation under Award Number MSN161649. The content is solely the responsibility of the authors and does not necessarily represent the official views of the AO Foundation or the National Institutes of Health.

CRediT authorship contribution statement
Kevin I. Rolnick : Investigation, Writing – original draft. Joshua A. Choe : Investigation, Writing – original draft. Ellen M. Leiferman : Investigation. Jaclyn Kondratko-Mittnacht : Investigation, Visualization. Anna E.B. Clements : Investigation, Visualization. Geoffrey S. Baer : Writing – review & editing. Peng Jiang : Writing – review & editing. Ray Vanderby : Funding acquisition, Resources, Conceptualization, Supervision, Writing - review & editing. Connie S. Chamberlain : Conceptualization, Supervision, Visualization, Formal analysis, Data curation, Writing - review & editing.

DATA AVAILABILITY
Data will be made available on request.

DECLARATION OF COMPETING INTEREST
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements
The authors acknowledge the technical support of Scott Liegel, B.S. and Katherine Klauser, B.S. for IHC and microscopy, and UW-Madison School of Medicine and Public Health TEM facility for TEM work.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.mbplus.2022.100124.

Keywords:
Periostin; Tendon; Scar; Healing; Collagen; Wound

Abbreviations:
ANOVA, analysis of variance; DAB, diaminobenzidine; ECM, extracellular matrix; H&E, hematoxylin and eosin; IHC, immunohistochemistry; MCL, medial collateral ligament; MTJ, musculotendinous junction; OCT, optimal cutting temperature; OSF-2, osteoblast specific factor-2; Postn, periostin; SMA, smooth muscle actin; TEM, transmission electron microscopy; WT, wild type
References

[1]. Suchak, A.A., Bostick, G., Reid, D., Blitz, S., Jonha, N., (2005). The incidence of Achilles tendon ruptures in Edmonton. Canada. Foot Ankle Int, 26 (11), 932–936.

[2]. Levenson, S.M., Geever, E.F., Crowley, L.V., Oates 3rd, J.F., Berard, C.W., Rosen, H., (1965). The Healing of Rat Skin Wounds. Ann. Surg., 161, 293–308.

[3]. Lin, T.W., Cardenas, L., Soslowsky, L.J., (2004). Biomechanics of tendon injury and repair. J. Biomech., 37 (6), 865–877.

[4]. Nishiyama, T., Kii, I., Kashima, T.G., Kikuchi, Y., Hoazama, A., Shimazaki, M., Fukayama, M., Kudo, A., (2011). Delayed re-epithelialization in peristin-deficient mice during cutaneous wound healing. PLoS ONE, 6 (4), e18410.

[5]. Shimazaki, M., Nakamura, K., Kii, I., Kashima, T., Amizuka, N., Li, M., Saito, M., Fukuda, K., Nishiyama, T., Kitajima, S., Saga, Y., Fukayama, M., Sata, M., Kudo, A., (2008). Peristin is essential for cardiac healing after acute myocardial infarction. J. Exp. Med., 205 (2), 295–303.

[6]. Yang, L., Serada, S., Fujimoto, M., Terao, M., Kobuk, Y., Kitaba, S., Matsu, S., Kudo, A., Naka, T., Murota, H., Katayama, I., (2012). Peristin facilitates skin sclerosis via PI3K/Akt dependent mechanism in a mouse model of sclerosis. PLoS ONE, 7 (7), e41994.

[7]. R.A. Norris, J.D. Potts, M.J. Yost, L. Junor, T. Brooks, H. Tan, S. Hoffmann, M.M. Hart, M.J. Kern, B. Damon, R.R. Markwald, R.L. Goodwin, Peristin promotes a fibroblastic lineage pathway in atroventricular valve progenitor cells, Developmental dynamics : an official publication of the American Association of Anatomists 238(5) (2009) 10526-63.

[8]. Norris, R.A., Damon, B., Mironov, V., Kasyanov, V., Ramamurthi, A., Moreno-Rodriguez, R., Trusk, T., Potts, J.D., Goodwin, R.L., Davis, J., Hoffman, S., Wen, X., Sugiy, K., Kern, C.B., Mjaatvedt, C.H., Turner, D.K., Oka, T., Conway, S.J., Molkenin, J.D., Forgacs, G., Markwald, R.R., (2007). Peristin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. J. Cell. Biochem., 101 (3), 695–711.

[9]. Noack, S., Seiffart, V., Willbold, E., Laggies, S., Winkel, A., Shahab-Osterloh, S., Florkemeier, T., Hertwig, F., Steinhoff, C., Nuber, U.A., Gross, G., Hoffmann, A., (2014). Peristin secreted by mesenchymal stem cells supports tendon formation in an ectopic mouse model. Stem Cells Dev, 23 (16), 1844–1857.

[10]. Minicucci, M.F., Santos, P.P., Rafacho, B.P., Goncalves, A.F., Ardisson, L.P., Batista, D.F., Azzevedo, P.S., Polegato, B.F., Okoshi, K., Pereira, E.J., Paiva, S.A., Zornoff, L.A., (2013). Peristin as a modulator of chronic cardiac remodeling after myocardial infarction. Clinics (Sao Paulo), 68 (10), 1344–1349.

[11]. Elliott, C.G., Wang, J., Guo, X., Xu, S.W., Eastwood, M., Guan, J., Leask, A., Conway, S.J., Hamilton, D.W., (2012). Peristin modulates myofibroblast differentiation during full-thickness cutaneous wound repair. J. Cell Sci, 125 (Pt 1), 121–132.

[12]. Ontsuka, K., Kobuk, Y., Shiraiishi, H., Serada, S., Ohta, S., Tanemura, A., Yang, L., Fujimoto, M., Arima, K., Suzuki, S., Murota, H., Toda, S., Kudo, A., Conway, S.J., Narisawa, Y., Katayama, I., Izuheka, K., Naka, T., (2012). Peristin, a matricellular protein, accelerates cutaneous wound repair by activating dermal fibroblasts. Exp. Dermatol., 21 (5), 331–336.

[13]. Chamberlain, C.S., Brounts, S.H., Sterken, D.G., Rolnick, K.I., Baer, G.S., Vanderby, R., (2011). Gene profiling of the rat medial collateral ligament during early healing using microarray analysis. J. Appl. Physiol., 111 (2), 552–565.

[14]. Chamberlain, C.S., Duenwald-Kuehl, S.E., Okotie, G., Brounts, S.H., Baer, G.S., Vanderby, R., (2013). Temporal Healing in Rat Achilles Tendon: Ultrasound Correlations. Ann. Biomed. Eng., 41 (3), 477–487.

[15]. H. Mutsuzaki, K. Kuwahara, H. Nakajima, Influence of peristin on the development of fibrocartilage layers of anterior cruciate ligament insertion, Orthop. Traumatol. Surg. Res. (2022) 103215.

[16]. Itto, N., Miyagoe-Suzuki, Y., Takeda, S., Kudo, A., (2021). Peristin is Required for the Maintenance of Muscle Fibers during Muscle Regeneration. Int. J. Mol. Sci., 22 (7)

[17]. Kuhn, B., del Monte, F., Hajjar, R.J., Chang, Y.S., Lebeche, D., Arab, S., Keating, M.T., (2007). Peristin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. Nat. Med., 13 (8), 962–968.

[18]. Yin, S.L., Qin, Z.L., Yang, X., (2020). Role of peristin in skin wound healing and pathologic scar formation. Chin. Med. J. (Engl), 133 (18), 2236–2238.

[19]. Wang, Y., Jin, S., Luo, D., He, D., Shi, C., Zhu, L., Guan, B., Li, Z., Zhang, T., Zhou, Y., Wang, C.Y., Liu, Y., (2021). Functional regeneration and repair of tendons using biomimetic scaffolds loaded with recombinant peristin. Nat. Commun., 12 (1), 1293.

[20]. Best, K.T., Nichols, A.E.C., Knapp, E., Hammert, W.C., Ketonis, C., Jonason, J.H., Awad, H.A., Loiselle, A.E., (2020). NF-kappaB activation persists into the remodeling phase of tendon healing and promotes myofibroblast survival. Sci. Signal, 13 (658)

[21]. Crawford, J., Nygard, K., Gan, B.S., O’Gorman, D.B., (2015). Peristin induces fibroblast proliferation and myofibroblast persistence in hypertrophic scarring. Exp. Dermatol., 24 (2), 120–126.

[22]. Kanisicak, O., Khalil, H., Ivey, M.J., Karch, J., Malik, B., D., Correll, R.N., Brody, M.J., SC, J.L., Aronow, B.J., Tallquist, M.D., Molkenin, J.D., (2016). Genetic lineage tracing defines myofibroblast origin and function in the injured heart. Nat. Commun., 7, 12260.

[23]. Rios, H., Koushik, S.V., Wang, H., Wang, J., Zhou, H.M., Lindsley, A., Rogers, R., Chen, Z., Maeda, M., Kruzhynska-Frejtag, A., Feng, J.Q., Conway, S.J., (2005). peristin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. Mol. Cell. Biol., 25 (24), 11131–11144.

[24]. Lindsley, A., Li, W., Wang, J., Maeda, N., Rogers, R., Conway, S.J., (2005). Comparison of the four mouse fascin phenotype defects, and an early-onset periodontal disease-like phenotype. Mol. Cell. Biol., 25 (24), 11131–11144.

[25]. Mosher, D.F., Johansson, M.W., Gillis, M.E., Annis, D.S., (2015). Peristin and TGF-beta-induced protein: Two

wound expression patterns during valvuloseptal morphogenesis. Gene expression patterns ; GEP, 5 (5), 593–600.

[26]. Norris, R.A., Kern, C.B., Wessels, A., Wirig, E.E., Markwald, R.R., Mjaatvedt, C.H., (2005). Detection of betaig-H3, a TGFbeta induced gene, during cardiac development and its complementary pattern with peristin. Anat. Embryol., 210 (1), 13–23.

[27]. Mosher, D.F., Johansson, M.W., Gillis, M.E., Annis, D.S., (2015). Peristin and TGF-beta-induced protein: Two
peas in a pod? Crit. Rev. Biochem. Mol. Biol., 50 (5), 427–439.

[27]. Oka, T., Xu, J., Kaiser, R.A., Melendez, J., Hambleton, M., Sargent, M.A., Lorts, A., Brunskill, E.W., Dorn 2nd, G.W., Conway, S.J., Aronow, B.J., Robbins, J., Molkentin, J.D., (2007). Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. Circ. Res., 101 (3), 313–321.

[28]. Chamberlain, C.S., Clements, A.E.B., Kink, J.A., Choi, U., Baer, G.S., Halanski, M.A., Hematti, P., Vanderby, R., (2019). Extracellular Vesicle-Educated Macrophages Promote Early Achilles Tendon Healing. Stem cells, 37 (5), 652–662.

[29]. Rasband, W.S., (1997). ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA.

[30]. A. International, ASTM E562-19E1: Standard Test Method for Determining Volume Fraction by Systemic Manual Point Count, Annual Book of ASTM Standards2019.