Tight Skin 2 Mice Exhibit Delayed Wound Healing Caused by Increased Elastic Fibers in Fibrotic Skin

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Rationale: The Tight Skin 2 (Tsk2) mouse model of systemic sclerosis (SSc) has many features of human disease, including tight skin, excessive collagen deposition, alterations in the extracellular matrix (ECM), increased elastic fibers, and occurrence of antinuclear antibodies with age. A tight skin phenotype is observed by 2 weeks of age, but measurable skin fibrosis is only apparent at 10 weeks. We completed a series of wound healing experiments to determine how fibrosis affects wound healing in Tsk2/+ mice compared with their wild-type (WT) littermates.

Method: We performed these experiments by introducing four 4mm biopsy punched wounds on the back of each mouse, ventral of the midline, and observed wound healing over 10 days. Tsk2/+ mice showed significantly delayed wound healing and increased wound size compared with the WT littermates at both 5 and 10 weeks of age. We explored the potential sources of this response by wounding Tsk2/+ mice that were genetically deficient either for the NLRP3 inflammasome (a known fibrosis mediator), or for elastic fibers in the skin, using a fibulin-5 knockout.

Conclusion: We found that the loss of elastic fibers restores normal wound healing in the Tsk2/+ mouse and that the loss of the NLRP3 inflammasome had no effect. We conclude that elastic fiber dysregulation is the primary cause of delayed wound healing in the Tsk2/+ mouse and therapies that promote collagen deposition in the tissue matrix in the absence of elastin deposition might be beneficial in promoting wound healing in SSc and other diseases.

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INTRODUCTION

SCLERODERMA (or systemic sclerosis, SSc) is a polygenic, autoimmune disorder of unknown etiology, characterized by the excessive and variable accumulation of extracellular matrix (ECM) proteins, vascular alterations, and production of auto-antibodies. It is a rare disease, and a potentially fatal one, due to consequent lung fibrosis with or without pulmonary arterial hypertension.¹ There is no cure for the disease and only palliative treatment is avail-

able. One of the factors limiting the treatment of SSc is the lack of understanding of the development of disease. Specific triggers of fibrosis are unclear because patients may have advanced disease before diagnosis, and skin biopsy samples from patients can only be investigated after the disease has been well established, highlighting the need for animal models that will allow investigation of early skin changes before the onset of fibrosis and an exploration of traits associated with SSc.

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A complex and incomplete literature surrounds the issue of wound healing in people with SSc. Because poor circulation due to Raynaud's syndrome is common in SSc, it is not surprising that many of them develop digital ulcers that exhibit slow and poor quality healing. Malnutrition contributes to poor healing,² and persons with SSc often suffer from malnutrition caused by gastrointestinal complications.^{3,4} Anecdotally, there are reports of surgical wounds that fail to heal properly in SSc patients, in body regions that are not especially vulnerable to a reduced blood supply. Keloid formation, instead of scarless wound resolution, is seen in individuals with SSc and is considered a wound healing defect.^{5,6} Few objective data exist to compare healing in affected versus unaffected skin, and although scleroderma has been characterized as an excessive wound healing response,⁷ distinguishing whether fibrosis plays an active role in delayed resolution of an open wound still needs to be determined.

Wound resolution is more appropriately studied in animal models, and there are several fibrotic mouse models of SSc, including chemically induced fibrosis and tight skin with a genetic basis. However, no mouse model is completely faithful to the full range of traits in SSc.^{8,9} The two popular genetic mouse models of disease are the *Tsk1* mouse^{10–12} and the *Tsk2* mouse.^{13,14} Each mouse strain bears a different homozygous lethal mutation that requires mice to be bred and evaluated as heterozygotes (e.g., *Tsk1*/+ and *Tsk2*/+). The *Tsk2*/+ mouse has a mutation in the *Col3a1* gene that leads to a missense mutation in the N-terminal peptide, marked increase in collagen accumulation, and loss of the subcutaneous fat layer in the dermis at 10 weeks of age compared with wild-type (WT) littermates.¹⁵ Transcription of the *Col1a1* gene and production of COL1A1 are also upregulated in fibroblasts isolated from *Tsk2*/+ mice^{15–17} at this age. The *Tsk2*/+ mouse recapitulates several facets of the human disease, including increased collagen and autoantibody production late in life.^{15,18} The *Tsk2*/+ mouse also has a classical tight skin phenotype and a small body stature. Most importantly, however, is that we have found that *Tsk2*/+ mice have dramatically increased elastin in the skin.¹⁵

Previous work in our laboratory has shown that the cardinal *Tsk2*/+ traits of fibrosis and tight skin are not dependent on the NOD-like receptor family, pyrin domain containing 3 (NLRP3) activation, or excess elastic fibers.¹⁵ In the present study, we consider the role of NLRP3 and elastic fibers in wound healing independently of fibrosis. We find

that *Tsk2*/+ mice have delayed wound healing as compared with WT littermates that is evident in both prefibrotic (5 weeks old) and postfibrotic (10 weeks old) mice. By crossing *Tsk2*/+ mice to NLRP3 inflammasome knockout (NLRP3-KO) mice or to elastic fiber knockout mice (Fibulin-5 (*Fbln5*)-KO), we determined that this delayed wound is due to excessive elastic fibers and not differential inflammasome activation.

CLINICAL PROBLEM ADDRESSED

An incomplete picture of wound healing in people with SSc prevents our understanding of the role of fibrosis on wound closure. By using an animal model that can be studied for both wound healing and fibrosis, and one where mice with selected genetic deficiencies can be used to probe individual pathways for healing, we have begun to assemble a more complete list of contributing factors for healing in the setting of dermal abnormalities.

MATERIALS AND METHODS

Ethics statement

All studies and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Drexel University, and conducted in accord with recommendations in the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 2011).

Mice

Tsk2/+ mice were obtained from Dr. Paul Christner (Thomas Jefferson University, Philadelphia, PA) with permission from the MRC Radiobiology Unit Laboratory and were housed at the Drexel University College of Medicine. The *Tsk2*/+ mice were originally bred onto the hybrid (C3H x B6) background. At Drexel, they were serially backcrossed to B6 mice. Fibulin-5-deficient mice were a kind gift from Dr. Hiromi Yanagisawa (University of Texas Southwestern Medical Center) and were bred to B6.*Tsk2*/+ mice. NLRP3-KO mice were obtained from Jackson Laboratories. For each experiment, age- and sex-matched WT littermates were used as controls. Mice were housed in individually ventilated autoclaved cages, fed Purina LabDiet® PicoLab® 5053 and 5058 ad libitum, and provided autoclaved water, acidified to pH 3. All studies were approved by the IACUC of Drexel University.

Tsk2/+ microsatellite genotyping

Mice were genotyped for the *Tsk2* locus using PCR of flanking markers: microsatellites *D1Mit233*, *D1Mit235*, and a microsatellite in the *Glutaminase*

gene (*Gls*). PCR products were separated by electrophoresis on a 3% agarose gel. Genotypes were confirmed by skin tightness assessment of every mouse.

Fibulin-5 deletion genotyping

Fibulin-5 deletion was detected using a two primer set PCR, whereby one primer set detected the WT allele and the other detected the KO allele. WT forward (common) primer 5'-AGCTAAGGATAACGAGGTGAGG-3', WT reverse primer 5'-TCAGATTACAGCGCTCACCA-3', and KO reverse primer 5'-AGCATCTATCCAAGCAACTACAG-3'. PCR products were separated by electrophoresis on a 3% agarose gel.

Wound healing: measurements and quantification

On Day 1, 5- and 10-week-old mice were anesthetized with isoflurane and their back hair removed with wax. On Day 0, mice were again anesthetized with isoflurane and four 4 mm full-thickness skin wounds were created with a sterile 4 mm biopsy punch. Wound pictures were taken from 6 inches directly above the mouse. Over days 1–10, mice were anesthetized daily and pictures taken and the wound area was traced using ImageJ software (<http://rsbweb.nih.gov/ij/>). After Day 10, mice were euthanized and back skin was taken for histological analysis. Analysis of wound closure was done with GraphPad Prism software.

Hydroxyproline assay

Mice were euthanized and the lower dorsal fur was removed by shaving. Two 8 mm punches were excised from each piece of skin, snap-frozen, weighed, and stored at -80°C . Protein was isolated for hydroxyproline assays.¹⁹ The optical density was read at 550 nm in triplicate and the hydroxyproline content was determined against a standard curve of hydroxyproline (Sigma-Aldrich).

Western blot

Skin was harvested and collagen content was determined by western blot analysis, as previously described.¹⁵ Briefly, skin was homogenized in the RIPA buffer (Sigma-Aldrich) containing 1% phosphatase inhibitor and 1% protease inhibitor (Sigma-Aldrich). The lysate was centrifuged at 8,000 g for 10 min at 4°C to pellet debris. The supernatant was collected. Approximately 75 μg of protein per sample was added to a reducing buffer, boiled, and then loaded onto an 8% SDS gel. Proteins were transferred to a polyvinylidene fluoride membrane, blocked in 5% nonfat milk in Tris-buffered saline, probed with goat anti-elastin (sc-17581) or goat anti- β -actin (#sc-1616), probed with a secondary antibody

donkey anti-goat (#sc-2020; Santa Cruz Biotechnology, Inc.), and developed using the SuperSignal West Dura ECL reagent (Thermo Scientific, Inc.). Band intensities were measured using ImageQuant TL Software (GE Healthcare Life Sciences).

RESULTS

Tsk2/+ mice have increased wound size and delayed healing

To examine wound healing in the Tsk2/+ mouse, we used two groups: 5- to 6-week-old animals that were considered prefibrotic, and 10-week-old animals that were considered postfibrotic by assessment of total skin collagen.¹⁵ Both male and female Tsk2/+ mice had delayed wound healing (Fig. 1, Table 1). Furthermore, we observed that within 2 days, wounds in Tsk2/+ mice become significantly larger than in WT mice in 5- and 10-week male mice and 10-week female mice. The maximum wound size in Tsk2/+ mice peaks at day 2 postwounding compared with the initial wound (day 0) in WT littermates (Fig. 1). This suggests that a factor relevant to their tight skin syndrome increases the wound size early in wound closure in Tsk2/+ mice.

Wound sizes and the subsequent healing time in mice are dependent on sex and age (Fig. 1). The sex difference is significant between WT mice at 5–6 weeks ($p < 0.0001$) and at 10 weeks ($p = 0.013$); it is even more pronounced between Tsk2/+ males and Tsk2/+ females ($p < 0.0001$). In females, 10-week-old Tsk2/+ mice have larger wounds than 5- to 6-week-old Tsk2/+ mice, yet in males, younger WT mice have larger wounds than older WT mice. The wounds in the majority of WT mice healed by 8 days, whereas most wounds in Tsk2/+ mice failed to close by day 10. As expected, a larger wound was linked to a longer 50% wound closure time (Table 1), however, as demonstrated by the female 5- to 6-week age group, Tsk2/+ mice still had delayed wound healing even when the wounds did not initially expand. Because both the prefibrotic (5–6 weeks) and postfibrotic (10 weeks old) mice display wound healing defects, we can conclude that delayed wound healing in Tsk2/+ mice is not a result of increased total collagen. This leads us to consider what early factors could be responsible for this phenotype.

A deficiency in the NLRP3 inflammasome does not improve wound healing in Tsk2/+ mice

Because fibrosis was likely not the driving factor behind delayed wound healing in Tsk2/+ mice, we explored if an inflammatory factor could be responsible by determining the effect of a deficiency

Figure 1. *Tsk2*^{+/+} mice have delayed wound healing as compared to wild-type littermates at both 5–6 and 10 weeks of age. Female (A) and male (B) littermates were genotyped as WT (loose skin) or *Tsk2*^{+/+} (tight skin). At 5–6 weeks or 10 weeks of age, mice had 4 mm wounds made in back skin. Measurements of residual wound size were made daily and are expressed as a percent of day 0. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

in NLRP3-KO on the wounding phenotype of the *Tsk2*^{+/+} mouse. NLRP3 inflammasome activation is required for skin fibrosis in the bleomycin-induced model of SSc,^{20–22} but not in *Tsk2*^{+/+} mice.¹⁵ To test if NLRP3 activation contributes to poor wound healing, *Tsk2*^{+/+} mice were crossed to NLRP3 knockout mice, and progeny with different *Tsk2* and *Nlrp3* genotypes were tested. Dorsal wounds were made at 10 weeks of age. The mice were grouped by genotype (*Tsk2.Nlrp3*-KO, *Tsk2.Nlrp3*-WT, WT.*Nlrp3*-KO, and WT.*Nlrp3*-WT) and stratified by sex. In two groups (female *Tsk2.Nlrp3*-KO and male WT.*Nlrp3*-KO), the complete deficiency of NLRP3 significantly lengthened the time to 50% wound healing (Fig. 2). In male animals, *Tsk2.Nlrp3*-KO mice had the same delayed wound healing and increased early wound size as *Tsk2.Nlrp3*-WT mice ($p=0.96$, Fig. 2), indicating that the loss of NLRP3 did not restore proper healing; female animals, *Tsk2.Nlrp3*-KO mice had modestly worse healing than female *Tsk2*.

Nlrp3-WT mice. In female mice with WT alleles at the *Tsk2* locus, *Nlrp3*-KO had no significant effect on healing times compared with WT.*Nlrp3*-WT ($p=0.61$). This result shows that the ability to form NLRP3+ inflammasomes does not play a role in the poor wound healing of *Tsk2*^{+/+} mice.

Elastic fibers are the primary cause of delayed wound healing in the *Tsk2*^{+/+} mouse

With both fibrosis and inflammasome activation eliminated as probable causes of delayed wound healing, we explored if elastic fiber dysregulation plays a role in *Tsk2*^{+/+} wound healing. We bred *Tsk2.Fbln5*-KO mice, and confirmed our previous report¹⁵ that elastic fiber loss had no effect on fibrosis as assessed by hydroxyproline at 20 weeks (Fig. 3). *Fbln5*-KO mice can make elastin, but do not assemble elastic fibers in the skin in either WT²³ or *Tsk2*^{+/+} mice.¹⁵

Dorsal wounds were made at 10 weeks of age in mice with the following genotypes: *Tsk2.Fbln5*-KO, *Tsk2.Fbln5*-WT, WT.*Fbln5*-KO, and WT.*Fbln5*-WT. The loss of *Fbln5* restored normal wound healing to *Tsk2*^{+/+} mice of both sexes (Figs. 4, 5). The wounds in both male and female *Tsk2.Fbln5*-KO mice close at a rate and to a degree that is very similar to WT mice. Furthermore, wounds in the *Tsk2.Fbln5*-KO mice did not expand as much after day 0, as they do in *Tsk2.Fbln5*-WT mice, and instead began closing similar to WT or WT.*Fbln5*-KO mice (Fig. 5). *Tsk2.Fbln5*-HET male and female mice healed similarly to *Tsk2.Fbln5*-WT indicating that hetero-

Table 1. Mean 50% wound closure time (days)

	Male	Female
WT at 5–6 weeks of age	4.71	2.32
WT at 10 weeks of age	3.22	3.89
<i>Tsk2</i> ^{+/+} at 5–6 weeks of age	9.15	7.56
<i>Tsk2</i> ^{+/+} at 10 weeks of age	9.85	7.51

Littermates were genotyped as WT (loose skin) or *Tsk2*^{+/+} (tight skin), and 4 mm full thickness skin wounds made at 5–6 weeks or 10 weeks of age and assessed daily. A best fit nonlinear regression was performed and the 50% wound closure time (in days) was interpolated for each group.

Figure 2. NLRP3 deficiency does not improve poor healing in *Tsk2* mice. Female (A) and male (B) littermates were genotyped as WT (loose skin) or *Tsk2*/+ (tight skin), and genotyped for expression of NLRP3. Four 4 mm wounds were made in back skin at 10 weeks of age. Measurements of residual wound size were made daily and are expressed as a percent of day 0. As measured in 2-way ANOVA, *Nlrp3*-WT mice did not differ significantly from *Nlrp3*-KO mice within each sex. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

zygosity for *Fbln5* is sufficient to cause delayed wound healing in *Tsk2*/+ mice (Fig. 6). Notably, all *Fbln5*-KO groups healed better than their *Fbln5*-WT counterparts (Fig. 4). WT.*Fbln5*-KO healed somewhat better (males) or considerably better (females) than WT.*Fbln5*-WT, indicating that normal skin elastin can inhibit early wound healing, in contrast to previous findings.²³ These results demonstrate that FBLN5 proteins or the skin elastic fibers containing FBLN5 and elastin, are responsible

for the two major features of delayed wound healing in *Tsk2*/+ mice: the expansion of the wound in the first 2 days of wound resolution, and the significantly delayed overall healing.

DISCUSSION

Mammalian wound healing comprises four overlapping phases: the immediate phase; the inflammatory phase; the proliferation, migration/

Figure 3. Fibrosis is unchanged in *Tsk2.Fbln5*-KO mice. (A) *Tsk2.Fbln5*-KO have the same excess collagen levels as *Tsk2.Fbln5*-HET mice. The 8 mm punches were excised from the back skin of 20 week male mice and analyzed for collagen content by hydroxyproline analysis. The hydroxyproline assay shows no significant difference in total collagen between *Tsk2*/+ samples either with or without fibulin-5, and significantly elevated collagen in both compared with WT (B6) littermates. (B) *Fbln5*-KO mice produce significantly less elastin as compared with *Fbln5*-HET mice. Protein isolated from mouse skin and analyzed by western blot. The gel was transferred to a membrane and probed with anti-elastin and anti-actin antibodies.

Figure 4. *Fbln5*-KO improves wound healing in both *Tsk2*/+ and wild-type littermates. Postwound skin pictures of females (left) and males (right) on day 0, 2, 6, and 10. Mice were anesthetized, hair was waxed, and full-thickness excisional wounds were created on the back of each animal with a 4 mm biopsy punch. Wound sizes were calculated using ImageJ. *Tsk2*/+ male mice with WT *Fbln5* exhibit an initial increase in wound size (maximum at day 2) followed by delayed healing when compared with wild-type mice. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

contraction phase; and the resolution phase.²⁴ The immediate phase occurs directly after injury.²⁵ During this stage, the platelets and surrounding immune cells release cytokines to aid the healing process. Together, immune cytokines, such as IL-1 and colony-stimulating factors, induce the forma-

tion of serum response factor, which leads to the increase in transcription of hundreds of genes important for the initiation of wound healing.²⁶ The inflammatory phase extends from day 0 to 6 post-wounding in mice.^{24,27} Next, the proliferation/migration/contraction phase begins at day 4 and

Figure 5. Elastic fiber deficiency improves poor healing in *Tsk2*/+ mice. Ten-week-old female (A) and male (B) littermates were genotyped for *Fbln5* and for *Tsk2* and had 4 mm dorsal skin wounds made at this time. Measurements of residual wound size were made daily and are expressed as a percent of day 0. As measured in 2-way ANOVA comparing male mice, the *Tsk2*/+ fibulin-5-sufficient mice had significantly different healing ($p < 0.0001$) compared with every other strain at each day, and the *Tsk2.Fbln5*-KO is similar to WT mice. In female mice, there is a significant difference due to *Tsk2* ($p < 0.0001$ day 2–8), and loss of *Fbln5* in *Tsk2*/+ mice rendered them statistically similar to WT.*Fbln5*-WT mice; in addition, there was a small but significant difference after day 5 between WT mice and WT mice bearing the *Fbln5*-KO. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 6. Wound healing in *Fbln5*-heterozygotes compared with *Fbln5*-WT littermates shows that one copy of *Fbln5* is sufficient to promote healing defect. Female (A) and male (B) littermates were genotyped as WT (loose skin) or *Tsk2*/+ (tight skin) and genotyped for *Fbln5*. Measurements of residual wound size in 10-week-old mice were made daily and are expressed as a percent of wound size on day 0. Wound healing of *Fbln5*-HET mice was graphed against *Tsk2.Fbln5*-WT mice and stratified by sex and *Tsk2* genotypes. A nonlinear regression determined that *Tsk2.Fbln5*-HET mice and *Tsk2.Fbln5*-WT mice heal at similar rates. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

stretches to day 14,²⁷ and is necessary for the permanent closure of the wound and the repair of damaged tissue. Alterations and inhibition of components in this process at any point can impede wound healing, and our studies attempted to determine which of these factors contributes to the significantly impaired healing seen in *Tsk2*/+ mice.

Collagen has a well-established role in early wound healing. During the proliferative phase, type III collagen (COL3A1) is secreted by migrating and proliferating fibroblasts, in and around the wound bed.²⁷ This collagen is vital for creating the provisional matrix during this phase.²⁷ Because the *Tsk2* mutation is in *Col3a1* (Long *et al.*, in revision, 2014), we anticipated that fibrosis itself would account for the poor wound healing in this strain, but deficient wound closure is seen before the accumulation of collagens in the skin, as reflected by the poor wound closure in 5-week-old *Tsk2*/+ mice, which do not yet display skin fibrosis.¹⁵ Type I collagen (COL1A1) is also important during the maturation stage when COL1A1 replaces the COL3A1 laid down in the granuloma tissue—COL1A1 fibrils are stronger and the most prevalent collagen in unwounded skin.²⁷ Mice bearing a mutant COL1A1 that is resistant to metalloproteinase cleavage are very deficient in wound healing.²⁸ However, excess collagen of this type is not the cause of the impaired wound healing in *Tsk2*/+ mice, as young, pre-fibrotic *Tsk2*/+ mice are not able to close dorsal wounds as well as their WT littermates.

Nlrp3 is a gene that encodes a pyrin-like protein, NLRP3, which interacts with apoptosis-associated proteins containing a caspase recruitment domain.²⁹ Activation of the NLRP3 inflammasome contributes to bleomycin-induced tissue injury because *Nlrp3*-KO mice injected with bleomycin do not develop fibrosis in the skin or lungs.^{20–22} During wound healing, the inflammasomes become activated and IL-1 β is cleaved when caspase-1 is processed to its mature form.²⁹ This process can occur in numerous cell types in the wound bed, including platelets and endothelial cells.²⁷ IL-1 β has many proinflammatory effects, including up-regulating the expression of collagens and TGF- β 1, required for wound healing. In the setting of excess fibrosis seen in *Tsk2*/+ mice, it might be expected that NLRP3 could contribute to poor wound healing by exaggerating the fibrotic response. However, *Tsk2.Nlrp3*-KO mice have the same amount of fibrosis as *Tsk2.Nlrp3*-WT littermates, and *Tsk2.Nlrp3*-KO still have impaired wound healing, not improved. Thus, the wound healing defect characteristic of *Tsk2*/+ mice is the same in tight skin animals whether or not they express NLRP3.

Elastic fibers have a less well-defined role in wound healing. *Fbln5* mRNA and elastic fibers appear late, after 14 days, in the wound healing process.²³ Work in a rabbit model found that ectopic elastin applied to the wound bed can decrease wound healing time³⁰; however, reduction of elastin fiber formation in *Fbln5*-KO mice either had no effect on mouse wound healing²³ or, in the present

study, genetic deletion of *Fbln5* significantly enhanced healing, and reduced the time to 50% wound closure (Fig. 5). Taken together, these results indicate that reducing elastic fibers can be beneficial to wound healing in mice, promoting earlier wound closure. Furthermore, we show that an overabundance of elastic fibers in Tsk2/+ skin has a detrimental effect on wound healing. Of course, further studies would be required to extend these results directly to people with fibrosis, as the Tsk2/+ model is a single-gene defect, and mouse skin has a substantially different architecture that does not adequately model the complexity of healing in the human being.

Whereas little is known regarding the rate of healing in SSc patients, it is known that SSc patients, especially those with severe, diffuse disease, are at a greater risk for developing skin ulcers³¹ and they show dysregulation of elastic fibers in their skin.^{32,33} Our studies in mice suggest that elastic fiber dysregulation can increase both the initial wound size and healing time. This is the first time elastic fibers have been shown to have a role in early wound healing processes, indicating that the overall health of the ECM and not just vascular insufficiency should be taken into account when considering the explanation for poor quality wound healing. Thus, therapies that promote collagen deposition in the tissue matrix in the absence of elastin deposition might be beneficial in promoting wound healing in SSc and other diseases characterized by chronic wounds.

INNOVATION

The ability to study wound closure in the Tsk2/+ model of a severe, fibrotic autoimmune human disease is novel, especially because we discovered that these mice exhibit a very significantly delayed dorsal wound response. Using this model, we teased apart the various factors that contribute to poor quality wound healing using genetic approaches and found that dermal elastic fibers, contrary to expectation, can impede wound closure when in great excess compared with normal skin.

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KEY FINDINGS

- The delayed wound healing in Tsk2/+ mice (a model of systemic sclerosis) is not related to their fibrosis *per se* because it is evident at ages when substantial fibrotic accumulation of the ECM has not yet occurred.
- The delayed wound healing in the Tsk2/+ mice is not due to the presence of inflammation that is dependent on the NLRP3 inflammasome. However, as might be predicted from the theory that a modest level of inflammation is required for optimal healing, all mice healed more slowly, sometimes significantly so, when they could not activate the NLRP3 pathway.
- The delayed wound healing in Tsk2/+ mice is due to excessive elastin- and fibulin-5-containing elastic fibers, because the *Fbln5*-KO restores healing kinetics and closure to near normal levels in Tsk2/+ male and female mice.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article. All authors contributed to the writing and the review of the article.

ABOUT THE AUTHORS

Kristen B. Long and **Chelsea M. Burgwin** contributed equally to this work. Kristen did her doctoral thesis on the Tsk2/+ mouse model in the Blankenhorn laboratory, and is currently a post-doctoral researcher at the University of Pennsylvania. Chelsea Burgwin is studying this mouse model for her doctoral dissertation work at the Drexel University in the Molecular Cell Biology and Genetics graduate program. **Dr. Richard Huneke** is a veterinarian and head of the University Laboratory Animal Resources at Drexel University College of Medicine, and was very helpful in assisting us in the practice of wound instillation and measurement in an ethical and humane manner. **Dr. Carol M. Artlett** is a contributing author with a long and substantial interest in SSc and fibrosis, and is currently Associate Professor in the Department of Microbiology and Immunology at Drexel University College of Medicine. **Dr. Elizabeth P. Blankenhorn** has a long-standing interest in genetics and animal models of complex diseases. She heads the Research Center of Excellence in Immunogenetics & Inflammatory Disease in the Institute for Molecular Medicine and Infectious Disease at Drexel University College of Medicine and is a Professor in the Department of Microbiology and Immunology.

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Abbreviations and Acronyms

B6 = C57Bl/6J

COL1A1 = collagen, type 1, alpha 1

COL3A1 = collagen, type 3, alpha 1

ECM = extracellular matrix

Eln = elastin

Fbln5 = fibulin-5

mRNA = messenger RNA

NLRP3 = NOD-like receptor family, pyrin domain containing 3

SSc = systemic sclerosis

Tsk = tight skin

WT = wild type