Dermal Fibroblast SLC3A2 Deficiency Leads to Premature Aging and Loss of Epithelial Homeostasis

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Skin homeostasis relies on fine-tuning of epidermis–dermis interactions and is affected by aging. While extracellular matrix (ECM) proteins, such as integrins, are involved in aging, the molecular basis of the skin changes needs to be investigated further. Here, we showed that integrin co-receptor, SLC3A2, required for cell proliferation, is expressed at the surface of resting dermal fibroblasts in young patients and is reduced drastically with aging. In vivo SLC3A2 dermal fibroblast deletion induced major skin phenotypes resembling premature aging. Knockout mice (3 months old) presented strong defects in skin elasticity due to altered ECM assembly, which impairs epidermal homeostasis. SLC3A2 dermal fibroblast loss led to an age-associated secretome profile, with 77% of identified proteins belonging to ECM and ECM-associated proteins. ECM not only contributes to skin mechanical properties, but it is also a reservoir of growth factors and bioactive molecules. We demonstrate that dermal fibroblast SLC3A2 is required for ECM to fully exert its structural and reservoir role allowing proper and efficient TGF-β localization and activation. We identified SLC3A2 as a protective controller of dermal ECM stiffness and quality required to maintain the epidermis to dermis interface as functional and dynamic.


INTRODUCTION
Intrinsic and extrinsic human skin aging involves a number of changes, such as reduced epidermal proliferation, impaired melanocyte functions, and decreased extracellular matrix (ECM) component biosynthesis, such as collagen types (McCullough and Kelly, 2006; Yaar et al., 2002). Skin homeostasis relies on reciprocal signaling between the epidermis and dermis, but the precise consequences of aging on this constant dialogue are not well known. Considering the great advances in big data access, potential exists for on this constant dialogue are not well known. Considering the great advances in big data access, potential exists for aging research while minimizing bias. Unfortunately, few studies have focused on skin aging, specifically on isolated dermal fibroblasts (DFs) from young and elderly patients. To our surprise, expression of the transmembrane protein SLC3A2 is modulated at the surface of these quiescent stromal cells during aging.

SLC3A2 (CD98hc, 4F2) is the ubiquitously expressed heavy subunit of heteromeric amino acid transporters that binds to an SLC7 family member. As such, SLC3A2 full-body knockout (KO) is lethal between embryonic days 3.5 and 9.5 (Tsumura et al., 2003). Besides fulfilling cellular amino acid requirements, SLC3A2 binds to β1 and β3 integrins and regulates their signaling properties in vitro (Fenczik et al., 1997). Its deletion induces defects in integrin-dependent cell behaviors, including fibroblast assembly, both in vitro and in vivo (Féral et al., 2007). SLC3A2, which is expressed at the cell surface of proliferative cells in multiple organs, specifically epithelial cells, is required for tissue homeostasis (Boulter et al., 2013; Bulus et al., 2012; Nguyen et al., 2011). In the skin, we have previously shown that keratinocytes rely on SLC3A2 for proper and efficient skin wound closure in adult mice, which was impaired during aging due to decreased SLC3A2 epidermal expression. However, to our knowledge, SLC3A2 has never been found in noncancerous stromal cells during aging.

Here we report that, in human skin, SLC3A2 is expressed in quiescent DFs and its loss correlates with aging. We constitutively deleted SLC3A2 in mouse dermal fibroblasts in vivo. To avoid targeting specific fibroblast subtypes (Lynch and Watt, 2018), we used global fibroblast promoter Fsp1 under Cre recombinase. Mice bearing this constitutive SLC3A2 DF deletion presented disrupted epidermal homeostasis, due to a major skin ECM defect, reminiscent of aged skin. Overall, we found that SLC3A2 loss in DFs induces premature skin aging. Consistently with in vivo ECM defects, dermal elastic modulus decreased from 4 kPa to 1 kPa in KO mice as young as 3 months old. A comparison of secretome from SLC3A2-deficient and SLC3A2-expressing DFs revealed a

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Abbreviations: DF, dermal fibroblast; ECM, extracellular matrix; KO, knockout; WT, wild type

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major age-related matrisome profile. ECM stiffness was reduced in the absence of dermal SLC3A2, as well as its bioactivity. As such, SLC3A2 is required for efficient TGF-β growth factor–loading in ECM. We found that dermis to epidermis intercommunications via SLC3A2 are key crosstalk to preserve skin homeostasis during aging.

RESULTS

Quiescent DFs express proliferation enhancer SLC3A2 in vivo

In the skin, transcriptional or translational analyses comparing DFs isolated from young and elderly patients are scarce (Jung et al., 2015; Won et al., 2012). We used RNA-sequencing data comparing DFs from patients of varying ages from 41 to 75 years (sequential biopsies, paired samples, GSE51518 (Jung et al., 2015), which allowed definition of intra-individual changes. We focused on the transmembrane protein SLC3A2, which is usually highly expressed on proliferative cells. Using this longitudinal age-related analysis, we found that SLC3A2 is expressed in resting DF cells. Then, we found that its expression was systematically reduced with age in these dermal cells (Figure 1a, 1b), which we confirmed on human tissue sections (Figure 1c). We aimed to decipher SLC3A2 function in resting DFs in vivo.

Dermal SLC3A2 loss leads to skin premature aging

We now showed that SLC3A2 is expressed not only in human skin epidermis (Boulter et al., 2013), but also in dermis. While epidermal keratinocyte SLC3A2 expression regulates skin homeostasis during aging (Boulter et al., 2013), we should decipher the contribution of DF SLC3A2 in this process. To do so, we constitutively deleted SLC3A2 gene in DFs in vivo. To avoid targeting specific fibroblast subtypes (Lynch et al. 2015), we used transgenic mice carrying constitutively active Cre recombinase under FSP1 promoter were crossed with mice carrying loxP-flanked alleles (SLC3A2<sup>fl/fl</sup>) (Féral et al., 2007). As expected, the SLC3A2 floxed allele was recombined specifically in the dermis and not in the epidermis of KO mice (Supplementary Figure S1a online). Accordingly, SLC3A2 protein was detectable at normal levels in primary keratinocytes from SLC3A2-deficient DF KO mice and absent in DFs (Supplementary Figure S1c and S1d, respectively). SLC3A2-deficient DF mice appeared healthy and fertile (Supplementary Figure S1b).

At 3 months old, we found a strong decrease in collagen fiber assembly in KO dermis in vivo (Figure 1d, quantified in Figure 1e) compared to wild type (WT), to the same extent as in elderly WT mice. Elastin fibers shortened in both young SLC3A2-deficient DF mice (3-month-old KO, 9.8 ± 1.5 μm) and elderly WT s (24- to 27-month-old WT, 9.6 ± 0.8 μm) compared to young WT (3-month-old WT, 16.98 ± 1.8 μm) (Figure 1g, 1g). Consequently, at 3 months old, KO mouse skin exhibited a 4-fold decrease in dermis elasticity compared to WT, as measured by atomic force microscopy (Figure 1h). The elastic modulus of 3-month-old KO (0.9 ± 0.38 kPa) and 24- to 27-month-old WT (1.3 ± 0.3 kPa) dermis was comparable. We found that SLC3A2 deletion leads to skin defects that strongly mimic premature aging.

Dermal fibroblast SLC3A2 is required for keratinocyte organization but is dispensable for keratinocyte stem cell maintenance

Reduction of dermal collagen and elastic fibers, caused by aging, induces ECM disruption. We demonstrated drastic dermal ECM defects in vivo after SLC3A2-deficiency in DFs. As skin integrity and function are maintained by epidermal organization, we should analyze the status of epidermal homeostasis in young dermal KO mice. First, SLC3A2 deficiency had little effect on gross skin morphology. Histologically, hematoxylin and eosin staining only showed weakly misoriented hair follicle (Figure 2a, resting phase of hair growth cycle). Second, immunofluorescent staining of K14, a basal monolayer marker, revealed a threefold increase of K14<sup>+</sup> clusters (i.e., when at least one K14<sup>+</sup> cell is located above the basal layer) in young KO versus young WT controls (Figure 2b, 2c), a phenotype usually observed only in elderly mice (here 24–27 months old, Figure 2b, 2c). Similarly, staining for K10, a marker for suprabasal differentiated keratinocyte layer, was observed in the basal layer of both young dermal KO and elderly WT mice, indicating a normal keratinocyte premature differentiation. As expected, K10 expression pattern was restricted to the suprabasal layer in young WT skin (Figure 2d, 2e). When analyzing mRNA levels of both K10 and K1 in full skin samples, KO presented much higher levels than WT (Figure 2g). Finally, immunofluorescent detection of later differentiation marker involucrin strengthened the premature differentiation phenotype (as observed with K10 staining on skin). No induction of hyper-differentiation was observed. Interestingly, the SLC3A2 DF KO-induced aging phenotype did not progress beyond the aged phenotype of WT mice, underscoring its premature aspect (Supplementary Figure S2 online). As cells from the outermost layer of the skin are continuously being shed, a constant replenishment is necessary to maintain homeostasis. Thus, we analyzed, by quantitative real-time reverse transcriptase–PCR, whether dermal SLC3A2 deficiency could drive loss of stem cell pools. Hair follicle stem cell markers (CD34, Lhx2, Lgr5, Lrig1, and K15) revealed no significant defect between KO and WT young skin (Figure 2h, confirmed by flow cytometry analysis, Figure 2i). Although SLC3A2 dermal deletion perturbs the overall skin epidermal organization in vivo, the stem cell niche is preserved.

Mechanically challenged keratinocytes failed to efficiently organize and proliferate in SLC3A2 DF-deficient environment in vivo

We used a physical challenge to remove the stratum corneum and force epidermal proliferation and stratification by several applications of adhesive tape on the back skin of young 3-month-old mice (KO and WT). Seven days later, hematoxylin and eosin and K14 staining revealed a strong tape-stripping–induced epidermal thickening in WT compared to unstimulated skin, but this increase was significantly reduced in KO mice (1.6-fold) (Figure 3a–3c). This was also associated with persistent mislocalization of K10<sup>+</sup> signal in basal layer of KO compared to WT-stripped mice (Figure 3d, arrows). Immunofluorescent staining for proliferating cell nuclear antigen, a cell proliferation marker, showed a significant decrease in proliferative keratinocytes in the basal
layer of stripped KO versus WT mice (Figure 3e, 3f). Strikingly, when keratinocytes were directly stimulated by 12-O-tetradecanoylphorbol-13-acetate topical skin application, no intrinsic proliferative defect was observed in the absence of DF SLC3A2 (Figure 3g, 3h). These results uncover a pivotal role of dermal SLC3A2 in maintaining a functional environment for keratinocyte-efficient response to external mechanical challenge.

Dermal fibroblast SLC3A2 is required to sustain WT keratinocyte proliferation in vitro
To decipher molecular mechanisms involved in epidermis–dermis interaction, we tested the ability of DFs (isolated from both WT and KO 3-month-old mice) to support primary WT keratinocyte proliferation in vitro as classical embryonic feeders do (Figure 4a, 4b), by colony-formation efficacy assay. While WT DFs sustained WT keratinocyte
Figure 2. Dermal SLC3A2 is required for epidermal keratinocyte organization and homeostasis. (a) Hematoxylin and eosin staining of representative back-skin sagittal sections of young WT and KO mice (scale bar = 200 μm). (b–f) Immunofluorescence staining of young WT and DF SLC3A2 KO (DermKO) and 24- to 27-month-old WT mice (Old WT) back-skin sections with antibody against basal keratinocyte marker K14 (b, c), differentiated keratinocyte marker K10 (d, e), and later differentiation marker involucrin (f). Note abnormal K14+ cluster (defined as a minimum of 1 K14+ above the basal layer) in epidermis of DF KO skin, as well as anomalous presence of K10+ staining in keratinocyte basal layer of DF KO skin. Arrowheads point to keratinocyte with the abnormal staining. Scale bar = 100 μm, n = 3 minimum per group. (g, h) Whole-skin mRNA expression of K10 and K1 (g) as well as epidermal stem cells markers from young WT and DermKO mice quantified by quantitative real-time reverse transcriptase–PCR (n = 4–8 per group, h). (i) No defect observed in α6+CD34+ epidermal stem cell population analyzed by flow cytometry on primary keratinocytes isolated from the dorsal epidermis of WT or DermKO mice. DF, dermal fibroblast; KO, knockout; WT, wild type. For all histograms, data represented are means, error bars are standard error of mean. *P > 0.05, **P < 0.01.
colony-formation efficacy with more than 150 (177 ± 14.4) clones formed after a 2-week coculture period, KO DFs did not support clonal proliferation (5.6 ± 0.7 clones formed) (Figure 4d, 4e). This defect was rescued when KO fibroblasts were reconstituted with full-length SLC3A2. Expression levels of SLC3A2 were confirmed by flow cytometry each cell type (Figure 4c). We showed that DF SLC3A2 is required to maintain keratinocyte undifferentiated in vitro. Low dermal stiffness forced keratinocyte differentiation As an external signal, ECM mechanical stiffness is able to govern cell fate (Janmey and Miller, 2011; Panciera et al., 2017; Schwartz, 2010; Vogel and Sheetz, 2006). The main phenotype observed in vivo after SLC3A2 dermal deletion corresponds to skin biomechanical properties, resembling aging-induced changes. We wondered whether KO low stiffness could solely be sufficient to induce impaired keratinocyte behavior. To do so, we used optimized culture conditions (i.e., WT embryonic feeder conditioned medium) and compared the differentiation status of primary undifferentiated WT keratinocytes plated on collagen-coated 4-kPa versus 1-kPa hydrogels, mimicking the in vivo young WT and KO dermal environment, respectively (Figure 4f, 4i). Forty-eight hours after plating undifferentiated WT keratinocytes on 1-kPa stiffness, 60% were differentiated, as shown by involucrin expression, a late suprabasal epidermal marker (Figure 4f, 4g). Conversely, WT stiffness (4 kPa) preserved keratinocyte differentiation status (only 5% were involucrin positive). The expected transcription activity mediated by YAP/TAZ, a nuclear relay for mechanical signals (Lee et al., 2014), was reduced on 1 kPa compared to 4 kPa. Specifically, CTGF mRNA expression, which controls production of fibrogenic factors, such as collagens, was decreased 2-fold in WT keratinocytes plated on 1 kPa hydrogel versus 4 kPa (mimicking KO vs. WT in vivo dermal environment) (Figure 4h). In vivo CTGF expression confirmed this with a 2-fold reduction of KO whole skin compared to WT (Figure 4i). Altogether, we demonstrate that proper dermal...
stiffness is required to maintain YAP/TAZ-mediated keratinocyte undifferentiated state, essential for the maintenance of skin homeostasis.

Absence of dermal SLC3A2 leads to age-associated secretome profile
The dermal ECM provides strength and resiliency to skin and maintains basal keratinocyte undifferentiated and proliferative. We investigated whether factors secreted by DFs could also modulate behavior of keratinocytes plated on 4-kPa hydrogels (mimicking young WT dermal environment). This time, we used conditioned medium from WT or KO DFs instead of the optimized culture conditions (i.e., embryonic feeder conditioned medium, Figure 4e, 4f). While WT conditioned medium preserved WT keratinocyte undifferentiated, involucrin signal was strongly induced, even on 4 kPa, when treated with KO conditioned medium (Figure 5a, 5b).

To identify implicated secreted factors, we performed a comparative mass spectrometry analysis of serum-free conditioned media from WT and KO DFs. A summary of the identified factors is shown in Table 1. Interestingly, we found that WT conditioned medium contained higher levels of CTGF (connective tissue growth factor) mRNA in keratinocytes plated on 4-kPa hydrogels compared to KO conditioned medium (Figure 5c, 5d). This suggests that WT DFs secrete factors that promote keratinocyte differentiation in response to increased stiffness.

Figure 4. DF SLC3A2 is required to sustain WT keratinocyte clonal proliferation in vitro. (a) Scheme of cell source for coculture experiments. (b) WT primary keratinocytes seeded on irradiated cells, either embryonic fibroblasts or WT DFs (SV40 large T antigen immortalized), showing WT DF capacity to sustain primary keratinocyte clonal proliferation. (c) Flow cytometry profile of immortalized DFs isolated from DermKO and age-matched WT mice confirming lack of endogenous murine SLC3A2 (mSLC3A2) expression in KO cells versus WT (left panel). Reconstitution of KO cells with human SLC3A2 (hSLC3A2) was also confirmed (right panel). (d) Colony-forming efficiency (CFE) of primary WT keratinocyte seeded on WT, KO DFs (isolated from back skin of WT or SLC3A2 KO DF mice) or KO DFs reconstituted for SLC3A2 expression (Rescue). Staining of 2-week culture plates with rhodamine B (representative images, experiment performed three times). (e) Quantification of clones that formed in each condition. Note that SLC3A2 KO DFs cannot sustain WT keratinocyte clonal expansion. (f) Forty-eight-hour primary WT keratinocyte culture (with WT embryonic feeders conditioned medium) on either 4-kPa or 1-kPa hydrogels, recapitulating in vivo stiffness of WT and KO dermis, respectively. Immunofluorescent staining of differentiation marker involucrin (green) with nuclei in DAPI (blue). (g) Percentage of involucrin-positive (inv+) keratinocytes from (f). (h) Analysis of CTGF mRNA expression (evaluated by quantitative real-time reverse transcriptase-PCR) of primary keratinocytes cultivated on hydrogels from panel (f) and whole back skin of WT and SLC3A2 dermal KO (DermKO) mice. Soft environment, associated with DF SLC3A2 deficiency, induces keratinocyte differentiation. DF, dermal fibroblast; KO, knockout; WT, wild type.
ultracentrifugated conditioned medium from WT versus KO DFs. Secretome analysis revealed that KO cells released an altered protein pattern in the extracellular milieu, both quantitatively and qualitatively, compared to WT cells. Among the 56 differentially secreted proteins identified (Supplementary Table S1 online), 77% were linked to ECM components (matrisome), which represented the most abundant altered biological processes (Figure 5c). Notably, for 12 components this major ECM dysregulation induced by SLC3A2 deficiency was associated with an age abundance change described previously (Waldera Lupa et al., 2015), such as the enzyme lysyl oxidase, responsible for the crosslinking of the elastin and collagen precursors (9.44-fold decrease in KO compared with WT cells).

To investigate possible interactions of differently regulated genes, data sets representing the 56 genes with altered expression profile were imported into the Ingenuity Pathway Analysis tool, as described in the Supplementary Materials and Methods (online). This analysis revealed five significant networks (Table 1). The highest score was obtained for cellular assembly and organization, cellular function and maintenance, and dermatological diseases and conditions. Molecules are represented as nodes, and the biological relationship between two nodes is represented as an edge. A solid line represents a direct interaction between the two gene products and a dotted line means there is an indirect interaction. All edges are supported by at least one reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base. The color of the node indicates whether the gene expression has an up-regulation (in red) or down-regulation (in green). Nodes are displayed using various shapes that represent the functional class of the gene product. Nodes are also placed in proper cell compartments (cytoplasm, membrane, extracellular space, other). DF, dermal fibroblast; KO, knockout; WT, wild type.
the ECM environment after SLC3A2 deletion in DFs (Figure 5).

Dermal SLC3A2 is required to maintain ECM reservoir capacity

Besides well-defined physical and biomechanical properties, the ECM is also a rich reservoir of growth factors, making them insoluble, unavailable, and not bioactive. A coordinate decrease of CTGF, TGF-β, and type I procollagen expression and content is a hallmark of aging skin and fibroblasts (Quan et al., 2013). Sequestration of latent TGF-β, by binding to LTBP1 in the ECM, is crucial for TGF-β proper mobilization and activation.

SLC2A3 dermal deficiency alters the ECM physical properties and the pattern of secreted proteins in the extracellular space. We asked whether ECM reservoir capacities could be compromised in the absence of SLC3A2. Our secretome analysis revealed a 2.52-fold decrease in LTBP1 secretion in KO compared to WT fibroblasts (Figure 5d and Supplementary Table S1), which was confirmed in vitro by Western blot (Figure 6a) and cell immunofluorescent staining (Figure 6b). Skin tissue sections, from WT and dermal KO skin, stained for LTBP1 by immunofluorescence, validated this result in vivo (Figure 6c). Using reporter cell line MFB-F11 (TGF-β-deficient but expressing secreted alkaline phosphatase under a downstream TGF-β effector SMAD responsive element) (Tesser et al., 2006), we observed mild decrease in TGF-β-secreted level in KO compared to WT DFs (Figure 6d). This reduction was strengthened when analyzing TGF-β released from the ECM generated by either KO or WT DFs after stretching (Figure 6e). Thus, we show that DF SLC3A2 contributes to generate an ECM with a reservoir capacity, particularly for TGF-β bioavailability. Altogether, we demonstrate that SLC3A2 is required to preserve ECM dynamic biophysical, mechanical, and biochemical properties coordinately with efficient TGF-β signaling during aging.

DISCUSSION

Recent studies have attempted to define the molecular signature of aging, specifically for mammalian stromal cells. Using these publicly available data, we focused our attention on SLC3A2, an integrin signaling enhancer, for which epithelial expression is required for tissue homeostasis, as shown in multiple organs including skin (Boulter et al., 2013; Bulus et al. 2012; Nguyen et al., 2011). Here, we first show that SLC3A2 was unexpectedly expressed on stromal cells, namely quiescent DFs (nonactivated). Second, its dermal expression decreased during aging in humans. In mice, DF SLC3A2 deficiency induced a strong premature aging skin phenotype due to major ECM stiffness dysregulation. Consequently, epidermal homeostasis was disrupted, as observed by premature keratinocyte differentiation in vivo. DF secretome analysis revealed that SLC3A2 is required for controlling ECM reservoir capacity by regulating TGF-β bioavailability through LTBP1 expression. We identified stromal SLC3A2 as a protective regulator of ECM's physical and biophysical properties during aging, thus maintaining the functional and dynamic interface between the epidermises and the underlying dermises.

SLC3A2, which is highly expressed in basal keratinocytes in both human and mouse skin, is crucial for epidermal homeostasis (Boulter et al., 2013; Lemaitre et al., 2011). We unexpectedly found that SLC3A2 is not only expressed in epithelial cells in vivo, but also in quiescent stromal DF cells. SLC3A2 is a cell proliferation booster, specifically during lymphocyte activation and cancer progression (Bajaj et al., 2016; Cantor et al., 2009). Thus, we define a role for stromal SLC3A2 in tissue homeostasis, independent of competitive proliferation.

To define the characteristic features of DFs in vivo, Waldera Lupa et al. (2015) isolated DFs from intrinsically aged human skin and revealed not only the classical senescence-associated secretory phenotype profile, but also an age-dependent pattern, specific to the skin. SLC3A2 is described as a long-term, age-associated, decreased protein, suggesting that it is intrinsically associated with DF aging. Our work, regarding both secretome profile and SLC3A2 expression pattern, is in good agreement with this study. SLC3A2 is not associated with senescent cell profile, but rather with aging pattern. An SLC3A2 expression decrease in DFs could be considered as an intrinsic hallmark of skin aging.

In the skin, the best described reciprocal signaling between epithelium and dermis involves the dermal papilla, a specialized population of fibroblasts required for hair follicle formation and cycle in the adult life (Watt and Fujiwara, 2011). This interaction is clearly based on epidermal integrins and basement membrane. Here, we used SLC3A2 dermal deficiency as a mean to decipher how a compromised dermis could influence epidermal homeostasis during aging. Physical and architectural features of the environment influence cell behavior through mechanical cues, features impaired during aging (DuFort et al., 2011). Our data showing that a compromised dermis induced epithelial YAP/TAZ activation defect leading to keratinocyte premature differentiation are consistent with recent work from S. Piccolo’s laboratory highlighting how YAP/TAZ participate in the epidermal stem cell fate decision (Totaro et al., 2017). Similarly, the ability of YAP/TAZ to transduce mechanical information to the nucleus is altered in mammalian epithelial cells due to microenvironment rigidity modulation, thus, leading to impaired mammal epithelial progenitor differentiation (Pelissier et al., 2014). Our work identifies dermal SLC3A2 as a key in vivo “architect” of the dermis mechanical maintenance, thus regulating epidermis homeostasis.
SLC3A2 expression. Our data indicate that SLC3A2 is a protective controller of dermal ECM stiffness and bioactivity for the maintenance of a functional and dynamic skin interface.

**MATERIALS AND METHODS**

**In vivo experiments**

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nice-Sophia Antipolis, Nice, France. SLC3A2 (CD98hc) conditional null mice, SLC3A2^fl/fl (Féral et al., 2007) were crossed with constitutively active FSP1Cre transgenic mice (The Jackson Laboratory, Bar Harbor, ME). All in vivo and ex vivo experiments, FSP1Cre, SLC3A2^fl/fl mice and age-matched SLC3A2^fl/fl (WT) littermate controls were used. Mouse line characterization is described in Supplementary Figure S1 and in the Supplementary Materials and Methods. For tape stripping, WT or KO young adult back skin was clipped and depilated using depilatory cream. Adhesive tape was applied and removed several times on the back skin of each mouse tested to remove the stratum corneum. Mice were sacrificed and back skin was collected and processed 7 days after tape stripping. Thickness of the epidermis was quantified using Image J software (National Institutes of Health, Bethesda, MD). For 12-O-tetradecanoylphorbol-13-acetate treatment, WT or KO young adult back skin was clipped and depilated using depilatory cream. Mice were sacrificed and collected at 7 days after 12-O-tetradecanoylphorbol-13-acetate treatment.

**Histology**

Tissue samples were processed and prepared as described in Boulter et al. (2013). Formalin-fixed, paraffin-embedded sections were used for all stainings. Primary and secondary antibodies are listed in the Supplementary Materials and Methods. Collagen fibers were revealed by Picrosirius red staining (Direct Red80; Sigma) (Supplementary Materials and Methods). Elastin staining was performed using Accustain Elastin stain from Sigma-Aldrich (HT25A-1KT) according to manufacturer’s instructions.

Besides its mechanical function, ECM biochemical properties are crucial to tissue homeostasis (reviewed in Mouet al., 2014; Watt and Fujiwara, 2011). ECM proteins bind bioactive molecules, such as enzyme and growth factors, and regulate their distribution and activation spatially and temporally (Hynes, 2009). The decline of the bioavailability of growth factor (insulin-like growth factor) with skin aging and its impact on epidermis differentiation has been suggested (Giangreco et al., 2008). Here, we clearly demonstrate the role of SLC3A2 in the regulation of ECM bioactivity. After dermal SLC3A2 deletion, a global ECM deregulation was observed both at the assembly (e.g., enzyme LOXL1, collagens, and elastin) and at the bioactivity (i.e., IL1R, LTBP1, and FBLN5) levels. Consequently, the localization and release of growth factor TGF-β were strongly impaired. Quan et al. (2010) reported a coordinate decrease of CTGF and TGF-β in skin fibroblasts during aging, leading to a type I procollagen decrease, which is consistent with our data. Indeed, we identified SLC3A2 as a potential upstream modulator of the CTGF/TGF-β/collagen axis in aging.

The concept of fibroblast heterogeneity has recently emerged and it is now clear that multiple functionally distinct fibroblast subpopulations exist within the dermis (Driskell et al., 2013; Driskell and Watt, 2015; Lynch and Watt, 2018). Nonetheless, no cell isolation based on a single marker is able to distinguish each DF subpopulation (Philippeos et al., 2018). SLC3A2 is expressed in fibroblasts present in both layers of the dermis: papillary and underlying reticular dermis. To insure targeting all SLC3A2-expressing DFs, we used fibroblast promoter FSP1, early expression of which allows targeting of DFs independently of dermal layers.

Together, our findings provide evidence for dermis–epidermis intercommunication during aging via dermal SLC3A2 expression. Our data indicate that SLC3A2 is a
Elastic modulus measurements
The mechanical properties of the samples were studied using a BioScope Catalyst atomic force microscope (Bruker Nano Surfaces, Santa Barbara, CA), as described in Boulter et al. (2013). For details, see Supplementary Materials and Methods.

Cell culture
Murine DFs were isolated from WT or KO young adult skin (Supplementary Materials and Methods). The preparation of conditioned media from WT or KO DFs and mass spectrometry analysis are described in the Supplementary Materials and Methods. Primary mouse keratinocytes were isolated from WT young adult skin, as described previously (Jensen et al., 2010). For clonogenic assay, primary keratinocytes and irradiated DFs were cocultured during 15 days at 32°C CO₂, 8% (as described by Jensen et al., 2010). For culture on hydrogels, primary keratinocytes were directly seeded on collagen-coated 1-kPa or 4-kPa hydrogels (Matrigen, Brea, CA) with 24-hour conditioned media from embryonic feeder, WT, or KO adult DFs. After 48 hours, cells were fixed (4% formaldehyde) or collected for RNA extraction. KO or WT DFs produced ECM during 7 days and ECM was stretched to quantify TGF-β activity in the ECM (Supplementary Materials and Methods).

TGF-β bioassay
Active TGF-β was quantified using MFB-F11 cells (provided by Tony Wyss-Coray), as described in Tesseret et al. (2006) and detailed in Supplementary Materials and Methods.

Quantitative PCR
RNAs were extracted from back-skin samples or primary keratinocytes and reverse transcription was performed as described in Boulter et al. (2013). Amplifications were achieved using specific primers (see Supplementary Table S2 online). Samples were normalized to rppl0 using the ΔΔCt method. Detailed procedure are in Supplementary Materials and Methods.

Western blotting
Whole-cell lysates were prepared and protein lysates were quantified and loaded on SDS-PAGE and analyzed by Western blotting, as described in Boulter et al. (2013). The primary antibody for Western blotting was anti-LTB1P1 antibody.

Statistical analysis
Cell culture experiments were performed at least three times. For animal experiments, Monte-Carlo simulation was used to determine group size. All animal experiments were performed in a blinded fashion. All quantifications represent mean ± standard error of the mean. Images are representative of experiments that have been repeated at least three times. Group comparison was performed using two-tailed unpaired Student t test.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.05.026.

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