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Vascular Basement Membrane Fragmentation in Keloids and the Expression of Key Basement Membrane Component Genes

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Background: Keloids are growing scars that arise from injury to the reticular dermis and subsequent chronic local inflammation. The latter may be promoted by vascular hyperpermeability, which permits the ingress of chronic inflammatory cells/factors. Cutaneous capillaries consist of endothelial cells that generate, and are anchored by, a vascular basement membrane (VBM). Because VBM blocks immune cells/factors ingress, we investigated whether keloids are associated with altered VBM structure and/or VBM component expression by local endothelial cells.

Methods: In total, 54 keloid (n = 27) and adjacent normal skin (n = 27) samples from 14 patients underwent transmission electron microscopy (TEM). Cross-sections of whole capillaries were identified. VBM thickness, continuity, and the number of layers in keloid and normal skin tissues were quantified. The differential expression of 222 previously reported VBM component genes in keloid and normal skin endothelial cells was analyzed using the GSE121618-microarray dataset.

Results: TEM images showed that keloid VBMs were significantly thinner than adjacent skin VBMs (0.053 versus 0.078 nm; $P < 0.001$). They were also greatly fragmented (continuity was 46% versus 85% in normal skin; $P < 0.001$) and had fewer (1.2 versus 2.4) layers ($P < 0.001$). Keloidal endothelial cells demonstrated downregulation of 22 genes, including papilin, laminin-α5, and laminin-α2, and upregulation of 28 genes, including laminin-β1, laminin-β2, laminin-γ1, and laminin-γ2.

Conclusions: VBMs are greatly fragmented in keloids. These changes support the notion that keloids are initiated/promoted, at least partly, by vascular hyperpermeability. *(Plast Reconstr Surg Glob Open 2024; 12:e6366; doi: 10.1097/GOX.0000000000006366; Published online 23 December 2024.)*

INTRODUCTION

Keloids are a dermatofibrotic disease that can be induced by cutaneous trauma, burns, surgery, vaccinations, and acne.[1] These scars grow relentlessly in the horizontal and vertical directions. They have an erythematous appearance in individuals with lighter skin tones and associate with pain, pruritus, contracture, and sometimes functional impairment. Because keloids decrease patients' quality of life, are resistant to monotherapies, and have high recurrence rates, research into the mechanisms that initiate and promote their growth is ongoing.[2]

A key feature of keloids is that they demonstrate chronic inflammation of the reticular dermis, as shown by high numbers of inflamed immune cells and strongly activated fibroblasts called myofibroblasts, pronounced angiogenesis, and elevated levels of growth factors such as transforming growth factor (TGF)-β and cytokines.[1,3,4]

A number of genetic,[5–7] systemic (eg, hypertension and female sex hormones), and local keloid risk factors have been identified.[8,9] The local factors include repeated/chronic infections due to acne/folliculitis and repetitive/sustained stretching or other mechanical forces on the wound/scar.[8] The importance of local mechanical forces is demonstrated by the fact that keloids have a strong predilection for body regions with strong mechanical forces on the skin tension (eg, the anterior chest) and they adopt body region-specific shapes: examples are the butterfly on the shoulder, the crab's claw on the anterior

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chest, and the dumbbell on the upper arm. As shown by our finite-element analysis, these shapes are driven by the predominant skin-stretching forces at particular body sites. Because immune and endothelial cells and myofibroblasts in wounds/scars are exquisitely sensitive to mechanical forces, skin-stretching activates these cells, thus eliciting and sustaining keloidogenic chronic inflammation in the reticular dermis.[10,11] Endothelial function may play a particularly crucial initiatory/promoting role in this inflammation: we hypothesize that the stretching tension on these cells induces vascular permeability that then permits the uninhibited influx of immune cells and factors into the wound/scar bed.[12,13] This notion is supported by several lines of evidence. First, conditions that associate with enhanced vascular permeability, namely, hypertension and pregnancy, are also risk factors for keloid formation and aggravation.[14–18] Second, cyclical stretching of skin flaps in vitro increases capillary permeability almost as strongly as histamine.[12] However, the mechanism that underlies the association between vascular permeability and keloid formation is not yet fully understood.[3,19]

Cutaneous capillaries are composed of tightly juxtaposed endothelial cells that line the lumen and are attached to the vascular basement membrane (VBM).[20,21] Pericytes are embedded in the VBM and make contact with the endothelial cells via cytoplasmic processes through holes in the VBM.[22,23] Structurally, BM/VBM is an ultrathin, high-density, sheet-like extracellular matrix that is produced by the underlying or enclosed cells; in the case of blood vessels, VBM is produced by both endothelial cells and pericytes.[24] Although their main constituents are type-IV collagen, laminin, perlecan, and nidogen, the genomics study of Jayadev et al[21] reported that BM consists of 222 matrix proteins and molecules that link the BM matrix to the anchored/enclosed cells. BM/VBM normally prevents the liberal extravasation of immune cells and immune and other molecules by acting as a physical barrier.[25] This barrier function is illustrated by the fact that immune cells extravasating from the blood vessel lumen into the surrounding tissue first accumulate between the endothelial cells and VBM before they can eventually transit the VBM through permissive low-protein regions.[26,27] Moreover, the VBM also tethers factors such as TGF-β and integrins. This activity prevents their tissue influx and shapes endothelial cell, pericyte, and nearby cell behavior.[28–30]

These observations led us to hypothesize that keloid formation and progression may be promoted by abnormalities in the VBM that result in vascular hyperpermeability. To test this notion, we examined keloid VBMs by transmission electron microscopy (TEM) and examined the VBM-associated gene expression in keloid endothelial cells.

Takeaways

Question: Are there structural changes in the vascular basement membrane (VBM), which regulates vascular permeability, in keloid tissue? If so, what differences exist in the expression of basement membrane-related genes in the endothelial cells, which produce basement membrane components, of keloid and normal skin?

Findings: Keloid VBMs were significantly thinner and more fragmented, and had fewer layers than adjacent skin VBMs. Keloidal endothelial cells demonstrated upregulation of 28 genes and downregulation of 22 genes.

Meaning: These changes support the notion that keloids are initiated/promoted, at least partly, by vascular inflammatory cells' hyperpermeability.

PATIENTS AND METHODS

Study Design and Ethics Approval

The prospective cross-sectional study was conducted in Nippon Medical School Hospital, Tokyo, Japan. It was approved by the institutional review board of Nippon Medical School and adhered to the principles of the Declaration of Helsinki (approval no. 29-05-760). Written informed consent to participate in the study was obtained from each study participant.

Patients and Tissues

In this prospective study, keloids and the adjacent healthy tissues were obtained from a convenience series of patients undergoing keloid resection in September 2017–March 2018 at Nippon Medical School Hospital. All samples were obtained from Japanese patients. All keloids were clinically diagnosed before surgery, and the diagnosis was confirmed by pathology of the resected tissue. Samples that were diagnosed by pathology as hypertrophic scars rather than keloids and samples that lacked normal skin around the lesion were excluded from the study. Clinical information, including sex, age, and site, was collected from the patient records.

Transmission Electron Microscopy

The tissue specimens were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 2 hours, washed overnight at 4°C in the same buffer, postfixed with 1% osmium tetroxide buffered with 0.1 M phosphate buffer for 2 hours, dehydrated in a graded series of ethanol, and then embedded in Epon 812. Ultrathin sections (80–90 nm thickness) were collected on copper grids, double-stained with uranyl acetate and lead citrate, and then examined by TEM (JEM-1400plus, JEOL, Tokyo). Each specimen was observed with Image J 1.53k (National Institutes of Health, Bethesda, MD). A total of 54 TEM images that captured the entirety of a capillary in keloid tissue (n = 27) and adjacent normal tissue (n = 27) were identified from 14 patients. The VBM thickness, VBM continuity, and the number of layers in the VBM were then determined, as follows. For VBM thickness, the VBM was measured at 20 randomly selected places around each capillary (Fig. 1). The mean was then calculated. VBM continuity was determined by measuring the perivascular circumference and then measuring the continuous sections of VBM as shown in Figures 2, and 3. VBM continuity (%) was then calculated

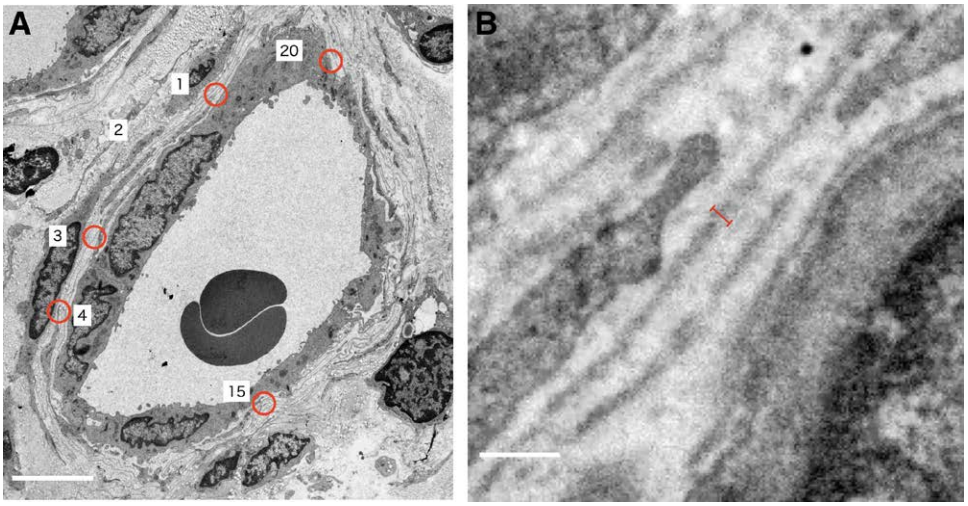

Fig. 1. Calculation of VBM thickness. For each patient, 2 TEM images that showed the entirety of a capillary in cross-section were randomly selected, one for the keloid sample and one for the adjacent normal skin. A, A representative image of a capillary. Scalebar, 5μm. Twenty regions on the VBM were selected randomly (red circles). B, Magnification of the area marked by the number 2 in part A. Scalebar, 0.3μm. The thickness of the VBM in each of the 20 sites was measured (red line). The mean of the 20 measurements served as the VBM thickness for the sample.

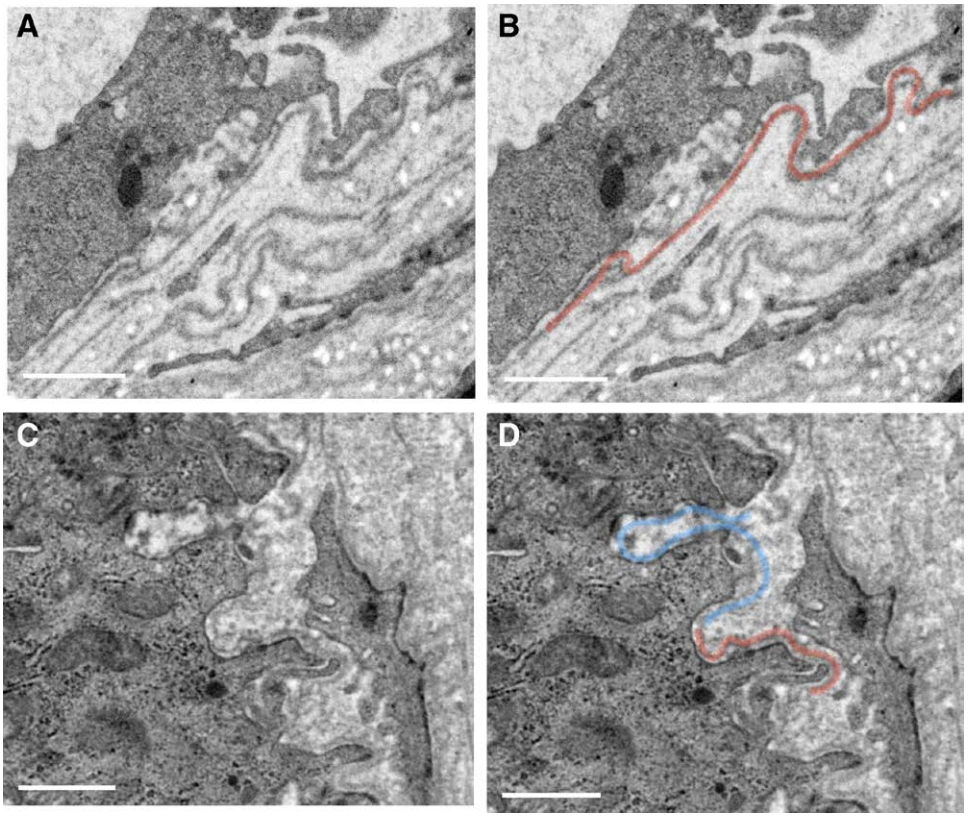

Fig. 2. Calculation of VBM continuity. For each patient, 2 TEM images that showed the entirety of a capillary in cross-section were randomly selected, one for the keloid sample and one for the adjacent normal skin. A and C, Representative images showing the VBM of capillaries. The parts of the VBM that were continuous (red line in [B] and [D]) and discontinuous (blue line in [D]) were identified. VBM continuity was calculated as follows: ([sum of total continuous VBM lengths]/total perivascular circumference) × 100. Scalebars in all images, 1μm.

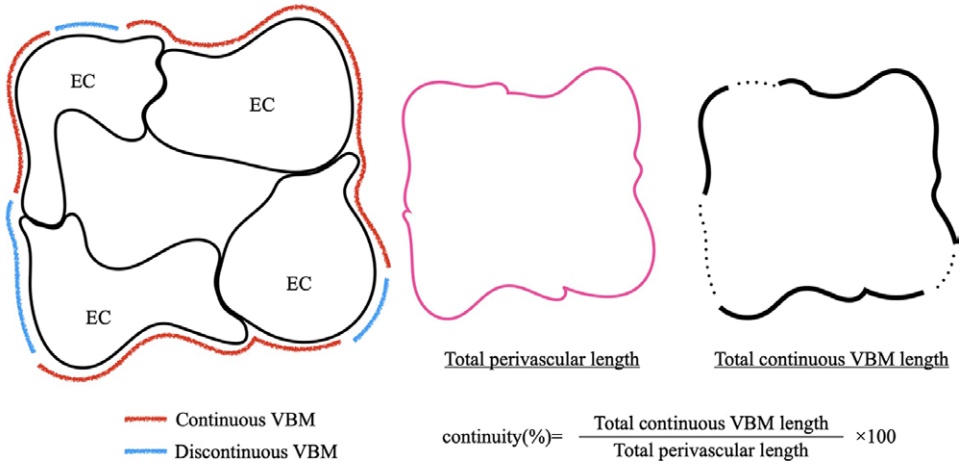

Fig. 3. Schematic depiction of the method with which VBM continuity was determined.

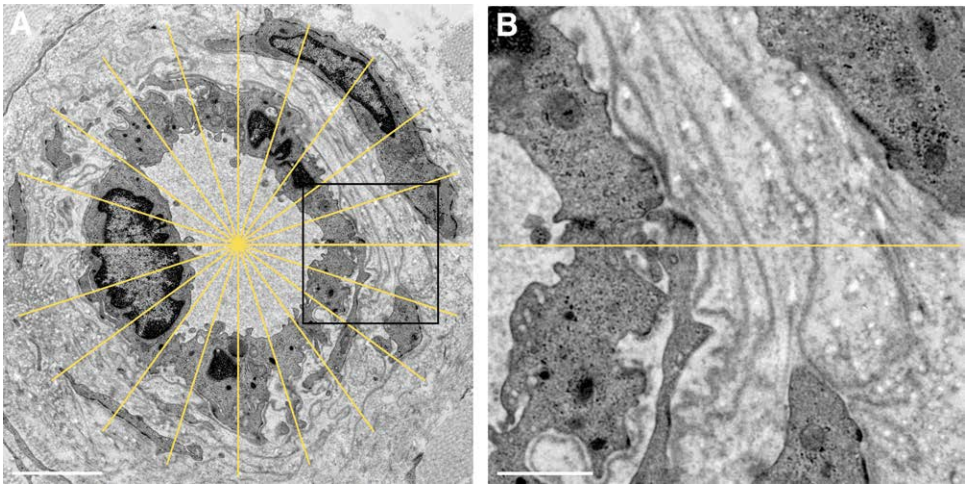

Fig. 4. Determination of the number of layers in the VBM. For each patient, 2 TEM images that showed the entirety of a capillary in cross-section were randomly selected, one for the keloid sample and one for the adjacent normal skin. A, A representative image of a capillary. Scalebar, 5μm. To determine the number of VBM layers, 20 equidistant lines were drawn radially from the central point of the capillary (yellow lines). B, Magnification of the area marked by the black box in part A. Scalebars, 1μm. The number of times VBM was encountered on each yellow line was counted. The mean of the 20 measurements/capillary was then calculated.

as follows: ([sum of total continuous VBM lengths]/total perivascular circumference) × 100 (Fig. 3). To determine the number of VBM layers, 20 equidistant lines were drawn radially from the central part of the capillary and the number of times VBM was encountered on each line was counted (Fig. 4). The mean of the 20 measurements/capillary was then calculated. All tissue-section selections and measurements were conducted by Y.S. in a blinded fashion.

VBM-related Gene Expression Analysis

Our GSE121618 dataset was retrieved from the Gene Expression Omnibus (GEO). This dataset contains the transcriptomes of endothelial cells from 5 keloid tissues and 6 normal skin samples from patients without keloids. The transcriptomes were determined by microarray analysis from $CD31^+$ vascular endothelial cells that were positively selected by magnetic-activated cell sorting. The microarray analysis was conducted with the Agilent-072363 SurePrint G3 Human GE v3 80×60K Microarray.[31] The online tool GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r) in GEO was used to identify which of 222 VBM component-encoding genes identified by Jayadev et al[21] were differentially expressed in keloid endothelial cells. Thus, differentially expressed genes (DEGs) with $|\log FC| \geq 1$ (ie, upregulated DEGs) or $|\log FC| \leq -1$ (ie downregulated DEGs) and *P* value less than 0.05 were identified. FC stands for fold change in expression relative to the normal skin samples. The *P* values were determined with the R package.

Table 1. Demographic and Clinical Characteristics of the 14 Patients and Their Keloids

Patient No	Age	Sex	Location	Duration of Morbidity (y)
1	46	Male	Chest	30
2	64	Male	Chest	18
3	25	Male	Chest	12
4	26	Male	Shoulder	14
5	11	Male	Chest	5
6	5	Female	Buttocks	2
7	68	Male	Chest	3
8	34	Female	Back	5
9	58	Male	Abdomen	2
10	36	Female	Abdomen	5
11	73	Male	Chest	60
12	50	Male	Neck	16
13	36	Male	Chest	7
14	49	Male	Chest	30

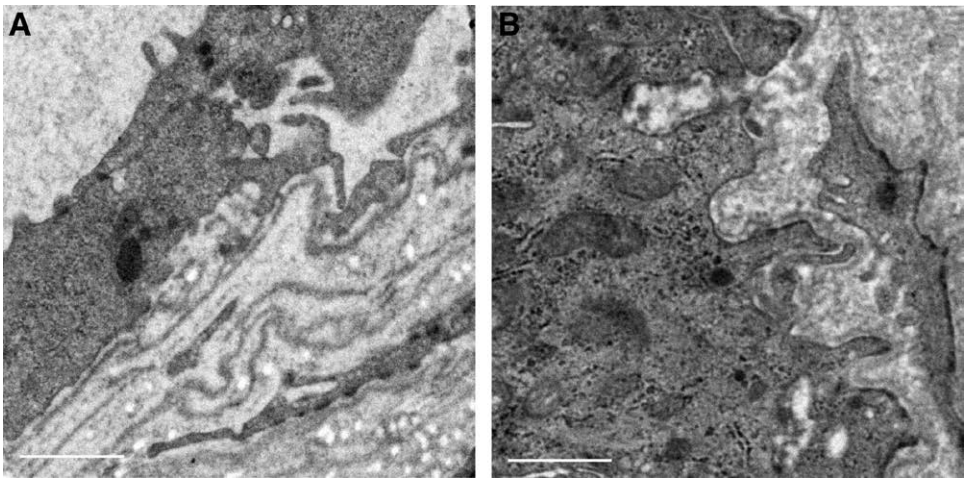

Fig. 5. Representative VBM from (A) adjacent normal tissue and (B) keloid tissue of a patient. The VBM in normal skin is continuous and thick. The VBM in keloid tissue is thin and fragmented. Magnification of both images, 1μm.

Table 2. Comparison of Keloid Tissue and Adjacent Normal Skin in Terms of Average VBM Thickness, VBM Continuity, and Number of Layers in the VBM of Capillaries

	Adjacent Normal Skin		Keloid		
Variable	n	Measurement	n	Measurement	*P**
VBM thickness, μm	27	0.0779 ± 0.0175	27	0.0532 ± 0.0052	<0.001
VBM continuity, %	10	85.3 ± 11.5	10	46.0 ± 14.35	<0.001
Mean no. layers in VBM	27	2.40 ± 0.85	27	1.18 ± 0.41	<0.001

The data were expressed as mean ± SD.
**P* values were determined by unpaired Student *t* test.

Statistical Analysis

VBM thickness, VB continuity, and the number of layers in the VBM were expressed as mean ± SD. The keloid tissues were compared with the normal adjacent tissues using Student independent-sample *t* tests. SPSS software (IBM SPSS statistics 29.0.1.0 for mac) was used. *P* values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Keloid VBM Demonstrates Marked Structural Changes

The mean age of the 14 keloid patients was 42 years, 79% were male, and their 27 keloids were mostly from the chest region (Table 1). The 54 samples of keloids and adjacent normal skin were subjected to TEM. VBM thickness and number of layers were determined in all samples.

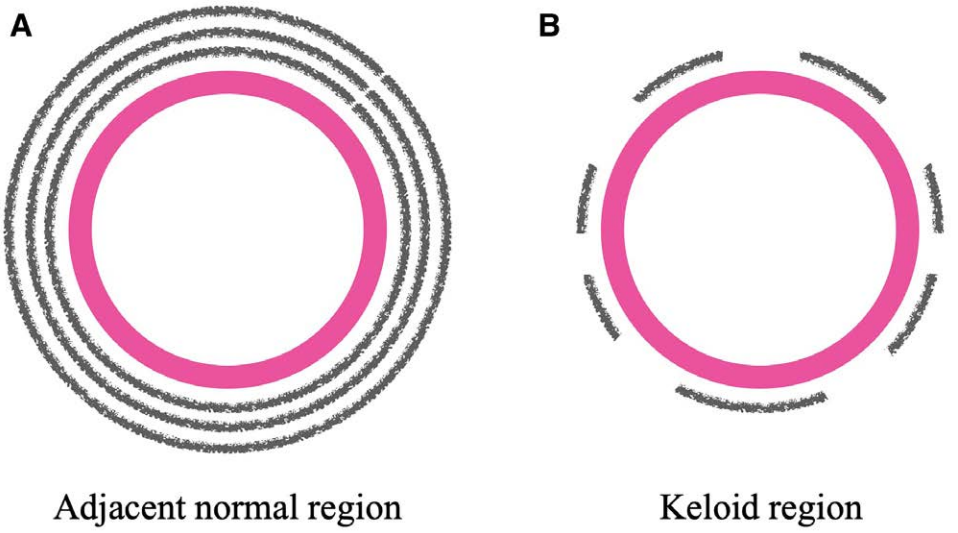

Fig. 6. Schematic depiction of the VBM in the adjacent normal skin (A) and the keloid (B). The pink circle signifies the endothelial cells lining the capillary lumen. The fuzzy black circles in the left image depict the three VBM layers in healthy skin capillaries.

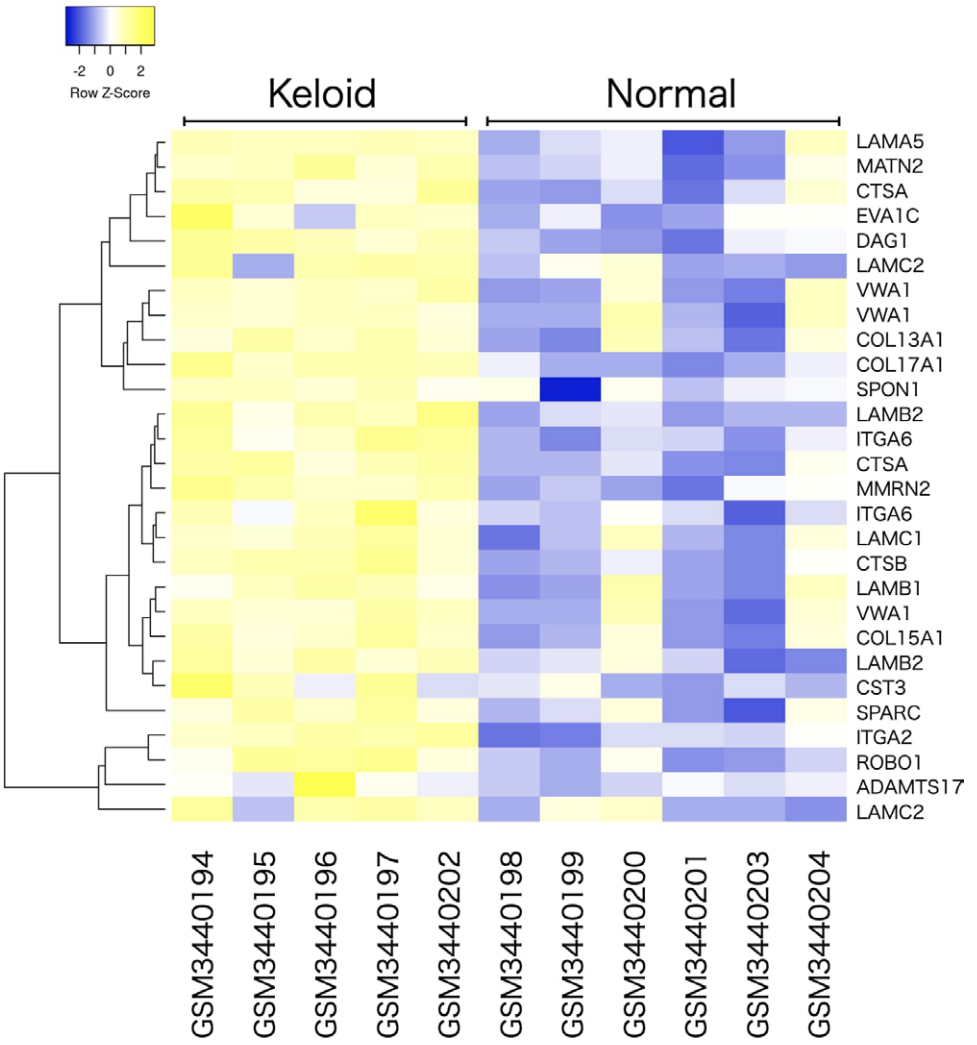

Fig. 7. The heat map of upregulated gene expression.

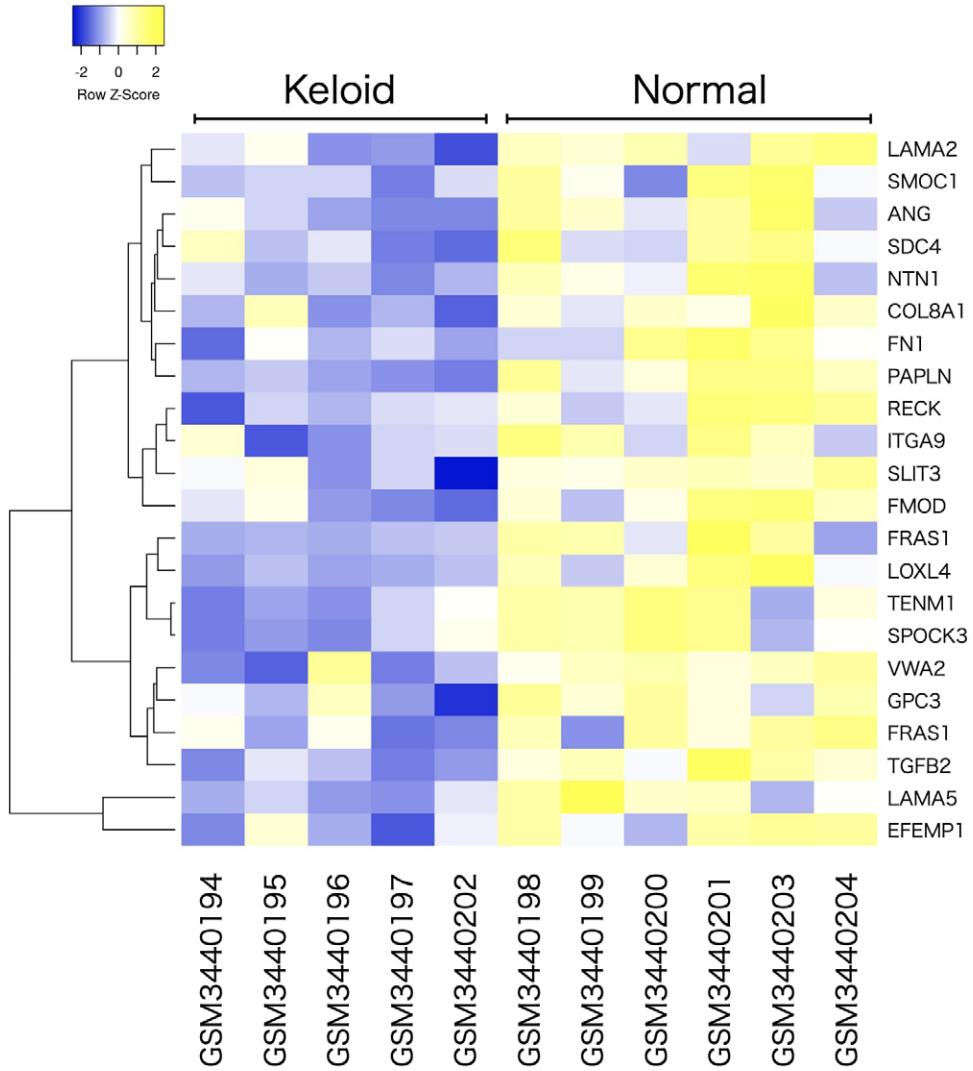

Fig. 8. The heat map of downregulated gene expression.

VBM continuity was determined in 10 randomly selected samples.

Representative cross-sectioned capillaries in keloids and the adjacent normal tissues are shown in Figure 5. The VBM in adjacent normal tissue was thick, continuous, and consisted of multiple layers. By contrast, the VBM in keloid tissue was significantly thinner (0.053 versus 0.078 nm), exhibited less continuity (46% versus 85%), and had fewer layers (1.2 versus 2.4 layers) (all $P < 0.001$) (Table 2;Fig. 6).

Keloid Endothelial Cells Demonstrate Altered Expression of Key VBM Components

Analysis of the 222 genes that encode BM matrix proteins and cell-surface interactor molecules[21] showed that 28 genes were significantly upregulated and 22 genes were significantly downregulated in endothelial cells in keloid tissue relative to skin endothelial cells from patients without keloids (Figs. 7, 8; Tables 3, 4). The most upregulated gene was *LAMC2,* which encodes the laminin-γ2 chain (3.5-fold upregulated). Other notable upregulated genes were *LAMA5* (encoding the laminin-α5 chain; 1.1-fold upregulated), *LAMB1* (encoding the laminin-β1 chain; 0.7-fold upregulated), *LAMB2* (encoding the laminin-β2 chain; 0.4-fold upregulated), and *LAMC1* (encoding the laminin-γ1 chain; 0.8-fold upregulated) (Table 3). The most downregulated gene was *PAPLN* (encoding papilin; 2.4-fold downregulated). Other notable downregulated genes were *LAMA5* (also encoding the laminin-α5 chain; 0.5-fold downregulated) and *LAMA2* (encoding the laminin-α2 chain; 1.1-fold downregulated) (Table 4). The upregulated *LAMA5* gene sequence differs from that of the downregulated *LAMA5* gene sequence.

DISCUSSION

The VBM provides essential structural support to the endothelial cells that line the capillary lumen and to the pericytes that are embedded in the VBM. It also acts as a barrier between cells/factors in the circulation and the

Table 3. Genes That Were Significantly (*P* < 0.05) Upregulated in Keloidal Endothelial Cells Compared With Normal Skin Endothelial Cells From Patients Without Keloids

Upregulation			
GENE_SYMBOL	**GENE_NAME**	***P***	**logFC**
LAMC2	Laminin, gamma 2	1.54E-02	3.46
COL17A1	Collagen, type XVII, alpha 1	1.25E-05	3.15
LAMC2	Laminin, gamma 2	1.75E-02	2.53
SPON1	Spondin 1, extracellular matrix protein	2.53E-02	2.42
COL13A1	Collagen, type XIII, alpha 1	8.4E-03	2.19
ROBO1	Roundabout, axon guidance receptor, homolog 1 (Drosophila)	2.68E-04	1.68
VWA1	von Willebrand factor A domain-containing 1	3.85E-02	1.46
VWA1	von Willebrand factor A domain-containing 1	7.82E-03	1.36
CTSB	Cathepsin B	1.33E-04	1.2
VWA1	von Willebrand factor A domain-containing 1	1.28E-02	1.19
ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	3.7E-04	1.14
MATN2	Matrilin 2	8.03E-04	1.11
COL15A1	Collagen, type XV, alpha 1	2.7E-03	1.07
LAMA5	Laminin, alpha 5	3.97E-03	1.05
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	7.47E-03	9.6E-01
ADAMTS17	ADAM metallopeptidase with thrombospondin type 1 motif, 17	4.53E-02	7.74E-01
MMRN2	Multimerin 2	9.67E-04	7.52E-01
LAMC1	Laminin, gamma 1 (formerly LAMB2)	1.08E-02	7.52E-01
LAMB1	Laminin, beta 1	3.81E-02	7.41E-01
CTSA	Cathepsin A	2.53E-03	7.26E-01
ITGA6	Integrin, alpha 6	7.92E-04	7.17E-01
DAG1	Dystroglycan 1 (dystrophin-associated glycoprotein 1)	1.1E-03	6.44E-01
ITGA6	Integrin, alpha 6	8.18E-03	6.17E-01
CTSA	Cathepsin A	2.22E-03	5.51E-01
CST3	Cystatin C	2.48E-02	5.37E-01
EVA1C	Eva-1 homolog C (*C. elegans*)	4.07E-02	4.37E-01
LAMB2	Laminin, beta 2 (laminin S)	7.81E-03	3.88E-01
LAMB2	Laminin, beta 2 (laminin S)	2.12E-02	3.56E-01

FC, fold change in keloidal endothelial cells relative to the gene expression in the skin endothelial cells from patients without keloids.

Table 4. Genes That Were Significantly (*P* < 0.05) Downregulated in Keloidal Endothelial Cells Compared Withh Normal-Skin Endothelial Cells From Patients Without Keloids

Downregulation			
GENE_SYMBOL	**GENE_NAME**	***P***	**logFC**
PAPLN	Papilin, proteoglycan-like sulfated glycoprotein	7.88E-05	−2.41
LOXL4	Lysyl oxidase-like 4	2.65E-03	−1.83
NTN1	Netrin 1	9.45E-03	−1.81
TGFB2	TGF-β2	3.77E-04	−1.78
FRAS1	Fraser extracellular matrix complex subunit 1	1.02E-02	−1.78
FRAS1	Fraser extracellular matrix complex subunit 1	1.77E-02	−1.55
FMOD	Fibromodulin	8.41E-03	−1.5
EFEMP1	EGF containing fibulin-like extracellular matrix protein 1	1.68E-02	−1.43
COL8A1	Collagen, type VIII, alpha 1	1.27E-02	−1.42
GPC3	Glypican 3	2.07E-02	−1.37
SMOC1	SPARC-related modular calcium binding 1	3.59E-02	−1.35
SLIT3	Slit homolog 3 (Drosophila)	9.7E-03	−1.25
FN1	Fibronectin 1	1.57E-02	−1.25
LAMA2	Laminin, alpha 2	3E-03	−1.07
SDC4	Syndecan 4	4.11E-02	−1.07
VWA2	von Willebrand factor A domain-containing 2	1.53E-02	−0.74
ANG	Angiogenin, ribonuclease, RNase A family, 5	1.89E-02	−0.58
LAMA5	Laminin, alpha 5	2.23E-02	−0.517
ITGA9	Integrin, alpha 9	4.2E-02	−0.5
SPOCK3	SPARC/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3	2.2E-02	−0.443
TENM1	Teneurin transmembrane protein 1	1.83E-02	−0.437
RECK	Reversion-inducing-cysteine-rich protein with kazal motifs	2.88E-02	−0.418

FC, fold change in keloidal endothelial cells relative to the gene expression in the skin endothelial cells from patients without keloid.

tissue surrounding the capillary.[32,33] The present study showed that the VBM in keloids is highly fragmented. Excessive angiogenesis during the healing process can trigger adverse responses in the wound, leading to the formation of keloids.[34] This abnormal VBM shape may also be observed during neovascular maturation and in blood vessels within tumors.[35,36] VBM is reconstructed through endothelial–pericytes interactions during neovascular maturation.[37] This suggests that neovascularization within the keloid may be indicative of incomplete maturation and remodeling.

Endothelial cells are the main generators of the VBM.[24] The keloid endothelial cells demonstrate very marked downregulation of the proteoglycan papilin, which is a key BM regulatory protein[21] that associates with BM remodeling.[38] In addition, multiple laminin subunits are up- or downregulated in keloid endothelial cells. Laminin is the most abundant noncollagenous BM protein. Because laminin mutations or knockouts have grave consequences and associate with many diseases, laminin is essential for BM structure and function.[20,39,40] It is composed of α, β, and γ chains, of which there are 5, 4, and 3 subtypes, respectively: consequently, laminin exists as 16 isoforms in humans. There are marked differences between tissues in terms of the predominant laminin isoforms: in cutaneous-capillary VBM, laminin-411 (α4β1γ1) and laminin-511 (α5β1γ1) dominate.[41,42] All 4 of the chains that constitute these isoforms are up/downregulated in keloid endothelial cells: laminin-α5 demonstrates up- and downregulation in different RNA sections, and laminin-β1 and laminin-γ1 are downregulated. It is unclear why part of LAMA5 is upregulated and another part is downregulated. However, these data suggest that the production of laminin-α5 may be upregulated, but laminin-α5 may not function properly because partial RNA sections in keloidal endothelial cells differ from that of control group. Our data also show that other laminin subtypes are upregulated in keloid endothelial cells, namely, laminin-β2 and laminin-γ2, whereas laminin-α2 is downregulated. Notably, laminin-α5 inhibits leukocyte migration through the VBM,[40] and the BM inhibits the influx of immune factors such as TGF-β.[43,44] Thus, the loss of laminin-α5 function by capillaries could lead to vascular hyperpermeability. The resulting untrammeled influx of immune cells and proinflammatory factors into the keloid scar could in turn promote the chronic inflammation that drives keloid initiation and growth.

The mechanisms that lead to VBM fragmentation in keloids is not yet known but we speculate that they reflect strong/repetitive mechanical forces on the endothelial cells. Multiple studies show that such forces alter the BM.[45–47] For example, Kanazawa et al[47] reported recently that continuous stretching of the rat soleus muscle reduced the width of the BM surrounding myocytes. Moreover, vascular endothelial cells are well known for their exquisite sensitivity to mechanical forces, and there is evidence that laminins in the VBM, particularly laminin-α5, participate in endothelial cell mechanosensing and mechanotransduction.[41,48] Thus, excessive mechanical forces on cutaneous capillaries could disrupt the VBM by physically tearing apart the structure and/or by overwhelming or disrupting endothelial cell production of VBM components such as laminin.

The possibility that VBM fragmentation could promote keloid fragmentation initiation/progression is supported by the fact that patients with diseases that result from abnormalities in BM-associated genes are more prone to keloidogenesis than the general population. These diseases include epidermolysis bullosa, which associates with mutations in genes encoding BM proteins, including proteins that form bridges with laminin-511.[49,50] Another disease is congenital muscular dystrophy, which can be caused by mutations in BM genes such as laminin-α2.[51,52] Moreover, Duchenne muscular dystrophy (DMD) associates with mutations in the cytoskeletal protein dystrophin, which links to BM laminin-α2 through the sarcolemma.[53] Patients with DMD are highly prone to keloids at surgical sites.[54] Notably, a mouse model of DMD exhibits irregular endothelial cell alignment, a discontinuous VBM, and a reduction in the BM components laminin and agrin.[55]

It is also possible that the VBM fragmentation in keloid endothelial cells is due to the chronic inflammation in the keloid tissue. For example, matrix metalloproteinases, which can disrupt all BM protein components,[56] are produced by inflammatory cells[57] and are highly upregulated in the chronically inflamed environment of keloids.[58] Alternatively, this mechanism acts together with mechanics-induced fragmentation of VBM. For example, the degradation of VBM by MMPs induces the release of small functional VBM components called matrikines that have been shown to impair endothelial cell survival and functions such as adhesion.[59] Thus, VBM fragmentation by the products of immune cells can further impair vascular endothelial function, thereby contributing to vascular hyperpermeability.

CONCLUSIONS

The present study showed that the VBM in keloids is thin, fragmented, and bears fewer layers than the VBM in adjacent normal tissue. The fragmentation of the VBM may be associated with incomplete neovascular maturation. Further elucidation of the mechanisms underlying neo-VBM remodeling within keloids is warranted.

LIMITATIONS

There are some limitations. First, a small sample size (14 samples) was adapted. Second, the samples obtained for TEM were different from those obtained for VBM-related gene expression analysis. Third, no validation experiments have been performed on the genes or proteins indicated by the microarray. The microarray data show that genes of TGF-β and fibronectin, which are upregulated in keloid tissue, were downregulated in keloidal endothelial cells. Genes and proteins considered important will require additional validation, such as immunostaining and qPCR. Fourth, there has been no comparison of VBM structure and basement membrane-related genes within keloids by site of origin. There is no mention of why these changes occur in the VBM. Further research is needed to elucidate

the mechanisms by which the VBMs are altered in keloids and how this leads to keloid formation.

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DISCLOSURE

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