Harnessing the Secretome of Hair Follicle Fibroblasts to Accelerate Ex Vivo Healing of Human Skin Wounds

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In skin homeostasis, dermal fibroblasts are responsible for coordinating the migration and differentiation of overlying epithelial keratinocytes. As hairy skin heals faster than nonhairy skin, we took bio-inspiration from the follicle and hypothesized that follicular fibroblasts would accelerate skin re-epithelialization after injury faster than interfollicular fibroblasts. Using both in vitro and ex vivo models of human skin wound closure, we found that hair follicle dermal papilla fibroblasts could accelerate closure of in vitro scratch wounds by 1.8-fold and epithelial growth capacity by 1.5-fold compared with controls \( (P < 0.05) \). We used a cytokine array to determine how the dermal papilla fibroblasts were eliciting this effect and identified two cytokines, sAXL and CCL19, that are released at significantly higher levels by follicular fibroblasts than by interfollicular subtypes. Using sAXL and CCL19 individually, we found that they could also increase closure of epithelial cells in a scratch wound by 1.2- and 1.5-fold, respectively, compared with controls \( (P < 0.05) \). We performed an unbiased transcriptional analysis, combined with pathway analysis, and postulate that sAXL accelerates wound closure by promoting migration and inhibiting epithelial differentiation of skin keratinocytes. Long term, we believe these results can be exploited to accelerate wound closure of human skin in vivo.

INTRODUCTION

As the interface of our body with our surroundings, skin provides us with external protection and internal homeostasis. When the skin barrier is compromised, the damage is usually healed via a series of coordinated events, including hemostasis and inflammation, re-epithelialization, and extracellular matrix remodeling (Velnar et al., 2009). However, in situations where any one of these processes is perturbed, a chronic or nonhealing wound can arise. It is thought that nonhealing wounds affect 1–2% of the population at any given time, whereas treatment costs account for 2–4% of the healthcare budget in industrialized countries (Guest et al., 2017). Not only are there grandiose economic effects of chronic wounds, but they can cause severe psychological and physical impact, negatively affecting quality of life of patients (Cole-King and Harding, 2001; Phillips et al., 1994).

A confounding issue with nonhealing wounds is the lack of therapeutics available that actively promote wound closure. In the U.S., PDGF-BB has been approved by the Food and Drug Administration for treatment of full-thickness skin wounds. Growth factors have also been approved for the treatment of chronic wounds in India, Japan, and Brazil; however, the European Medicines Agency does not currently have an approved growth factor for the treatment of skin wounds. Rather, the only European Medicines Agency–approved treatment for chronic wounds is a birch bark extract for which the mechanism of action remains elusive (Barret et al., 2017). Thus, there is a pressing medical need to identify new therapeutics that can safely and effectively accelerate skin wound closure.

To identify new therapeutics that could potentially aid wound closure, we sought to take bio-inspiration from skin itself. One difference in skin across body sites is its ability to heal. For example, scalp skin containing large terminal hair follicles has superior healing compared with trunk skin, making it a gold standard site for harvesting skin for split thickness grafts (Mimoun et al., 2006; Weyandt et al., 2009; Wyrzykowski et al., 2015). Grafting of hair-bearing scalp skin into chronic venous ulcers has also been shown to promote wound closure, over and above closure observed when skin plugs from abdominal skin (containing small vellus hair follicles) are used (Alam et al., 2019; Jiménez et al., 2012; Martínez et al., 2016). Although both of these aforementioned studies are in human skin, mouse skin containing hair follicles in an active growth state has also been shown to heal quicker than skin with hair follicles in a resting state. This is due in part to quicker re-epithelialization and to increased angiogenesis and matrix deposition in the dermis (Ansell et al., 2011).

The observation that actively growing hair follicles can promote increased wound closure is intriguing, raising the question of how the follicle enables this phenomenon. In vitro studies have shown that the hair dermis releases...
cytokines, which promote vascularization (Bassino et al., 2015). However, a series of studies using transgenic mice have confirmed that hair follicle stem cells have a key role in providing a source of cells for re-epithelialization after skin injury (Ito et al., 2005; Levy et al., 2007). Although re-epithelialization is a key step in cutaneous wound repair, it requires the synergistic involvement of both epidermal and dermal cell populations, with dental fibroblasts providing cues that direct the migration and differentiation of overlying epithelial cells (Ghetti et al., 2018; Werner and Smola, 2001). With the knowledge that skin fibroblasts are heterogeneous (Driskell et al., 2013; Driskell and Watt, 2015; Harper and Grove, 1979; Philippeos et al., 2018; Rinkevich et al., 2015; Sorrell and Caplan, 2004), and taking inspiration from the accelerated wound closure observed on the scalp compared with other body sites, we postulated that hair follicle fibroblasts might have an undetermined role in accelerating wound re-epithelialization.

To assess whether hair follicle fibroblasts could promote wound re-epithelialization, we first had to isolate subtypes of fibroblasts from scalp skin for growth in culture. In human skin, fibroblasts are categorized by their location within the dermis. For example, papillary fibroblasts (PFi) are located in the upper dermis, adjacent to the epithelium, whereas reticular fibroblasts (RFi) are found in the lower dermis. Within the hair follicle, there are two subsets of dental fibroblasts known as dental papilla fibroblasts (DPFi) and dental sheath fibroblasts. Although interfollicular fibroblasts can be isolated by digestion followed by FACS (Korosec et al., 2019), DPFi currently cannot be enzymatically digested using standard protocols, and a microdissection approach is therefore required for their isolation (Topouzi et al., 2017). To keep equivalent methodology throughout, we isolated PFi, RFi and DPFi subtypes from scalp skin using microdissection and set out to evaluate their wound-promoting potential using in vitro and ex vivo human skin wound healing models. After finding that DPFi promote the fastest wound closure of the three subtypes, we used cytokine arrays to determine the DPFi secretome and identify factors that were causing the aforementioned effect.

RESULTS

The DPFi secretome promotes faster keratinocyte (KC) re-epithelialization both in vitro and ex vivo

Before initiating wound healing assays, we first isolated matched sets of DPFi, PFi, and RFi from human scalp skin. A recent study assessing DPFi, PFi, and RFi in mouse skin concluded that these three subtypes are derived from two lineages with a common progenitor (Driskell et al., 2013). After assessing expression in scalp skin (Supplementary Figure S1a), we compared the expression pattern of these three subtypes against one another in vitro to ensure we were culturing distinct fibroblast populations and that culture conditions were not causing reversion to a homogeneous fibroblast lineage (Supplementary Figure S1b). We assessed expression of PDGFP, PDP4 (CD26), α-SMA, and CNN1, which have previously been used to distinguish between fibroblast subtypes (Driskell et al., 2013; Jahoda et al., 1991; Janson et al., 2013; Janson et al., 2012). We found that PFi cultures express significantly higher amounts of PDGFP and CD26 than RFi and DPFi cells, whereas comparatively α-SMA and CNN1 were significantly expressed in DPFi cultures when compared with PFi and RFi (Supplementary Figure S1c). Taken together, this demonstrates that the DPFi, PFi, and RFi cultures established from human scalp skin exhibit distinct characteristics from one another, and based on this we concluded that we were growing three different fibroblast subtypes in vitro.

We next wanted to test our hypothesis that DPFi would accelerate re-epithelialization more than PFi or RFi. To collect the fibroblast secretome, we incubated KC growth media (Epilife, Thermo Fisher Scientific, Waltham, MA) with fibroblast subtypes for 48 hours, conditioning the Epilife with factors secreted by the fibroblasts (termed conditioned medium [CM]). Fibroblast CM was then placed onto KCs, and using a scratch assay technique, we generated monolayer scratch wounds in KCs in vitro (Figure 1a and b). We quantified the observed migration and proliferation of KCs across the scratch wound and found significant differences between the four CM conditions, DPFi, PFi, RFi, and unconditioned Epilife (control). DPFi CM promoted 1.8-fold more wound closure with a mean re-epithelialized area of 0.46 mm² (standard deviation [SD] 0.17) by 9 hours compared with Epilife (0.25 mm², SD 0.15), PFi CM (0.23 mm², SD 0.07), and RFi CM (0.32 mm², SD 0.13; P < 0.05) (Figure 1c, Supplementary Figure S2a, and Supplementary Table S1).

To assess the time-course of re-epithelialization, we measured closure area at several time points before complete closure. We modeled re-epithelialization as a logistic growth process, where area initially grows rapidly before slowing to a stop, as described in Materials and Methods. Briefly, re-epithelialization or growth capacity (in mm²) describes the capability of the different conditions to close the wound, whereas the initial growth rate describes how rapidly the different conditions approach the growth capacity. We found that DPFi CM produced a faster initial growth rate (1.05 mm², SD 0.06; Figure 1d) and the highest growth capacity (0.42 mm², SD 0.03; Figure 1e) of all the groups. This suggests that DPFi CM is able to increase both the amount of re-epithelialization and the speed at which it is achieved. RFi CM also increased the growth capacity of KCs significantly more than PFi (0.33 mm², SD 0.03 vs. 0.23 mm², SD 0.02), perhaps reflective of their known role in wound closure (Driskell et al., 2013).

Next, we assessed if the results obtained in the in vitro model could be replicated in ex vivo human skin. To evaluate this, we performed a wound healing assay known as a punch within a punch, using skin from abdominoplasties (Figure 1f and g). DPFi CM, PFi CM, RFi CM, and Epilife were applied topically and daily onto the punch wound for 6 days. We found that DPFi CM closed the initial wound by 1.83 mm² (SD 0.52) after 6 days, which was significantly more than PFi CM, RFi CM, and Epilife, which all closed the initial wound by less than 1.48 mm² (SD 0.34; Figure 1h, Supplementary Figure S2c, and Supplementary Table S2). Surprisingly, the initial growth rate of DPFi CM was significantly lower than PFi CM and RFi CM (1.11 hr⁻¹ [SD 0.22] vs. 1.54 hr⁻¹ [SD 0.30] and 1.54 hr⁻¹ [SD 0.32], respectively; Figure 1i), whereas the DPFi CM growth capacity was 1.62 mm² (SD 0.08), which was significantly higher than PFi CM,
RFi CM, and Epilife (Figure 1j). These results demonstrate that the DPFi CM has the capacity to accelerate both scratch wound closure in vitro and punch wound closure ex vivo. However, although DPFi CM contains the whole DPFi secretome, it is likely that specific elements of the secretome are responsible for the observed effect.

Figure 1. DPFi CM promotes faster KC re-epithelialization in vitro and ex vivo. (a) Schematic illustrates the scratch assay method in a 6-well plate, where the KCs are wounded with a p200 pipette tip. (b) Representative phase contrast images of the KC scratch assay incubated with DPFi CM and Epilife. The red dotted line indicates the edge of the wound over a period of 9 hours. Bar = 500 μm. (c) The graphs outline re-epithelialized area in mm² in the in vitro model over 9 hours when incubated with Epilife, DPFi CM, PFi CM, and RFi CM. (d, i) Initial growth rate results of the (d) in vitro model and (i) ex vivo model, demonstrate the ability of Epilife, DPFi CM, PFi CM, and RFi CM to reach growth capacity. (e, j) Growth capacity results of the (e) in vitro model and (j) ex vivo model, which describes the capacity of Epilife, DPFi CM, PFi CM, and RFi CM to close the wound. (f) Schematic illustrates the ex vivo punch assay with an 8-mm diameter outer punch and 2-mm diameter inner punch (the wound). (g) Representative images of the ex vivo model with topically applied DPFi CM and Epilife. The red dotted line indicates the edge of the wound over a period of 6 days. Bar = 5 mm. (h) The graphs outline re-epithelialized area in mm² in the ex vivo model over 6 days when incubated with Epilife, DPFi CM, PFi CM, and RFi CM. All comparisons in graphs (d), (e), (i), and (j) are significant (P ≤ 0.05) unless labeled otherwise. CI, confidence interval; CM, conditioned medium; DPFi, dermal papilla fibroblast; KC, keratinocyte; ns, not significant; PFi, papillary fibroblast; RFi, reticular fibroblast.
DPFi-specific cytokines promote faster wound closure than the control in vitro

Thus far, we found that DPFi CM could promote significantly faster re-epithelialization of in vitro and ex vivo wounds compared with controls. We postulated that growth factors or cytokines specifically released by DPFi were responsible for this observed effect. To investigate the components of the fibroblast secretome, we performed a human cytokine antibody array, comparing the cytokines present in the CM obtained from DPFi, PFi, and RFi cultures. The following three comparisons were made: PFi CM versus DPFi CM, RFi CM versus DPFi CM, and RFi CM versus PFi CM. When comparing PFi CM versus DPFi CM, we identified three cytokines significantly over-represented in DPFi CM (Figure 2a and c), whereas in the RFi CM versus DPFi CM comparison, eight cytokines were identified as significantly released by DPFi (Figure 2b and c). Of these, two were uniquely present in DPFi CM versus both PFi and RFi (Figure 2c). These were CCL19 and sAXL. Antibodies against these cytokines also revealed CCL19 expression in DPFi in vivo (Supplementary Figure S3).

Because RFi CM increased KC growth capacity over and above PFi CM, we also compared RFi CM with PFi CM, identifying just one cytokine that was significantly released by RFi CM, IL-6 (Supplementary Figure S4a and b).

To assess whether CCL19, sAXL, and IL-6 could affect KC re-epithelialization, we first tried a range of concentrations and selected the optimal concentration for further analysis (data not shown). We then tested individual cytokines for their efficacy in promoting KC re-epithelialization in a scratch wound assay. IL-6, released by RFi, promoted a KC re-epithelialization of 0.24 mm² (SD 0.12) by 9 hours (Supplementary Figure S4c). This and the growth capacity of IL-6 were no different to the Epilife control, whereas the initial growth rate of IL-6 was actually significantly lower than Epilife (Supplementary Figure S4d and e). sAXL and CCL19 promoted wound closure at 0.31 mm² (SD 0.08) and 0.37 mm² (SD 0.09), respectively, by 9 hours, which were significantly faster than Epilife (0.25 mm², SD 0.15; Figure 2d, Supplementary Figure S2b, and Supplementary Table S1). The initial growth rate of sAXL (0.84 hr⁻¹, SD 0.04) and CCL19 (0.83 hr⁻¹, SD 0.04) were significantly higher than Epilife (0.77 hr⁻¹, SD 0.05), as were the growth capacities of both sAXL (0.32 mm², SD 0.02) and CCL19 (0.37 mm² SD 0.02) compared with Epilife (0.27 mm², SD 0.03; Figure 2e and f). These results suggest that the RFi CM specific cytokine IL-6 does not accelerate re-epithelialization, and hence we did not conduct further experiments with IL-6. However, as both the DPFi CM—specific cytokines, sAXL and CCL19, enhanced KC re-epithelialization in vitro, we decided to pursue analysis of these cytokines using ex vivo models.

Although the data so far has all assessed re-epithelialization as representative of wound healing, dermal remodeling is also a key component of wound closure. To assess the effect of sAXL and CCL19 on dermal fibroblasts, we used alamar blue to measure fibroblast metabolic activity after the addition of cytokines (Supplementary Figure S5). Neither sAXL nor CCL19 had any effect on DPFi, which was perhaps to be expected as DPFi release both of these cytokines. Although PFi and RFi both proliferated much faster than DPFi, the added cytokines did not have any effect on these fibroblasts either. Therefore, although sAXL and CCL19 can accelerate epithelial cell migration, they do not promote proliferation of dermal fibroblasts when used at the same concentration.

sAXL and CCL19 promote faster wound closure in ex vivo human skin

After determining that sAXL and CCL19 could accelerate KC migration in vitro faster than controls, we next investigated whether they would modulate wound healing in human skin ex vivo. Using human skin in the punch within a punch assay, we compared the abilities of sAXL and CCL19 in promoting ex vivo wound re-epithelialization (Supplementary Figure S6). We also assessed the efficacy of PDGF-BB, the Food and Drug Administration approved therapeutic for full-thickness wounds. Factors were applied topically onto the punch wound at 24-hour intervals (Figure 3a). We found that six days after application, sAXL reduced the initial wound opening by 1.77 mm² (SD 0.22), which was significantly more the Epilife-treated wounds, which closed by 1.44 mm² (SD 0.28; Figure 3b, Supplementary Figure S2d, and Supplementary Table S2). The same effect was observed with CCL19, with significantly increased wound closure in comparison with the control (1.86 mm², SD 0.4; Figure 3b, Supplementary Figure S2d, and Supplementary Table S2). The time-course of wound closure analysis showed that although CCL19 and sAXL both had increased growth capacity relative to Epilife (1.54 mm², SD 0.06, and 1.54 mm², SD 0.08, for CCL19 and sAXL, respectively, compared with 1.18 mm², SD 0.04, for Epilife; Figure 3d), the initial growth rates were not significantly different between conditions (Figure 3c). With PDGF-BB, topical application decreased the initial wound by 1.55 mm² (SD 0.57), but this result was not significant compared with the control (Figure 3b, Supplementary Figure S2d, and Supplementary Table S2). The growth capacity of PDGF-BB was 1.3 mm² (SD 0.08), significantly lower than sAXL and CCL19, perhaps suggesting that sAXL and CCL19 could be superior therapeutic alternatives to PDGF-BB for use after injury (Figure 3d).

sAXL promotes KC migration while inhibiting KC differentiation

As sAXL was more abundant in DPFi CM than CCL19, we wanted to understand how sAXL regulates signaling pathways in KCs. We therefore used a microarray to perform an unbiased transcriptional analysis where we compared sAXL, DPFi CM, and Epilife on scratch wound transcription in KCs in vitro (Figure 4a). Raw data was analyzed with a one-way analysis of variance identifying 2,574 genes that were significantly and differentially regulated between conditions (Figure 4a). Principal component analysis showed that sAXL and DPFi clustered more closely together than Epilife, thus sharing less variance (Figure 4b). Specifically, variance between Epilife medium and both DPFi CM and sAXL was on the 1st principle component, whereas variance between the biological repeats (P1 and P2) was on the 2nd principle component.

To help determine unique genes involved in accelerated wound closure in vitro, upregulated and downregulated
gene lists of sAXL and DPFi CM versus Epilife were plotted in a Venn diagram (Figure 4c). Using this, we identified 1,222 genes upregulated and 570 downregulated in both DPFi CM and sAXL treated KCs in comparison with KCs treated with the Epilife control. We believe that these gene lists encompass genes that are enabling accelerated scratch wound closure as a result of their differential regulation (Supplementary Table S3). We used Ingenuity Pathway Analysis software to identify pathways activated in response to the genes uniquely regulated by DPFi CM and sAXL, identifying three main pathways, the Hippo pathway, the ephrin pathway, and the epidermal growth factor pathway (Figure 4d). Activation of YAP1, a member of the Hippo pathway, can promote migration of cells while blocking KC
differentiation. In addition, the epidermal growth factor receptor was also upregulated in KCs, predicted to promote cell cycle progression but simultaneously block KC differentiation. 

\textit{EPHA4}, a member of the ephrin pathway, was the most highly upregulated gene in KCs in sAXL and is known to promote cell migration, cell movement, and adhesion of epithelial cells.

To validate transcriptional changes, we performed reverse transcriptase PCR using \textit{EPHA4}, \textit{SOS1}, \textit{IL33}, and \textit{CCL20} primers (Figure 4e and Supplementary Table S3). To see if these genes would also be differentially regulated ex vivo, we isolated RNA from the leading edge of the epidermis of ex vivo punches treated with Epilife, sAXL, CCL19, and PDGF-BB. Here, only the \textit{EPHA4} results were able to be duplicated (Supplementary Figure S7), highlighting the ephrin pathway’s involvement in the wound healing process.

**DISCUSSION**

In this bio-inspired study, we set out to identify if the secretome of hair follicle fibroblasts could accelerate wound closure over and above interfollicular fibroblasts. CM from three distinct fibroblast subtypes found in human scalp skin (DPFi, PFi, and RFi) were assessed, revealing that DPFi CM significantly accelerated wound re-epithelialization both in vitro and ex vivo. Normally in a wound, there are no hair follicles to promote faster re-epithelialization; however, hairy skin does heal faster than hairless skin (Mimoun et al., 2006; Weyandt et al., 2009; Wyrzykowski et al., 2015). The role of hair follicle epithelial cells in wound re-epithelialization has been well described (Ito et al., 2005), whereas our work shows that the hair follicle dermis has a paracrine effect on KC re-epithelialization during wound closure. Whether or not this is a pure physiological process helping to explain how hairy skin heals faster than nonhairy skin or just an observation we can exploit to promote wound closure remains to be determined. Certainly, here, our main goal was to take bio-inspiration from hairy skin to identify new therapeutics to promote wound closure.

Previously, lineage tracing studies in murine skin demonstrated that RFi migrate to a wound bed before PFi (Driskell et al., 2013), suggesting that RFi have a role in wound healing. Our results show that RFi CM promotes a significantly higher growth capacity in KCs in vitro than the control, supporting this proposal. IL-6, which was found to be released by RFi at higher levels than PFi, also promoted a
Figure 4. Microarray analysis reveals unique genes in response to sAXL and DPFi CM in vitro. (a) Normalized intensity values of 2,574 genes differentially expressed in KCs in response to sAXL, DPFi CM, and Epilife. (b) PCA plot showing variance on two components. Component 1 shows treatment variance whereas component 2 shows biological sample variance. (c) Four-way Venn of the upregulated and downregulated genes in sAXL and DPFi CM versus Epilife. (d) RT-PCR analysis on an in vitro wound assay. (e) Top pathways involved in regulating wound closure. Significance is displayed in the graph (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001) as determined by a one-way ANOVA and the error bars represent mean ± SD. ANOVA, analysis of variance; CM, conditioned medium; DPFi, dermal papilla fibroblast; KC, keratinocyte; PCA, principal component analysis; RT-PCR, reverse transcriptase–PCR; SD, standard deviation.
significant increase in KC growth capacity when compared with the control. Potentially, this result represents a normal physiological response to wound healing, where RFi in the wound bed likely release IL-6 to promote re-epithelialization.

Growth factors have been widely used to accelerate cutaneous wound closure. Here, we identified the cytokines sAXL and CCL19, released by DPFin, and showed that they can accelerate human skin wound healing both in vitro and ex vivo. CCL19 has been associated previously with healing of murine oral wounds (McGrory et al., 2004), whereas expression is downregulated in nonhealing diabetic wounds (also in mice [Finley et al., 2016]). To our knowledge, there has not been a connection made between sAXL, the extracellular domain of AXL that is cleaved at the cell surface (Korsunov, 2012; O’Bryan et al., 1995), and cutaneous wound healing. Therefore, this seemed an intriguing cytokine to study here as a novel therapeutic to promote wound closure. To understand how sAXL can promote faster re-epithelialization, we performed a transcriptional analysis, identifying activation of the ephrin signaling pathway in response to sAXL. Ephrin signaling has also been associated with wound closure, wherein upregulation of ephrinB1 and ephrinB2 following injury in mouse skin leads to a down-regulation of adherens junctions and increased migration (Nunan et al., 2015).

It is important to note that there are limitations in our study with respect to donor sex, age, and location of biopsies used. All of the CM collected for in vitro experiments were from male fibroblasts taken from occipital scalp biopsies. The in vitro scratch wounds were performed on scalp skin KCs from male donors aged 34–64 years, whereas the ex vivo punch within a punch wounds were conducted on abdominal tissue from female donors aged 25–45 years. As there are known differences in skin healing based on age (Gosain and DiPietro, 2004; Holt et al., 1992), sex (Adams et al., 2008), and body location (Mimoun et al., 2006), experiments assessing the effect of sAXL and CCL19 on different body locations and with different sexes will provide a more comprehensive view of the role of these cytokines in wound closure. Furthermore, although this study demonstrates that sAXL and CCL19 promote re-epithelialization of human skin in vitro and ex vivo after injury, in vivo studies will need to be performed before translation to fully understand the role of these cytokines in the complex process of wound healing.

In summary, in this body of work we took bio-inspiration from studies demonstrating that hairy skin heals faster than nonhairy skin to develop our hypothesis. We went on to show that hair follicle fibroblasts could accelerate re-epithelialization after wounding faster than interfollicular fibroblasts and identified specific factors in the DPFin secretome responsible for the observed effect. We next demonstrated that these factors, sAXL and CCL19, used individually or in combination with one another could also accelerate wound closure more than the currently used therapeutic PDGF-BB. We therefore propose that these cytokines are potential therapeutic solutions for human skin wound closure that are superior to existing treatments.

MATERIALS AND METHODS

Human skin biopsies

For all in vitro experiments, cells isolated from occipital scalp skin biopsies were used. These were taken from the occipital scalp of male patients aged 34–64 years undergoing surgical proceedings after receiving written informed consent using Imperial College Research Ethics Committee approved consent forms. Tissue is held under Imperial College Healthcare Tissue Bank HTA license 12275 and used in the Imperial College Healthcare Tissue Bank approved project R15055.

For the ex vivo experiments, human abdominal skin with adipose tissue from female patients aged 25–45 years was purchased from Caltag Medsystems (Buckingham, United Kingdom).

Isolation and cell culture of fibroblasts and KCs

DPFin cells were isolated and maintained as previously described (Topouzi et al., 2017). To isolate PFi and RFi from the papillary and reticular dermis, the skin biopsy was separated into two pieces using a scalpel blade to cut adjacent to the epidermis. An explant method was used for culture of DPFin, PFi, and RFi. Comparatively, KCs were isolated from occipital skin using enzymatic digestion. Cultures of DPFin, PFi, and RFi were used to obtain CM, the components of which were identified using a RayBio C-Series human cytokine antibody array C1000 (RayBiotech, Peachtree Corners, GA). Further details regarding cell culture can be found in the Supplementary Materials and Methods.

In vitro and ex vivo wound models

To assess the ability of the fibroblast secretome to accelerate wound healing, CM was obtained from DPFin, PFi, and RFi from three patients. To conduct the in vitro scratch assay, 6-well plates containing confluent KCs were scratched once with a p200 pipette, creating a wound approximately 6 × 10^4 µm^2 (every wound was measured to get an accurate measurement for each repeat). CM or recombinant proteins (Supplementary Table S4) were added to wounds, which were imaged hourly, at the same location on the scratch, from 0 to 9 hours to capture wound closure.

To conduct the ex vivo wound assay, human abdominal skin from four patients was used, following a protocol adapted from Nasir et al. (2019). A 2-mm diameter biopsy punch was used to create wounds approximately 3 mm^2 in small circular pieces of skin, known as a punch within a punch. CM or recombinant proteins were added daily into the center of wounds, which were imaged every 24 hours for 6 days to capture wound closure. Images were analyzed using Image J to determine the wound area. The contrast on each image was changed, which enabled clear distinction of the epidermis from underlying dermis and visualization of the wound edge.

Re-epithelialization model

Wound area was measured over time by imaging the wound, and re-epithelialized area was calculated as the reduction in wound area over time. We modeled the change in re-epithelialization occurring in ex vivo and in vitro models over time as a logistic growth process. In this model, the epithelium surrounding the wound grows from an initial area, a_0, to a growth capacity, K, which may or may not be sufficient to close the wound. The growth is initially exponential (with a growth rate r) but is gradually reduced as the growth capacity is approached. Such a model is commonly used to model growth dynamics in biological systems from cell or animal populations to tumor growth. The
governing equation for re-epithelialized area with respect to time (t) is:

\[ a(t) = \frac{K}{1 + \left(\frac{k}{K} - 1\right)e^{-kt}} \]

We used the nonlinear curve-fitting package LMFIT (https://lmfit.github.io/lmfit-py/) to estimate the parameters \((a_0, K, \text{and } r)\). The Levenberg-Marquardt algorithm of least-squares nonlinear regression was performed on each treatment group, with technical repeats averaged for each patient, giving each fitting regression 38 (in vitro) or 18 (ex vivo) degrees of freedom. The fitting algorithm provides an estimation of the standard error of each parameter, which enabled a pairwise comparison of parameters across the treatment groups. False-discovery rate adjustments to account for multiple comparisons were made to the corresponding P-values obtained from two-tailed t-tests accounting for unequal variances. Statistical tests were performed using the python package StatsModels (https://www.statsmodels.org). To test the differences between groups in terms of end-point re-epithelialization, we used a linear mixed model (using R package nlme) to account for both technical and biological repeats. This model predicts re-epithelialization area based on fixed effects (treatment group) and nested random effects (patient and technical replicate). By using a linear mixed effects modeling approach, we could incorporate all technical replicate data while avoiding pseudoreplication. Post-hoc pair-wise analysis with Tukey corrections for multiple comparisons was used to test for differences between the mean re-epithelialization between groups.

Statistical analyses

The number of biological replicates used for each wound healing experiment is indicated in the respective methods section, where in each case at least three technical replicates were used. Data are presented as the mean and standard deviation. Statistical significance was assessed using one-way analysis of variance and a Tukey multiple-comparison post-hoc test unless otherwise stated. Differences were considered statistically significant at a P-value \(\leq 0.05\).

Data availability statement

Microarray data has been deposited in the Gene Expression Omnibus Reference GSE131615.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: HT, CAH; Data Curation: HT, CJB; Formal Analysis: HT, CJB; Funding Acquisition: CAH; Investigation: HT; Methodology: HT, CAH; Project Administration: CAH; Resources: GW; Software: CJB; Supervision: CAH; Validation: HT, CJB; Visualization: HT, CJB; Writing - Original Draft Preparation: HT; Writing - Review and Editing: HT, CJB, GW, CAH.

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.2019.09.019.

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SUPPLEMENTARY MATERIALS AND METHODS

Isolation and culture of papillary fibroblasts (PFi) and reticular fibroblasts (RFi)

After separation of the papillary and reticular dermis using a scalpel, remaining hair fibers were removed with washmaker’s forceps. The pieces of skin were placed into separate 35-mm plates, chopped into small pieces using scissors, and equally distributed around a dry plate. Once the tissue pieces adhered to the base of the plate, DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% antibiotic-antimycotic (ABAM; Gibco) was added and dishes were incubated at 37 °C with 5% CO2. After 10 days, cells had migrated from the reticular and papillary pieces of skin, which are now termed PFi and RFi. PFi and RFi were maintained in DMEM 10% FBS and grown at 37 °C and 5% CO2.

Keratinocyte (KC) isolation and culture

Occipital scalp skin was washed in DMEM with 2% ABAM for 20 minutes for cleaning before dissection. Adipose tissue was cut off the skin, and the rest of the tissue was placed in dispase overnight at 4 °C. After overnight incubation, the epidermis was peeled off the dermis using sterile forceps and placed in 5 ml 1% trypsin in a 37 °C water bath. The solution was vortexed every 5 minutes to ensure that cells were freed from the epidermis. The reaction was quenched using 5 ml Defined Trypsin Inhibitor (Gibco). A cell strainer with 40-µm pore sizes was used to remove any pieces of tissue. Cells were then centrifuged into a pellet at 200g for 8 minutes. The supernatant was removed and Epilife (Gibco) with Epilife Defined Growth Supplement (EDGS, Gibco) and 1% ABAM was added to the cells. The cells, which are KCs, were then plated at a density of 5,000 cells/cm² in flasks precoated using a coating matrix kit (Gibco). KCs were maintained in Epilife with EDGS and grown at 37 °C and 5% CO2.

Conditioned medium (CM) collection

Dermal papilla fibroblast (DPFi), PFi, and RFi cells from human occipital scalp skin were seeded at a density of 6,000 cells/cm² in DMEM supplemented with 10% FBS. After 48 hours, the cells were washed two times with phosphate buffered saline (PBS; Gibco) and Epilife supplemented with EDGS was added to the cultures. Epilife media conditioned by DPFi, PFi, or RFi was collected 48 hours later. The media was filtered through a 0.22-µm pore sized filter to remove cell debris and stored at −20 °C until used. Unconditioned Epilife media, which was used as a control, was subject to the same treatment but placed in a culture plate containing no cells.

KC scratch wound assay

Six-well plates were precoated using a coating matrix kit. KCs were plated at a density of 6,000 cells/cm² using Epilife supplemented with EDGS. At confluency, a p200 pipette tip was used to scratch the middle of the well to create a wound approximately 6 × 10⁵ µm². KCs were washed two times with PBS to remove debris. CM obtained from DPFi, PFi, and RFi from three patients as well as a control with just Epilife supplemented with EDGS was added onto scratched KCs. Three technical replicates were tested simultaneously for each biological replicate and images were taken at 10 timepoints, from time 0 to 9 hours, using a Leica phase contrast microscope at ×5 magnification. Images were analyzed using Image J software where the edge of the wound was traced and the wound area was automatically calculated in mm².

Ex vivo wound model

Human abdominal skin with adipose tissue was collected from three patients and used for an ex vivo wound model following a protocol adapted from Nasir et al. (2019). The tissue was washed in DMEM supplemented with 2% ABAM for 30 minutes. Then, it was moved to DMEM supplemented with 1% ABAM for the rest of the procedure. Subcutaneous fat was removed to obtain a sheet of epidermis with a thin dermis below. A series of 2-mm diameter partial thickness wounds were made using a Stiefel biopsy punch, and the epidermis and papillary dermis were removed from these punches using forceps and fine scissors. Surrounding these 2-mm punches, a series of 8-mm diameter full wounds were made, resulting in a doughnut shape. With this technique we created a punch within a punch to assess wound closure of the inner 2-mm wound. The donuts were then transferred to the top of a 0.45-µm nylon membrane (Millipore, Burlington, MA) sitting on nonwoven gauze in a 6-well plate. To the nonwoven gauze in each well was added 1.5 ml of William’s E media (Life Technologies, Thermo Fisher Scientific) supplemented with 1% Penicillin/Streptomycin (Gibco), 2 mM L-glutamine (Gibco), 10 µg/ml insulin (Sigma, St. Louis, MO), and 10 ng/ml hydrocortisone (Sigma). Seven conditions were tested simultaneously, with at least five technical replicates for each condition. The conditions tested along with the concentration of the recombinant proteins used are shown in Supplementary Table S4. Daily, 5 µl solutions of the recombinant proteins in Epilife with EDGS (control) were pipetted into the center of the wound. Media was changed daily with excess media being removed from the well and replaced with 1 ml fresh media. Images were taken for 6 days every 24 hours with a Leica stereo microscope. The images were analyzed using Image J where the edge of the wound was traced and the wound area was automatically calculated in mm². RNA was also isolated from the punch within a punch epithelium. Briefly, 3 days after the initial 2-mm wound was created, we used a 4-mm punch to excise the edge of the wound along with the healed area. Samples were placed in dispase for 30 minutes, after which time the re-epithelialized tissue was separated from the underlying dermis. This RNA was used for ex vivo validation of the microarray data.

Human cytokine antibody array

RayBio C-Series human cytokine antibody array C1000 (RayBiotech) was used to analyze the CMs obtained from DPFi, PFi, and RFi to determine the components of the CMs. The protocol and the reagents used were ones provided by the kit supplier. All the solutions were prepared according to the manufacturer’s instructions. The protein analyzer plugin for Image J was used to analyze the cytokine array antibody membranes. Once the normalized values were obtained, the following three comparisons...
were made: DPFi versus PFi, DPFi versus RFi, and PFi versus RFi, by calculating the fold change (log₂) and the P-value (log₁₀) using a t-test with unequal variance. All analysis was performed with two biological replicates.

**Microarray analysis and validation**

Raw data from the microarray was analyzed using the commercial software package GeneSpring GX 14.9 (Agilent Technologies, Santa Clara, CA). The intensity values of the samples were normalized and summarized using RMA algorithm. Parametric tests with the P-value set at 0.05 were performed to determine significant differential expression between samples. Entities were chosen on a fold change cut off of ≥2. Venn diagrams enabled identification of genes which were uniquely upregulated or downregulated in KCs after exposure to sAXL and DPFI CM but not Epilife. Pathway analysis on these specific genes was performed using Ingenuity Pathway Analysis (Agilent). To validate the directional changes of genes identified as significantly and differentially expressed, we performed reverse transcriptase–PCR. RNA extraction was performed using a QiaShredder and RNeasy Mini kit (Qiagen, Hilden, Germany) following manufacturer’s instructions to obtain RNA from fresh tissue, DPFi, PFi, and RFi. cDNA was synthesized using OligoDT primers and SuperScript III (Life Technologies). For the reverse transcriptase–PCR, PowerUP SYBR Green Master Mix (2X; Life Technologies) was used with primers designed against sequences in the University of California, Santa Cruz database (Supplementary Table S5). Reverse transcriptase–PCRs were run on an ABI 7500 Fast RealTime PCR with the cycles as follows: 2 minutes at 50 °C and 2 minutes at 95 °C followed by 35 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Expression analysis was performed relative to GAPDH using the ddCT algorithm, with expression in fresh tissue used as a baseline comparison (value=1). Reverse transcriptase–PCR was performed using cDNA from three biological replicates, and the relative expressions were consistent in all patients. Statistical analysis was performed using one-way analysis of variance test.

**Silencing analysis**

Cells grown on coverslips or 10-μm frozen skin sections cut on a cryostat were fixed with 4% paraformaldehyde for 10 minutes then washed with PBS three times. They were then permeabilized and blocked using 5% goat serum with 0.3% Triton X in PBS for 20 minutes and then incubated with the primary antibody overnight at 4 °C. Antibodies used included chicken anti-vimentin (1:200, Abcam, Cambridge, United Kingdom), mouse anti-PDPN (1:1,000, Abcam), mouse anti-CD26 (1:100, Bio-Rad, Hercules, CA), rabbit anti-collagen I (1:200, Abcam), mouse anti-α-SMA (1:200, Abcam), chicken anti-K14 (1:1,000, Abcam), rabbit anti-Ki67 (1:1,000, Abcam), mouse anti-AXL (1:100, Abcam), and mouse anti-MIP3b (also known as CCL19, 1:200, Abcam). The following day, antibodies were washed off and samples were incubated with the secondary antibodies which included goat anti-mouse 488 (1:200, Molecular Probes, Eugene, OR), goat anti-rabbit 546 (1:200, Molecular Probes), and goat anti-chicken 633 (1:200, Molecular Probes). They were washed three times with PBS and mounted onto glass slides using prolong gold antifade reagent with DAPI (Life Technologies) for visualization. The slides were imaged on a Leica confocal microscope. All analysis was performed with at least two biological replicates.

**Alamra blue assay**

To assess proliferation characteristics of fibroblast subtypes, cells were seeded at 6,000 cells per cm² in DMEM 10% FBS. At 24 hours, the medium was changed to either DMEM 10% FBS with 2 μg/ml sAXL, DMEM 10% FBS with 0.5 ng/ml CCL19, or DMEM 10% FBS only as a control. At 24 hours, 48 hours, and 168 hours, 100 μl alamar blue reagent (Invitrogen, Thermo Fisher Scientific) was added directly to cells in 1 ml culture medium. The cells were incubated for 3 hours at 37 °C, protected from direct sunlight. After 3 hours, 100 μl aliquots were taken in triplicate and their absorbance was measured at 570 nm, using 600 nm as a reference wavelength. A standard curve was calculated; therefore, the fluorescence intensity was proportional to cell number. All analysis was performed with at least two biological replicates.
Supplementary Figure S1. DPFi, PFi, and RFi show differences in expression and morphology. (a) Immunofluorescence staining of fresh tissue with PDPN (green, left two panels) shows expression in the papillary dermis and hypodermis, whereas staining with CD26 (green, right two panels) is expressed throughout the dermis. Nuclei are stained with DAPI (blue). Bar = 130 μm. (b) Immunofluorescence analysis of DPFi, PFi, and RFi at passage 4 using VIM (cyan), COL1 (red), PDPN (green), CD26 (green), α-SMA (green), and DAPI (blue). Bar = 130 μm. (c) RT-PCR analysis showing the relative expression of PDPN, CD26, α-SMA, and CNN1 in DPFi, PFi, and RFi at passage 4, with expression relative to expression in whole skin. Significance is displayed in the graphs (*P < 0.05, **P < 0.01, ***P < 0.001) as determined by one-way ANOVA and the error bars represent (b) mean ± SD and (c) maximum and minimum values. ANOVA, analysis of variance; DPFi, dermal papilla fibroblast; PFi, papillary fibroblast; RFi, reticular fibroblast; SD, standard deviation.
Supplementary Figure S2. Re-epithelialized area in mm² in vitro and ex vivo at the last timepoint. 
(a, b) Re-epithelialized area in mm² at 9 hours in the in vitro experiments. 
(c, d) Re-epithelialized area in mm² at 6 days in the ex vivo experiments. 
Significance is displayed in the graphs (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001) as determined by a linear mixed model in which treatment was considered a fixed effect and patient a random effect. Error bars represent mean ± SD. DPFi, dermal papilla fibroblast; PFi, papillary fibroblast; RFi, reticular fibroblast; SD, standard deviation.
Supplementary Figure S3. sAXL and CCL19 expression in dermal fibroblasts in vivo. Immunofluorescence staining of skin and hair follicles with sAXL (green), CCL19 (green), and DAPI (blue) indicating expression of CCL19 in the papillary dermis and the dermal papilla. Bar = 150 μm.
Supplementary Figure S4. IL-6 is significantly present in RFi CM vs PFi CM. (a) Membrane of the cytokine antibody array with DP Fi CM, PFi CM, and RFi CM. IL-6 is shown in the purple box. (b) The volcano plots show the cytokines present in RFi CM versus PFi CM. Fold change (log2) is shown on the x-axis and P-value (log10) on the y-axis. The red dotted lines show the cut off values, which for the P-value is 0.05 and for the fold change is ranging from –2 to +2. The black dots represent the cytokines that were not found to be significant. The yellow dots represent the cytokines that appear to be significant in the PFi CM and the green dot (IL-6) represents the cytokine that was significantly present in the RFi CM. (c) The graphs outline re-epithelialized area in mm² in the in vitro model over a period of 9 hours when incubated with Epilife, DP Fi CM, PFi CM, RFi CM, and IL-6. (d) Initial growth rate results of the in vitro model demonstrate the ability of Epilife, DP Fi CM, PFi CM, RFi CM, and IL-6 to reach the growth capacity. (e) Growth capacity results of the in vitro model, which describes the capacity of Epilife, DP Fi CM, PFi CM, RFi CM, and IL-6 to close the wound. All comparisons in graphs (d) and (e) are significant (P ≤ 0.05) unless labeled otherwise. CI, confidence interval; CM, conditioned medium; DP Fi, dermal papilla fibroblast; ns, not significant; PFi, papillary fibroblast; RFi, reticular fibroblast.
Supplementary Figure S5. Alamar blue assay in fibroblasts in vitro. (a, b) Number of DPFi, PFi, and RFi cells at day 0, day 1, day 4, and day 7 after addition of (a) sAXL and (b) CCL19. No differences between sAXL and controls, or CCL19 and controls, within a cell type were deemed significant. Error bars represent mean ± SD. DPFi, dermal papilla fibroblast; PFi, papillary fibroblast; RFi, reticular fibroblast; SD, standard deviation.
Supplementary Figure S6. Ex vivo re-epithelialization after topical application of sAXL. Immunofluorescence staining of ex vivo skin biopsies with sAXL applied daily onto the wound at day 3 after wounding (top panel) and day 6 after wounding (bottom panel) with K14 (green), Ki67 (red), VIM (pink) and DAPI (blue), shows re-epithelialization has occurred across the wound bed. Bar = 500 μm.
Supplementary Figure S7. RT-PCR analysis of microarray on ex vivo wound healing model. RT-PCR data from the edge of the wound of day 3 samples of ex vivo punch assays using EPHA4, SOS1, IL33, and CCL20 primers. Significance is displayed in the graph (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001) as determined by a one-way ANOVA and the error bars represent mean ± SD. ANOVA, analysis of variance; CM, conditioned medium; DPFI, dermal papilla fibroblast; RT-PCR, reverse transcriptase-PCR; SD, standard deviation.
### Supplementary Table S1. P-Values and Significant Differences between Conditions in the In Vitro Model

| Comparison         | P-value    | Result |
|--------------------|------------|--------|
| DPfi - CCL19       | 0.656343   | FALSE  |
| Epilife - CCL19    | 0.000258   | TRUE   |
| IL6 - CCL19        | 0.038504   | TRUE   |
| PFi - CCL19        | 6.27E-05   | TRUE   |
| RFi - CCL19        | 0.029047   | TRUE   |
| sAXL - CCL19       | 0.223863   | FALSE  |
| Epilife - DPfi     | 5.70E-07   | TRUE   |
| IL6 - DPfi         | 0.008341   | TRUE   |
| PFi - DPfi         | 8.22E-08   | TRUE   |
| RFi - DPfi         | 0.001393   | TRUE   |
| sAXL - DPfi        | 0.099516   | FALSE  |
| IL6 - Epilife      | 0.258474   | FALSE  |
| PFi - Epilife      | 0.684461   | FALSE  |
| RFi - Epilife      | 0.104521   | FALSE  |
| sAXL - Epilife     | 0.018822   | TRUE   |
| PFi - IL6          | 0.149757   | FALSE  |
| RFi - IL6          | 0.869392   | FALSE  |
| sAXL - IL6         | 0.302484   | FALSE  |
| RFi - PFi          | 0.041112   | TRUE   |
| sAXL - RFi         | 0.007492   | TRUE   |

The table shows the P-values and statistical t-test results at the last timepoint of the in vitro re-epithelialization model complementing Figures 1c and 2d. Results on the tables are shown as TRUE (significant) or FALSE (not significant). Significance was defined as $P < 0.05$. All P-values have been adjusted for multiple comparisons using the Tukey method. Abbreviations: DPfi, dermal papilla fibroblast; PFi, papillary fibroblast; RFi, reticular fibroblast.

### Supplementary Table S2. P-Values and Significant Differences between Conditions in the Ex Vivo Model

| Comparison         | P-value    | Result |
|--------------------|------------|--------|
| CCL19 - AXL        | 0.521403   | FALSE  |
| DPfi - AXL         | 0.521403   | FALSE  |
| Epilife - AXL      | 0.008244   | TRUE   |
| PDGF - AXL         | 0.10927    | FALSE  |
| PFi - AXL          | 0.07309    | FALSE  |
| RFi - AXL          | 0.003946   | TRUE   |
| DPfi - CCL19       | 0.979349   | FALSE  |
| Epilife - CCL19    | 0.000629   | TRUE   |
| PDGF - CCL19       | 0.027284   | TRUE   |
| PFi - CCL19        | 0.013451   | TRUE   |
| RFi - CCL19        | 0.000518   | TRUE   |
| Epilife - DPfi     | 0.000629   | TRUE   |
| PDGF - DPfi        | 0.02732    | TRUE   |
| PFi - DPfi         | 0.010965   | TRUE   |
| RFi - DPfi         | 0.000518   | TRUE   |
| PDGF - Epilife     | 0.347385   | FALSE  |
| PFi - Epilife      | 0.521197   | FALSE  |
| RFi - Epilife      | 0.66544    | FALSE  |
| PDGF - PDGF        | 0.762032   | FALSE  |
| RFi - PDGF         | 0.190386   | FALSE  |
| RFi - PFi          | 0.318493   | FALSE  |

The table shows the P-values and statistical t-test results at the last timepoint of the ex vivo re-epithelialization model complementing Figures 1h and 3b. Results on the tables are shown as TRUE (significant) or FALSE (not significant). Significance was defined as $P < 0.05$. All P-values have been adjusted for multiple comparisons using the Tukey method. Abbreviations: DPfi, dermal papilla fibroblast; PFi, papillary fibroblast; RFi, reticular fibroblast.
**Supplementary Table S4. Factors Added to In Vitro and Ex Vivo Wounds**

| Condition | Concentration | Supplier |
|-----------|---------------|----------|
| sAXL      | 2 µg/ml       | R&D Systems |
| CCL19     | 0.5 ng/ml     | BioLegend |
| PDGF-BB   | 5 ng/ml       | BioLegend |
| DPFi CM   | 48-hour conditioned medium | Human occipital dermal fibroblasts |
| PFi CM    | 48-hour conditioned medium | Human occipital dermal fibroblasts |
| RFi CM    | 48-hour conditioned medium | Human occipital dermal fibroblasts |
| Epilife   | Prepared as advised by supplier with EDGS | Gibco Life Technologies |

Abbreviations: CM, conditioned medium; DPFi, dermal papilla fibroblast; EDGS, Epilife Defined Growth Supplement; PFi, papillary fibroblast; RFi, reticular fibroblast.

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**Supplementary Table S5. Primer Sequences Used in RT-PCR**

| Gene     | Sequence (5' to 3')                      |
|----------|------------------------------------------|
| GAPDH    | F- CGTCTTCACCACCCATGGAGA<br>R- CCGGCCATCACGCCACAGTTT |
| EPHA4    | F- GTGAACAGCAAACCTTGTGC<br>R- ATGGAGGGGGGTAAACCGTACG |
| SOS1     | F- CCTGCTACCTTACACCCGAAG<br>R- CGACTCACCACAGCTACTCTTC |
| IL33     | F- CTGTGAGTCTTGGGTTGAGTACC<br>R- GCTATCGTGGAAACCTTGG |
| CCL20    | F- GCCATAGAAGGCTGTGAC<br>R- ACAAGTCCGTCGAGGAC |
| PDPN     | F- GCCACCAATGCTACCTCACGGAGAAA<br>R- TTTGCAGCGGCGTCATCACCC |
| CD26     | F- TACCTCTGCTGGGTGGTGGT<br>R- GTTGATTACAGCTCCTGCG |
| α-SMA    | F- GTTGACAGAAGCAGACAGCGAAC<br>R- GGCCACACCAAGCTACATTG |
| CNN1     | F- CCAAGAGATAGACACCACAG<br>R- TGGACTCACCTGTGATGGG |

Abbreviation: F, forward; R, reverse; RT-PCR, reverse transcriptase—PCR.

1 Primer set was taken from Hagh et al. (2015).
2 Primer set was taken from Janson et al. (2012).