

Fibroblast Heterogeneity in Healthy and Wounded Skin

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Fibroblasts are the main cell type in the dermis. They are responsible for the synthesis and deposition of structural proteins such as collagen and elastin, which are integrated into the extracellular matrix (ECM). Mouse and human studies using flow cytometry, cell culture, skin reconstitution, and lineage tracing experiments have shown the existence of different subpopulations of fibroblasts, including papillary fibroblasts, reticular fibroblasts, and fibroblasts comprising the dermal papilla at the base of the hair follicle. In recent years, the technological advances in single-cell sequencing have allowed researchers to study the repertoire of cells present in full-thickness skin including the dermis. Multiple groups have confirmed that distinct fibroblast populations can be identified in mouse and human dermis on the basis of differences in the transcriptional profile. Here, we discuss the current state of knowledge regarding dermal fibroblast heterogeneity in healthy mouse and human skin, highlighting the similarities and differences between mouse and human fibroblast subpopulations. We also discuss how fibroblast heterogeneity may provide insights into physiological wound healing and its dysfunction in pathological states such as hypertrophic and keloid scars.

Mammalian skin comprises two layers, a stratified epithelium, the epidermis, and an underlying connective tissue, the dermis, including the subcutaneous fat. The dermis is a structural scaffold that gives strength and elasticity to the skin. It is composed mainly of type I and type III collagens and other collagen subtypes, proteoglycans, and elastin (Watt and Fujiwara 2011). Within the dermis are embedded blood and lymphatic vessels, nerve cells, immune cells,

and appendageal structures, which are specialized invaginations of the epithelium: hair follicles, sebaceous, and sweat glands that penetrate the dermis. The dermis microstructure differs between human and mouse skin. In mice, there is a relatively high density of hair follicles and the dermo-epidermal junction is relatively flat. In humans, the density of hair follicles is lower, except in a few locations such as scalp and face, and the epidermis projects into the dermis via rete ridges (Fig. 1).

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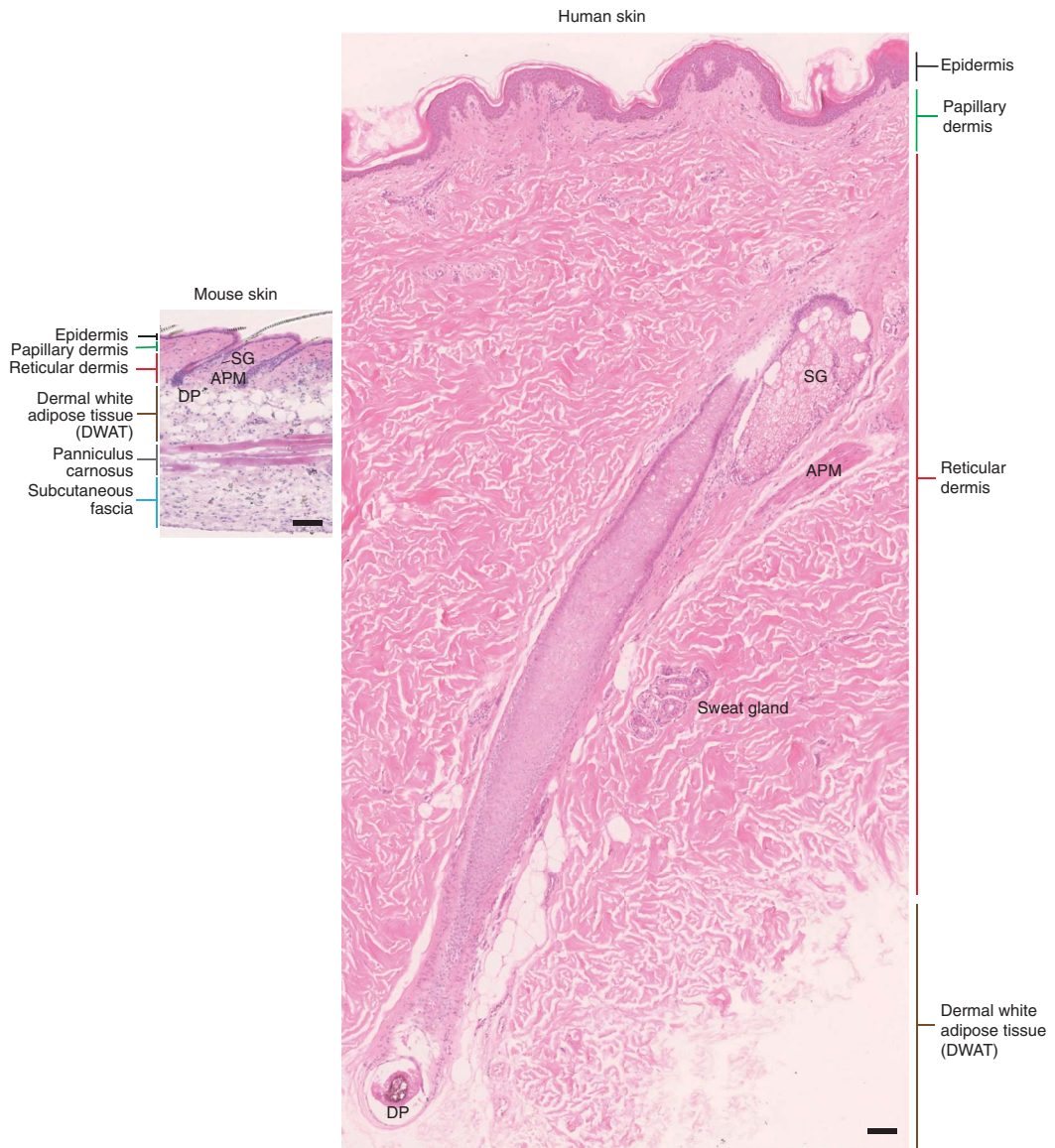


Figure 1. Comparison of mouse and human body skin. The epidermis and dermis are thicker in human than in mouse skin. In most body sites, mouse body skin has a higher density of hair follicles compared to human body skin and the sweat glands are segregated to non-hair-bearing regions. In both mouse and human skin, the dermis is composed of a papillary and a reticular layer. Underneath the dermis, in mice, the subcutaneous fat is composed of a dermal with adipose tissue (DWAT), a panniculus carnosus (striated muscle), and the fascia beneath. In human, the dermis is separated from the underlying fascia by a large (many centimeters in the truck area) layer of subcutaneous fat (DWAT). Scale bars, 100 μm . (SG) Sebaceous gland, (APM) arrector pili muscle, (DP) dermal papilla.

In both mouse and human, the dermis is composed of two distinct layers identified by histology (Meigel et al. 1977). The upper, papillary dermis lies immediately beneath the epidermis and reaches a depth of 300–400 μm in humans, depending on age and body site. The papillary dermis is composed of poorly oriented collagen bundles that support the overlying epidermis (Watt and Fujiwara 2011). The lower dermis, called the reticular dermis, is located underneath the papillary dermis (Woodley 2017) and over subcutaneous fat tissue (Driskell et al. 2014). Reticular dermis contains thicker, directionally oriented collagen bundles (Sorrell and Caplan 2004).

Fibroblasts are the main cell type in the dermis and are responsible for the synthesis and deposition of structural proteins such as collagen and elastin, which are integrated into the extracellular matrix (ECM) (Watt and Fujiwara 2011; Shaw and Rognoni 2020; Plikus et al. 2021). In vitro studies in human (Harper and Grove 1979; Schönherr et al. 1993; Sorrell and Caplan 2004; Sorrell et al. 2007; Janson et al. 2012; Woodley 2017) and in vivo studies in mouse skin (Driskell et al. 2013; Kaushal et al. 2015; Rinkevich et al. 2015; Jiang et al. 2018; Abbasi et al. 2020; Leavitt et al. 2020; Goss et al. 2021) have shown the existence of different subpopulations of fibroblasts, including papillary fibroblasts, reticular fibroblasts, arrector pili muscle (APM) fibroblasts, and fibroblasts comprising the dermal papilla and sheath fibroblasts at the base of the hair follicle. Lineage-tracing studies in mice have defined markers of different fibroblast subpopulations and shown how functionally distinct fibroblasts behave during development, wound healing, and fibrosis. Single-cell RNA sequencing (scRNA-seq) studies (Philippeos et al. 2018; Tabib et al. 2018; Joost et al. 2020; Solé-Boldo et al. 2020; Vorstandlechner et al. 2020; Deng et al. 2021; Reynolds et al. 2021) have also revealed heterogeneity in mouse and human skin.

In this review, we discuss the current state of knowledge regarding dermal fibroblast heterogeneity in healthy mouse and human skin, and how different fibroblast subpopulations contribute to wound healing.

FIBROBLAST HETEROGENEITY IN HEALTHY AND WOUNDED ADULT MOUSE SKIN

Heterogeneity of Fibroblasts in Healthy Mouse Skin

Some of the earliest evidence for fibroblast heterogeneity in mouse skin came from studies of the dermal papilla at the base of the hair follicles (Kishimoto et al. 2000; Shimizu and Morgan 2004; Rendl et al. 2008). Mice have several distinct hair follicle types that differ in length, thickness, and in the presence or absence of kinks in the hair shaft (Schlake 2007). Although some genes are expressed in all dermal papillae, the transcription factor Sox2 is specifically expressed in the dermal papillae of guard/awl/auchene follicles (Driskell et al. 2009, 2011). Another example of a specific subset of dermal fibroblasts are the cells of the APM (Fujiwara et al. 2011), which express the $\alpha 8\beta 1$ integrin that mediates adhesion to nephronectin deposited by keratinocytes in the bulge of the hair follicle.

Fibroblast Populations during Mouse Skin Development

In characterizing APM and dermal papilla fibroblasts, flow cytometry, cell culture, skin reconstitution, and lineage-tracing techniques were applied, leading us to consider whether the same methodology could be used to characterize fibroblast populations within the papillary and reticular dermis of the mouse. For this, we made extensive use of Pdgfr α expression as a pan-fibroblast marker (Kalluri and Zeisberg 2006; Collins et al. 2011). Focusing on mouse back skin, we found that at embryonic day 12.5 (E12.5), there was a multipotent mesenchymal cell progenitor expressing Pdgfr α , Dlk1, and Lrig1. At E14.5, a subset of these cells differentiated into Sox2⁺ dermal papillae (Pdgfr α ⁺, Cd26⁻, Sox2⁺). At approximately E16.5, the mesenchymal progenitors differentiated into papillary (Pdgfr α ⁺, Dlk1⁻, Blimp1⁺, Lrig1⁺) and reticular fibroblasts (Pdgfr α ⁺, Dlk1⁺, Blimp1⁻). The papillary fibroblasts formed the dermal papillae of zigzag hairs and the APM, whereas the

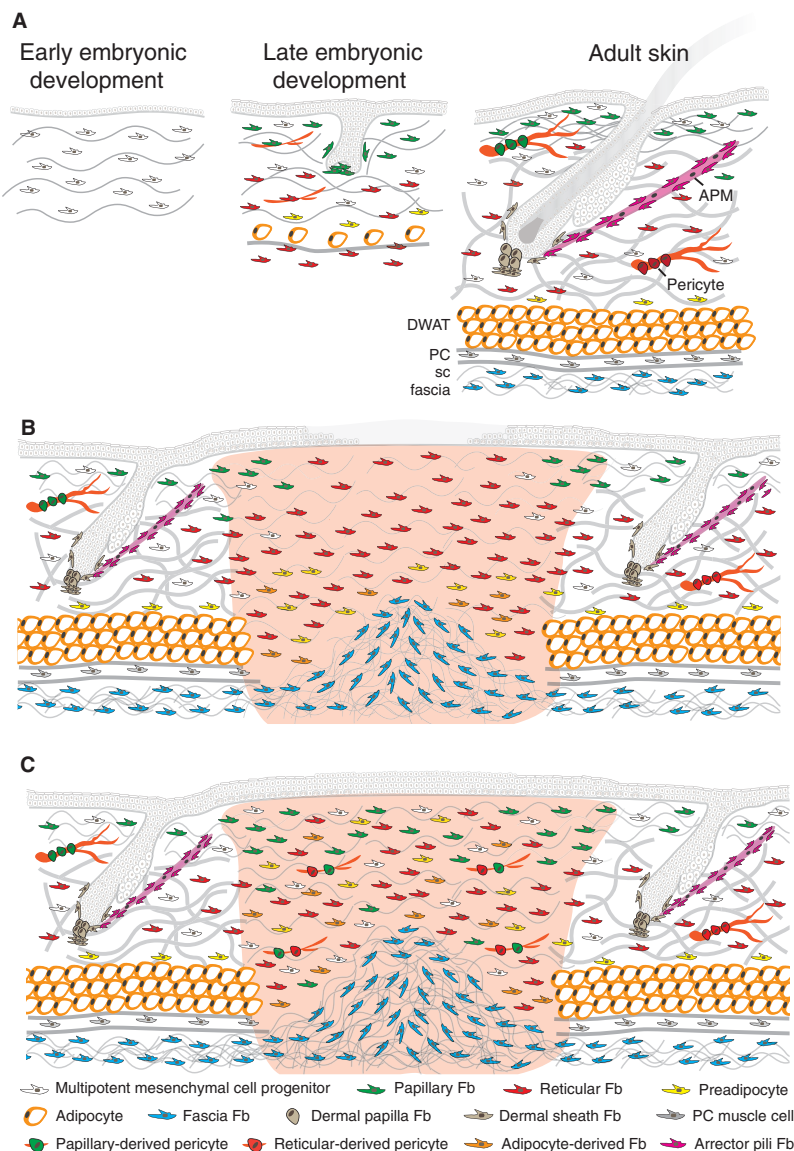


Figure 2. Dermal cell organization during mouse skin development (A) and early (B) and advanced adult wound healing (C). In early embryonic development, there are multipotent mesenchymal cell progenitors present in the dermis. In late embryonic development, the mesenchymal progenitors have differentiated into papillary and reticular fibroblasts (Fb). The papillary fibroblasts form the dermal papillae of developing hair follicles, whereas the reticular fibroblasts gave rise to preadipocytes and mature adipocytes. In mouse adult skin, many hair follicles project into the dermis. There are specific fibroblasts in the dermal papilla at the base of each follicle, in the dermal sheath around each follicle, and in the APM that connects to the hair follicle bulge. The papillary dermis is composed of multipotent mesenchymal cell progenitors, papillary fibroblasts, and papillary derived pericytes. The reticular dermis is composed of multipotent mesenchymal cell progenitors, reticular fibroblasts, preadipocytes, and reticular derived pericytes. The subcutaneous fat is mainly composed by mature adipocytes in the dermal white adipose tissue (DWAT), panniculus carnosus (PC), muscle cells in the panniculus muscle layer, and fascia fibroblasts in the subcutaneous (sc) fascia. (A) In wounded adult skin, the initial wave of dermal repair is dominated by reticular fibroblasts; papillary fibroblasts from the upper dermis follow later. Fascia fibroblasts collectively migrate into the wound to contribute to the repair of deep full thickness wounds in the early phase. Also, preadipocytes and adipocytes in the lower dermis start to be recruited. At this stage, fibroblasts are highly proliferative and start to secrete collagen fibers. Reepithelialization is not complete. (B) In the advanced phase of wound healing, reepithelialization is complete and reticular fibroblasts, papillary fibroblasts, preadipocytes, adipocytes, and multipotent mesenchymal cell progenitors have migrated into the wound and are randomly distributed. More fascia fibroblasts from the subcutaneous fascia may have migrated and expanded into the lower area of deep full thickness wounds. At this stage, collagen fibers are secreted and remodeled by fibroblasts.

reticular fibroblasts gave rise to pre- and mature adipocytes (Fig. 2A; Table 1; Driskell et al. 2013).

Using similar approaches, Longaker's laboratory identified distinct mouse embryonic fibroblast lineages within the dorsal dermis. The Engrailed-1 (En1)-positive (En1⁺) lineage is the primary contributor to connective tissue deposition and organization during embryonic development, cutaneous wounding, radiation fibrosis, and cancer stroma formation (Rinkevich et al.

2015). Cd26 is highly expressed by the En1⁺ fibroblast lineage. Cd26-specific fibroblast ablation results in a diminution of connective tissue deposition in wounds in adult murine skin (Rinkevich et al. 2015). In contrast, En1⁻ fibroblasts enhance dermal development and regeneration (Jiang et al. 2018).

Paired-related homeobox 1 (Prrx1)-positive (Prrx1⁺) and Prrx1-negative (Prrx1⁻) mouse embryonic fibroblast lineages have been de-

Table 1. Experimentally validated markers of dermal cell populations in mouse and human

Cell type	Mouse markers	Human markers	References
Pan-fibroblast	Pdgfra* , Pdgfrβ*, Col1a1 , Vim , Cd90 , Lum , Dcn	PDGFRα* , COL1A1 , FAP , VIM , CD90 , LUM , DCN	Saalbach et al. 1998; Kalluri and Zeisberg 2006; Collins et al. 2011; Philippeos et al. 2018
Dermal papilla fibroblast	Crabp1, Alkphos, Promolin1, Blimp1 , Lef1	VERSICAN, MZF1, MEF2C, BLIMP1 , LEF1	Higgins et al. 2013; Telerman et al. 2017; Heitman et al. 2020
Dermal sheath fibroblast	A-Sma* , Acan, Myh10, Mlck, My19	A-SMA* , GREM2, SM22, MYH11, MYL9 , MEF2C	Higgins et al. 2013; Niyama et al. 2018; Heitman et al. 2020
Papillary fibroblast	Lrig1, Blimp1, Cd39 , Cd26* , Col6a5 , Col23a1 , Apccdd1, Hspb3 , Fap	APCDD1, COL18A1, ENPP2, COL6A5 , CD39 , CD26* , COL23A1 , HSPB3 , WIF1	Driskell et al. 2013; Rinkevich et al. 2015; Jiang et al. 2018; Philippeos et al. 2018; Korosec et al. 2019; Solé-Boldo et al. 2020
Reticular fibroblast	Dlk1, Sca1*, Prrx1	A-SMA* , MGP, PPARγ, CNN1, COL11A1, TGM2, CD36, CTHRC1, CXCL1, EMGN, MGST1	Janson et al. 2012; Driskell et al. 2013; Rinkevich et al. 2015; Jiang et al. 2018; Philippeos et al. 2018; Korosec et al. 2019; Solé-Boldo et al. 2020
Arrector pili muscle fibroblast	A-Sma* , Itga8, Sm22α, Npnt	Not described in human	Fujiwara et al. 2011
Preadipocyte fibroblast	Cd34 , Sca1 , Pdgfra*	CD34 , PDGFRα* , CD24, CD29	Rodeheffer et al. 2008; Festa et al. 2011; Berry and Rodeheffer 2013; Rivera-Gonzalez et al. 2014
Adipocyte	Adiponectin , Perilipin , Pparγ, Fabp4	ADIPONECTIN , PERILIPIN	Festa et al. 2011; Rivera-Gonzalez et al. 2014
Pericyte	A-Sma* , Cd146 , Rgs5, Tbx18 , Pdgfrβ* , Ng2, Pdgfra*	A-SMA* , CD146 , RGS5 , TBX18 , PDGFRβ*	Guimarães-Camboa et al. 2017; Yamazaki et al. 2017; Goss et al. 2021

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Table 1. Continued

Cell type	Mouse markers	Human markers	References
Fascia fibroblast	CD26* , En1, Sca1*, Nov , Gpx3	A-SMA*, CD26* , CD44, NOV	Correa-Gallegos et al. 2019
Dermo-hypodermal junction fibroblast	Not described in mouse	KLF9, NTN9	Korosec et al. 2019; Haydout et al. 2020

Shared population markers between mouse and human are highlighted in bold, while markers, which are not population-specific, have been highlighted with an asterisk. (ACAN) Aggrecan core protein, (ALKPHOS) alkaline phosphatase, (APCDD1) adenomatous polyposis coli down-regulated 1, (BLIMP1) B lymphocyte-induced maturation protein-1, (CD146) cluster of differentiation 146 (melanoma cell adhesion molecule), (CD24) cluster of differentiation 24 (small cell lung carcinoma cluster 4 antigen), (CD26) cluster of differentiation 26 (dipeptidyl peptidase 4), (CD29) cluster of differentiation 29 (integrin β 1), (CD34) cluster of differentiation 34 (hematopoietic progenitor cell antigen), (CD39) cluster of differentiation 39 (ectonucleoside triphosphate diphosphohydrolase 1), (CD44) cluster of differentiation 44 (homing function and Indian blood group system), (CD90) cluster of differentiation 90 (Thy-1 cell surface antigen), (CNN1) Calponin-1, (COL11A1) collagen, type XI α 1 chain, (COL18A1) collagen, type XVIII α 1 chain, (COL1A1) collagen, type I α 1 chain, (COL23A1) collagen, type XXIII α 1 chain, (COL6A1) collagen, type VI α 1 chain, (CRABP1) cellular retinoic acid-binding protein 1, (CTHRC1) collagen triple helix repeat-containing 1, (CXCL1) growth-regulated α protein, (DCN) decorin, (DLK1) delta-like noncanonical Notch ligand 1, (EN1) Engrailed homeobox 1, (ENPP2) ectonucleotide pyrophosphatase/phosphodiesterase 2, (FAP) fibroblast activation protein (prolyl endopeptidase), (GPX3) glutathione peroxidase 3, (GREM2) Gremlin 2 (DAN family BMP antagonist), (HSPB3) heat shock protein β 3, (ITGA8) integrin subunit α 8, (KLF9) Krüppel-like factor 9, (LEF1) lymphoid enhancer-binding factor 1, (LRIG1) leucine-rich repeats and immunoglobulin-like domains protein 1, (LUM) Lumican, (MEF2C) myocyte enhancer factor 2C, (MEF2C) myocyte enhancer factor 2C, (MGST1) microsomal glutathione S-transferase 1, (MLCK) myosin light-chain kinase, (MYH10) myosin heavy chain 10, (MYH11) myosin heavy chain 11, (MYL9) myosin light chain 9, (MZF1) myeloid zinc finger 1, (NG2) neural/glia antigen 2 (melanoma-associated chondroitin sulfate proteoglycan), (NOV) nephroblastoma overexpressed, (NPNT) nephronectin, (NTN9) Neurturin 9, (PDGFR- α) platelet-derived growth factor receptor α , (PDGFR- β) platelet-derived growth factor receptor β , (PRRX1) paired related homeobox 1, (RGS5) regulator of G-protein signaling 5, (SCA1) stem cell antigen 1, (SM22) smooth muscle protein 22, (TBX18) T-box transcription factor 18, (TGM2) protein-glutamine γ -glutamyltransferase 2, (VIM) Vimentin, (WIF1) WNT inhibitory factor 1, (A-SMA) α smooth muscle actin.

scribed within the ventral dermis (Leavitt et al. 2020). Similar to En1⁺ fibroblasts in the dorsal dermis, Prrx1⁺ delineates at least two distinct embryonic fibroblast populations (Leavitt et al. 2020). Prrx1⁺ fibroblasts from the ventral lineage have been shown to be scar-forming fibroblasts. Those cells are responsible for most of the collagen production in the dermis following radiation, wounding, and in tumor stroma formation (Hu et al. 2018).

Fibroblast Populations in Adult Mouse Skin

In recent years, scRNA-seq of adult mouse skin has confirmed the existence of a wide variety of fibroblast subtypes. Joost et al. (2020) identified seven subtypes, four of which were significantly expanded in either anagen or telogen, demonstrating that the composition of fibroblasts is in-

fluenced by the hair cycle. Markers of the four major fibroblast subpopulations have provided information about their distribution within the skin. F1 (Sparc^{hi}/Dcn⁺) and F2 (Sparc^{lo}/Dcn⁺) are uniformly distributed in the dermis, with F1 being enriched in anagen and F2 in telogen. F3 (Gpx3⁺/Plac8^{lo}) are spatially segregated to the hypodermis, and F4 (Gpx3⁺/Plac8^{hi}) are located within the adventitia (Joost et al. 2020). It is hypothesized that F1 and F2 represent a single fibroblast population that shows changes in gene expression in response to different stages of the hair cycle (Joost et al. 2020).

Pericytes Emerge from Fibroblast Populations

Pericytes are perivascular cells embedded within the basement membrane of blood vessels and can be found within arterial, venous, and lym-

phatic vascular channels in most tissues (Hall 2006; Paquet-Fifield et al. 2009). Pericytes interact with endothelial cells to regulate blood flow via vessel constriction, maintain vessel wall integrity, regulate vascular permeability, and control angiogenesis through directing endothelial cell proliferation and migration (Thomas et al. 2017). Pericytes express a range of markers including Rgs5 (Bondjers et al. 2003), Pdgfr β (Armulik et al. 2011), Nestin (Birbrair et al. 2013), Tbx18 (Guimarães-Camboa and Evans 2017), Cd146 (Dvoretzkiy et al. 2019), and neural/glial antigen 2 (Ng2) (Armulik et al. 2011). However, none of these markers is pericyte specific as they can also be expressed by other perivascular cell populations, including adventitial fibroblasts and endothelial cells.

Although a small proportion of embryonic pericytes in E15.5 mouse skin have a hematopoietic origin (Yamazaki et al. 2017), the lineage relationships of most pericytes were unknown until recently. Goss et al. discovered that Ng2⁺ pericytes can be categorized into four subpopulations based on expression of Pdgfr α and Pdgfr β . Lineage tracing shows that Ng2⁺ cells do not give rise to dermal fibroblasts and that the origins of Ng2⁺ pericytes depend on their location (Goss et al. 2021). Thus, papillary fibroblasts (Lrig1⁺ lineage) give rise to pericytes in the upper dermis, whereas pericytes in the lower dermis are primarily derived from reticular Dlk1⁺ fibroblasts. Notwithstanding the different origins of skin pericytes, most scRNA-seq studies in mouse skin have identified only one pericyte population (Ge et al. 2020). One potential explanation is that because there are no pericyte-specific markers and clustering methods are based on marker expression, pericyte heterogeneity may be underrecognized.

Mouse Fibroblast Heterogeneity in Physiological Wound Healing

In mouse skin, the wound healing process depends on the size of the wound. Both large (>1 cm²) and small (<1 cm²) wounds heal by a combination of contraction and reepithelialization, which is followed by the formation of new connective tissue leading to scar formation.

However, large wounds also contain a regenerative zone, which is rich in fibroblasts in which hair follicle neogenesis occurs (Ito et al. 2007) resulting in regenerative wounds (Fig. 3). Furthermore, the size of the wound that is required for hair follicle neogenesis depends on the model organism; for example, the African spiny mouse is capable of full regeneration of dorsal skin wounds (Seifert et al. 2012).

Loss of Postnatal Fibroblast Quiescent State on Wounding

Dermal maturation is governed by a tight balance of fibroblast proliferation, quiescence, and ECM deposition. There is a switch in fibroblast behavior from highly proliferative in embryonic dermis to quiescent in postnatal skin, associated with ECM deposition (Rognoni et al. 2018). On wounding, this postnatal quiescent state is lost, leading to the activation, proliferation, and migration of different fibroblast lineages at the wound site (Fig. 2B; Shaw and Martin 2016). Intriguingly, in addition to depositing/remodeling ECM in the wound bed, fibroblasts are able to acquire a dermal papilla or adipocyte fate in response to distinct signals promoting hair follicle and DWAT regeneration (Gay et al. 2013; Plikus et al. 2017; Lim et al. 2018).

Adult Fibroblast Populations within the Wound Bed

Lineage-tracing experiments have revealed that, on wounding, mouse fibroblasts in the lower dermis are the first to repopulate the damaged tissue and deposit ECM. Fibroblasts in the papillary dermis move in later and have a role in remodeling and/or appendage regeneration (Driskell et al. 2013; Rognoni et al. 2016). Hair follicle neogenesis in healing wounds occurs predominantly in the central wound bed, suggesting the existence of tissue repair zones with differential regenerative potential (Ito et al. 2007).

New technologies such as scRNA-seq have identified 12 fibroblast populations in the wound bed of mouse skin at 12 d post-wounding, which not only differ in transcription factor

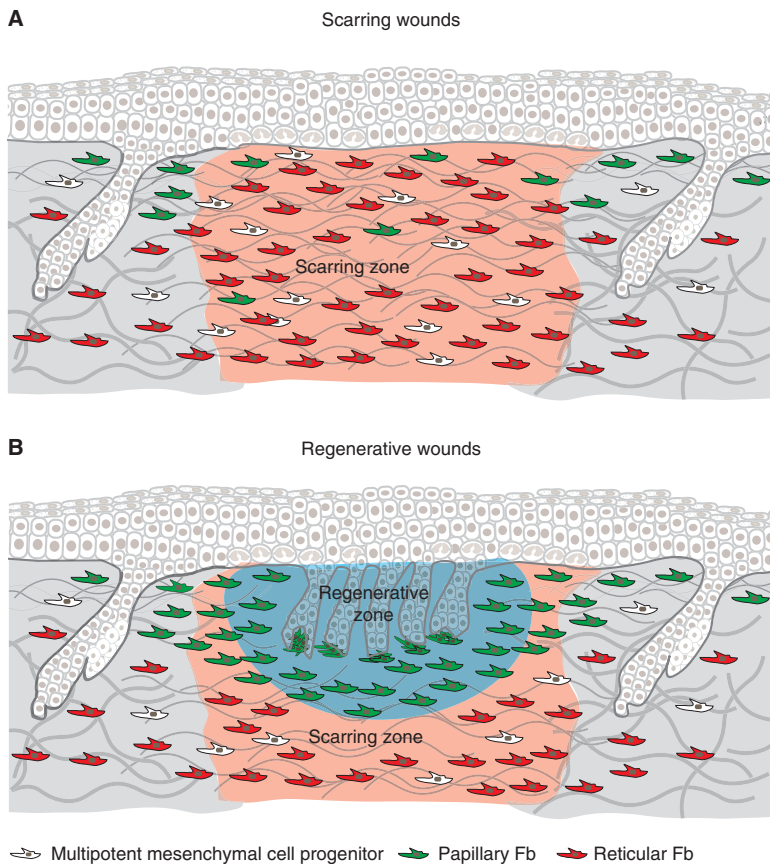


Figure 3. Dermal wound healing zones in scarring and regenerative wounds. In mouse skin, the wound healing process differs depending on the size of the wound. Both large (>1 cm²) and small (<1 cm²) wounds heal by a combination of contraction and reepithelialization, which is followed by the formation of new connective tissue leading to scar formation (A). However, large wounds also contain a regenerative zone that is rich in fibroblasts in which hair follicle neogenesis occurs (B). Conversely, a small wound (<1 cm²) repairs without de novo hair follicle formation, resulting in scarring (A).

and signaling pathway marker/receptor/ligand expression but also in cell cycle state and spatial distribution (Guerrero-Juarez et al. 2019). Furthermore, an scRNA-seq comparison of late-stage (18 d post-wounding) large regenerative and fibrotic wounds identified the presence of three shared myfibroblast populations (Gay et al. 2020). Group 1 and group 2 share an ECM degradation, organization, and preadipocyte signature, although group 2 differs in the inclusion of a proliferation profile. Group 3 displays a transcriptional profile associated with smooth muscle contraction and laminin and

collagen interaction (Gay et al. 2020). Within fibrotic wounds, group 3 myofibroblasts are the predominant population (Gay et al. 2020). However, the functional significance of this cellular diversity requires further study, including the role of the wound bed microenvironment in controlling the properties of different fibroblast populations.

Sophisticated multiomics approaches are offering further insights into fibroblast phenotypes (Mascharak et al. 2021). By combining scRNA-seq and ATAC-seq, a recent study revealed that the wound bed microenvironment

induces significant chromatin rearrangement in recruited fibroblasts, generating a central, upper dermal regenerative zone and a peripheral, lower dermal scarring zone. Additionally, pharmacological intervention targeting Runx1 and retinoic acid signaling can modulate wound repair outcomes, indicating that the regenerative capacity of fibroblasts can be modulated (Fig. 3; Abbasi et al. 2020). Application of Runx1 inhibitors to healing wounds reduces de novo hair follicle formation and regenerative potential, whereas application of exogenous retinoic acid has the opposite effect, augmenting regeneration and resulting in an increase in new hair follicles (Abbasi et al. 2020).

Dermal–Epidermal Communication during Wound Healing

Spatial fibroblast segregation and interaction with the epidermis are essential for establishing a regenerative zone in the upper dermis, underscoring the importance of the local microenvironment. Key epidermal signals promoting a regenerative niche in the upper dermis include the Wnt/ β -catenin and Sonic hedgehog (Shh) pathway (Lichtenberger et al. 2016; Lim et al. 2018). In contrast, sustained activation of Wnt/ β -catenin signaling in dermal fibroblasts is insufficient for hair follicle neogenesis, inhibits adipocyte differentiation, and induces fibrotic lesions (Hamburg-Shields et al. 2015; Mastrogiannaki et al. 2016; Rognoni et al. 2016). Conversely, constitutive overexpression of the canonical Wnt/ β -catenin transcription factor Lef1 in dermal cells primes the wound bed toward more regenerative skin repair (Phan et al. 2020).

Fibroblast-specific inhibition of β -catenin signaling in mice enhances hair follicle regeneration by preventing the early expansion of lower (profibrotic) dermal cells, whereas Lef1 depletion in dermal cells impairs hair follicle neogenesis, emphasizing its key role in promoting a papillary/dermal papilla fate in wound bed fibroblasts (Rognoni et al. 2016; Phan et al. 2020). Dermal Hedgehog (Hh) activation enables hair follicle regeneration by inducing dermal papilla gene expression and conversion of profibrotic Wnt/ β -catenin signaling, promoting

active wound fibroblasts to follow a dermal papilla fate in the upper dermis (Lim et al. 2018).

Different Fibroblast Populations Are Influenced by the Local Wound Microenvironment

scRNA-seq has revealed heterogeneous expression of dermal papilla signature genes within active wound bed fibroblasts, suggesting that additional factors such as spatial distribution, cell-cell/ECM interactions, and other signaling pathways are essential for controlling cell plasticity. For example, Wnt/ β -catenin signaling is particularly high in scar-forming wounds (Rognoni et al. 2016), and this prolonged Wnt activity is promoted by macrophages that phagocytize and degrade the Wnt inhibitor SFRP4 in the lower wound bed dermis (Gay et al. 2020). A specific macrophage subset promotes proliferation of an activated adipocyte precursor population during wound repair, providing a striking example of how functional heterogeneity is regulated at the cellular level within the skin (Shook et al. 2018).

Increased mechanical tension in the lower wound bed promotes scar formation by reactivating expression of *En1* in reticular fibroblasts (Mascharak et al. 2021), consistent with the role of *En1* in inducing a profibrotic fibroblast fate during embryonic skin development (Rinkevich et al. 2015). Blocking mechanotransduction by pharmacological or genetic inhibition of Yes-associated protein (YAP) prevents *En1* activation and promotes wound regeneration, including recovery of skin appendages and mechanical strength, thereby preventing scarring (Mascharak et al. 2021). The importance of the local microenvironment is also apparent during wound induced angiogenesis, in which the location of the regenerating blood vessel determines which fibroblast subpopulation gives rise to blood vessel-associated Ng^{2+} pericytes (Goss et al. 2021).

Several fibroblast subpopulations contribute to dermal regeneration, and scRNA-seq and lineage tracing have revealed that interfollicular fibroblasts are the major source of wound bed fibroblasts. The specialized fibroblast subpopu-

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lations of the dermal papilla, pericytes, dermal sheath, and APM make little contribution to wound repair (Kaushal et al. 2015; Abbasi et al. 2020; Goss et al. 2021). In contrast, adipocytes of the dermal white adipose tissue (DWAT) can differentiate into wound bed myofibroblasts in response to TGF- β signaling in early wound repair (Zhang et al. 2019). These cells subsequently convert to quiescent adipocytes during the later wound resolution phase when exposed to BMP signals from regenerating wound bed hair follicles (Plikus et al. 2017). It is currently unclear whether this is a response to extrinsic signals or whether adipocytes derived from myofibroblasts maintain an anatomical memory and convert back to their original state.

Another fibroblast population that is relevant to wound repair resides in the fascia beneath the panniculus muscle and contributes to the repair of deep full thickness wounds. Fascia fibroblasts up-regulate the cell-cell adhesion molecule N-cadherin and Cx43 on wounding and enter the wound bed with a swarm-like behavior (Jiang et al. 2020; Wan et al. 2021) in contrast to the individual migration of papillary and dermal fibroblasts (Rognoni et al. 2018). During this coordinated migration, fascia fibroblasts pull their surrounding matrix environment, including immune cells, blood vessels, and nerves, into the wound bed enabling them to quickly seal the wound and provide a provisional wound bed matrix.

A further aspect of wound healing is the effect of aging. Skin aging induces changes in fibroblast composition, lineage identity, and microenvironment, significantly delaying skin repair after wounding (Rognoni et al. 2016; Salzer et al. 2018; Shook et al. 2018). The progressive decrease in the number of fibroblasts during skin aging is balanced by increased formation of membrane protrusions (Marsh et al. 2018). Recent single-cell transcriptomic analyses of wound bed fibroblasts from young and aged mice has revealed distinct subpopulations of fibroblasts with different (proinflammatory) cytokine expression signatures (Mahmoudi et al. 2019). After tissue repair, wound bed fibroblasts reestablish quiescence to maintain skin homeostasis.

FIBROBLAST HETEROGENEITY IN HUMAN HEALTHY AND WOUNDED ADULT SKIN

Human Healthy Fibroblast Heterogeneity

Lineage-tracing studies performed in mice (Driskell et al. 2013; Rinkevich et al. 2015; Jiang et al. 2018) identified distinct fibroblast subpopulations in the dermis, leading to the hypothesis that the same is true in human dermis. Because it is not possible to perform lineage-tracing studies in humans, most of our understanding of human fibroblast subpopulations comes from cell culture experiments and single-cell transcriptional profiling.

Subpopulations and Markers

Human fibroblasts express a range of conserved markers including LUM, DCN, VIM, PDGFR α , and COL1A2 (Philippeos et al. 2018). We performed spatial transcriptional profiling (comparing gene expression in microdissected papillary and reticular dermis) and compared this to single-cell sequencing of a total of 184 cells isolated from human dermis (Philippeos et al. 2018). This revealed that there were at least four populations of fibroblasts present in the dermis. We were able to define cell surface markers that permitted the prospective isolation of populations that showed different functions in both interferon release assays and in supporting the growth of keratinocytes in organotypic 3D cultures (Philippeos et al. 2018).

Technological advances in single cell transcriptomics now permit us to analyze tens of thousands of cells in a single experiment and studies by multiple groups have confirmed that distinct fibroblast populations can be identified in human dermis on the basis of differences in transcriptional profile. Solé-Boldo et al. (2020) analyzed in excess of 15,000 cells derived from sun-protected skin of two young and three older male donors. They identified four subpopulations of fibroblasts. RNA-scope showed one of these to be preferentially localized in the papillary dermis, two in the reticular dermis, and one with a more widespread distribution but a preference for vasculature (Table 2). Additionally,

Table 2. Summary of putative markers for fibroblast subpopulations present in normal human skin

Study	Skin sites	Cluster name	Markers	Inferred function/pathways
Solé-Boldo et al. 2020	Inguinoiliac area	1	CTHRC1	Secretory-reticular
		2	CCL19, APOE	Proinflammatory-reticular
		3	APCDD1, COL13A1, COL23A1, COL18A1	Secretory-papillary
	Trunk area	9	ASP, POSTN, COL11A1, COL24A1	Mesenchymal, cartilage, bone development
		FB1	MFAP5, FAP, COL12A1, LOX, DPP4	ECM assembly, wound healing, angiogenesis
		FB2	APOE	Antimicrobial immune response, leukocyte migration
Vorstandlechner et al. 2020	Trunk area	FB3	APCDD1, WIF1	Cartilage development, leptin signaling
		FB4	B4GALT1	Growth factor response
		FB5	CXCL1, APOE	Antimicrobial immune response, leukocyte migration
	Arm area	FB6	ABCDD1, CXCL1, WIF1	Interferon γ , p38, NF- κ B signaling
		0	SERP2, DPP4, PCOLCE2, CD55, WIF1, NKD2	Negative regulation of signaling pathways, regulation, and sequestering of BMP and protein localization to extracellular matrix
		1	FMO1, LSP1, MYOC, IGFBP3, ITM2A, CYGB, C7, AADAC, RAMP2	Negative regulation of cell movement, lipid clearance, and stress response
Tabib et al. 2018	Arm area	2	SERP2, DPP4, PCOLCE2, CD55	Negative regulation of signaling pathways, regulation and sequestering of BMP, and protein localization to extracellular matrix
		3	SERP2, DPP4	Development of tendon, muscle, circulatory system, and heart
		4	CRABP1, TNN, DPEP1, COL11A1	Negative regulation of signaling pathways, regulation and sequestering of BMP, and protein localization to extracellular matrix
	Arm area	5	FMO1, LSP1, MYOC, IGFBP3 ITM2A, CYGB, and C7	Negative regulation of signaling pathways, regulation and sequestering of BMP, and protein localization to extracellular matrix
		6	SERP2, DPP4, PCOLCE2, CD55, PRG4, LINC01133, FMO1, LSP1, MYOC, IGFBP3 ITM2A, CYGB, C7	Not defined
		7	SFRP4, C2orf40, ANGPTL7	Not defined
Reynolds et al. 2021	Breast area	Fb1	TNFAIP6, IL6, PTGES	Not defined
		Fb2	CXCL3, CXCL12	Not defined
		Fb3	TNFAIP6, IL6, PTGES, CD82, CCL19, TNC	Not defined

Continued

Table 2. Continued

Study	Skin sites	Cluster name	Markers	Inferred function/pathways		
He et al. 2020	Extremity areas	FB1	APOD, PTGDS, MFAP4, PCOLCE	Not defined		
		FB2	MFAP5, SFRP2, WISP2, POSTN, MFAP4,			
		FB3	APCDD1, POSTN, MXRA5, PCOLCE, MFAP5			
Acensi3n et al. 2021	Multiple body areas	A	ELN, MMP2, QPCT, SFRP2	Dermal cell and extracellular matrix (ECM) homeostasis		
		A1	IGFBP6, PII16, SLPI, WISP2			
		A2	APCDD1, COL18A1, COMP, NKD2			
		A3	ELN, RGCC, SGCA, WIF1			
		A4	FBN1, PCOLCE2, PRG4, SFRP4			
		B	APOE, C7, CYGB, IGFBP7			
		B1	CCL2, ITM2A, SPSB1, TNFAIP6			
		B2	CCDC146, CCL19, CD74, TNFSF13B			
		C	DKK3, TNMD, TNN, SFRP1			
		C1	COL11A1, DPEP1, TNMD, WFDC1			
		C2	COCH, CRABP1, FIBIN, RSPO4			
		C3	ASPN, F2R, GPM6B, POSTN			
		C4	ANGPTL7, APOD, C2 or f40, TM4SF1			
						Immune surveillance and promoting inflammation
						Specialized subpopulations, such as dermal papilla cells (type C2) and dermo-hypodermal junction fibroblasts

Solé-Boldo et al. compared the transcriptional profile of young and old fibroblast populations and uncovered, via gene ontology analyses, an age-dependent loss of the functional annotations of each cluster, reflecting a reduction in associated genes. Furthermore, the papillary fibroblast subpopulation within aged fibroblasts presented a more reticular gene signature while the reticular fibroblast subpopulation signature was less pronounced. Therefore, aging results in a loss of fibroblast identities (Solé-Boldo et al. 2020). Vorstandlechner et al. (2020) looked at 4764 cells isolated from the abdomen of three healthy donors. Tabib et al. (2018) isolated a total of 8622 cells from six samples of dorsal sun-exposed forearm skin from both male and female subjects. Clustering identified a total of eight cellular populations within the fibroblasts (Table 2), which could be grouped into two major clusters defined by expression of SFRP2/DPP4 and FMO1/LSP1. Tabib et al. (2018) found that staining for SFRP2/DPP4 revealed elongated cells and FMO1/LSP1 was expressed by rounder cells.

More recently, Reynolds et al. analyzed in excess of 500,000 cells derived from embryonic human skin, adult skin, and skin from patients with the inflammatory skin diseases eczema and psoriasis. Healthy adult skin was derived from mammoplasty patients. They identified three fibroblast subpopulations and two pericyte subpopulations (Reynolds et al. 2021). Human pericytes express ACTA2 and RGS5 (Cho et al. 2003; Paquet-Fifield et al. 2009). scRNA-seq of healthy human skin has yet to identify more than one pericyte population; however, analysis of atopic dermatitis and psoriasis samples has revealed the presence of a second pericyte population that is proposed to play a role in leukocyte recruitment and TNF- α -mediated signaling (Reynolds et al. 2021).

He et al. analyzed 39,042 cells from lesional and nonlesional skin of five patients with atopic dermatitis and seven controls (He et al. 2020). They identified three subpopulations of fibroblasts in healthy skin (Table 2). Additionally, they identified a population of fibroblasts that was only present in lesional skin of patients with atopic dermatitis. This was identified by the expression of

COL6A5, COL18A1, and CCL2. We have previously observed COL6A5 expression in the papillary dermis of healthy skin (Philippeos et al. 2018) and it is possible that these differences reflect a difference in body site or degree of sun exposure.

All single-cell sequencing approaches use complex statistical transformations to reduce the dimensionality of the input data and permit visualization and analysis. A limitation of the analysis is that the number of clusters identified varies according to the methods of normalization, the dimensionality reduction approach, and the specific parameters chosen for the analysis. It is therefore not surprising that there are differences in the numbers of clusters identified between different studies. To address this, Acensión et al. (2021) reanalyzed data published by Tabib et al. (2018), Solé-Boldo et al. (2020), He et al. (2020), and Vorstandlechner et al. (2020). The data were integrated, with the exception of the He et al. data set, which had low cell viability. On this basis, three classes—axes—of fibroblasts were identified, which were designated A–C. Within each class, numerous subpopulations were defined (Table 2). Most of these subpopulations were reproducible across observed datasets, although it remains to be determined to what extent these reflect true subpopulations rather than different cell states or tissue contexts such as different anatomical sites.

Certain fibroblast populations are not well represented within single-cell sequencing data sets and this may reflect difficulties in extraction of cells—for example, hair follicles with associated dermal papillae and dermal sheaths—or degradation of cells during processing—for example, preadipocytes and adipocytes.

Analysis of gene expression patterns within the different fibroblast subpopulations permits the identification of transcriptional markers, expressed in one or more of the clusters. The identity of these markers varies considerably across the different studies (Table 2). It is desirable to identify cell surface markers for the different human fibroblast subpopulations because this allows prospective isolation for therapeutic and experimental approaches, including in vivo targeting of specific subpopulations, for example, using monoclonal antibodies. Based on analysis

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of 184 cells, we identified CD39 and CD26 (DPP4) as markers of functionally distinct fibroblast subpopulations (Philippeos et al. 2018). In our data, CD26 was coexpressed with MFAP5. Vorstandlechner et al. have confirmed that CD26 (DPP4) is preferentially expressed on the same subpopulation of fibroblasts as MFAP5 (Table 2). Tabib et al. (2018) also found DPP4 expression restricted to a subpopulation of fibroblasts. DPP4 is thus a promising therapeutic target for specific fibroblast cell therapy.

Spatial Localization

Many of the markers identified do not have well-characterized antibodies that can be used for immunolocalization in skin sections. For this reason, many groups have taken advantage of methodological advances in RNA FISH approaches, such as RNAscope (Bio-technique), which permit visualization of transcripts in intact tissue sections. Solé-Boldo et al. looked at the localization of the four different clusters that they identified using RNAscope (Table 2). They found the secretory–papillary population to be preferentially localized in the papillary dermis; the secretory–reticular was preferentially located in the reticular dermis; the mesenchymal population was found in the reticular dermis and in association with hair follicles; and the proinflammatory population was present in both papillary and reticular dermis and also in association with the vasculature.

Fibroblasts can be found both embedded in the dermis itself and in intimate association with the basement membrane of the epidermis, hair follicles, sweat glands, and blood vessels. It seems probable that fibroblasts in these differing contexts will manifest differences in their function and that this will be reflected in gene expression profiles.

Recently, researchers have highlighted another level of complexity within human fibroblast heterogeneity, namely, the fibroblasts from the junction of the dermis and subcutaneous tissue (dermo–hypodermal junction fibroblasts) (Haydont et al. 2020). The existence of a fibroblast population showing adipocyte-like molecular characteristics within the lower reticular

dermis has previously been reported in human skin (Korosec et al. 2019). These cells have distinct expression signatures and functional differences from dermal papillary and reticular fibroblasts. They show specific expression of genes involved in ECM synthesis processing and tissue skeleton organization.

What Is the Function of the Different Human Fibroblast Populations?

Gene ontology and pathway analysis provide some insight into the properties of fibroblasts in the identified clusters. Solé-Boldo et al. (2020) found that three out of four of their populations displayed high levels of expression of markers associated with ECM organization, cell adhesion, and collagen fibril organization. In contrast, population 2 possessed a proinflammatory expression profile. In addition, populations 3 and 9 expressed a mesenchymal profile that included markers of muscle and bone development (Table 2).

Reanalysis of existing single-cell data sets has led to the proposal that there are three classes of fibroblasts present in the human dermis that differ in their potential functions (Ascensión et al. 2021). The first, type A, is responsible for ECM maintenance and synthesis; the second, type B, is implicated in immune surveillance and promotion of inflammation; and the third, class C, incorporates numerous different fibroblasts with specialized functions including the dermal papilla.

It is recognized that fibroblasts derived from the upper dermis better support the growth of the epidermis in cell culture (Sorrell et al. 2004; Lee and Cho 2005; Janson et al. 2012). We have shown that fibroblasts with this property can be prospectively isolated using CD90 and CD39 surface markers (Philippeos et al. 2018).

Developmental Programs Regulating Fibroblast Identity

In keeping with classical heterotopic transplantation experiments (Sengel 1990), there is evidence that fibroblasts from different regions of the body have different transcriptional programs (Shaw

and Rognoni 2020). Fibroblasts present at different sites in the adult are derived from different embryological origins including neural crest, lateral plate mesoderm, and dermatomyotome (Le Lièvre and Le Douarin 1975; Houzelstein et al. 2000; Sriram et al. 2015). This is reflected in differences in the pattern of expression of Hox genes, which are known master regulators of positional identity during body morphogenesis (Rinn et al. 2006, 2008).

Fibroblasts derived from the palms and soles have the ability to induce keratin 9 expression in keratinocytes, whereas fibroblasts derived from nonplantar sites do not (Yamaguchi et al. 1999). Androgen receptors have been reported to be expressed more highly in regions of the scalp susceptible to male pattern hair loss (Hibberts et al. 1998). In keeping with this observation, hair follicles transplanted from the posterior scalp to the anterior scalp retain their resistance to androgen-induced miniaturization (Orentreich 1959).

Intrinsic and Extrinsic Regulatory Mechanisms Specifying Fibroblast State

For human tissues including skin, a large fraction of our knowledge of fibroblast identity is derived from single-cell sequencing studies. These represent a snapshot of identity at a specific point in developmental time and space. Fibroblasts can be found both embedded in the dermis itself but also in intimate association with the basement membrane of the epidermis, hair follicles, sweat glands, and APM.

The details of how the transcriptional identity of fibroblasts in these differing tissue contexts is specified remains to be determined. However, cell culture experiments support an interplay between the function and identity of fibroblasts and neighboring cells in the tissue. Coculture experiments have shown reciprocal interactions between keratinocytes and fibroblasts (Maas-Szabowski et al. 1999; Werner et al. 2007). Interactions with ECM elements are also important—fibroblasts cultured in conventional monolayer conditions show differences in gene expression and morphology compared with those cultured in collagen gels (Eckes et al. 1993).

Human Fibroblast Heterogeneity in Physiological Wound Healing

Wound healing in human skin is the result of dynamic and highly regulated processes involving multiple cell types. In the local wound area, a well-defined and coordinated series of events take place: hemostasis, inflammation, new tissue formation, and tissue remodeling.

The Role of Fibroblasts on Wounding

On wounding, fibroblasts migrate into the wounds to restore as much as possible of the skin architecture by producing new ECM and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound. Additionally, fibroblasts release growth factors and inflammatory cytokines to modulate immune cell functions.

In humans, typical wound healing results in scar formation, which can be caused by fibroblast subpopulation dysfunction and decreased proliferation and/or fibroblast depletion. Although the mechanisms used by mammals to promote rapid wound closure and inflammation are fundamental for the prevention of skin infection and dehydration, the resulting scar formation can also be unfavorable owing to the lack of skin appendages, which leads to defects in the skin's biological and physiological functions. For example, hair follicles and sebaceous glands confer sensory and thermoregulatory functions on the skin (Chen et al. 1997; Li et al. 2011). Therefore, the capability to restore the original state of a healthy skin is highly valued.

Fibroblast Heterogeneity in Pathological States Including Scar and Keloid

Single-cell transcriptional profiling provides an understanding of the cellular populations present in a scar—the end point of cutaneous wound healing. Deng et al. (2021) compared 21,488 cells derived from keloid scars and 19,167 cells derived from normal scars. They found that fibroblasts, endothelial cells, and blood vessel-associated smooth muscle cells accounted for the majority of cells within scars.

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Fibroblasts made a lesser contribution to keloid scars versus normal scars, which may reflect the expansion of vasculature in keloid scars (Deng et al. 2021). Bioinformatic analysis revealed four clusters and 13 distinct fibroblast populations across the normal scar and keloid scar samples. When the normal scar and keloid scar populations were compared, there was a significant increase in a population of fibroblasts in keloids that corresponded to the subpopulation previously characterized by Solé-Boldo et al. (2020) as mesenchymal and characterized by the expression of POSTN, ASPN, and COL11A1. Other populations identified by Solé-Boldo et al. were relatively decreased. Receptor–ligand analysis found evidence of altered signaling pathways including POSTN and TGF β 1.

We suggest that dysfunction, decreased proliferation, and/or depletion of fibroblast subpopulations can play different roles in human wound healing, resulting in different repair outcomes, such as the formation of normal scars in physiological tissue repair and fibrosis or ulcers in pathological tissue repair. Understanding dermal fibroblast heterogeneity is challenging, but it is critical to obtaining mechanistic insights into the human wound healing process. These insights could form the basis of developing therapeutics targeting specific subpopulations in wound healing and scarring.

DISCUSSION

Fibroblast Subpopulations in the Human and Mouse

In both human and mouse, scRNA-seq studies have identified multiple distinct populations of fibroblasts. This is in keeping with earlier studies performed via lineage tracing in mice and in cultures of human cells. Despite the anatomical differences between mouse and human skin, many fibroblast population markers are conserved across species (Table 1). This similarity between mouse and human fibroblast subpopulation transcriptomic profiles is reflected in the conservation of signaling pathways (Rognoni et al. 2016; Philippeos et al. 2018; Tabib et al. 2018).

Lessons for Human Wound Healing from the Mouse

Single-cell transcriptomic and epigenetic studies represent a snapshot of cellular identity at a point in developmental time and space. They do not permit us to disentangle the relative contributions of intrinsic transcriptional programs, spatial context within the tissue, and developmental origins. These questions are of particular relevance during development and wound healing, both of which are periods of dynamic change associated with highly coordinated migration and differentiation of multiple cell populations. It is challenging to study dynamic processes in the human; however, powerful genetic and lineage-tracing approaches permit us to do so in the mouse. For this reason, a great deal of our understanding of development and wound healing in the human is inferred from studies in the mouse.

Supporting the similarities in transcriptomic profiles of fibroblasts from mouse and human is the fact that the fundamental principles of dermal maintenance, development, and wound healing are broadly conserved across mammalian species. In the adult steady-state mouse dermis, fibroblasts rarely divide and those that are lost through aging or extrinsic damage are not replaced by division or migration of fibroblasts from neighboring regions (Scharffetter-Kochanek et al. 2000; Wlaschek et al. 2001; Naylor et al. 2011). This is likely to be the case in the human dermis because fibroblast division is rarely observed in histological sections in the steady state and there is a decrease in fibroblast numbers in the dermis with age (Marsh et al. 2018; Solé-Boldo et al. 2020).

During wound healing in the mouse dermis, inflammatory signals lead to an extensive process of cell division and fibroblast migration. Fibroblasts from the reticular dermis migrate into the wound environment first, with fibroblasts from the papillary dermis migrating subsequently. In support of the hypothesis that a similar process is operational in the human dermis, there is an expansion of POSTN⁺ASPN⁺COL11A1⁺ fibroblasts in keloid scars (Deng et al. 2021); in healthy skin, this population is preferentially localized to the lower dermis, sug-

gesting that it may correspond to the reticular population of fibroblasts present in the mouse. The appearance of a regenerative zone in the papillary dermis in the mouse (Driskell et al. 2013; Rognoni et al. 2016) does not appear to have a direct correlation in the human because human scars are devoid of hair follicles and other cutaneous appendages. Finally, recent studies have suggested a key role for fascia-derived fibroblasts in healing of mouse wounds (Jiang et al. 2020). This seems less likely to be relevant in the human because at many body sites, particularly on the trunk, the dermis is separated from the underlying fascia by many centimeters of subcutaneous fat.

Open Questions and Ongoing Work

The existence of multiple fibroblast populations has raised a number of important questions that are the subject of ongoing investigation. One unresolved question is to what extent the fibroblast populations present in the human dermis correspond to those within the mouse. This is important because, by necessity, mouse models are used to model dynamic processes such as development and wound healing that are not straightforward to study in humans. Although not all specific markers of fibroblast subpopulations are conserved between human and mouse, it is possible that transcriptional profiles or master transcriptional regulators will be conserved across species.

A second important question is the spatial localization of fibroblast populations within the dermis, in the steady state, in the wound environment, and in scars. Studies in the mouse have shown a distinction between papillary fibroblasts and reticular fibroblasts; however, when the localization of the different fibroblast populations identified by RNA sequencing is assessed through RNA FISH or protein staining in human skin, some populations are not exclusive to either the papillary or the reticular dermis (Philippeos et al. 2018). It is likely that novel spatial transcriptomic approaches will clarify the localization of fibroblasts within the dermis both in terms of depth and with respect to other structures such as the epidermis, hair follicles, glan-

dular structures, and blood vessels, and this is a major focus of ongoing work (Heibel et al. 2020; Ji et al. 2020). The spatial localization of different fibroblast populations within the dermis will likely reflect differences in function. Gene ontology-based approaches have given clues as to these different functions; however, functional experiments through genetically modified mice and organotypic culture of human fibroblasts will be required to understand these processes in greater detail.

It is not clear to what extent the observed differences in transcriptional profiles reflect self-perpetuating intrinsic transcriptional programs versus a consequence of signals arising from ECM or neighboring cells in the tissue context. These questions can be addressed through transplantation, culture, and lineage-tracing experiments. It is possible that the observed transcriptional profiles represent a superposition of different contributory elements. It will also be important to understand whether the identity of fibroblast subpopulations is conserved for an individual clone of cells or whether cells can switch identity from one population to another. Finally, it will be of great importance to understand how fibroblast populations are modified in scarring, malignancy, and aging, because manipulation of fibroblast identity or ablation of specific populations has the potential to offer new therapeutic approaches to treatment.

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