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SFRP2/DPP4 and FMO1/LSP1 define major fibroblast populations in human skin

Tracy Tabib[1], **Christina Morse**[1], **Ting Wang**[2], **Wei Chen**[2], **Robert Lafyatis**[1]

[1]Division of Rheumatology and Clinical Rheumatology, Department of Medicine, University of Pittsburgh

[2]Division of Pulmonary Medicine, Allergy and Immunology, Department of Pediatrics, School of Medicine, University of Pittsburgh

Abstract

Fibroblasts produce matrix, regulate inflammation, mediate reparative processes, and serve as pluripotent mesenchymal cells. Analyzing digested normal human skin by single cell RNA-seq (scRNA-seq), we explored different fibroblast populations. T-distributed stochastic neighbor embedding and clustering of scRNA-seq data from six biopsies revealed two major fibroblast populations, defined by distinct genes, including SFRP2 and FMO1, expressed exclusively by these two major fibroblast populations. Further subpopulations were defined within each of the SFRP2 and FMO1 populations, as well as five minor fibroblast populations, each expressing discrete genes: CRABP1, COL11A1, FMO2, PRG4 or C2ORF40. Immunofluorescent staining confirmed that SFRP2 and FMO1 define cell types of dramatically different morphology. SFRP2+ fibroblasts were small, elongated, and distributed between collagen bundles. FMO1+ fibroblasts were larger, and distributed in both interstitial and perivascular locations. Differential gene expression by SFRP2+, FMO1+ and COL11A1+ fibroblasts suggests roles in matrix deposition, inflammatory cell retention, and connective tissue cell differentiation, respectively.

INTRODUCTION

Fibroblasts secrete extracellular matrix, mediating reparative and fibrotic processes. Fibroblasts play key roles in healing wounds but also as the mediators of fibrosis. In addition, they regulate inflammation, by anchoring leukocytes and regulating immune cell functions. In lymphoid tissues they contribute to secondary lymphoid organ structure and in non-lymphoid tissues to the development of tertiary lymphoid structures (Barone et al., 2016). In carcinogenesis fibroblasts have an emerging role, as cancer associated fibroblasts support and regulate tumor cell growth (Ohlund et al., 2014). Thus, fully understanding the complexity of normal dermal fibroblasts is key to understanding their roles in a wide variety of pathological conditions.

Corresponding author: Robert Lafyatis, 200 Lothrop St., BST S720, Pittsburgh, PA 15260, Telephone: 412-383-8123, lafyatis@pitt.edu.

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Dissecting fibroblast functional heterogeneity has lagged behind understanding of inflammatory cell types due to a lack of discrete markers and limitations of technologies such as fluorescence-activated cell sorting on enzymatically digested tissues. Thus, we have no comprehensive understanding of the repertoire of tissue fibroblasts. Due to skin accessibility and complexity as a connective tissue, dermal mesenchymal cells have been a focus by several groups. Dr. Watt's group has described different well-defined cell populations in normal skin. These studies have shown that arrector pili/smooth muscle cells selectively express ITGA8 and NPNT (Fujiwara et al., 2011). Further this group has defined a series of markers that are stable or dynamic for dermal papilla (CRABP1), papillary (DPP4/CD26), and reticular (PDPN, SCA1/ATXN1) fibroblasts (Driskell et al., 2013, Driskell and Watt, 2015).

Recent murine studies have advanced several alternative approaches to understanding mesenchymal cell heterogeneity in various tissues and of profibrotic cell progenitors. One approach to understanding mesenchymal cell heterogeneity is single cell cloning. A recent study showed that different functional, clonal populations could be characterized on the basis of HAS2/MMP10 versus COL1A1/DCN/MMP2 expression (Hiraoka et al., 2016). However, a major limitation to cell cloning is uncertainty regarding the stability of cell phenotypes upon expansion of cells in vitro. In addition, it may be difficult to identify rare, discrete mesenchymal cell types.

Several groups have begun to examine cellular heterogeneity in mouse embryonic mesenchymal cells directly using single cell analyses. Singhal et al, analyzing cultured embryonic mesenchymal cells by flow cytometry, distinguished six subpopulations of cells based on CD73 (NTSE), CD146 (MCAM), CD90, PDPN, CD24 and CD38 (Singhal et al., 2016). This heterogeneity was stable for 4–6 passages. Rinkevich et al, using single cell microfluidic gene expression analysis, showed that a population of embryonic lineage fibroblasts purified from adult murine skin expresses COL1A1, COL3A1, FBN1, PDGFRA, VIM, DCN, S100A4 and other genes (Rinkevich et al., 2015). Engrailed positive versus Engrailed negative cell types could be further distinguished based on expression of other sets of genes (transcriptomes). Most significantly, mice in which Engrailed derived cells were deleted showed less scarring after wound healing. In addition, DPP4 was shown to be a marker of Engrailed positive cells and its inhibition led to decreased wound scarring. These murine studies have clarified the importance of understanding fibroblast heterogeneity in skin, and have provided several transcriptome datasets that can aid in identifying fibroblast subpopulations in human skin.

Using advances in scRNA-seq technology, allowing thousands of single cells to be analyzed in a single experiment, we examined single cell transcriptomes of cell populations from whole skin without pre-purifying fibroblast populations. We identify multiple discrete dermal fibroblast populations, including two major and five minor fibroblast types, strongly suggesting underlying functional heterogeneity.

RESULTS

Detection of skin transcriptomes corresponding to epithelial, endothelial and mesenchymal cell types in normal skin.

Using scRNA-seq, we examined gene expression in all cells obtained from enzymatically-digested skin from six healthy control (HC) skin samples. Dorsal mid-forearm skin samples were analyzed from both male and female subjects of varying ages (Supplementary Table 1). We examined a total of 8,522 cells from six subjects (1135–1748 cell/sample; Supplementary Table 1). To gain power to detect rare cell types, and to examine the reproducibility of cell types between subjects, cell transcriptomes from all the samples were grouped and analyzed together.

Combined cell-gene count matrices were analyzed by principal component analysis (PCA) and the statistical significance of principal components analyzed using Jackstraw (Chung and Storey, 2015). Statistically significant principal components were used for t-SNE dimensional reduction and visualization, and for clustering. Cells were clustered using an unsupervised graph based clustering algorithm (SLM clustering, described in methods), which in total identified 19 distinct clusters of cells, distinguished by color (Figure 1a). Cells from each subject were also indicated by different colors (Figure 1b), showing that each cluster included cells from each biopsy. SLM clusters contained genes well known to be expressed by various cell types in the skin, permitting the cells in each cluster to be identified (Figures 1c and 2). Desmin (DES) clearly identified a cell cluster of smooth muscle cells; Keratin 1 (KRT1) and Keratin 14 (KRT14) as clusters of keratinocytes; Von Willebrand Factor (VWF) as endothelial cells; Collagen, type 1, alpha 1 (COL1A1) as fibroblasts; Regulator Of G-Protein Signaling 5 (RGS5) as pericytes (also on some endothelial cells); premelanosome protein (PMEL) as melanocytes; T-cell surface glycoprotein CD3 delta chain (CD3D) as T cells; Allograft inflammatory factor 1 (AIF1) as macrophage/dendritic cells; Immunoglobulin J chain (IGJ) as B cells; Secretoglobin Family 1B Member 2 (SCGB1B2P) as secretory (glandular) cells. These and other markers provided strong transcriptome signatures for each cell cluster (Supplementary Table 2 and Supplementary Table 3), shown graphically in feature plots (Figure 2). More detailed examination showed series of known markers expressed by designated cell types: VWF, CLDN5, CDH5 by endothelial cells, RGS5, TPM1, TPM2, CNN1, CALD1 by pericytes (Paquet-Fifield et al., 2009); DES, ITGA8 and NPNT by arrector pili-smooth muscle cells (5): and AIF1, HLA-DRB, CD1C, IL1B by macrophages and dendritic cells (Supplementary Table 2 and 3).

Cells in three SLM clusters: #0, #3 and #4, expressed COL1A1 and COL1A2 and grouped together on the t-SNE analysis (Figure 1). Only pericyte clusters, SLM clusters #6 and #10, also expressed low levels of COL1A1 and COL1A2, while many cell types expressed other putative fibroblast markers, such as Fibroblast specific protein 1 (FSP1/S100A4) and Vimentin (VIM) (Strutz et al., 1995), and thus these were not good markers for fibroblasts (Supplementary Figure 1). PDGFRA, another fibroblast marker (Collins et al., 2011), though detectably expressed in only a fraction of the fibroblasts, was seen in all fibroblast groupings and no other group. CD34, a marker for dermal fibroblasts, was also expressed by

endothelial cells (Nazari et al., 2016). Expression of COL1A1 and COL1A2, the two genes making up type I, collagen, correlated highly (R^2=0.86).

ScRNA-seq transcriptomes indicate discrete subpopulations of fibroblasts.

In order to understand dermal fibroblast heterogeneity in more detail, we extracted the transcriptome data of the fibroblasts in SLM clusters #0, #3, and #4 (2,742 cells) and reanalyzed the data. T-SNE and SLM clustering of just these cells showed eight clusters (referred to as fibroblast SLM clusters, Supplementary Figures 2 and 3). Cluster #2 contained mainly cells from subject SC33, and further analysis by hierarchical clustering (Figure 3), and feature and violin plots, indicated that this cluster did not represent a discrete cell type. Instead, a large group of cells defined by fibroblast SLM clusters #0, #2, #3 and #6 expressed SFRP2 and DPP4 (SFRP2+ fibroblasts, 1,671 cells, Figure 4a). Subsets of cells within this larger group could be distinguished based on further marker genes: a subset of cells defined by fibroblast SLM clusters #0, #2, and #6 expressed PCOLCE2 and CD55 (Figure 4b); a subset defined by cluster #0 expressed WIF1, and NKD2 (Figure 4c); and a subset that included part of cluster #6 expressed PRG4 (Figure 5a and b) and LINC01133. These genes also grouped together on hierarchical clustering (Figure 3).

A second large group of cells (536 cells) defined by fibroblast SLM clusters #1 and #5 (Supplementary Figure 2) expressed FMO1, LSP1, MYOC, IGFBP3 ITM2A, CYGB, and C7 (Figures 3 and 5). A subset of these cells showed expression restricted to cluster #1, including AADAC and RAMP2 (Figures 3, 5c and 5d). In addition, two relatively small populations of cells within fibroblast SLM cluster #4 could be distinguished. One (Figure 3, cluster #4A) was defined by CRABP1, a marker of pluripotent dermal papilla cells, and TNN; the second (cluster #4B) was defined by COL11A1 and DPEP1 expression (Figure 5e and 5f). These cell types also showed discrete gene clusters on hierarchical clustering (Figure 3).

Finally, two other small cell populations, delineated sharply from the main groupings of cell on the t-SNE analysis and clustering. One of these expressed SFRP4 (Figure 5a and 5b) and grouped closely with PRG4+ cells on the t-SNE (Supplementary Figure 2). The other expressed ANGPTL7, C2orf40 (Fibroblast SLM cluster #7, 33 cells, Figures 3 and 5). Some of these ANGPTL7/C2orf40+ cells also expressed SFRP4 (Figure 5).

In summary, in normal human skin we could distinguish two major fibroblast populations showing SFRP2/DPP4 and FMO1/LSP1 markers. Additional markers defined subpopulations of fibroblasts within each of these major cell groups. In addition, distinct marker genes defined five minor cell populations: CRABP1, COL11A1, PRG4, ANGPTL7 and SFRP4. Only one of the minor populations, CRABP1+ dermal papilla cells, has been well defined previously.

Correlations with murine fibroblast markers.

Lineage markers described of murine dermal fibroblasts: NT5E (CD73), MCAM (CD146), CD90 (THY1) and ICAM1, did not stratify the human dermal fibroblast cell types (Supplementary Figure 4, (Singhal et al., 2016)). CD26/DPP4, a marker of profibrotic fibroblasts, was expressed primarily by SFRP2+ cells (Figures 3 and 4a). The distribution

IGFBP2, LEPR and COL23A1 on Engrailed (EN1)-negative expressing progenitors (Rinkevich et al., 2015) were associated with NKD2/WIF1 expressing cells (Figures 3, 4c and Supplementary Figure 5). Other described murine markers were not specific for fibroblasts or fibroblast subpopulations (see Supplementary Results).

Immunofluorescence confirms two major fibroblast populations in human dermis.

We examined cell protein expression of marker genes by immunofluorescence (IF). IF staining of SFRP2, a marker expressed by the most common major cell population, showed cytoplasmic expression in many small, cells. Depending on the orientation of the cells in the tissue, IF staining showed very small cells with small nuclei or very elongated cells with elongated nuclei (Figure 6A). These cells resided in between connective tissue bundles. IF staining of DPP4 stained the same small cells (Figure 6A and Supplementary Figure 6), and double IF confirmed that SFRP2 and DPP4 stained the same cells (Figure 6D). Thus, SFRP2 and DPP4 expression identify this major dermal fibroblast population, seen as a discrete fibroblast population on fibroblast SLM (clusters #0/2/3/6, Supplementary Figure 2) and hierarchical clustering (Figure 3).

In contrast, IF staining of FMO1 showed nuclear staining of relatively large nuclei of cells found throughout the dermis (Figure 6B). LSP1, a gene co-expressed by these cells (Figure 3) stained the cytoplasm of the same cell population (Figure 6B). These cells co-stained with LSP1, which showed cytoplasmic staining, thus confirming this second major dermal fibroblast population (Figure 6e). FMO1/LSP1 stained an entirely discrete population of cells compared to staining with SFRP2 and DPP4 (Figure 6, panels d and f). Thus, FMO1 expression identifies this second major dermal fibroblast population seen on fibroblast SLM (clusters #1 and 5, Supplementary Figure 2A) and hierarchical clustering (Figure 3).

Correlations with markers of proliferation and sex.

None of the fibroblast subsets showed increased markers of proliferation as assessed by S and G2 phase markers, and PCNA expression (Supplementary Results and Supplementary Figure 7). To understand possible sex related differences in healing we examined selectively expressed genes by fibroblasts from male and female subjects. Several matrix associated genes: VIM, MFAP4 and FBLN1, were expressed slightly higher in female than male fibroblasts (Supplementary Results, Supplementary Table 4, and Supplementary Figure 8).

Gene Ontology (GO) Enrichment Analysis.

We examined the relationship between the genes in clusters defining the principal cell types using human GO biological processes (Supplementary Results and Supplementary Table 5). Biological processes attributed to SFRP2/DPP4+ cells included negative regulation of signaling pathways, regulation and sequestering of BMP, and protein localization to extracellular matrix; FMO1+ fibroblast processes included negative regulation of cell movement, lipid clearance, and stress response; COL11A1+ fibroblast processes included development of tendon, muscle, circulatory system and heart.

DISCUSSION

Our studies shed light on fibroblast heterogeneity in human skin, which we can now clearly identify by transcriptome markers. We find that there are two major fibroblast populations defined by distinct transcriptomes, and immunofluorescently by SFRP2 and FMO1 staining. The observed transcriptomes also define several other fibroblast populations (Supplementary Figure 9). Within each of the major fibroblast populations there is further heterogeneity, with several subpopulations within each the SFRP2+ and FMO1+ fibroblast populations. In addition, we identify five minor cell populations, each of these populations expressing distinctive genes. Because these populations include few cells, we speculate that they may include progenitor or stem cell populations. Since different cell populations expressed the vast majority of genes at similar levels, the genes that are uniquely or selectively expressed by each cell type likely point to specialized functions and progenitor potentials.

Vimentin and FSP1/S1004 have been proposed as discrete fibroblast markers but were also highly expressed by macrophage/dendritic cells, smooth muscle cells, pericytes and T cells. CD34 was a better pan-fibroblast marker, but was also expressed by endothelial cells. PDGFRA expression was most discrete for fibroblasts. Although it was not detected on many of the fibroblasts, this was likely due to low expression. Type 1 collagen genes, both COL1A1 and COL1A2, were the most consistent fibroblast markers, expressed only at low levels on pericytes.

SFRP2 expression defined a distinct subpopulation of fibroblasts that appear thin and tubular with narrow elongated nuclei and cytoplasmic extensions. Although SFRP2+ cells are small, they are found at a very high frequency compared to the other major fibroblast population, defined by FMO1 expression. They also expressed higher levels of several of the more common matrix genes, including type I collagen, fibrillin and fibronectin, as well as, DPP4, a marker of murine dermal mesenchymal cells destined to play a major role in fibrosis (Rinkevich et al., 2015). In mice DPP4+ cells deposit matrix during embryogenesis and wound healing, as well as post-radiation fibrosis. SFRP2+/DPP4+ cells have morphological features of cells variously described as telocytes or dendrocytes (Rusu et al., 2012). These mesenchymal cells were first described by light and electron microscopy and then associated with CD34 expression. However, we show that CD34 expression is expressed by both SFRP2+ and FMO1+ fibroblasts. Since other skin cells do not express SFRP2, these cells can now be readily distinguished from other dermal cells by both gene expression and immunofluorescence.

The SFRP2+ cell population included two subpopulations: one expressing of WIF1, COMP and NKD2 (WIF1+ cells), the other expressing PCOLCE2, CD55 and FSTL3. WIF1+ cells expressed the highest levels of type I collagen. Notably, WIF1 has been implicated in the fibrotic systemic sclerosis skin disease. Counterintuitively, higher WIF1 gene expression in whole skin biopsies is associated with lower skin scores, suggesting that this fibroblast subtype disappears in fibrotic skin in systemic sclerosis (Rice et al., 2015). Possibly these cells transition into another cell type under the influence of local cytokines, as has been suggested for CD34+ cells (Nazari et al., 2016).

FMO1+ cells were larger with much larger nuclei,. Expression of COL1A1 and COL1A2 was lower in these cells, suggesting that their function is not primarily in matrix production. Associated GO processes included negative regulation of cell migration and motility. This is consistent with CXCL12 expression by these cells, suggesting that they may act like bone marrow stromal cells, to retain white blood cells within the dermis by anchoring CXCR4 expressing cells (Sugiyama et al., 2006). In the bone marrow, CXCL12-expressing cells are essential for maintaining hematopoietic stem cells, but are also progenitor cells for osteoblasts and adipocytes (Omatsu et al., 2010).. A subgroup of the FMO1+ cells selectively expressed a small additional gene cluster, including AADAC and RAMP2.

We identified several rare cell types in the skin. One of these expressed CRABP1, a marker for dermal papilla cells, residing at the base of the hair follicle where they influence differentiation of stem cells in the hair follicle bulge (Collins and Watt, 2008). Dermal papilla cells are closely related to “skin-derived precursors”, which are pluripotent cells able to differentiate into neural, adipocyte and smooth muscle (Biernaskie et al., 2009, Toma et al., 2001). These cell represented ~ 5% of the fibroblast cells, and our data show a broader set of genes expressed uniquely by these cells, including TNN and ASPN. T-SNE grouping indicates that these CRABP1+ cells are closely related to a previously unrecognized cell type expressing COL11A1, DPEP1 and RBP4, an adipokine, as well as a retinol binding protein (Yang et al., 2005). RBP4 regulates adipocyte progenitor differentiation (Muenzner et al., 2013), and GO analysis suggests that these COL11A1+ cells are involved in tendon, muscle and connective tissue development, suggesting that these may also represent pluripotent cells.

Three other rare cell populations appeared closely related. SFRP4-expressing cells (SFRP4+, 92 cells) included a subset of cells expressing C2orf40 and ANGPTL7 (C2orf40+, 33 cells). SFRP4+C2orf40- cells clustered adjacent to C2orf40+ cells, although grouping separately on t-SNE. Finally, PRG4 + cells (59 cells) grouped immediately adjacent to SFRP4+/ C2orf40- cells on t-SNE, but showed a pattern of expression on hierarchical clustering overlapping more with PCOLCE2+ fibroblasts. This pattern of gene expression suggests that SFRP4+/ C2orf40- cells might be progenitors of PCOLCE2+ and/or SFRP2+ cells. These several rare subpopulations of cells appear to represent true rare cell types rather than doublet cell transcriptomes, as the marker genes for these cells are not found in any other cell type isolated from the skin. In general, we did not identify doublet cell transcriptomes, although these would be anticipated at a low frequency ~1.2% in the experiments.

Previously defined markers in murine skin did not define well fibroblast heterogeneity in human skin. Most surprising was the lack of a recognizable stratification between reticular and papillary fibroblasts. CD26/DPP4, a marker for papillary fibroblasts in neonatal mice, clustered hierarchically immediately adjacent to SFRP2, together defining one of the major cell types in the skin. These SFRP2+ cells were found in both reticular and papillary dermis. LRIG1, another marker for papillary fibroblasts (Driskell et al., 2013), was found in a similar distribution, sparing FMO1+ fibroblasts. SCA1/ATXN1, a marker for reticular fibroblasts was expressed across the fibroblast cell types throughout the dermis. We did not detect DLK1, another dynamic marker for reticular fibroblasts. These results are not

unexpected since CD26/DPP4 and SCA1/ATXN1 are expressed more diffusely in adult murine skin, and LRIG1 and DLK1 expression becomes undetectable (Rognoni et al., 2016). We speculate that different SFRP2+ fibroblast subpopulations may account for the different matrices seen in papillary and reticular dermis areas, but we have not been able yet to identify a discrete marker by immunofluorescence. Another alternative is that the papillary and reticular matrices are established during development but do not require continued specialized fibroblast types for their maintenance.

Although we cannot be certain that we captured all of the mesenchymal cell types in human skin, several observations suggest that we have captured most or all of the fibroblast subpopulations. First, we captured 2,742 fibroblasts including CRABP1+ pluripotent dermal papillary cells, making it unlikely that we failed to capture cell types simply because they are rare. Second, we captured all expected cell populations in the skin with the exception of adipocytes. We saw very few cells expressing markers of adipocytes (9 of 8,522 cells): perilipin (PLPN), adiponectin (ADIPOQ). As the biopsies contained recognizable subcutaneous fat, we would have expected these cells to be present at higher numbers. Likely adipocytes died during preparation of the cells for single cell RNA-seq or failed to pass through the 70 micron filter of the cell lysate. However, one or more of the rare subpopulations of cells may represent adipocyte progenitor/preadipocytes (Festa et al., 2011, Rodeheffer et al., 2008) or fat derived stem cells (Dykstra et al., 2017, Festa et al., 2011, Rodeheffer et al., 2008), RBP4+ cells would be a possible candidate for the latter.

In summary, human dermal fibroblasts represent a complex group of cells, but in particular showing two major populations. Discrete gene expression by these and several minor fibroblast populations points to different functional roles in regulating matrix deposition, inflammation, and likely serving as pluripotent mesenchymal stem cells.

METHODS

Preparation of single cell libraries, sequencing and analysis.

Methods are detailed in Supplementary methods. Dorsal forearm skin biopsies were obtained under a protocol approved by the University of Pittsburgh Institutional Review Board. Each subject gave written informed consent. Biopsies were digested, cell suspensions loaded into the Chromium instrument (10X Genomics), and the resulting barcoded cDNAs used to construct libraries. RNA-seq was performed on each sample, obtaining ~200 million reads/sample. Cell-gene UMI counting matrices were generated and analyzed using Seurat (Satija et al., 2015) to identify distinct cell populations (Macosko et al., 2015) and hierarchically clustered, using Cluster 3.0 (de Hoon et al., 2004).

Immunofluorescent staining.

Single and dual antibody staining using tyramide signal amplification were performed on formalin fixed, paraffin embedded human forearm skin biopsies. Antibody stains were performed using mouse anti-SFRP2 (1:1000; EMD Millipore, Temecula, CA); rabbit anti-FMO1(1:1000; Prestige Antibodies, Sigma, St. Louis, MO); rabbit anti-LSP1(1:1000;

Prestige Antibodies, Sigma, St. Louis, MO); rabbit anti-DPP4/CD26 (1:40; Abcam, Cambridge, MA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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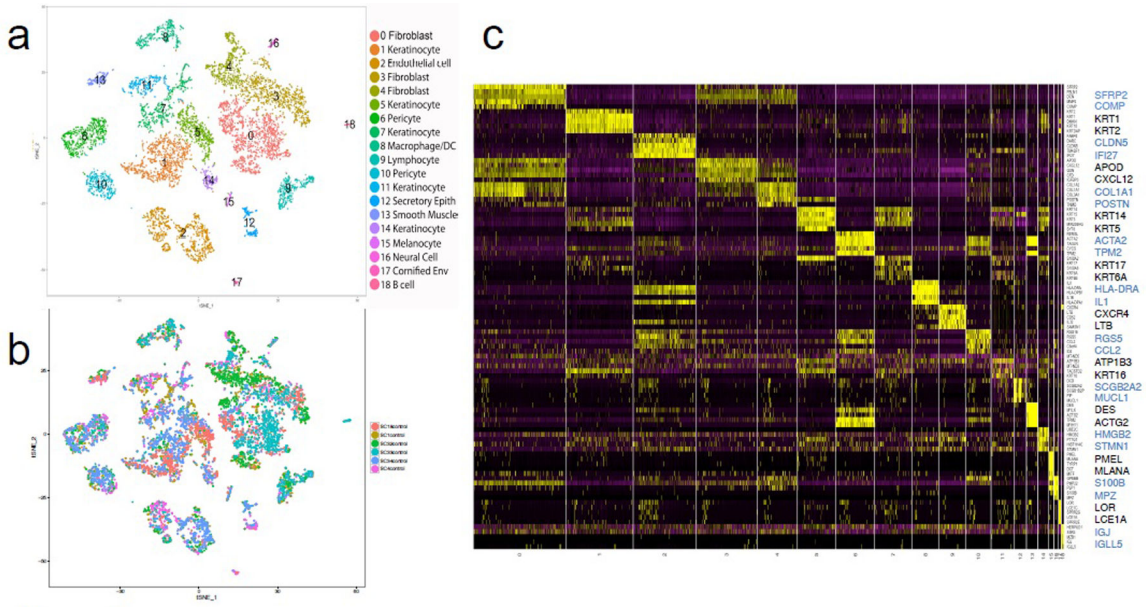

Figure 1. T-SNE shows groupings and SLM clustering of normal skin cell populations.

Panel a: Each point represents a cell. Two-dimensional t-SNE shows dimensional reduction of cell transcriptomes. Cells are colored by K-nearest neighbors graph based on Euclidean distance in PCA space using a smart local moving algorithm (SLM) to iteratively group cells. Panel b: Cells are grouped by t-SNE as in panel A, but are colored according to the subject identity. Panel c shows transcriptomes of 8,522 cells from six normal skin biopsies clustered using Seurat (SLM clustering). Each column represents a cell. The five genes most differentially expressed between each cluster are shown, and two of these five genes are enlarged to help identify each cluster. Cluster numbers, indicated at the bottom are as shown in Figure 1a, t-SNE.

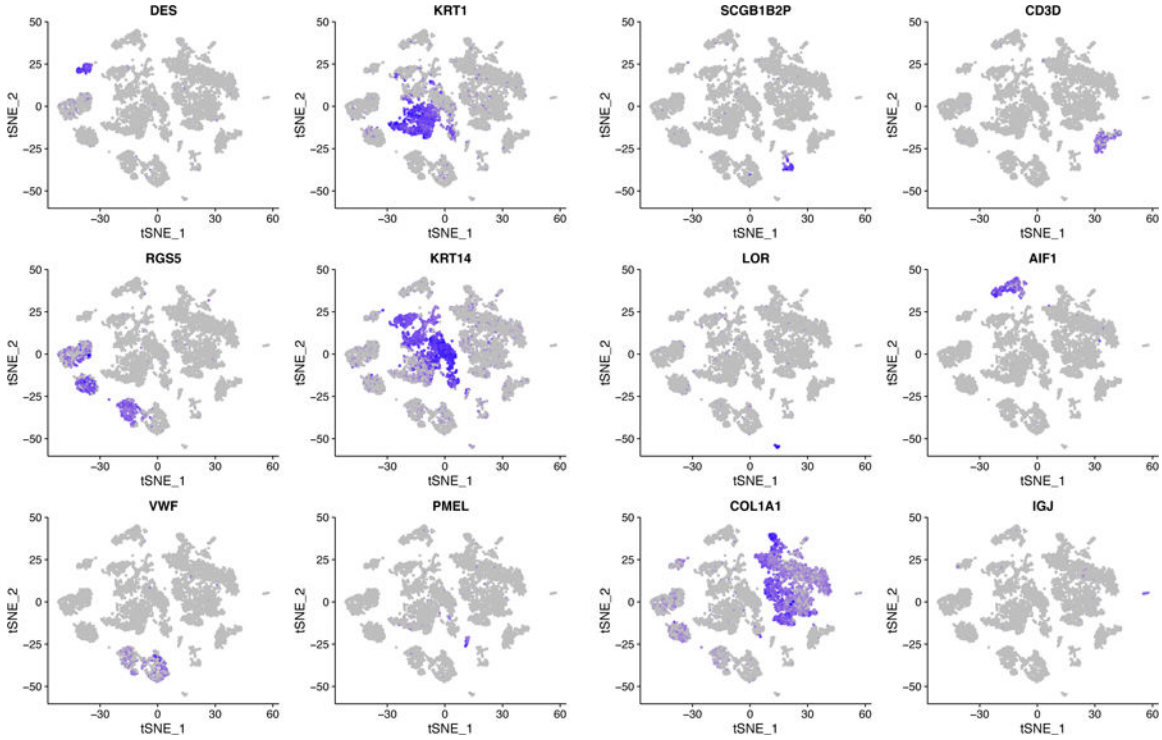

Figure 2. Feature plots of genes defining different cell types in normal skin.

The intensity of purple color indicates the normalized level of gene expression. DES: smooth muscle/arrector pili; RGS5: pericytes; VWF: endothelial cells; KRT1 and KRT14: keratinocyte populations; PMEL: melanocytes; SCGB1B2P: gland cells; LOR: cornified keratinocytes; COL1A1: fibroblasts; CD3D: T cells; AIF1: macrophages/dendritic cells; IGJ: B cells

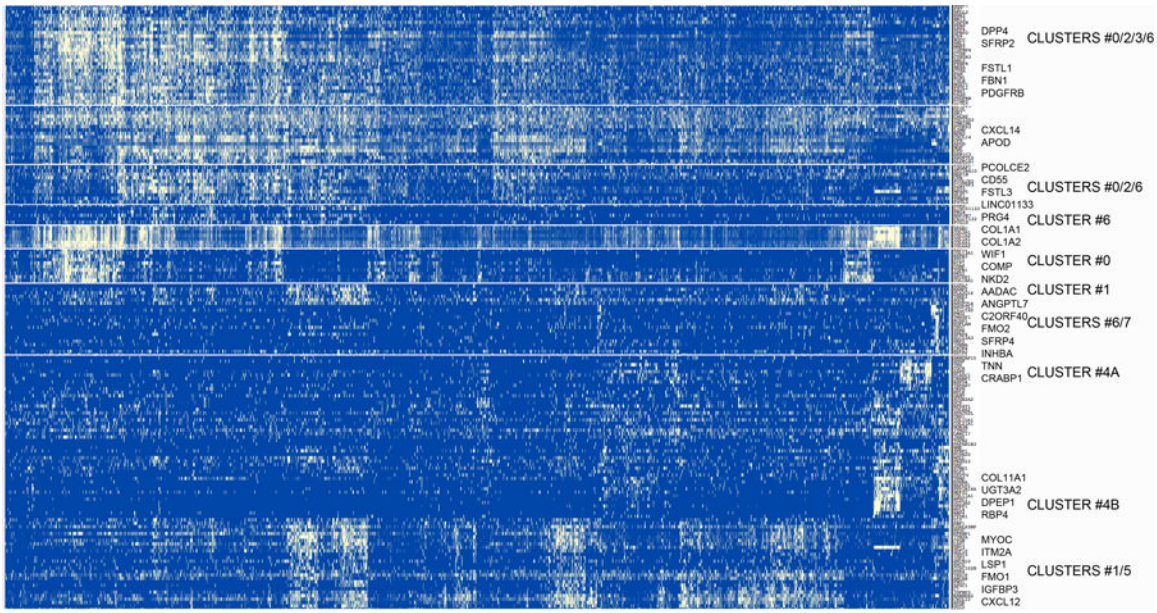

Figure 3: Hierarchical clustering of fibroblast transcriptomes from normal skin.

Fibroblasts in SLM cluster #0, 3 and 4 were hierarchically clustered. Genes identified to define cell types selected by t-SNE and SLM clustering, as well as visual inspection were used to identify clusters showing gene expression discrete to various cell types. Names of genes associated with each cluster are enlarged to the right of the clustering. The SLM clusters that define the different clusters and cell types are shown to the far right.

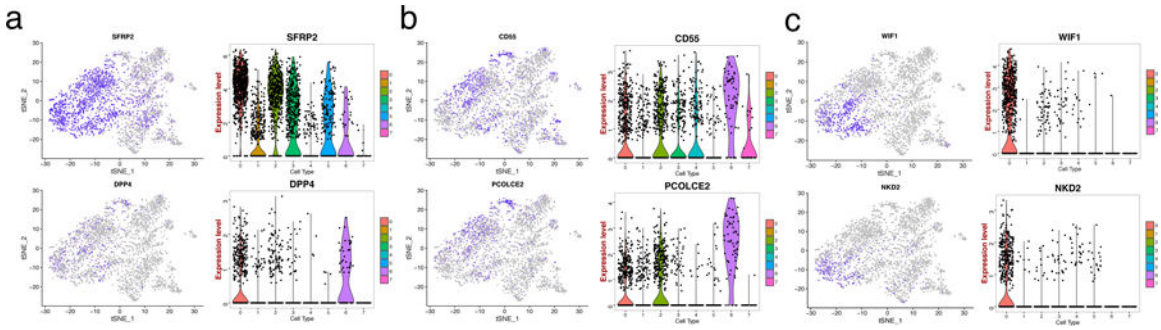

Figure 4. Gene expression by SFRP2+ fibroblasts and CD55/PCOLCE2 and WIF1/NKD2 fibroblast subpopulations.

Intensity of purple color indicates the level of gene expression on t-SNE feature plots for SFRP4 and DPP4 (Panel a), CD55 and PCOLCE2 (Panel b) and WIF1 and NKD2 (Panel c). Violin plots adjacent to each t-SNE plot show the expression level of genes in each of the SLM clusters shown in Figure 5

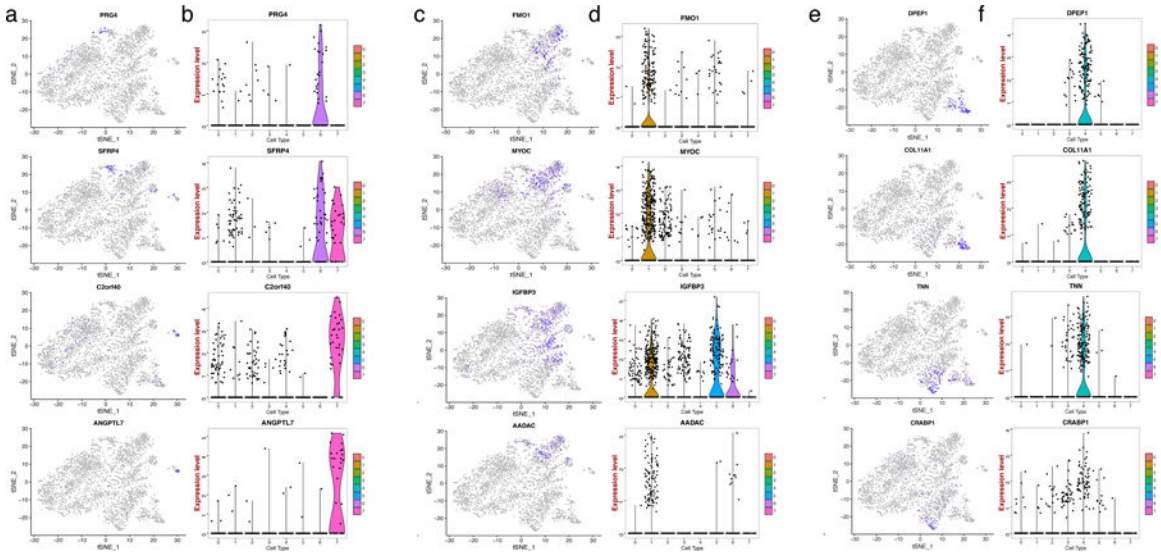

Figure 5. Gene expression by fibroblast subpopulations.

Intensity of purple color indicates the level of gene expression on t-SNE feature (a, c, e). Violin plots adjacent to each t-SNE plot (b, d, f) show the expression level of genes in each of the SLM clusters shown in Figure 5.

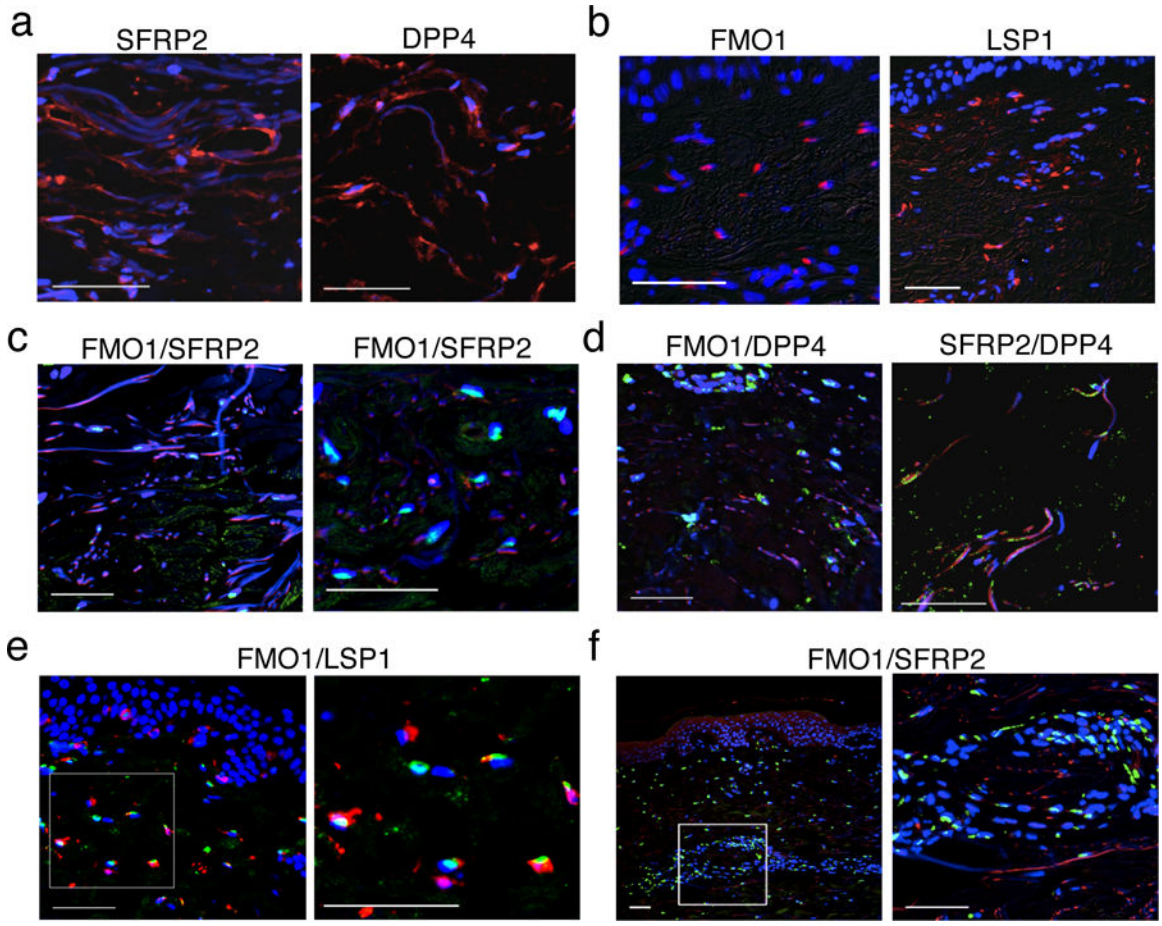

Figure 6. Immunofluorescent staining of normal skin showing two distinct major populations of dermal fibroblasts.

Single IF staining of SFRP2 and DPP4 detects cytoplasmic staining of morphologically smaller, elongated cells (panel a, red). IF staining of FMO1 and LSP1 detects nuclear (FMO1) and cytoplasmic (LSP1) staining of larger, more round cells (panel b, red). Double IF staining is shown of FMO1 (green) and SFRP2 (red, panel c); FMO1 (green), DPP4 (red) and SFRP2 (red) DPP4 (green, panel d); FMO1 (green) and LSP1 (red, panel e); and FMO1 (green) and SFRP2 (red, panel f) Dual staining with higher magnification insets are included for FMO1 and LSP1 (panel e) and FMO1 and SFRP2 (panel f). For all panels nuclei are counterstained with DAPI (blue). Scale bar=50 μM.