Schwann Cells in the Ventral Dermis Do Not Derive from Myf5-Expressing Precursors

Haizea Iribar, Virginia Pérez-López, Usue Etxaniz, Araika Gutiérrez-Rivera, and Ander Izeta

INTRODUCTION

The embryonic origin of lineage precursors of the trunk dermis is somewhat controversial. Precursor cells traced by Myf5 and Twist2 (Dermo1) promoter activation (i.e., cells of presumed dermomyotomal lineage) have been reported to generate Schwann cells. On the other hand, abundant data demonstrate that dermal Schwann cells derive from the neural crest. This is relevant because dermal precursors give rise to neural lineages, and multilineage differentiation potential qualifies them as adult stem cells. However, it is currently unclear whether neural lineages arise from dedifferentiated Schwann cells instead of mesodermally derived dermal precursor cells. To clarify these discrepancies, we traced SOX2+ adult dermal precursor cells by two independent Myf5 lineage tracing strains. We demonstrate that dermal Schwann cells do not belong to the Myf5+ cell lineage, indicating that previous tracing data reflected aberrant cell fate transitions and that bona fide Myf5+ dermal precursors cannot transdifferentiate to neural lineages in physiological conditions.

RESULTS

A SOX2+ Cell Population Traced by Myf5-creSor Expression Retains Neural Competence in Ventral Trunk Dermis

To trace the lineage of precursor cells in the dorsal and ventral dermis, we chose the same transgenic mouse line that had been previously used to express cre recombinase under the control of the Myf5 promoter (Myf5-creSor).
Myf5-creSor mice by fluorescence-activated cell sorting (FACS) through EYFP expression, put them into differentiation media, and quantified their neural progeny by immunofluorescence and flow cytometry (Figure 1B). In the ventral dermis, we noticed the existence of a small and previously over-looked Myf5<sup>+</sup> cell population (1.9% ± 2.2%, n = 15) that was difficult to reconcile with a lateral plate mesoderm origin of precursor cells in this region (Ohtola et al., 2008). Besides a morphology consistent with neural-competent, dedifferentiated Schwann cells (lower panel of Figure 1B; see also Figures S1 and Etxaniz et al., 2014), the Myf5<sup>+</sup> cells expressed neural precursor cell marker NESTIN (Figure S1). To determine whether the Myf5<sup>+</sup> cells from ventral skin presented in vitro neural differentiation capabilities, we isolated cell fractions from Myf5<sup>creSor</sup>,R26EYFP<sup>+/−</sup> mice by fluorescence-activated cell sorting (FACS) through EYFP expression, put them into differentiation media, and quantified their neural progeny by immunofluorescence with anti-GFAP and anti-βIII TUBULIN antibodies (Figures 1C and 1D). Furthermore, the differentiated cultures of the Myf5<sup>+</sup> fraction presented a characteristic bipolar glial morphology that was corroborated by co-staining with markers p75NTR, GFAP, βIII TUBULIN, and S100β (Figures S1A–S1P). Interestingly, Myf5<sup>+</sup> cells expressed SOX2, a transcription factor associated with dermal stem cells (Figures S1Q–S1T). These data suggested that a SOX2<sup>+</sup> neural precursor cell population (unexpectedly traced by Myf5 expression) retained neural competence in mouse ventral dermis.

To further characterize in vitro differentiated cells, we determined the expression of key markers of the Schwann cell lineage (Etxaniz et al., 2014) by real-time qRT-PCR (Figure S2). We selected the genes Ngfr (coding for p75NTR), Cdh19 (CADHERIN 19), Egr1 (KROX20), Gap43 (GAP43), Ncam1 (CD56), S100b (S100β), and Egr2 (KROX20) to discriminate between the different stages of Schwann cell lineage determination (Figures S2A and S2B). Analysis of mRNA expression for these genes demonstrated that markers specific of Schwann cell precursors (SCP), such as Cdh19, as well as genes shared by SCPs and immature Schwann cells (such as Egr1 and Gap43) were upregulated in differentiated Myf5<sup>+</sup> cells, indicating that significant numbers of cells remained in precursor state in culture. The more differentiated cells seem to belong to the non-myelinating Schwann lineage as shown by the expression patterns of Ngfr, Gap43, Ncam1, S100b, and Egr2 (Figure S2C). In all, these data suggested that Myf5<sup>creSor</sup> cells might belong to the Schwann lineage and give rise to Schwann cells in vitro.
In Situ Localization of Ventral Myf5-creSor Cells Corroborates Their Schwann Cell Identity

To investigate the identity and localization of Myf5+ cells in the dermis, we first analyzed ventral skin sections of adult Myf5cre/+;R26TdTomato mice. Myf5-creSor+ cells presented a regular fiber-like pattern (Figures 2A and 2B) reminiscent of Schwann cells of the superficial cutaneous nerve network (Gresset et al., 2015). In fact, a distinct subset of Myf5+ cells in subepidermal location showed a unique morphology, with long processes expanding from the cell body (Figure 2B, open arrowheads). This is characteristic of terminal Schwann cells (teloglia) that ensheath nociceptive nerve endings at the dermo-epidermal interface (Gresset et al., 2015). To better understand the spatial distribution of Myf5+ cells, we performed whole-mount ventral dermis analyses (Fujiwara et al., 2011; Tschachler et al., 2004) of the Myf5cre/+;R26EYFP strain. Myf5+ cells (detected with anti-GFP) formed a subepidermal nerve plexus stained positive to p75NTR (E; red), S100 (F; red), and SOX10 (G and G'; red) glial markers. (H–J) A subset of Myf5+ cells (GFP, green) expressed the non-myelinating SC marker INTEGRIN α1 (H; red; arrowhead), while other Myf5+ cells co-localized with myelin basic protein. (I) Some Myf5+ cells (GFP, green) co-localized with myelin basic protein (MBP, red), and thus constitute myelinating SCs (arrows). A subset of Myf5+ cells lacked co-localization with MBP, and thus constitute non-myelinating SCs (arrowhead). (J) An example of non-myelinating Myf5+ cells traced by TdTomato reporter (red, arrowhead). MBP staining is shown in green (arrow). All sections were counterstained with Hoechst 33258 (blue). Scale bars represent 50 μm in (A), (C), and (D), 25 μm in (B), 20 μm in (E), (F), (G), and (J), and 10 μm in (G'), (H), and (I).
MBP⁺ myelinating Schwann cells were also detected, although GFP expression was greatly diminished (Figure 2I, arrows).

At the deep dermis, thicker NF200⁺ and PGP9.5⁺ nerve bundles (Figures 3A, 3B, and 3D) were ensheathed by Myf5⁺ cells that co-localized with glial markers GFAP, p75NTR, S100b, and SOX10 (Figures 3C–3G). These nerves form the subcutaneous plexus at the level of the dermal panniculus carnosus muscle (Naldaiz-Gastesi et al., 2016), which was also traced by this construct (GFP, green; open arrowheads), as well as thick nerve bundles that co-stained with GFAP (C; red), PGP9.5 (D; red), p75NTR (E; red), S100 (F; red), and SOX10 (G and G; red). Analysis of MBP (H and H; red) revealed some co-localization of Myf5⁺ cells (GFP, green), indicative of myelinating (MBP⁺) Schwann cells (arrows). Non-myelinating Schwann cells (arrowheads) were also clearly detected. All sections were counterstained with Hoechst 33258 (blue). Scale bars represent 50 µm in (A), 25 µm in (B) to (H), and 20 µm in (H').

In the hair follicles, glial cells forming the mechanosensory lanceolate complex (Li and Ginty, 2014) were also Myf5-creSor⁺ (Figure 4A). Hair-follicle-associated Myf5⁺ cells were p75NTR⁺, S100b⁺, and NESTIN⁺ (Figures 4B–4E), as described for the lanceolate complex glial cells (Johnston et al., 2013). In addition, Myf5⁺ cells were occasionally detected in three other locations (Figures S3–S5): (1) perivascular Myf5⁺ cells (Figure S3) were consistent with a pericyte identity, as they displayed a characteristic morphology (Figures S3C and S3D; arrows) and co-expressed pericytic markers α-smooth muscle actin (αSMA) and platelet-derived growth factor receptor β (PDGFRβ); (2) dermal nerve cells ensheathing axons in SOX2⁺ touch domes (Reinisch and Tschachler, 2005) were also Myf5⁺ (Figure S4); and
hair-follicle melanocytes were adjacent to the dermal papilla (Figure S5).

In all, these results demonstrated that the small (but reproducible) population of ventral Myf5+ cells are mainly composed of Schwann cells, and also includes rare cells of possible mesenchymal origin such as pericytes, an identity associated with dermal stem cell subsets in human skin (Etxaniz et al., 2014; Feisst et al., 2014; Ruetze et al., 2013; Yamanishi et al., 2012), as well as hair-follicle-associated melanocytes.

**Sox2 Expression Levels Correlate with Neural Competence of Mouse Ventral Precursors**

The neural competence of dermal precursor cells isolated from human foreskin (and mouse dorsal skin) is regulated by the expression levels of SOX2 (Etxaniz et al., 2014). To test whether this is also the case for mouse ventral dermis, we separated dermal sphere cells from Sox2+/EGFP mice by FACS according to their EGFP expression levels into SOX2-high, -medium, -low, and -negative populations (Figure 5). The endogenous Sox2 mRNA levels correlated with EGFP, as determined by qRT-PCR (Figure 5B). In vitro differentiation of freshly isolated cell populations and immunofluorescence analyses with anti-GFAP, anti-βIII TUBULIN, and anti-GFP antibodies (surrogate for SOX2; Figures 5D–5G) demonstrated that neural competence was restricted to SOX2 medium and SOX2 high cell fractions, and correlated with the Sox2 mRNA levels of each fraction. Morphology of the SOX2+ cells in the differentiated cultures was consistent with a neural identity (Figures 5E and 5G–5I). This was corroborated by co-expression in differentiated cells of markers PGP9.5,
Figure 5. Neural Competence of Ventral Precursors Correlates with Sox2 Expression Levels

(A) Ventral dermis-derived sphere cultures from Sox2EGFP/+ mice were sorted by FACS into SOX2-negative (NEG), -low (LOW), -medium (MED), and -high (HIGH) subpopulations according to their EGFP levels.

(B) Sox2 mRNA levels of separated cell fractions measured by qRT-PCR. Log2 relative quantification (LOG2 RQ) is shown in comparison with the NEG fraction. Mean values and SEM are represented (n = 3). Statistical significance (two-tailed unpaired t test): ***p = 0.0003 for comparison of HIGH versus UNS, *p = 0.0113 for HIGH versus LOW, *p = 0.0113 for HIGH versus MED, and ***p = 0.0003 for UNS versus MED. No statistical significance (ns) was found in all other comparisons.

(C) Sox2 mRNA levels measured in the UNS and Myf5+ cell fractions measured by qRT-PCR. The mean RQ value is represented for each fraction in comparison with the Myf5+/C0 cell fraction. Error bars indicate SEM (n = 4). Statistical analysis value (two-tailed unpaired t test) was not significant (p = 0.0654).

(D) In vitro glial differentiation of UNS, NEG, LOW, MED, and HIGH populations isolated from Sox2EGFP/+ dermal cultures. Quantification of the percentage of GFAP+ cells in relation to the total number of cells. Error bars indicate SEM (n = 3). Statistical significance (two-tailed unpaired t test) values were **p = 0.0021 for comparison of UNS versus HIGH fraction and *p = 0.0149 for HIGH versus MED. No statistical significance was found in any other comparisons.

(E) Representative images of in vitro differentiated cultures of the UNS, NEG, LOW, MED, and HIGH cell fractions, stained with anti-GFP (Sox2EGFP, green) and anti-GFAP (red).

(F) In vitro neuronal differentiation of unsorted Sox2EGFP/+ dermal cultures. Quantification of the percentage of βIII TUBULIN+ cells in relation to the total number of cells and GFP+ cells are shown. Error bars indicate SEM (n = 3).

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In vitro of NF200 + dermal nerves (Figure 6E). Lanceolate ending peripheral axons that sprouted out from a dense network to the total number of cells and GFP + cells are shown. Error bars indicate SEM (n = 3). Scale bars represent 25 μm in (E), (H), (I), (L), and (M), and 50 μm in (G) and (K). See also Figure S6.

**Myf5** Cells in the Ventral Dermis Partially Overlap with Resident SOX2 + Cells

To define the relationship between SOX2 + and Myf5 + cells in the ventral dermis, we analyzed skin biopsies of Sox2 +/EGFP mice in dermal whole-mount preparations. At the subepidermal level, SOX2 + cells (stained with anti-GFP) were associated with the cutaneous peripheral nerve plexus, where they ensheathed NF200 + axons and co-stained with glial marker S100β (Figures 6A–6C). In hair follicles, SOX2 expression was localized in the lanceolate complexes (arrows in Figure 6D; Figures 6D–6F). Each receptor was individually innervated by a subset of peripheral axons that sprouted out from a dense network of NF200 + dermal nerves (Figure 6E). Lanceolate ending SOX2 + cells co-stained with NESTIN and S100β (Figures 6G–6H). Additionally, SOX2 + cells were occasionally detected at the dermal papilla/dermal cup of some hair follicles (arrowheads; Figures 6D and 6G). SOX2 + pericytes were not detected. Since both SOX2 + and Myf5 + cells of ventral dermis overlap in similar dermal compartments, and to ascertain whether these markers were present in the same cells, we crossed Myf5 +/EGFP; R26ERTomato mice with Sox2 +/EGFP. The analysis of whole-mount samples from the ventral dermis of Myf5 +/EGFP; R26ERTomato; Sox2 +/EGFP triple transgenic mice confirmed that lanceolate ending Myf5 + cells (seen as TdTomato + cells in these experiments) co-expressed SOX2 (arrows in Figures 6I and 6J). Both markers were also co-expressed by Schwann cells of the cutaneous plexus, although this was more difficult to visualize due to the apparently lower SOX2 expression levels (data not shown).

**Schwann Cells in the Ventral Dermis Are Not Traced by a More Restricted Myf5 Lineage Strain**

Tracing of Schwann cells by Myf5 expression would in principle be unexpected. Of note, a cause for concern with the Myf5tm3loxcreSor mouse model is that transgene expression is constitutive, and widespread when crossed with R26YFP (Eppig et al., 2015 and data not shown). To clarify whether tracing by the Myf5-creSor transgene was truly indicative of Myf5 + cell lineage or was otherwise aberrant, we used a second cre-expressing strain (B195AP-cre) that traces a more restricted subset (17.6% in the dorsal dermis) of bona fide Myf5 + cells (Naldaiz-Gastesi et al., 2016). In the ventral dermis, 0.7% of sphere cells were B195AP + (EYFP +), as assessed by flow cytometry (Figure 7A). In vitro glial differentiation of B195AP-positive and -negative cell fractions showed that only the cells negative for Myf5 expression were able to generate GFAP + cells. Furthermore, B195AP + cells gave rise to MYH2 + myotubes in vitro (Figures 7B and 7C), which is consistent with tracing of panniculus carnosus-derived muscle satellite stem cells by this strain (Figures 7D and 7E; see also Naldaiz-Gastesi et al., 2016). Interestingly, SOX2 expression co-localized with B195AP + cells (Figures 7F and 7H). Finally, no co-localization of B195AP + cells was observed in situ with nerves and Schwann cells, as detected by absence of co-expression or vicinity with cells expressing markers PGP9.5, p75NTR, S100β, and NF200 (Figures 7G–7J). These results suggest that ventral dermal Schwann cells traced by the Myf5-creSor transgene do not belong to the Myf5 + cell lineage, and are most likely explained by aberrant cre recombination expression in the Myf5-creSor mice.

**DISCUSSION**

In the last few years, dermal stem/precursor cells and different subsets of fibroblasts have been isolated and variously named by independent research groups. Clearly, the field is in need of harmonization and clarification. This is due in part to the developmental regionalization of the dermis and to the variety of ill-described dermal stem cell niches, but also to the lack of markers that uniquely distinguish stem cells.

In this article, we demonstrate that the neural-competent cells in ventral dermis are Schwann cells aberrantly traced by the Myf5-creSor construct. This is certainly true for ventral dermis and seems probable for dorsal dermis.
as well, although most dorsal dermal cells are traced by Myf5 expression, and this fact complicates the matter of discriminating among multiple components of the cell fractions. Tracing by Myf5 expression by a second strain (B195AP-cre) showed that Schwann cells do not originate in a bona fide Myf5+ cell lineage. Thus the tracing by Myf5-creSor mice (Jinno et al., 2010) does not actually represent the existence of a Myf5+ cell population that generates Schwann cells. A similar picture may emerge from Dermo1 (Twist2)+ cell fate analyses (Krause et al., 2014), since the tracing construct used by these authors also presents widespread expression (including neural tissue), similar to Myf5-creSor mice (Eppig et al., 2015).

Adult dermal stem cells of diverse niches are all considered to be SOX2+ (a marker that, once more, is not specific for dermal stem cells [Agabalyan et al., 2016]). In mouse dorsal skin, the diverse SOX2+ cell niches are well characterized (Biernaskie et al., 2009; Clavel et al., 2012; Driskell et al., 2009; Lesko et al., 2013) but the expression levels of this transcription factor are cell-context dependent and highly dynamic. Besides, Schwann cell dedifferentiation in response to wounding accounts for the majority of SOX2+ cells populating the wound bed (Johnston et al., 2013). In this context, we showed here that cell populations aberrantly traced by the Myf5-creSor construct seem to be coincident with those previously described as SOX2+ by diverse groups. In our hands, in situ SOX2+ compartments matched between ventral and dorsal skin of Sox2EGFP mice (data not shown).
In conclusion, aberrantly traced Myf5-creSor Schwann cells generate neural lineages (mostly Schwann cells) upon in vitro differentiation in a SOX2-level-dependent manner. This report sheds light on the identification of adult ventral trunk neural precursor cells and demonstrates that they correspond to dedifferentiated peripheral glia, which derive from the neural crest (Gresset et al., 2015).

EXPERIMENTAL PROCEDURES

Animals
Mice (8- to 12-weeks-old) were used in accordance with the relevant Spanish and European guidelines after approval by the Biobonostia Animal Care Committee. Transgenic lines (described in Table S1) were purchased from JaxMice with the exception of Sox2+/EGFP mice, which were donated by Dr. K. Hochedlinger (Harvard University) and B195AP-cre mice, a gift of Dr. J.J. Carvajal (Centro Andaluz de Biología del Desarrollo).

Cell Isolation, Culture, and Differentiation
Animals were euthanized by CO2 inhalation and the dorsal and ventral skin carefully dissected. Dermal cells were processed and put in suspension culture as described by Etxaniz et al. (2014). Dermal sphere cells were separated by FACs (as detailed below), plated onto 12-mm coverslips coated with extracellular matrix (ECM) secreted from 804G cells, growth in adherence, and differentiated in Schwann medium for an additional 7–12 days as described previously (Gago et al., 2009). For neuronal differentiation, dermal spheres were disaggregated and directly plated onto...
were collected for expansion as described by Etxaniz et al. (2014). Cells were separated according to their SOX2 expression levels. Tissue samples were embedded in OCT compound, Sakura) and 5 μm-thick cryostat sections were cut. The staining was performed as described by Jinno et al. (2010).

Immunofluorescence on Coverslips, Frozen Skin Sections, and Dermal Whole Mounts
Dermal sphere cultures and in vitro differentiated cells were fixed, permeabilized, stained, and imaged as described by Etxaniz et al. (2014). Tissue samples were embedded in Tissue-Tek (OCT compound, Sakura) and 5 μm-thick cryostat sections were cut. The staining was performed as described by Jinno et al. (2010). Antibodies used are detailed in Table S4, Dermal whole-mount immunofluorescence has been described elsewhere (Fujiwara et al., 2011; Tschachler et al., 2004).

Image Acquisition
Images were acquired on an LSM510 META confocal microscope (Zeiss) using the ZEN 2008 sp2 software package (v. 4.2), in addition to an Eclipse 80i fluorescence microscope (Nikon) using NIS elements-AR software packages (v.3.2).

Cell Sorting
Dermal spheres at day 7 of proliferation were dissociated to a single-cell suspension with 0.25% trypsin-EDTA solution (Sigma-Aldrich), resuspended in sorting buffer, and analyzed in a FACSAria III (Becton Dickinson) with TOPRO exclusion of viable populations. Cell fractions from Myf5−/−;R26EYFP mice were sorted as EYFP-positive and -negative, and SOX2+EGFP samples were separated according to their SOX2 expression levels. Cells were collected for expansion as described by Etxaniz et al. (2014).

RNA Extraction and Real-Time qPCR
Total RNA was extracted from sorted cells using a miRNeasy micro kit with the automatic QIACube workstaton (Qiagen). Reverse transcription was performed using RNA to cDNA High Capacity Kit (Applied Biosystems). The cDNA of selected genes (SOX2 expression analysis) was specifically amplified (14 cycles) using a PreAmp Master Mix Kit (Applied Biosystems). TaqMan probe and SYBR green-based gene expression analyses were performed using 96- and 384-well plates on 7900HT (Applied Biosystems) and Light Cycler 96 (Roche) real-time PCR systems, respectively. Relative quantification analyses were carried out by using the RQ (2 −ΔΔCt) method (Livak and Schmittgen, 2001). The significance threshold was set at a fold change of 2. TaqMan probes and SYBR green primer sequences are listed in Tables S2 and S3, respectively.

Statistical Analyses
Statistical analysis was carried out using GraphPad Prism software v5.01. For multiple group comparisons, a two-tailed unpaired t test was performed. The number of biological replicates (n) for each experiment and average ± SEM are indicated when applicable, and statistical signficance is indicated by *p < 0.05, **p < 0.01, and ***p < 0.001.

SUPPLEMENTAL INFORMATION
Supplemental Information includes six figures and four tables and can be found with this article online at https://doi.org/10.1016/j.stemcr.2017.09.010.

AUTHOR CONTRIBUTIONS
H.I. performed most of the experimental work. V.P.-L. helped in cell culturing, mouse genotyping, and histological characterization. U.E. performed some of the initial cell culture and differentiation experiments. A.G.-R. was responsible for mouse colony handling and co-directed experimental work. A.I. directed and financed the project. H.I. and A.I. wrote the manuscript, which was approved by all authors prior to submission.

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REFERENCES
Agabalyan, N.A., Hagner, A., Rahmani, W., and Biernaskie, J. (2016). SOX2 in the skin. In Sox2—Biology and Role in Development and Disease, H. Kondoh and R. Lovell-Badge, eds. (Academic Press), pp. 281–300.
Agabalyan, N.A., Rosin, N.L., Rahmani, W., and Biernaskie, J. (2017). Hair follicle dermal stem cells and skin-derived precursor cells: exciting tools for endogenous and exogenous therapies. Exp. Dermatol. 26, 505–509.
Biernaskie, J., Paris, M., Morozova, O., Fagan, B.M., Marra, M., Penny, L., and Miller, F.D. (2009). SKPs derive from hair follicle precursors and exhibit properties of adult dermal stem cells. Cell Stem Cell 5, 610–623.
Christ, B., Huang, R., and Scaal, M. (2007). Amniote somite derivatives. Dev. Dyn. 236, 2382–2396.
Clavel, C., Grisanti, L., Zemla, R., Rezza, A., Barros, R., Sennett, R., Mazloom, A.R., Chung, C.-Y., Cai, X., Cai, C.-L., et al. (2012). Sox2 in the dermal papilla niche controls hair growth by fine-tuning BMP signaling in differentiating hair shaft progenitors. Dev. Cell 23, 981–994.

Driskell, R.R., Giangreco, A., Jensen, K.B., Mulder, K.W., and Watt, F.M. (2009). Sox2-positive dermal papilla cells specify hair follicle type in mammalian epidermis. Development 136, 2815–2823.

Dupin, E., and Sommer, L. (2012). Neural crest progenitors and stem cells: from early development to adulthood. Dev. Biol. 366, 83–95.

Eppig, J.T., Blake, J.A., Bult, C.J., Kadin, J.A., and Richardson, J.E. (2015). The Mouse Genome Database (MGD): facilitating mouse as a model for human biology and disease. Nucleic Acids Res. 43, D726–D736.

Etxaniz, U., Pérez-San Vicente, A., Gago-López, N., García-Domínguez, M., Iribar, H., Aduriz, A., Pérez-López, V., Burgoa, I., Irizar, H., Muñoz-Culla, M., et al. (2014). Neural-competent cells of adult human dermis belong to the Schwann lineage. Stem Cell Reports 3, 774–788.

Feist, V., Brooks, A.E., Chen, C.J., and Dunbar, P.R. (2014). Characterization of mesenchymal progenitor cell populations directly derived from human dermis. Stem Cells Dev. 23, 631–642.

Fernandes, K.J.L., McKenzie, I.A., Mill, P., Smith, K.M., Akhaban, M., Barnabé-Heider, F., Biernaskie, J., Junek, A., Kobayashi, N.R., Toma, J.G., et al. (2004). A dermal niche for multipotent adult skin-derived precursor cells. Nat. Cell Biol. 6, 1082–1093.

Fujiwara, H., Ferreira, M., Donati, G., Marciano, D.K., Linton, J.M., Sato, Y., Hartner, A., Sekiguchi, K., Reichardt, L.F., and Watt, F.M. (2011). The basement membrane of hair follicle stem cells is a muscle cell niche. Cell 144, 577–589.

Gago, N., Pérez-López, V., Sanz-Jaka, J.P., Cormanzena, P., Eizaguirre, I., Bernad, A., and Izeta, A. (2009). Age-dependent depletion of human skin-derived progenitor cells. Stem Cells 27, 1164–1172.

Garcia-Parra, P., Naldaiz-Gastesi, N., Maroto, M., Padin, J.F., Goicochea, M., Aiastui, A., Fernandez-Morales, J.C., Garcia-Belda, P., Lacalle, J., Alava, J.L., et al. (2014). Murine muscle engineered from dermal precursors: an in vitro model for skeletal muscle generation, degeneration, and fatty infiltration. Tissue Eng. Part C Methods 20, 28–41.

Gresset, C., Couplier, F., Gerschenfeld, G., Jourdon, A., Matiesc, G., Richard, L., Vallat, J.-M., Charnay, P., and Topilko, P. (2015). Boundary caps give rise to neurogenic stem cells and terminal glia in the skin. Stem Cell Reports 5, 278–290.

Hunt, D.P., Jahoda, C., and Chandran, S. (2009). Multipotent skin-derived precursors: from biology to clinical translation. Curr. Opin. Biotechnol. 20, 522–530.

Jessen, K.N.R., Misky, R., and Lloyd, A.C. (2015). Schwann cells: development and role in nerve repair. Cold Spring Harb. Perspect. Biol. 7, a020487.

Jinno, H., Morozova, O., Jones, K.L., Biernaskie, J.A., Paris, M., Hosokawa, R., Rudnicki, M.A., Chai, Y., Rossi, F., Marra, M.A., et al. (2010). Convergent genesis of an adult neural crest-like dermal stem cell from distinct developmental origins. Stem Cells 28, 2027–2040.

Joannides, A., Gaughwin, P., Schwiening, C., Majed, H., Sterling, J., Compston, A., and Chandran, S. (2004). Efficient generation of neural precursors from adult human skin: astrocytes promote neurogenesis from skin-derived stem cells. Lancet 364, 172–178.

Johnston, A.P.W., Naska, S., Jones, K., Jinno, H., Kaplan, D.R., and Miller, F.D. (2013). Sox2-mediated regulation of adult neural crest precursors and skin repair. Stem Cell Reports 1, 38–45.

Krause, M.P., Dworski, S., Feinberg, K., Jones, K., Johnston, A.P.W., Paul, S., Paris, M., Peles, E., Bagli, D., Forrest, C.R., et al. (2014). Direct genesis of functional rodent and human schwann cells from skin mesenchymal precursors. Stem Cell Reports 3, 85–100.

Lesko, M.H., Driskell, R.R., Kretzschmar, K., Goldie, S.J., and Watt, F.M. (2013). Sox2 modulates the function of two distinct cell lineages in mouse skin. Dev. Biol. 382, 15–26.

Li, L., and Ginty, D.D. (2014). The structure and organization of lanceolate mechanosensory complexes at mouse hair follicles. Elife 3, e01901.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408.

Millar, S.E. (2005). An ideal society? Neighbors of diverse origins interact to create and maintain complex mini-organs in the skin. PLoS Biol. 3, e372.

Naldaiz-Gastesi, N., Goicoechea, M., Alonso-Martín, S., Aiastui, A., López-Mayorga, M., García-Belda, P., Lacalle, J., San José, C., Araúzo-Bravo, M.J., Trouilh, L., et al. (2016). Identification and characterization of the dermal panniculus carnosus muscle stem cells. Stem Cell Reports 7, 411–424.

Ohtola, J., Myers, J., Akhtar-Zaidi, B., Zuzindlak, D., Sandesara, P., Yeh, K., Mackem, S., and Atit, R. (2008). beta-Catenin has sequential roles in the survival and specification of ventral dermis. Development 135, 2321–2329.

Olivera-Martinez, I., Villet, J.P., Michon, F., Pearston, D.J., and Dhouraylli, D. (2004). The different steps of skin formation in vertebrates. Int. J. Dev. Biol. 48, 107–115.

Reinisch, C.M., and Tschachler, E. (2005). The touch dome in human skin is supplied by different types of nerve fibers. Ann. Neurol. 58, 88–95.

Ruetze, M., Knauer, T., Gallinat, S., Wenck, H., Achterberg, V., Maerz, A., Deppert, W., and Knott, A. (2013). A novel niche for skin derived precursors in non-follicular skin. J. Dermatol. Sci. 69, 132–139.

Tschachler, E., Reinisch, C.M., Mayer, C., Paiha, K., Lassmann, H., and Weninger, W. (2004). Sheet preparations expose the dermal nerve plexus of human skin and render the dermal nerve end organ accessible to extensive analysis. J. Invest. Dermatol. 122, 177–182.

Yamanishi, H., Fujiwara, S., and Soma, T. (2012). Perivascular localization of dermal stem cells in human scalp. Exp. Dermatol. 21, 78–80.