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Identification of epidermal progenitors for the Merkel cell lineage

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In *Development* **137**, 3965-3971, Tbc1d10c immunofluorescence labelling of keratinocytes in touch domes of mouse skin was shown in Fig. 1B-D,H,L (p. 3967). Subsequently, the authors have found that the Tbc1d10c antibody used in this study appears to be labelling keratinocytes non-specifically by cross-reacting with another antigen. The labelling of touch dome cells with the marker CD200 remains valid (Fig. 1).

The authors wish to stress that these findings do not change the conclusions of the study, as subsequent analyses make use of the CD200 marker.

Identification of epidermal progenitors for the Merkel cell lineage

Seung-Hyun Woo^{1,*}, Magda Stumpfova^{1,*}, Uffe B. Jensen⁴, Ellen A. Lumpkin^{2,3} and David M. Owens^{1,2,†}

SUMMARY

Epithelial stem cells in adult mammalian skin are known to maintain epidermal, follicular and sebaceous lineages during homeostasis. Recently, Merkel cell mechanoreceptors were identified as a fourth lineage derived from the proliferative layer of murine skin epithelium; however, the location of the stem or progenitor population for Merkel cells remains unknown. Here, we have identified a previously undescribed population of epidermal progenitors that reside in the touch domes of hairy skin, termed touch dome progenitor cells (TDPCs). TDPCs are epithelial keratinocytes and are distinguished by their unique co-expression of $\alpha 6$ integrin, Sca1 and CD200 surface proteins. TDPCs exhibit bipotent progenitor behavior as they give rise to both squamous and neuroendocrine epidermal lineages, whereas the remainder of the $\alpha 6^+$ Sca1⁺ CD200⁻ epidermis does not give rise to Merkel cells. Finally, TDPCs possess a unique transcript profile that appears to be enforced by the juxtaposition of TDPCs with Merkel cells within the touch dome niche.

KEY WORDS: Skin, Epidermis, Touch dome, Tbc1d10c, CD200, Mouse

INTRODUCTION

In the hairy skin of mice and humans the interfollicular epidermis (IFE) contains specialized epithelial structures termed touch domes (TDs) that consist of epidermal keratinocytes in juxtaposition with neuroendocrine Merkel cells (Moll et al., 1996a; Johnson et al., 2000; Johnson, 2001; Halata et al., 2003; Pinkus, 1902; Reinisch and Tschachler, 2005; Smith, 1977). Merkel cells are putative sensory mechanoreceptor cells that are thought to be required for the perception of light touch stimuli (Maricich et al., 2009). Merkel cells express numerous neuronal proteins, suggesting that the Merkel cell lineage is derived from neural crest stem cells (Halata et al., 2003). This idea was initially supported by cell lineage-tracing studies that identified *Wnt1*-expressing neural crest stem cells as the site of Merkel cell origin (Szedler et al., 2003). However, multiple recent reports comparing murine lineage-tracing models using a *Wnt1* or cytokeratin 14 (*Krt14* or *K14*) Cre driver indicate that Merkel cells are derived from the proliferative keratinocyte (*Krt14*-expressing) layer of skin and not the neural crest (Morrison et al., 2009; Van Keymeulen et al., 2009). In support of these findings, conditional deletion of the *atoh1* helix-loop-helix transcription factor *Atoh1* in *K14-Cre* mice was shown to abolish Merkel cell development, whereas in *Wnt1-Cre* mice it had no effect on the development or maintenance of Merkel cells.

Since Merkel cells are post-mitotic (Merot and Saurat, 1988; Moll et al., 1996b; Vaigot et al., 1987), an epidermal progenitor pool would presumably be required to maintain this lineage during homeostasis. However, such a progenitor population has not been determined. Here we show that the progenitors for epidermal Merkel cells reside within the TD and demonstrate that these cells

are uniquely qualified to give rise to Merkel cell lineages in the IFE of hairy skin. We also provide the first evidence of an adult epithelial progenitor population with the capacity to give rise to neuroendocrine and squamous lineages. Collectively, these findings are crucial for our understanding of normal skin neurobiology and skin disease states such as Merkel cell carcinoma.

MATERIALS AND METHODS

Mice, EdU and antibodies

K14Cre (Dassule et al., 2000) (Jackson Laboratories), *R26R^{lacZ}* (Soriano, 1999) (Jackson Laboratories), FVB (Taconic Farms) and *Nude-Foxn1^{nu}* mice (Harlan) were maintained under IACUC protocols according to ICM guidelines.

For proliferation and lineage-tracing studies, FVB mice received a single or six (twice per day for 3 days) intraperitoneal injections of 20 mg/kg 5-ethynyl-2'-deoxyuridine (EdU) (Invitrogen) (Jensen et al., 2008).

Antibodies for FACS analysis were $\alpha 6$ integrin-PE, CD34-APC, CD200 purified and Sca1-Pe-Cy7 all from BD Biosciences and Alexa Fluor 680 anti-rat IgG (Invitrogen). Antibodies for tissue staining were β -galactosidase (Abcam), CD200-Alexa Fluor 647 (eBioscience), Ctgf (L-20; Santa Cruz), cytokeratin K1 (Covance), cytokeratin K10-488 [Covance; K10 antibody was directly conjugated to Alexa 488 using the Apex Alexa Fluor 488 Antibody Labeling Kit as per manufacturer's instructions (Invitrogen)], cytokeratin K8 (TROMA-I, DSHB), cytokeratin K14 (Covance), connexin 43 (N-19; Santa Cruz), NF-H (Millipore), Sca1-FITC (eBioscience), Cav2.1 (Millipore) and Alexa Fluor 488 anti-rat IgG, Alexa Fluor 488 anti-rabbit IgG, Alexa Fluor 594 anti-rat IgG, Alexa Fluor 594 anti-rabbit IgG, Alexa Fluor 633 anti-rabbit IgG and Alexa Fluor 647 anti-goat IgG all from Invitrogen.

Tissue analysis

Dorsal skin specimens were obtained from 8-week-old FVB mice for all analyses except for EdU labeling, which was performed in 3-week-old FVB mice. Skins were harvested at 1 and 16 hours or 1, 3 or 8 days following the last EdU injection. Skin grafts were harvested at 2, 4 and 6 weeks post-grafting. All tissue specimens were embedded in O.C.T. medium and cryopreserved.

For detection of β -galactosidase (β -gal), skin and skin graft specimens were harvested and fixed in 0.2% glutaraldehyde followed by overnight staining with X-gal (Invitrogen). Tissues were then washed in PBS to visualize positive X-gal staining.

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Immunostaining on histological skin cryosections was conducted as previously described (Jensen et al., 2008). EdU incorporation was detected using the Click-iT EdU Alexa Fluor 488 Imaging Kit (Invitrogen). Fluorescent images were captured using a Zeiss inverted Axiovert 200M or a Zeiss 510 META laser-scanning confocal microscope.

Flow cytometry

Epithelial keratinocytes were isolated from 8-week-old *K14Cre;R26^{lacZ}* mice and labeled with antibodies to $\alpha 6$ integrin (PE-conjugated; BD Biosciences), Sca1 (PE-Cy7-conjugated; BD Biosciences) and CD34 (APC-conjugated; BD Biosciences) surface proteins (Jensen et al., 2008). In addition, cells were labeled with antibodies against murine CD200 (BD Biosciences) followed by detection with Alexa Fluor 680 (Invitrogen). Live cells were subjected to FACS analysis using a FACSAria II sorter (BD Biosciences) and FlowJo Software (Tree Star). For each sorting experiment, typically three dorsal skins were pooled to generate $5\text{--}6 \times 10^7$ epidermal cells. From this population, a total of 3.5×10^7 viable $\alpha 6^+$ cells was typically identified and subsequently sorted into $\alpha 6^+$ Sca1⁺ CD200⁺ TD ($0.8\text{--}1 \times 10^6$ cells) and $\alpha 6^+$ Sca1⁺ CD200⁻ IFE^{ATD} (1×10^7 cells) populations. EdU incorporation was visualized in FACS-sorted TD cells using the Click-iT EdU Alexa Fluor 488 Flow Cytometry Kit (Invitrogen) (Jensen et al., 2008).

Skin reconstitution assay

FACS-sorted keratinocytes were mixed with 2×10^6 neonatal dermal fibroblasts and surgically implanted on the dorsal fascia of recipient Nude mice using silicon grafting chambers (Jensen et al., 2008). For each FACS-sorted population, three replicate grafts containing either 2.6×10^5 (TD and IFE^{ATD} groups) or 2.5×10^6 (IFE^{ATD} group) keratinocytes per graft were generated. This entire experimental procedure was repeated twice. Chambers were removed 1 week after cell injection and reconstituted skin was typically observed within 2 weeks.

Microarray analysis

Total RNA was isolated from FACS-sorted TD and IFE^{ATD} populations using TRIzol (Invitrogen) and double-stranded cDNA was synthesized and transcribed into biotin-labeled cRNA using the GeneChip 3' IVT Express Kit (Affymetrix) (50 ng total RNA/sample). Biotin-labeled cRNA (11 μg /sample) was hybridized on Mouse Genome 430 2.0 Array Chips (Affymetrix), which were scanned using the Affymetrix GeneChip Scanner 3000. Raw microarray values were quantified using GeneChip Operating Software (Affymetrix) and processed using TIGR MultiExperiment Viewer v4.0 Software (Saeed et al., 2006) to identify genes differentially regulated in TD versus IFE^{ATD} keratinocytes by at least 2-fold, using a paired Student's *t*-test ($P < 0.05$). Each group was examined in triplicate. Differentially regulated genes were functionally grouped by gene ontology using the ClueGO feature of Cytoscape v2.7.0 (Bindea et al., 2009). Microarray data have been deposited at Array Express with Accession Number E-MEXP-2925.

Reverse transcription (RT)-PCR analysis

Total RNA from FACS-sorted TD and IFE^{ATD} cells was used to generate single-stranded cDNA using the SuperScript III cDNA Synthesis Kit (Invitrogen). TD and IFE^{ATD} cDNA samples were subjected to PCR analysis (Jensen et al., 2008) (see Table S1 in the supplementary material).

RESULTS AND DISCUSSION

Expression of Tbc1d10c and CD200 marks the cutaneous TD compartment

To further characterize TD cells we conducted immunofluorescence labeling for Tbc1d10c, which we previously found to label epithelial progenitor cells residing in the upper isthmus of hair follicles (HFs) (Jensen et al., 2008), and for the immunosuppressive surface protein CD200 (Hoek et al., 2000). Immunolabeling for Tbc1d10c was conducted in conjunction with cytokeratin 8 (K8) as a marker for IFE Merkel cells (Moll et al., 1996a; Johnson et al., 2000; Johnson, 2001; Halata et al., 2003).

As expected, K8⁺ Merkel cells were detected in TDs and were innervated with NF-H⁺ (Nefh – Mouse Genome Informatics) sensory afferents (Fig. 1A). Similar to our previous findings (Jensen et al., 2008), Tbc1d10c was not detected in basal keratinocytes in the majority of the IFE. However, we detected Tbc1d10c expression in columnar keratinocytes confined to the TD (Fig. 1B–D). The expression of Tbc1d10c was restricted to epithelial keratinocytes within the boundary of underlying Merkel cells, and therefore to TD keratinocytes. Tbc1d10c expression was continuous in the granular layer of the IFE (Fig. 1B,C), suggesting that Tbc1d10c might play a role in late-stage IFE differentiation. CD200 also marked an identical region to Tbc1d10c in the TD, but was absent from the remainder of the IFE basal layer (Fig. 1E–G; see Fig. S1 in the supplementary material). CD200 was also highly expressed in the dermal Schwann cells (Fig. 1F,G) that support the fibers innervating Cav2.1-expressing (Cacna1a – Mouse Genome Informatics) Merkel cells (Piskowski et al., 2008) (Fig. 1E,G). Epidermal Cav2.1⁺ Merkel cells were also modestly labeled for CD200 (Fig. 1F,G). Collectively, these data identify Tbc1d10c and CD200 as bona fide TD markers and demonstrate a level of phenotypic discrimination for TD cells as compared with the remainder of the IFE.

To assess proliferation, we first analyzed skin sections for incorporation of the modified nucleoside EdU. EdU labeling was observed in Tbc1d10c⁺ TD keratinocytes in skin sections harvested 1 hour following a single EdU injection (see Table S2 in the supplementary material), indicating that basal TD keratinocytes are actively cycling, whereas no EdU labeling was observed in underlying mature Merkel cells or suprabasal keratinocytes within the TD (see Table S2 in the supplementary material). By 3–8 days following single EdU administration, EdU-labeled cells were observed in suprabasal TD keratinocytes but not in Merkel cells (see Table S2 in the supplementary material). At 24 hours following six EdU injections, EdU labeling was primarily observed in basal Tbc1d10c⁺ TD keratinocytes (Fig. 1H,I) and, to a lesser extent, in suprabasal TD keratinocytes, but not in mature Merkel cells (see Table S2 in the supplementary material). However, by 3–8 days following six EdU administrations, EdU labeling was still present in basal Tbc1d10c⁺ TD keratinocytes and had expanded into underlying Merkel cells as well as suprabasal differentiated keratinocytes (Fig. 1J,K; see Table S2 in the supplementary material), illustrating that basal Tbc1d10c⁺ TD cells contribute to Merkel and squamous lineages during homeostasis. Finally, no significant overlap was observed between the Tbc1d10c⁺ compartment and the cytokeratin K10⁺ differentiated compartment within the TD (Fig. 1L,M). These observations collectively indicate that the Tbc1d10c compartment in the TD is actively cycling and confirm that mature Merkel cells are post-mitotic.

Purification of TD keratinocytes by flow cytometry

To further characterize TD keratinocytes, we employed a cell-sorting strategy using $\alpha 6$ integrin, Sca1 (Ly6a – Mouse Genome Informatics) and CD34 surface markers, as previously described (Jensen et al., 2008), in conjunction with CD200. From the total population of epithelial cells, $\alpha 6$ integrin and CD34 surface levels were used to positively select for all ($\alpha 6^{\text{low}} + \alpha 6^{\text{high}}$) basal keratinocytes in the IFE and HFs (Fig. 1N) (Jensen et al., 2008). Within the $\alpha 6^+$ population, Sca1 and CD34 surface levels were then used to positively isolate the entire proliferative layer of the IFE, including TD keratinocytes, and exclude all regions of the HF except the infundibulum (Fig. 1O) (Jensen et al., 2008). The CD34⁺ population was used as a benchmark for gating Sca1⁻

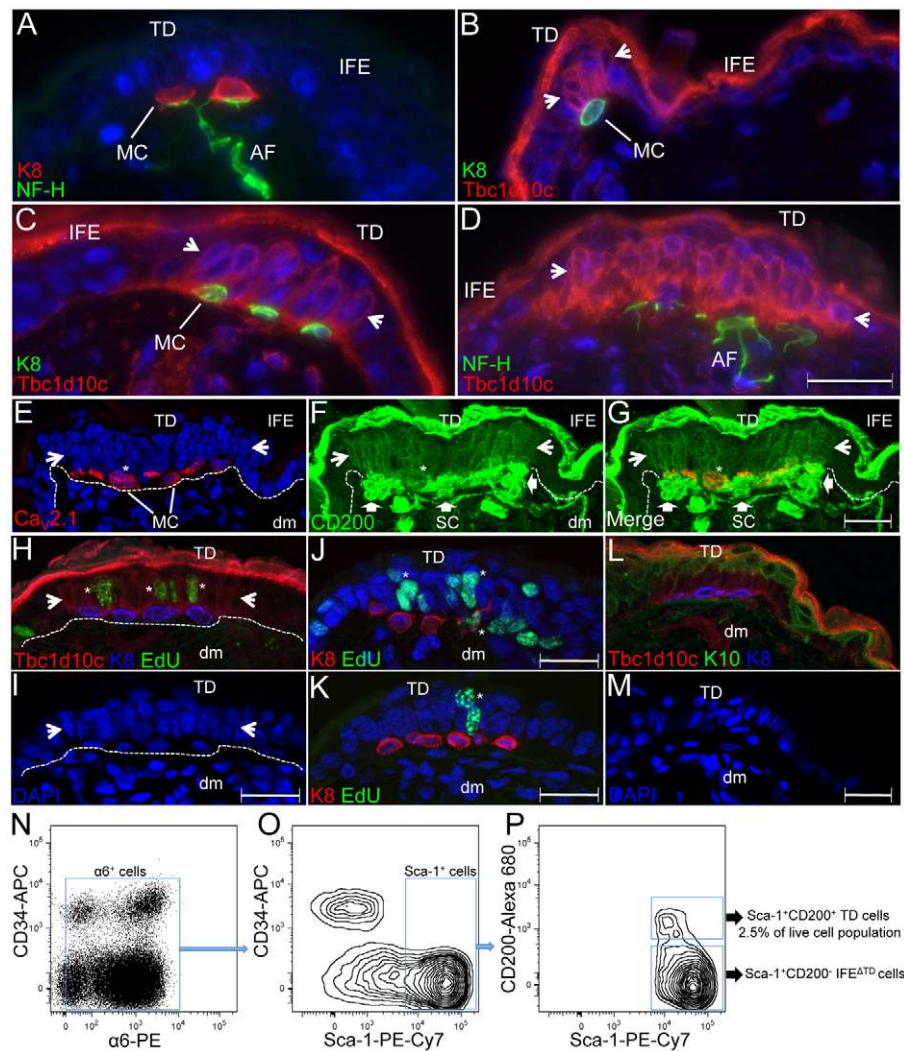


Fig. 1. Immunofluorescence labeling and FACS detection of touch domes (TDs). (A-M) K8 (A-C,J-L), NF-H (A,D), Tbc1d10c (B-D,H,I,L), Ca ν 2.1 (E,G), CD200 (F,G), EdU (H,J,K) and K10 (L) fluorescence labeling in mouse skin sections. Asterisks designate EdU⁺ basal columnar (H) and suprabasal (J,K) keratinocytes and Merkel cells (J) or Ca ν 2.1⁺ CD200⁺ Merkel cells (E-G). Line arrowheads designate TD borders (B-I) and block arrowheads designate Schwann cells (F,G). Dashed line designates the epidermal-dermal junction. E-G, H,I and L,M represent a series of the same image. AF, afferent fiber; dm, dermis; IFE, interfollicular epidermis; MC, Merkel cell; SC, Schwann cell. Scale bars: 60 μ m in A-D; 20 μ m in E-M. (N-P) FACS dot (N) and contour (O,P) plots showing detection of α 6 integrin, CD34, Sca1 and CD200 in epithelial keratinocytes.

from Sca1⁺ cells because Sca1 is not expressed in any region of the HF lower than the infundibulum (Jensen et al., 2008). Finally, the Sca1⁺ pool was gated based on CD200 levels into Sca1⁺ CD200⁺ TD and Sca1⁺ CD200⁻ IFE^{ATD} (IFE minus TD) populations (Fig. 1P; see Fig. S1 in the supplementary material). Flow cytometry-based quantification of these populations revealed that α 6⁺ Sca1⁺ CD200⁺ TD cells represent ~2.5% of the total live epithelial cell population in hairy skin (Fig. 1P). Almost 100% of sorted TD cells were K14⁺ (see Fig. S2 in the supplementary material) and this population also contained EdU⁺ proliferating keratinocytes (see Fig. S1 in the supplementary material).

Epidermal TDs harbor bipotent progenitor cells

To further assess the capacity of TD cells to give rise to long-term epidermal lineages, keratinocytes were harvested as previously described (Jensen et al., 2008) from *K14Cre;R26R^{lacZ}* reporter mice, FACS sorted into TD and IFE^{ATD} populations and implanted onto the dorsal fascia of recipient Nude mice (Jensen et al., 2008). Skin grafts were recorded and harvested at 2, 4 and 6 weeks post-grafting to ensure long-term reconstitution of skin lineages and exclude any contribution from differentiated cell populations. Consistent with previous grafting studies using Sca1⁺ cells (Jensen et al., 2008), skin grafts derived from TD and IFE^{ATD} cells did not

develop hair as determined by gross inspection (Fig. 2A-C), but were able to reconstitute IFE by 2 weeks post-grafting as visualized by X-gal staining (Fig. 2D-F).

The presence of β -gal-expressing cells in the skin grafts was utilized to trace *K14Cre;R26R^{lacZ}*-derived cells. β -gal was present in both TD (Fig. 2G-R) and IFE^{ATD} (see Fig. S2 in the supplementary material) grafts at 4 and 6 weeks post-implantation (data not shown), indicating that both populations contain long-term epidermal progenitor cells. However, β -gal⁺ K8⁺ Merkel cells were only present in TD grafts (Fig. 2G-J) and not in IFE^{ATD} or dermal fibroblast (DF) control grafts (see Fig. S2 in the supplementary material). By 4 weeks post-implantation, K8⁺ Merkel cells were positioned at the bottom of the IFE within reconstituted TDs and were highly innervated by NF-H⁺ afferent fibers (Fig. 2S-V), indicating that Merkel cells derived from *K14Cre;R26R^{lacZ}* TD cells are likely to be functional mechanoreceptors. Since Merkel cells are also CD200⁺ (Fig. 1E-G), we cannot rule out the possibility of contaminating Merkel cells in the FACS-sorted TD population. However, over 99% of FACS-sorted TD cells were found to be K14⁺ (see Fig. S2 in the supplementary material), indicating that little to no Merkel cell contamination is present in the TD population. The absence of Merkel cells in TD grafts harvested at 2 weeks (Fig. 2W) confirmed that post-mitotic Merkel cells

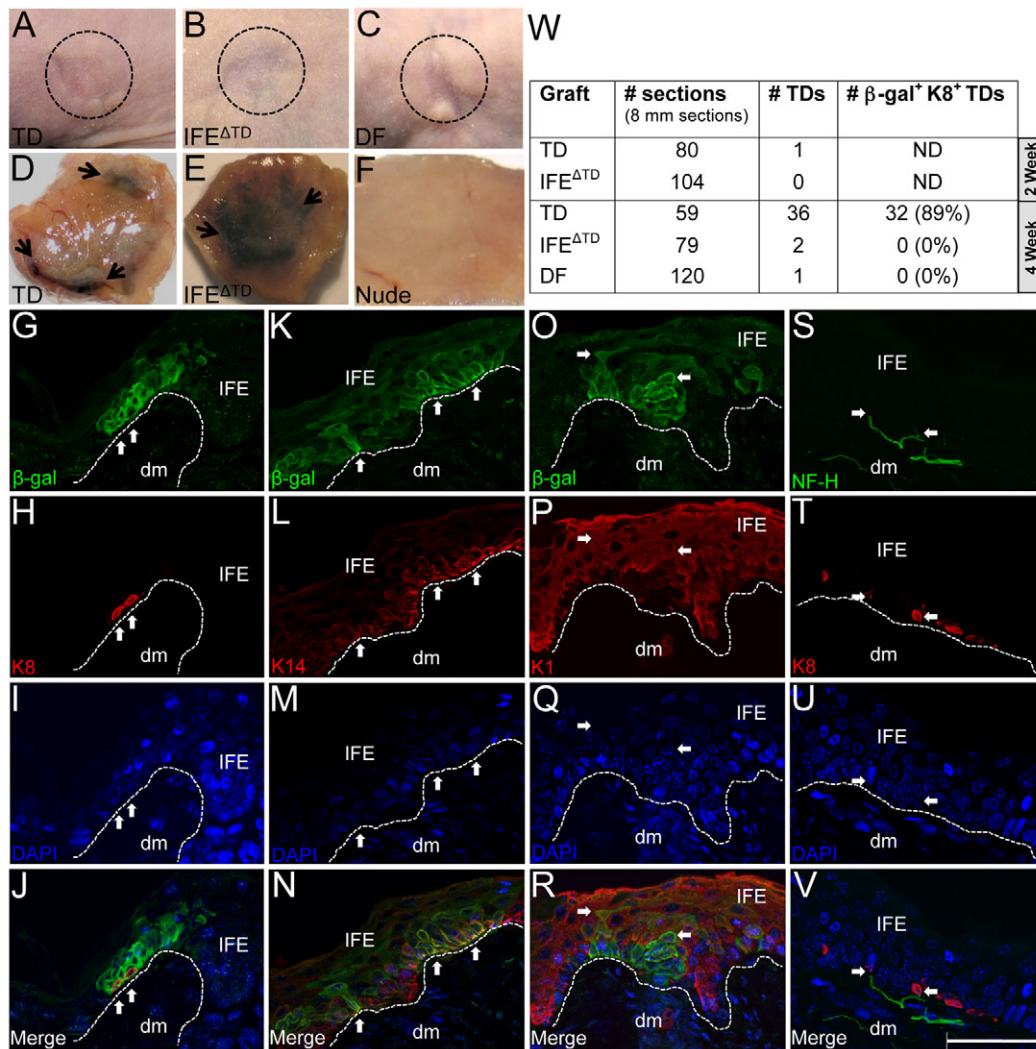


Fig. 2. Skin grafts and lineage analysis of TD progenitor cells (TDPCs). (A-F) Mouse skin grafts captured 6 weeks post-grafting (A-C) and X-gal-stained (arrowheads) 2-week grafts (D-F). Circle designates graft perimeter. There was no X-gal staining in Nude skin (F). (G-V) Four-week TD grafts. Arrows point to β-gal⁺ K8⁺ Merkel cells (G-J); β-gal⁺ K14⁺ basal (K-N) and β-gal⁺ K1⁺ suprabasal (O-R) keratinocytes; and NF-H⁺ afferents innervating K8⁺ Merkel cells (S-V). Each column represents the same image; merged panels demonstrate colabeled cells for each staining combination (J,N,R) or NF-H⁺ afferent fibers (V). The dashed line designates the epidermal-dermal junction. Scale bar: 50 μm. (W) Quantification of TDs (#TDs) in 2-week (upper) and 4-week (lower) grafts (average section length was 8 mm) and the number (with percentage) of TDs that co-expressed β-gal and K8 in each group. DF, dermal fibroblast control; dm, dermis; IFE, interfollicular epidermis; ND, not detected.

do not persist in the grafts. In grafts harvested at 4 weeks, Merkel cell-containing TDs were frequently detected in TD skin grafts and 89% of these TDs co-expressed β-gal and K8 (Fig. 2G-J,W). By contrast, very few reconstituted TDs were observed in IFE^{ΔTD} grafts, none of which expressed β-gal, indicating that they were derived from host Nude skin (Fig. 2W; see Fig. S2 in the supplementary material). In addition, β-gal⁺ epidermal cells colabeled with K14⁺ (proliferative) (Fig. 2K-N) and K1⁺ (differentiated) (Fig. 2O-R) keratinocyte markers throughout the IFE of TD skin grafts. Collectively, these results show that FACS-sorted TD cells possess bipotent progenitor capacity by their ability to give rise to permanent Merkel and squamous epidermal lineages. Moreover, these TD progenitor cells (TDPCs) appear to be uniquely qualified to give rise to the Merkel lineage.

Importantly, the absence of reconstituted follicular or sebaceous lineages shows that the commitment to epidermal lineages is strictly maintained in TD and IFE^{ΔTD} cells, even under regenerative conditions. This restricted lineage commitment to the IFE is consistent with the contribution of epidermal stem/progenitor cells during homeostasis (Fig. 1). Therefore, these observations pertaining to skin regeneration might be highly reflective of the progenitor capacity of TD and IFE^{ΔTD} populations during homeostasis.

Microarray analysis confirms a distinct transcriptome in TDPCs

We conducted microarray RNA expression analysis in order to confirm that FACS-sorted TD cells are localized to the TD region of the IFE and to determine whether the TD keratinocyte-derived transcript profile was distinct from that of IFE^{ΔTD} cells. Total RNA was isolated from FACS-sorted TD and IFE^{ΔTD} populations and subjected to microarray expression analysis. The expression of 1572 genes was altered in TD cells compared with IFE^{ΔTD} cells (Fig. 3), indicating that TD cells were phenotypically distinct within the total IFE pool. Out of 709 upregulated genes, the two largest clusters comprised genes functioning in cell communication (36%) and neurological processes (15%) as determined by ClueGo analysis (Fig. 3C). Only 10% of upregulated genes, including *Cd200*, are enriched in purified Merkel cells (Haerberle et al., 2004), suggesting that the expression profile of mature Merkel cells is distinct from that of their epidermal progenitors.

To validate the microarray data, the RNA levels of three upregulated genes, *Ctgf*, *Foxc1* and *Fzd7* (Fig. 3A), were analyzed by RT-PCR analysis. Positive expression for all three genes was detected by PCR in TD cells but not in IFE^{ΔTD} cells (Fig. 4A), indicating that these transcripts might be unique to TD cells. Equal levels of cytokeratin K14 expression were detected between TD

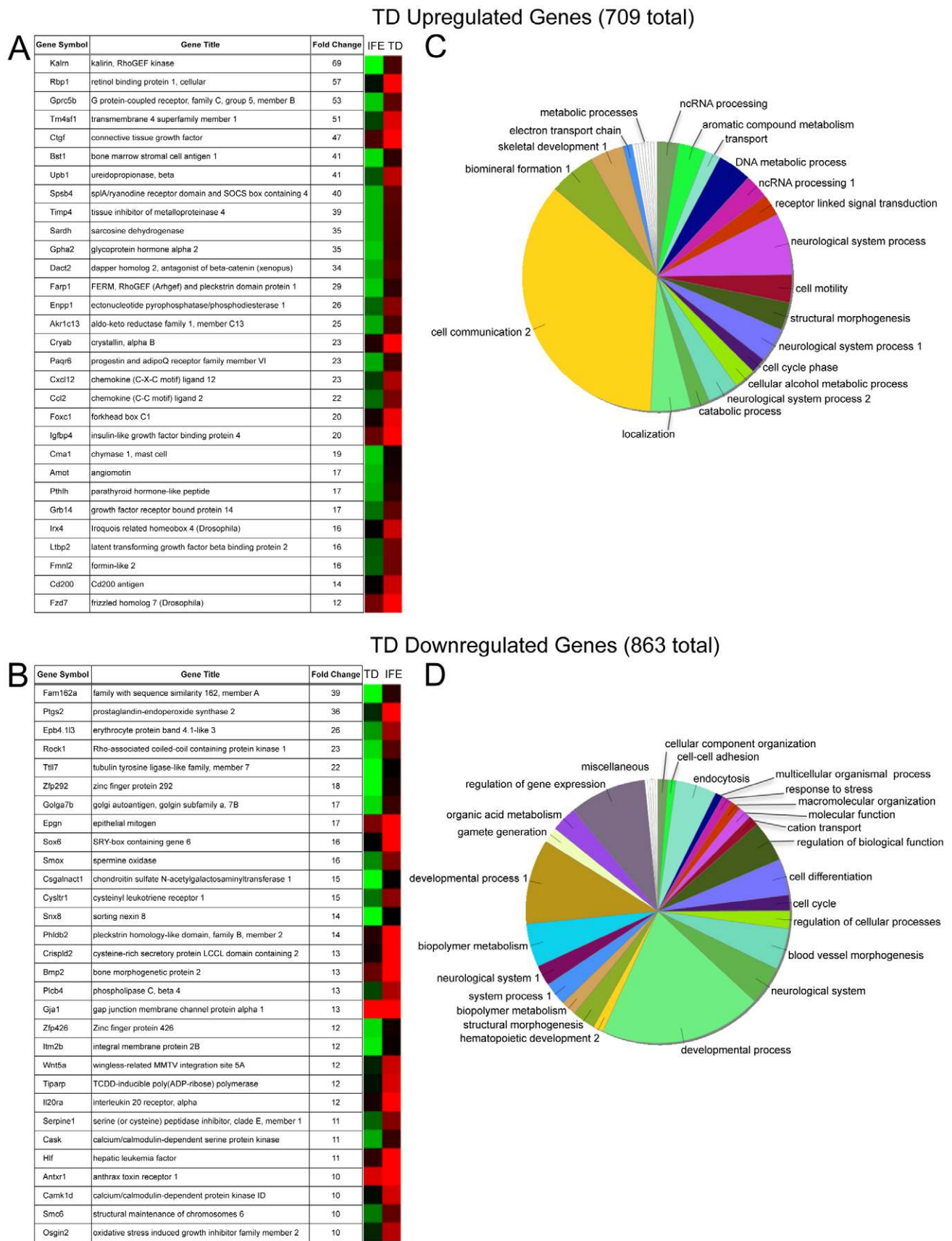


Fig. 3. Microarray RNA expression analysis of TDPCs. (A,B) Top 30 upregulated (A) and downregulated (B) transcripts in FACS-sorted mouse TD cells compared with IFE^{TD} cells, ranked by fold change. Each list is accompanied by a heat map expression analysis. **(C,D)** Pie charts depicting gene expression cluster overviews for TD upregulated (C) and downregulated (D) genes.

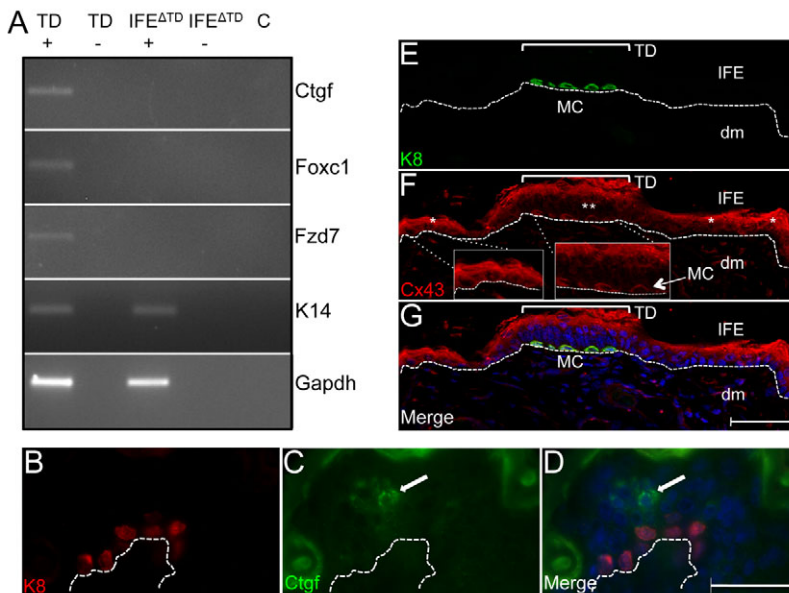


Fig. 4. RT-PCR and immunofluorescence analysis of TD regulated genes. (A) RT-PCR analysis of the TD enriched genes *Ctgf*, *Foxc1* and *Fzd7* with (+) or without (-) reverse transcriptase. *Gapdh*, *K14* and water (C) were included as controls. (B-G) *Ctgf* (arrow) and K8 colabeling (B-D) or connexin 43 and K8 colabeling (E-G) in mouse skin. The dashed line designates the basement membrane. Asterisks designate connexin 43 labeling in the IFE (*) and TD (**). Insets (F) are at 1.5× magnification. dm, dermis; IFE, interfollicular epidermis; MC, Merkel cell. Scale bars: 20 μm in B-D; 40 μm in E-G.

and IFE^{ATD} cells, confirming the presence of basal keratinocytes in the FACS-sorted TD population (Fig. 4A). Expression of the upregulated gene *Ctgf* was localized to TD keratinocytes residing directly above Merkel cells (Fig. 4B-D), whereas expression of the downregulated gene *Gjal* (connexin 43) was largely absent from TD keratinocytes as compared with the remainder of the IFE (Fig. 4E-G). However, connexin 43 expression was detected in epidermal Merkel cells (Fig. 4E-G). Collectively, these results indicate a TD residence for FACS-sorted α6⁺ Sca1⁺ CD200⁺ cells.

Our results provide evidence for a progenitor niche within epidermal TDs that give rise to Merkel and squamous lineages under homeostatic and regenerative conditions and suggest that this population might also be a site of origin for disease states such as Merkel cell carcinoma (MCC). However, unlike in mouse, a rare population of Merkel cells is found in human epidermis outside of TDs and hair follicles and is increased in number in response to sun exposure (Moll et al., 1990), suggesting that this unique Merkel cell population in human epidermis might be the origin of MCC. Although the etiology of MCC is unknown, several studies have implicated a role for Merkel cell polyomavirus (MCPyV) infection as a causal event (Feng et al., 2008; Katano et al., 2009; Andres et al., 2010). One intriguing possibility is that an abundance of CD200 provides immune privilege not only to normal TDPCs but also to MCPyV-transformed TDPCs, thereby contributing to MCC pathogenesis. However, any potential role for TDPCs or CD200 in MCC pathogenesis remains purely speculative.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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