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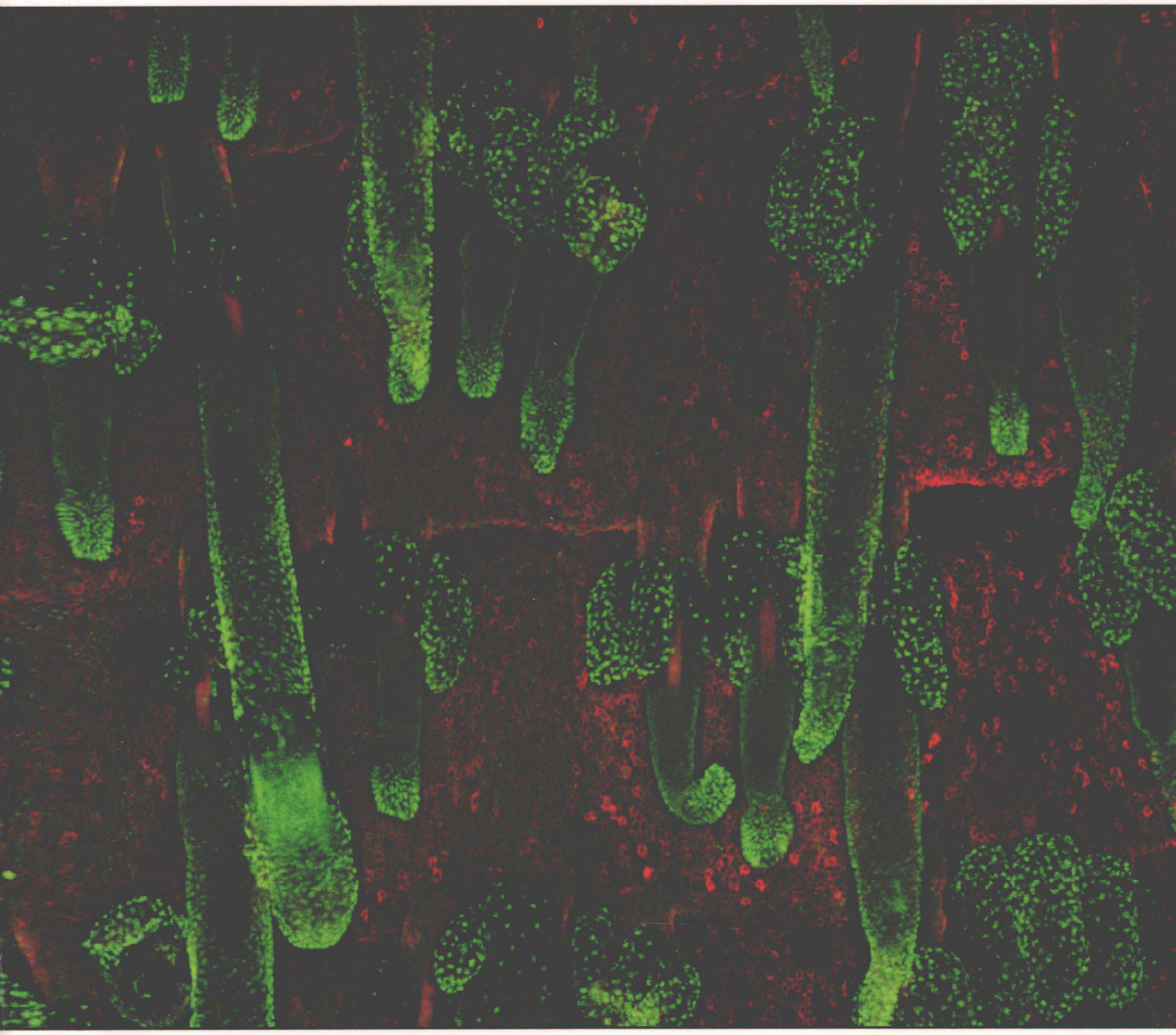
The Skin and Its Diseases



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The Skin and Its Diseases

A subject collection from *Cold Spring Harbor Perspectives in Medicine*

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Preface

The Italian philologist and physician Geronimo Mercuriali published the first textbook on skin and its diseases in 1572. Today, however, we are at a nexus in modern biology where we possess unparalleled understanding of the molecular and genetic basis of skin homeostasis and worldwide access to patients and animal models with which to correlate our knowledge and develop therapies for human diseases. This nexus means that laboratory experiments are having a greater direct impact on clinical practice than ever before. Our motivation for publishing *The Skin and Its Diseases* is our belief that the skin is the quintessential model vertebrate tissue. We feel that this compendium of current knowledge is useful for both further studies of the skin itself and insights into related changes in other tissues. Our hope is that this book will catalyze such interactions and stimulate further research in basic science and clinical/translational medicine.

We are well aware that this research topic is enormous and have endeavored to assemble a representative slice of our current knowledge. Although it does not do justice to some emerging and well-developed areas and lacks contributions from certain leaders in the field, the diversity of knowledge in this compendium will whet your appetite for exploring deeper.

The chapters in the book are organized around the major cell types in the skin and the diseases that affect them, including epidermis, dermis, and cutaneous epithelial specializations such as the touch receptor. The topics range from traditional dermatologic strongholds, such as psoriasis and cutaneous oncology, to newer approaches, such as the use of embryonic-stem-cell-based therapies. The content in each chapter may not completely align with that in other chapters. We have purposely given freedom to each author to review the field as they see fit. We feel that the apparent areas of conflict provide motivation for additional experimentation.

We thank Barbara Acosta and her colleagues at Cold Spring Harbor Laboratory Press for their support. Barbara's expertise in helping us put the book together and her patience with the inevitable delays are greatly appreciated. We thank our families for putting up with us as we assembled this reference. We would like to especially thank past and current members of the cutaneous biology community for their conversations, critiques, and insights that have spurred us to investigation. We feel fortunate to have such wonderful colleagues and hope that this book will motivate readers to contribute to new understanding of the skin and its diseases.

Anthony E. Oro
Fiona M. Watt

Contents

Markers of Epidermal Stem Cell Subpopulations in Adult Mammalian Skin

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The epidermis is the outermost layer of mammalian skin and comprises a multilayered epithelium, the interfollicular epidermis, with associated hair follicles, sebaceous glands, and eccrine sweat glands. As in other epithelia, adult stem cells within the epidermis maintain tissue homeostasis and contribute to repair of tissue damage. The bulge of hair follicles, where DNA-label-retaining cells reside, was traditionally regarded as the sole epidermal stem cell compartment. However, in recent years multiple stem cell populations have been identified. In this review, we discuss the different stem cell compartments of adult murine and human epidermis, the markers that they express, and the assays that are used to characterize epidermal stem cell properties.

Mammalian skin comprises of two distinct layers—the epidermis and the underlying dermis (Fig. 1). As the skin's outer layer, the epidermis provides the barrier function protecting mammals from environmental influences such as physical, chemical, or thermal stress, and also against dehydration (Proksch et al. 2008; Fuchs 2009). The epidermis is a multilayered epithelium consisting of the interfollicular epidermis (IFE) and associated hair follicles (HFs), sebaceous glands (SGs), and eccrine sweat glands. Keratinocytes are the main epidermal cell type. Several other cell types, such as Merkel cells, melanocytes, and Langerhans cells, are also found in mammalian epidermis. Merkel cells are neuroendocrine cells that lie in so-called touch domes within the IFE and are responsible for the touch sensory function of the skin (Van Keymeulen et al. 2009; Woo et al. 2010). Melanocytes are specialized pigment cells that produce melanin granules, which are taken up by keratinocytes and protect against sunlight-induced DNA damage (Rabbani et al. 2011; Chang et al. 2013). Langerhans cells, which are epidermal dendritic cells, are part of the adaptive immune response and, hence, a critical element of the skin barrier (Romani et al. 2010).

A basement membrane separates the epidermis from the underlying collagen-rich dermis (Watt and Fujiwara 2011). The dermis plays an important role in epidermal development (Alonso and Fuchs 2006; Blanpain and Fuchs

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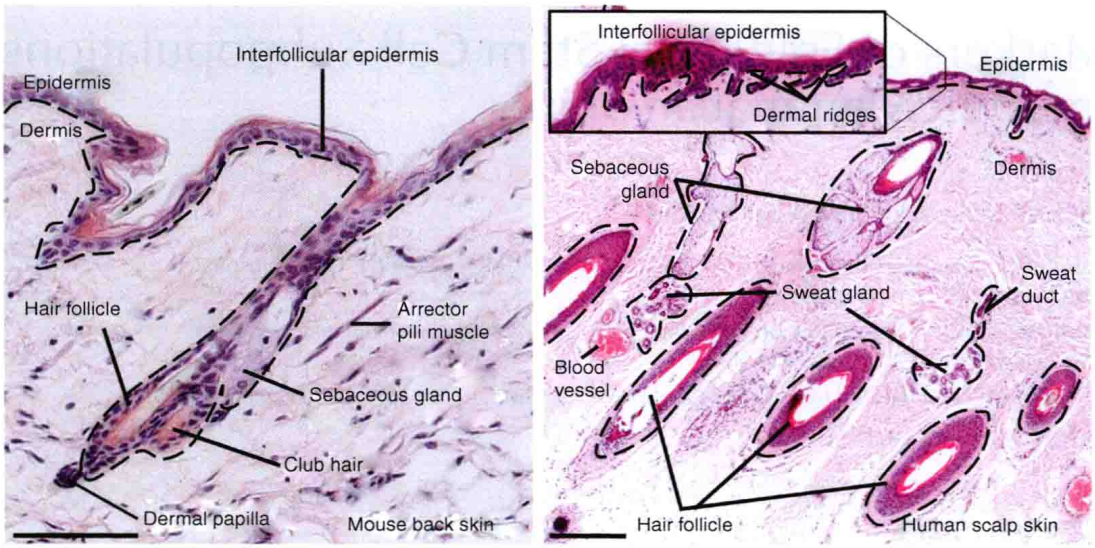

Figure 1. Histology of mammalian skin. Adult mouse (*left*) and human skin (*right*) stained with hematoxylin. Note the absence of eccrine sweat glands in mouse back skin. Dashed lines indicate position of the basement membrane. Scale bars, 100 μm (mouse skin) and 500 μm (human skin).

2009), as epidermal–dermal interactions are, for example, critical for HF formation during embryogenesis (Messenger 1993; Botchkarev and Kishimoto 2003). Each region of the dermis contains fibroblasts, some of which have specialized functions, for example in the dermal papilla (DP). DP cells stimulate epidermal cells to grow downward and form the HF (Alonso and Fuchs 2006; Driskell et al. 2011). The DP remains an integral part of the HF base throughout the hair cycle (Messenger 1993; Botchkarev and Kishimoto 2003; Alonso and Fuchs 2006). Several other cell types, such as nerves, lymphatic cells, endothelial cells, as well as different types of bone-marrow-derived immune cells (e.g., macrophages, mast cells, T, and B cells), are also present in the dermis (Kalluri and Zeisberg 2006; Arwert et al. 2012). The arrector pili muscle is a smooth muscle resident in the dermis connecting the HF with the IFE and is responsible for pilo-erection ("goosebumps") to prevent heat loss (Fujiwara et al. 2011). The subcutaneous fat layer is formed by intradermal adipocytes (Schmidt and Horsley 2012). Skin adipocytes play a role in the regulation of HF cycling (Festa et al. 2011).

Because the differentiated cells of the epidermis are dead, frequently anucleate cells, epidermal maintenance depends on proliferation of stem cells, which are cells with an extensive self-renewal capacity and the ability to produce daughter cells that undergo further differentiation. Stem cells have been identified in human non-hair-bearing skin (Barrandon and Green 1987; Jones and Watt 1993). However, historically, in the mouse, epidermal maintenance was attributed to a single population of epidermal stem cells residing in a compartment of the lower HF known as the bulge (Cotsarelis 2006; Blanpain and Fuchs 2009), where the arrector pili muscle contacts the HF basement membrane (Fujiwara et al. 2011). This view has changed over the last decade as evidence of stem cell pools outside the bulge has accumulated. In this review, we will focus on the different epidermal stem cell subpopulations identified in human and murine skin.

TOOLS TO STUDY EPIDERMAL STEM CELLS

Different assays have been developed to study adult epidermal stem cells. As these tools have recently been extensively reviewed (Fuchs and

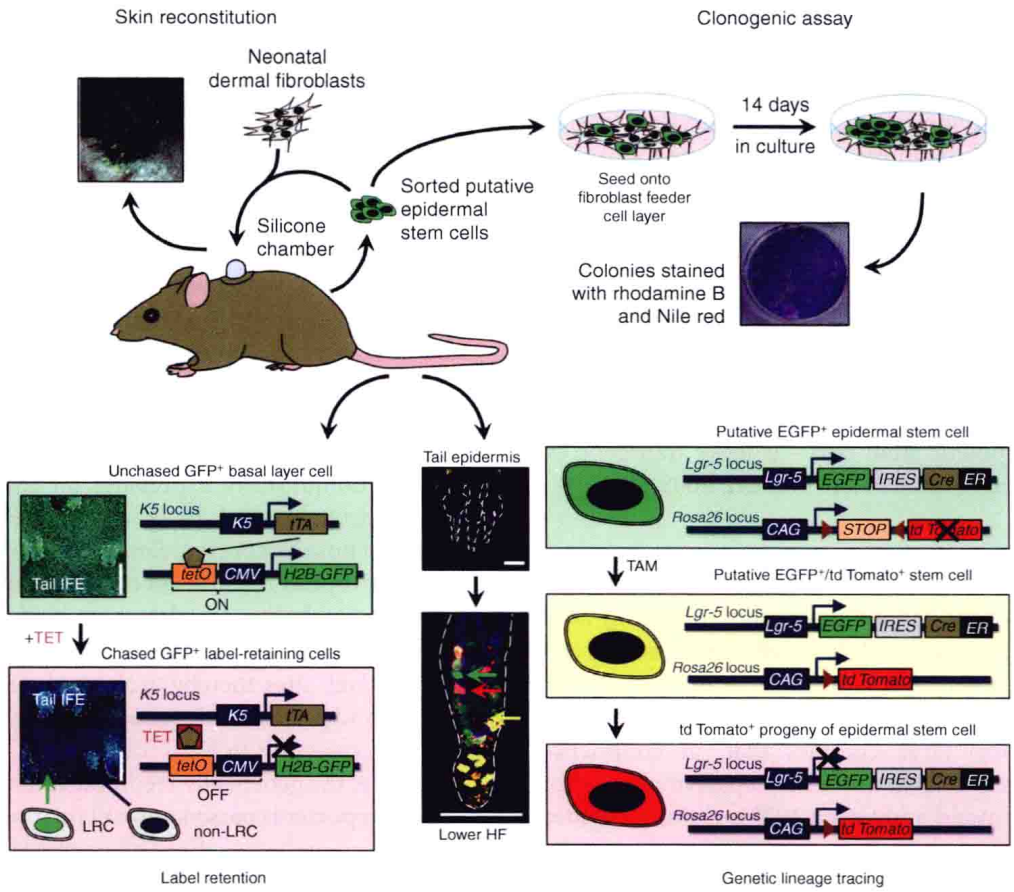

Figure 2. Strategies to study epidermal stem cells and their markers. Disaggregated epidermal cells can be either mixed with neonatal murine dermal fibroblasts and grafted onto immunocompromised mice to study their skin reconstitution potential in vivo or they can be seeded onto a feeder cell layer to study their clonogenic potential in culture. Slowly cycling cells in vivo can be identified through DNA label retention, either by injecting nucleotide labels such as 5-bromo-2-deoxyuridine (BrdU) or by using genetic approaches such as the tetracycline-regulated H2B-GFP system. (Images based on data from Mascre et al. 2012; reproduced, with permission, from C. Blanpain and *Nature* © 2012, Macmillan.) Genetic lineage tracing enables fate mapping of epidermal stem cells and their progeny during tissue homeostasis. CAG, chicken β-actin promoter with CMV enhancer; CMV, cytomegalovirus promoter; EGFP, enhanced GFP; ER, tamoxifen-inducible mutated estrogen receptor; GFP, green fluorescent protein; HF, hair follicle; H2B, histone H2B; IFE, interfollicular epidermis; IRES, internal ribosome entry site; K, keratin; LRC, label-retaining cell; TAM, tamoxifen; TET, tetracycline; tetO, tetracycline operator; tTA, tetracycline transactivator. Scale bars, 100 μm.

Horsley 2011; Snippert and Clevers 2011; Kretzschmar and Watt 2012), we will only give an overview of the four major techniques used in epidermal stem cell research (Fig. 2).

Label Retention

A dogma established by pioneers of hemopoietic stem cell research is that adult stem cells are infrequently dividing (slowly cycling), quiescent cells, which therefore retain radioactively labeled nucleotides, such as tritiated thymidine or 5-bromo-2-deoxyuridine (BrdU) (Till and McCulloch 1961). In skin, such cells—so-called label-retaining cells (LRCs)—were identified in the HF bulge by DNA-label pulse-chase experiments (Cotsarelis et al. 1990; Braun et al. 2003). A major drawback of this technique is that certain types of postmitotic terminally differentiated cells efficiently retain DNA labels and re-

main in the tissue for a long period of time (Snippert and Clevers 2011; Steinhauser et al. 2012). To enable isolation of live LRCs using flow cytometry, Fuchs and colleagues elegantly adapted the pulse-chase technique to visualize LRCs using green fluorescent protein tagged histone (H2BGFP), which is tetracycline-dependent and expressed in a tissue-specific manner (Tumbar et al. 2004). This approach has now been used extensively and has generated important insights into epidermal LRC, although one potential caveat—found when using the H2BGFP transgenic mouse model to isolate hematopoietic stem cells—is leaky transgene expression (Challen and Goodell 2008).

Clonogenic Assays

One of the earliest approaches to identify adult human epidermal stem cells was to isolate cells from tissue and culture them in vitro. A subpopulation of the cells that attached proliferated to form large colonies that, at confluence, merged to form a stratified epidermal cell sheet (Rheinwald and Green 1975). Cultured epidermal sheets have been used extensively as autografts to treat burns victims, establishing that stem cells survive in culture (Green 2008). Formation of self-renewing clones has been used as an in vitro readout of stem cells, first in human epidermis and subsequently in mice (Barrandon and Green 1987; Jones and Watt 1993; Morris and Potten 1994).

Skin Reconstitution

Adult stem cells are not only defined by their capacity to self-renew, but also by their potential to produce all types of differentiated cells within their tissue (multipotency). This can be assessed by performing skin reconstitution assays in which repair of a skin wound or reconstitution from disaggregated cell populations is evaluated (Jensen et al. 2010).

Genetic Lineage Tracing

The first description of genetically modified mice laid the foundation for many powerful approaches in stem cell biology (Jaenisch and Mintz 1974). Lineage tracing using the Cre-*loxP* system (Hoess and Abremski 1984; Kretzschmar and Watt 2012) enables genetic labeling of stem cells and their progeny in intact, undamaged tissue using fluorescent and other reporters (Zinyk et al. 1998).

The first report of lineage tracing in mouse skin involved a transgenic mouse harboring Cre recombinase fused to a tamoxifen-inducible mutated estrogen receptor expressed under the control of the epidermal basal layer-specific *keratin 14* promoter (K14CreER^{t}) (Vasioukhin et al. 1999). This mouse line was crossed with a mouse ubiquitously expressing an inactive *LacZ* reporter flanked by a *loxP-STOP-loxP* sequence (Rosa26reporter, *Rosa26R*) (Soriano 1999). On topical application of tamoxifen (or its active metabolite 4-hydroxy-tamoxifen, 4-OHT) to double transgenic mice, Cre recombines the *loxP* sites thereby excising the STOP sequence and permanently (genetically) activating *LacZ* expression in K14^{+} cells. When these cells divide, the genetically recombined and active *LacZ* reporter is passed onto all their progeny. The label can be visualized by assaying for β-galactosidase (β-gal), and, thus Vasioukhin et al. (1999) were able to show that the K14^{+} basal layer of murine epidermis contained β-gal^{+} stem cells that give rise to β-gal^{+} differentiating suprabasal cells. Subsequently, mice expressing fluorescent reporters, such as enhanced green fluorescent protein (*Rosa26EGFP*) (Mao et al. 2001), enhanced yellow fluorescent protein (*Rosa26EYFP*), enhanced cyan fluorescent protein (*Rosa26ECFP*) (Srinivas et al. 2001), tdTomato (*R26tdTomato*) (Madisen et al. 2010), and multicolor confetti (*Rosa26Confetti*) (Snippert et al. 2010b), as well as bicistronic knock-in mice carrying CreER^{T2} combined with a fluorescent marker (e.g., EGFP) (Barker et al. 2007) have been introduced. These have led to even more sophisticated approaches to fate map epidermal stem cells. However, it is important to carefully assess each mouse model used for lineage tracing to exclude possible compartmental or spatiotemporal misexpression of the promoter driving Cre recombinase (Snippert and Clevers 2011; Kretzschmar and Watt 2012).

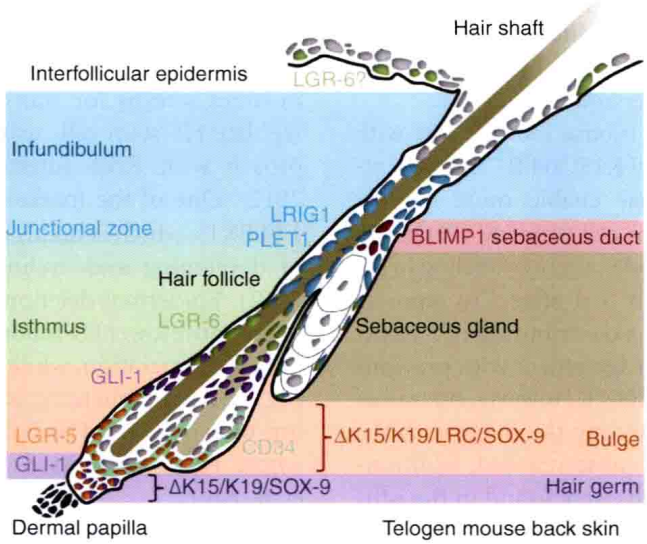

Figure 3. Markers of epidermal stem cell subpopulations in murine adult skin. Schematic of epidermal stem cell pools in murine telogen (hair follicle resting phase) back skin.

Studies have used a combination of these techniques to define the location and features of adult epidermal stem cells (Fig. 3).

STEM CELLS OF THE HAIR FOLLICLE BULGE AND GERM

In murine skin, bulge stem cells in the lower HF were initially identified through DNA or histone label-retention studies (Cotsarelis et al. 1990; Braun et al. 2003; Tumbar et al. 2004). Slowly cycling and therefore label-retaining stem cells are almost entirely localized to the bulge region and are only rarely found elsewhere within the epidermal basal layer (Braun et al. 2003). This might reflect the fact that the lower HF compartment is not continuously regenerated, but subject to cycles of hair growth, regression, and rest. In contrast, actively cycling stem cells in the permanent portion of the epidermis (comprising the upper HF, SG, and IFE) are required to ensure a continuous supply of differentiated progeny (Watt and Jensen 2009).

Over the last decade, several groups have identified markers of the HF bulge stem cell niche in mouse and human skin. Cluster of differentiation 34 (CD34) and keratin 15 (K15) are the most widely used markers of murine bulge stem cells. $CD34^+$ bulge cells are infrequently dividing and label retaining as well as able to self-renew in culture to form colonies (Trempus et al. 2003). Significant overlap is found between expression of CD34 and K15 (Lyle et al. 1998), which is expressed at low levels throughout the epidermal basal layer and enriched in the bulge (Troy et al. 2011; Xiao et al. 2013). There are two distinct layers of $CD34^+$ bulge stem cells (Blanpain et al. 2004), one of which expresses high levels of α6 integrin and is attached to the basement membrane, whereas the other, which appears only after the first hair cycle, is suprabasal and expresses low levels of α6 integrin (Blanpain et al. 2004).

A truncated version of the human *K15* promoter (*K15*) has been used to specifically target the bulge stem cell population (Morris et al. 2004). Lineage tracing showed that *K15* targeted cells contribute to all epidermal lineages during normal HF cycling. By expressing EGFP under the control of the *K15* promoter, it was shown that $EGFP^+/\alpha6$ $integrin^+$ keratinocytes are slowly cycling in vivo and have high proliferative potential in culture (Morris et al. 2004). Also, in skin reconstitution assays sorted K15EGFP expressing cells were able to generate all epidermal compartments, suggesting that this cell popula-

tion is indeed multipotent, notwithstanding the caveat that founder line integration site and copy number can lead to nonbulge expression of this promoter (Petersson et al. 2011).

A new transgenic mouse founder line with low expression levels of K15CreER^{T2} was recently created, which may enable more specific tracking of bulge stem cell progeny (Petersson et al. 2011). In this model, there is labeling of SG and upper HF within 5 d after Cre reporter activation, suggesting a contribution of *K15* expressing bulge cells, in agreement with previous studies (Morris et al. 2004). However, no trail of labeled progeny connecting the bulge with the upper HF and SG was observed and, additionally, Cre expressing cells were found in the isthmus region of the upper HF (Petersson et al. 2011), preventing firm conclusions to be drawn about the contribution of bulge stem cells to the normal homeostasis of the upper HF and SG. Studies using *K19CreER*^{T} mice to target the bulge have failed to show labeling of the upper HF and SG (Morris et al. 2004; Youssef et al. 2010; Lapouge et al. 2011).

SRY (sex determining region Y)-box 9 (SOX-9) is another marker of the bulge stem cell compartment (Vidal et al. 2005; Nowak et al. 2008). SOX-9 is expressed in the developing hair placodes at embryonic day (E) 15.5 and marks slowly cycling and CD34^{+} HF stem cells that give rise to the entire pilosebaceous unit (Vidal et al. 2005; Nowak et al. 2008). Using two different transgenic mouse lines expressing epithelial-specific Cre (Y10Cre and K14Cre) transgenes, it has been shown that conditional deletion of *Sox-9* causes alopecia and loss of the SG (Vidal et al. 2005; Nowak et al. 2008). Mice with epidermal-specific loss of *Sox-9* lack postnatal expression of crucial bulge markers such as CD34 and K15 and show complete loss of label-retaining bulge cells and actively cycling matrix cells during hair morphogenesis in neonates. These data indicate that *Sox-9* is a functional stem cell marker that is indispensible for hair homeostasis (Vidal et al. 2005; Nowak et al. 2008).

Over the past decade, several other functional bulge stem cell markers have been described, such as transcription factor 3 (TCF-3) (Nguyen et al. 2006), LIM homeobox 2 (LHX2) (Rhee et al. 2006), and nuclear factor of activated T cells, cytoplasmic 1 (NFATC1) (Horsley et al. 2008). Recently Fuchs and colleagues have performed in utero screens for transcription factors that regulate HF stem cells using a knockdown approach with RNA interference (Chen et al. 2012). One of the markers identified is T-box 1 (TBX1), which is highly enriched in the bulge of developing and cycling HFs (Chen et al. 2012). Epidermal deletion of *Tbx1* causes loss of the entire lower HF following multiple rounds of hair regeneration, while the IFE, SG, and upper HF remain phenotypically normal, suggesting that only the HF bulge stem cell pool—where *Tbx1* is expressed—is exhausted (Chen et al. 2012).

Since its first description as a marker of stem cells in the crypt base of the murine small intestine, leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR-5) has been shown to be a marker of adult stem cells in numerous epithelial tissues (Barker et al. 2007, 2010; Huch et al. 2013). In skin, LGR-5 marks stem cells in the lower HF bulge and hair germ during telogen and in the lower outer root sheath during anagen (Fig. 4) (Jaks et al. 2008). During telogen, the hair germ is quiescent, like the bulge, but at the onset of anagen it is the first compartment of the lower HF to be triggered to proliferate (Greco et al. 2009; Rompolas et al. 2012). Toftgård and coworkers found that LGR-5^{+} stem cells are the first HF cells to proliferate upon Wnt-dependent induction of anagen and contribute to HF maintenance during adult homeostasis (Jaks et al. 2008). Interestingly, some LGR-5^{+} cells in the lower outer root sheath of anagen HFs are not lost during later catagen, but remain in the hair germ and contribute to HF growth during the next anagen, suggesting that these hair germ cells retain stem cell properties similar to conventional bulge stem cells (Jaks et al. 2008; Hsu et al. 2011). One model to explain these findings is that there is bicompartmentalization within the lower HF, whereby the HF bulge contains a pool of quiescent stem cells, which act as a reserve cell population and only become activated to replace the loss of rapidly cycling stem cell residing in the hair germ during periods of hair growth (Jaks

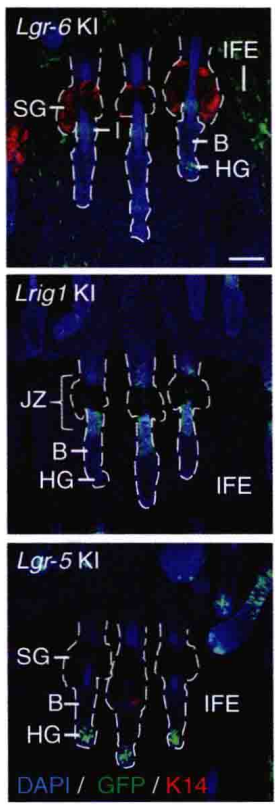

Figure 4. Genetically engineered mice carrying a bicistronic expression cassette containing a fluorescent reporter (such as green fluorescent protein) and a tamoxifen-inducible Cre recombinase under the control of an epidermal stem cell pool-specific promoter—such as the *Lgr-6*, *Lrig1*, and *Lgr-5* knock-in mice—enable sorting of the stem cells and lineage tracing of their progeny. Whole mounts of murine tail epidermis stained for GFP (stem cell marker), K14 (basal layer marker) and DAPI (nuclei marker) are shown. B, bulge; DAPI, 4′,6-diamidino-2-phenylindole; GFP, green fluorescent protein; HG, hair germ; IFE, interfollicular epidermis; JZ, junctional zone; K14, keratin 14; KI, knock-in; SG, sebaceous gland. Scale bars, 100 μm.

et al. 2008; Greco et al. 2009; Greco and Guo 2010; Zhang et al. 2010; Hsu et al. 2011).

Components of the Hedgehog signaling pathway, such as Sonic Hedgehog (SHH) and zinc finger protein GLI-1, are expressed in the hair bulge and germ (Levy et al. 2005; Brownell et al. 2011). Morgan and colleagues showed that SHH^+ stem cells are present in the hair placode during embryogenesis and contribute to the establishment of the hair bulge stem cell compartment (Levy et al. 2005). Lineage tracing using *ShhGFPCre* showed that SHH^+ cells in the lower HF do not give rise to IFE cells, suggesting compartmentalization of epidermal stem cells during homeostasis (Levy et al. 2005). In adult skin, SHH is highly expressed in the hair germ (HF matrix during anagen) and SHH expressing cells generate all HF layers except for the outer root sheath (Youssef et al. 2010; Lapouge et al. 2011). GLI-1^+ cells are found in the lower bulge and germ, as well as in the upper bulge (Brownell et al. 2011). Interestingly, stem cells expressing *Gli-1* not only gave rise to differentiated cells in all layers of the lower HF, but labeled progeny were also found in the isthmus and junctional zone adjacent to the SG (Brownell et al. 2011). Finally, robust contribution to IFE regeneration on wounding has shown that GLI-1^+ stem cells are multipotent (Brownell et al. 2011).

Characterizing HF stem cell markers in human skin has been challenging because of the lack of robust tools for lineage tracing (Rochat et al. 1994). Nevertheless, Vogel and coworkers determined the distribution of LRCs in human anagen HF by grafting human skin onto immunocompromised mice and performing BrdU labeling, thereby confirming the existence of LRC in the human HF bulge (Ohyama et al. 2006). The authors subsequently isolated cells from different HF regions by microdissection and performed microarray analysis. This led to the identification of a population of bulge cells with high colony-forming efficiency expressing the marker CD200, which is enriched in stem cells (Ohyama et al. 2006). Using flow cytometry sorting for different putative bulge stem cell markers, Inoue et al. (2009) were able to show that human bulge stem cells are enriched for both CD200 and K15, but negative for CD34 and CD271. Human bulge stem cells are the main focus of studies on hair regeneration defects such as androgenetic alopecia (AGA) (Paus and Cotsarelis 1999). A study by Cotsarelis and colleagues comparing the features of HF cell subpopulations in bald and nonbald scalp from AGA patients indicates heterogeneity within the HF stem cell compartment in human skin similar to mouse skin (Garza et al. 2011).

Bulge stem cells enriched for K15 were retained in balding skin, but HF progenitors in the germ expressing CD200 and CD34 were lost, contributing to the defect in hair regeneration (Garza et al. 2011).

STEM CELLS OF THE UPPER HAIR FOLLICLE AND SEBACEOUS GLANDS

Work by Ghazizadeh and Taichmann (2001) initially suggested that epidermal homeostasis in the mouse is mediated by multiple stem cells with restricted lineages. Fate mapping through transfection of murine keratinocytes with a *LacZ* expressing retrovirus provided in vivo evidence for the presence of long-lived progenitors in SG and IFE, as both showed β-gal labeling solely to their compartment, independent of labeling in the HF bulge.

Placenta-expressed transcript protein 1 (PLET1), the first specific marker of HF stem cells to be described outside the bulge, was identified through antibody labeling (Nijhof et al. 2006). Besides expressing α6 integrin and K14, PLET1$^+$ keratinocytes are negative for the hair bulge markers CD34 and K15 and are infrequently BrdU label retaining (Nijhof et al. 2006). In vitro assays, such as colony-forming efficiency assays and serial passage of colonies, identified PLET1$^+$ cells as stem cells with similar proliferative capacity to bulge stem cells (Nijhof et al. 2006).

Ghazizadeh and Taichmann (2001) initially proposed the existence of a distinct population of SG stem cells. Later, Fuchs and coworkers suggested that a reservoir of cells in the HF adjacent to the SG, marked by the transcription repressor B lymphocyte-induced maturation protein 1 (BLIMP1, expressed by the *Prdm1* gene), contains sebocyte progenitors (Horsley et al. 2006). Lineage tracing using a constitutively active Cre expressed under the control of the *Prdm1* promoter indicated that BLIMP1$^+$ cells give rise to differentiated lipid-producing sebocytes. Also, mice with epidermal-specific deletion of *Prdm1* displayed postnatal SG hyperplasia, suggesting a role for BLIMP1 in controlling the transition of quiescent stem cells to proliferative progenitor cells (Horsley et al. 2006). However, this study is controversial because BLIMP1 is expressed in terminally differentiated cells of all epidermal compartments, including the SG (Magnúsdóttir et al. 2007; Lo Celso et al. 2008; Cottle et al. 2013; Page et al. 2013).

Leucine-rich repeats and immunoglobulin-like domain protein 1 (LRIG1) is expressed by actively cycling stem cells in the HF junctional zone (adjacent to the SG and infundibulum) and SG (Fig. 4) (Jensen et al. 2009; Page et al. 2013). LRIG1$^+$ stem cells are enriched for PLET1, but are negative for bulge stem cell markers such as CD34 or LGR-5 and only show low expression of α6 integrin (Jensen et al. 2009; Page et al. 2013). Stem cells enriched for LRIG1 contribute to all epidermal lineages in skin reconstitution assays and feed into the SG and infundibulum in lineage-tracing experiments (Jensen et al. 2009; Page et al. 2013). On wounding, progeny of LRIG1$^+$ stem cells are rapidly recruited to the site of injury and contribute permanently to tissue regeneration (Page et al. 2013).

The isthmus, a region just above the HF bulge, contains another stem cell population marked by the *Lgr* family member LGR-6 (Fig. 4) (Snippert et al. 2010a). Lineage-tracing experiments have shown that LGR-6$^+$ stem cells maintain adult homeostasis of the upper HF, SG, and IFE (Snippert et al. 2010a). Upon injury, LGR-6 stem cell progeny readily contribute to wound healing, as well as to hair neogenesis when grafted onto nude mice (Snippert et al. 2010a). During postnatal hair morphogenesis, epidermal *Lgr-6* expression is mainly detectable in the isthmus region, but LGR-6$^+$ cells are also found in the IFE basal layer, periphery of the SG and the lower HF (Snippert et al. 2010a). Recent work has shown (Page et al. 2013) that this expression pattern is maintained in adult life, which might explain the robust and rapid labeling of IFE and SG during tamoxifen-induced lineage-tracing experiments, as reported by Snippert et al. (2010a). In their 2010 study, Clevers and colleagues concluded that "LGR-6 marks the most primitive epidermal stem cell" (Snippert et al. 2010a). This holds true provided that the scattered LGR-6$^+$ cells within all epidermal compartments (outside the isthmus region) are indeed stem cells. Thus, *Lgr-6* expression

might mark stem cells that postnatally migrate into all epidermal compartments to maintain adult homeostasis of the entire tissue.

Its remains unclear whether LGR-6$^+$ cells in the isthmus are also enriched for GLI-1, as detection of either endogenous protein has been largely unsuccessful. Given that GLI-1$^+$ cells give rise to progeny in the isthmus and junctional zone, but not to sebocytes, it suggests that both markers are coexpressed at least in some cells in the upper HF (Snippert et al. 2010a; Brownell et al. 2011).

In human skin, no markers of stem cells residing in either the junctional zone or SGs have been described so far. However, in agreement with some work on BLIMP1 expression in mouse SGs (Magnúsdóttir et al. 2007; Lo Celso et al. 2008; Cottle et al. 2013), Sellheyer and Krahl (2010) identified BLIMP1 as a marker of terminally differentiated sebocytes, not as a sebocyte progenitor marker.

STEM CELLS OF THE INTERFOLLICULAR EPIDERMIS

Although the evidence for stem cells in human IFE was established many years ago from clinical applications of cultured epidermis, there has been considerable debate about whether or not there are stem cells in mouse IFE (Jones et al. 2007). Lineage tracing with the AhCreER^T transgenic mouse line (where CreER is ubiquitously expressed in all tissues) has been interpreted as evidence for the existence of a single pool of unipotent progenitors. In contrast, lineage analysis using CreER driven by *K14* or the promoter of the differentiation marker involucrin (*Ivl*) argue for the existence of a slow cycling stem cell population that gives rise to a more rapidly cycling progenitor cell population (Clayton et al. 2007; Mascre et al. 2012). The observation that mouse tail IFE is characterized by two distinct differentiation programs (parakeratotic scales and orthokeratotic interscales) that are maintained by distinct populations in the IFE basal layer with different proliferation rates suggests the need to reinterpret the earlier studies (Gomez et al. 2013). One candidate for a marker of mouse IFE stem cells is LGR-6 (Fig. 4) (Snippert et al. 2010a; Sotiropoulou and Blanpain 2012; Page et al. 2013).

Basal layer keratinocytes are not the only IFE cells to express K14. The touch dome is a distinct compartment within the IFE that contains mechanosensory Merkel cells and specialized keratinocytes (touch dome keratinocytes) (Woo et al. 2010). The developmental origin of Merkel cells was long attributed to the neural crest (Szeder et al. 2003), but lineage tracing using epidermal basal layer markers such as K14 has provided evidence that they are of epidermal origin (Morrison et al. 2009; Van Keymeulen et al. 2009; Woo et al. 2010). K17$^+$ stem cells within the touch dome maintain Merkel cell homeostasis and also contribute to the differentiated keratinocytes above the touch dome (Doucet et al. 2013). Differentiated Merkel cells express the transcription factor SOX-2, and epidermal deletion of *Sox-2* results in a reduction in Merkel cell number (Lesko et al. 2013).

Because techniques for culturing human IFE stem cells in vitro are available (Rheinwald and Green 1975), flow cytometry combined with clonal analysis can be used to identify stem cell markers. IFE basal layer cells are heterogeneous and some cells differentiate within a few rounds of division, whereas others (the putative stem cells) show extensive self-renewal capacity, both in culture (Barrandon and Green 1987) and following engraftment into immune compromised mice (Barrandon et al. 1988; Jones et al. 1995).

The first in vitro–characterized marker of human IFE stem cells was high expression of β1 integrin ECM receptors (Jones and Watt 1993). These cells are located in clusters in human IFE in vivo (Jones et al. 1995). Human epidermal stem cells are also reported to express high levels of α6 integrin (Li et al. 1998) and low levels of the transferrin receptor (CD71) (Tani et al. 2000) and desmoglein-3 (DSG3) (Wan et al. 2003). Cells expressing high levels of β1 integrin are enriched for other markers, including the Notch ligand delta-like 1 (DLL1) (Lowell et al. 2000) and melanoma chondroitin sulphate proteoglycan (MCSP) (Legg et al. 2003).

Single-cell gene expression profiling has been used to identify further markers of human