Imaging Skin Epidermal Stem Cells: A Review

Hilda Amalia Pasolli
The Rockefeller University New York, NY
pasolla@rockefeller.edu

Nature has provided us with a wonderful outer surface, the skin. We humans are fascinated with our epidermis and hair. We spend an exorbitant amount of time and money on skin and hair maintenance. We try hard to keep it soft, smooth, radiant, tan or pale, clean or made-up. We try to remove hair from some areas as hard as we try to keep it on others. Only when our skin is afflicted by disease, burns, wounds, or scratches is it apparent that this amazing outer shell protects us from dehydration and infections. To perform this crucial protective function and to be able to respond to injuries, skin needs to undergo self-renewal to repair damaged tissue and to replace old cells. For this formidable task, skin depends on stem cells. Stem cells have the ability to self-perpetuate and to give rise to differentiating cells that constitute one or more tissue types.

Epidermis and hair follicles

Epidermis is composed of layers, the outermost of which is the skin surface. The innermost layer, the basal one, is composed of proliferative cells, which attach to the underlying basement membrane. The basal layer stratifies to give rise to the differentiated cell layers of the spinous layer, granular layer and the stratum corneum (Figure 1). This wonderful architecture is maintained through a diversity of intercellular junctions such as adherens junctions, desmosomes, and tight junctions while hemidesmosomes and focal adhesions provide attachment to the basement membrane. Epidermis homeostasis is maintained by balancing cellular proliferation in the basal layer with the outward flux of terminally differentiating cells moving outward to form the spinous, granular and stratum corneum layers, which will eventually be sloughed from the skin surface.

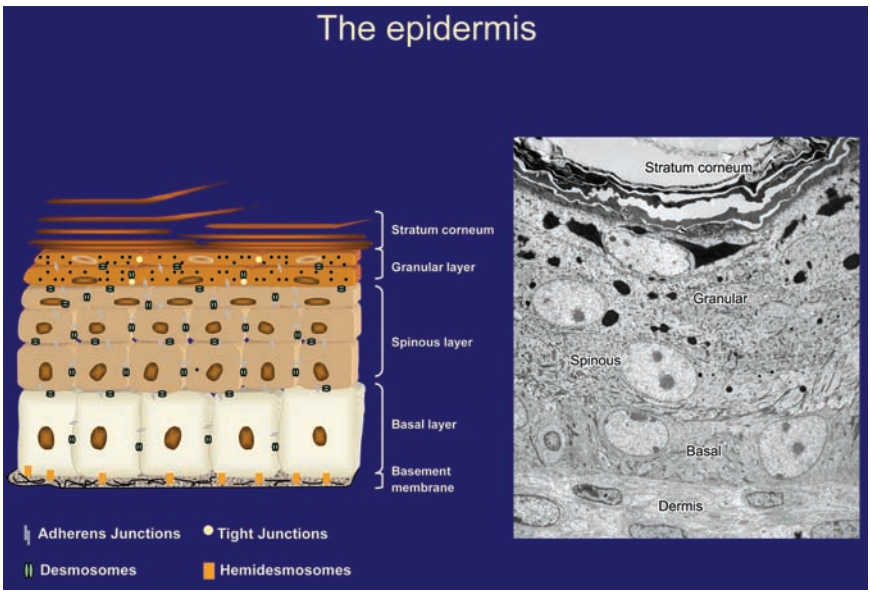


Figure 1. Mammalian skin consists of the epidermis and dermis, separated by a basement membrane. The epidermis is a stratified squamous epithelia that is composed of several cell layers. Resting on the basement membrane is the basal layer (BL), consisting of proliferating, transit-amplifying cells (see text). The basal layer stratifies to give rise to differentiated cell layers of the spinous layer (SL), granular layer (GL) and the stratum corneum (SC).Epidermal cells is kept together by different types of intercellular junctions (adherens junctions, desmosomes, tight junctions) and attach to the basement membrane by hemidesmosomes. At the right, micrograph of mouse epidermis as viewed with the transmission electron microscope.

One of the most remarkable features of mammalian epidermis is its ability to generate appendages, such as hair follicles, sebaceous glands, and sweat glands. I will focus on the description of hair follicles. Hair follicles are amazingly complex structures (Figure 2A): they are composed of an outer root sheath (ORS) that is contiguous with the epidermis, an inner root sheath (IRS), and the hair shaft itself. The IRS and hair shaft are each composed of three different layers. The hair bulb, at the base, contains actively proliferating undifferentiated cells, the matrix, which gives rise to the IRS and hair shaft cell layers. The matrix encloses a pocket of specialized mesenchymal cells called the dermal papilla (DP).

The lower segment of each hair follicle cycles through periods of active growth (anagen), regression (catagen), and rest (telogen) (Figure 2B). During anagen matrix cells divide rapidly. As they move upward they differentiate, giving rise to the IRS and hair shaft layers. During catagen the lower follicle undergoes apoptotic cell death. The DP moves upward until it rests beneath the bulge, where it remains during telogen. The follicle can then prepare for the next cycle of growth. The bulge resides in the ORS, in a small niche below the sebaceous gland, at or near the insertion of the arrector pili muscle.

How to identify stem cells: label retention

One of the most distinctive attributes of stem cells of the hair follicle is that they divide infrequently. This is most probably in order to preserve their proliferative potential and to avoid errors in DNA sequence that could occur during replication. In their search for skin stem cells, Cotsarelis and colleagues (Cotsarelis et al, 1990) made a major breakthrough when they noticed that mouse skin stem cells that retained a DNA label ([^{3}H] thymidine) were located in the hair follicle bulge. Cells that rarely divide (such as stem cells) will be able to keep their DNA label and hence can be identified as label-retaining cells (LRCs). On the contrary, cells that are actively proliferating will soon 'dilute out' the DNA label in subsequent cell divisions. The bulge is an attractive location for skin epithelial stem cells for several reasons: it resides at the base of the permanent part of the follicle where stem cells are in a convenient position to regenerate the hair at the end of each hair cycle, and they are in a close and contiguous position to reconstitute wounded epidermis. The deep position of the bulge also guards the stem cells from mechanical stress and from sunlight-induced mutations, which is crucial for cells that must maintain an intact genome for many years.

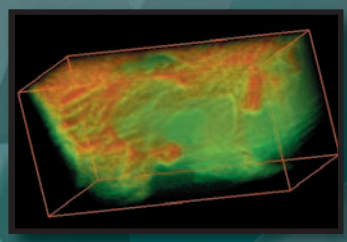
Label-retaining cells work as stem cells

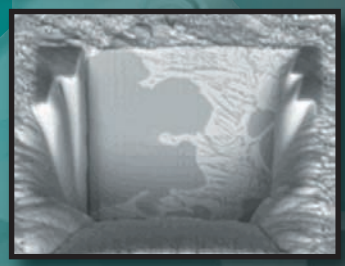
Following the identification of LRCs, the next challenge was how to verify that these cells can indeed function as stem cells. Purifying and characterizing LRCs as stem cells required a method to isolate these cells and permit their manipulation for prospective therapeutic use. The Fuchs and Cotsarelis labs utilized fluorescence activated cell-sorting (FACS) to sort bulge cells from mouse hair follicles based on specific labels.

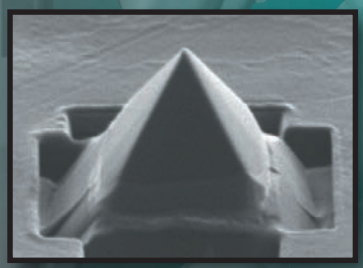
The Fuchs lab induced for a limited time the expression of a keratin 14 (K14) promoter-driven transgene, encoding a long-lived histone tagged with green fluorescent protein (GFP) (Tumbar et al, 2004).

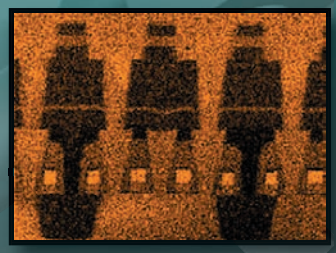
Power Tool for Grownups

MultiBeam - look - build - analyze - in real time









JIB-4500

NanoFabrication

Another
Extreme imaging
solution

JEOL

Stability • Productivity • Performance
www.jeolusa.com • salesinfo@jeol.com
978-535-5900

Who says you can't afford to have it all? High-resolution imaging, bitmap milling, TEM sample prep, and high-speed slicing and sampling in real time with this all-in-one SEM/FIB. The new JEOL MultiBeam combines the world's most popular low vacuum LaB_6 electron column with high resolution ion beam optics. Utilize the full power of the MultiBeam power tool for your next nanofabrication project. For more information visit www.jeolusa.com/multibeam.

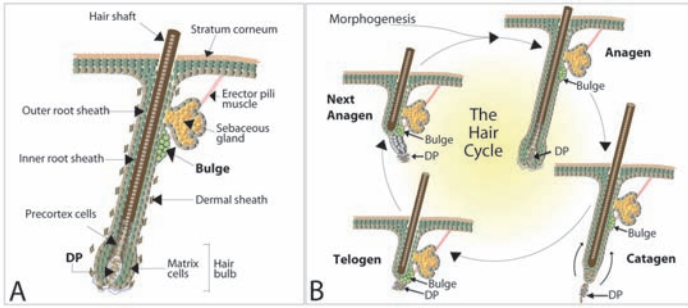


Figure 2: Schematic of an anagen (A) and cycling (B) hair follicle. Hair follicles undergo periodic cycling from anagen (hair producing) through catagen (regression), and into telogen (resting). (A) In the anagen hair follicle the dermal papilla (DP) is enclosed within the hair bulb, and the bulge is nestled below the sebaceous gland. (B) During the hair cycle, the proximity of the DP and the bulge varies, with the closest contact occurring at telogen as the follicle prepares for a new anagen. Reprinted by permission from Cold Spring Harbor Laboratory Press: Laura Alonso and Elaine Fuchs, **G***enes Dev. 2003; 17: 1189-1200*

The isoform keratin 14 is specifically expressed in the basal cells of epidermis and ORS cells, which includes bulge cells. Histones are proteins that bind to the DNA. Therefore we could make all cycling keratin 14 expressing cells GFP-positive. By administering a drug to the mice, we could turn off the expression of the transgene and wait for months to 'wash out' the GFP label from actively dividing cells. We found, as predicted, that the GFP-label-retaining cells were located in the hair bulge. Then, standard FACS could be used to isolate and characterize the cells expressing high levels of GFP.

In addition to this, research by the Morris and Cotsarelis group showed that the intermediate filament protein keratin 15 (K15) was primarily expressed in the bulge region and relatively rare in other skin epithelial cells (Morris et al, 2004). They proceeded and marked LRCs. They engineered mice in which the K15 promotor drives the expression of GFP, thus also allowing FACS-based sorting of cells into those expressing high levels of GFP. More recently, the purification of bulge stem cells have been accomplished in our lab by FACS based on the expression of bulge surface markers such as α6 integrin and CD34 coupled with K14-GFP expression (Blanpain et al, 2004).

The new methods to purify bulge cells allowed researchers to perform clonal analyses and engraftment and demonstrate the two defining features of stem cells, namely self-renewal and pluripotency. When cultured bulge cells and dermal cells were grafted onto the back of *Nude* mice (*Nude* mice are a mice strain which due to a genetic defect lack hair), they were able to regenerate epidermis , hair follicles and sebaceous glands showing that our bulge-derived skin cells were indeed multipotent stem cells (Blanpain et al, 2004). The Cotsarelis group showed that the K15 bulge cells could produce all the epithelial components of the anagen follicle (Morris et al, 2004).

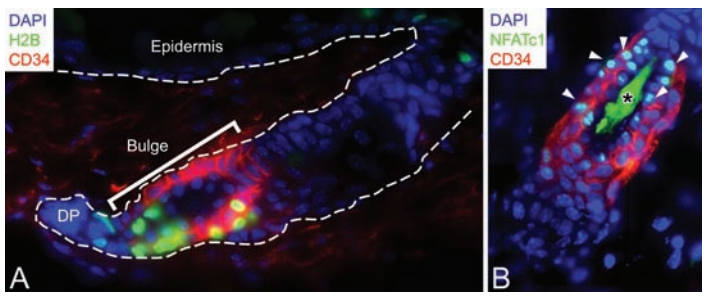


Figure 3. Bulge stem cell markers. *Hair follicles in resting phase (telogen). Skin stem cells reside in their niche, the region of the hair follicle known as the bulge. Slow-cycling cells retain the label for histone 2B-green fluorescent protein (A) The stem cells express a surface marker, CD34. Fig. B: NFATc1 is expressed in bulge stem cells (arrowheads) DAPI stains all nuclei. Asterisk indicates hair shaft autofluorescence. DP, dermal papilla.*

These methods have allowed to not only identify and image bulge LRCs, but also to characterize their gene expression by performing microarray analyses. Approximately 150 genes are preferentially expressed in the bulge relative to the proliferating basal cells of the epidermis. Although each of these procedures purifies slightly different cell populations, the array data are in quite good agreement, enabling researchers to exploit this information to learn more about follicle stem cells. Analysis of genes expressed by bulge stem cells allowed researchers to identify further markers of this population such as the transcription factors Lhx2, NFATc1, and Sox9 (Horsley et al, 2008, Rhee et al, 2006) and to elucidate how these transcription factors act to maintain the bulge stem cell niche.

Skin epidermal stem cells: future challenges

Bulge cells are so far the best characterized stem cells in skin. We know that they can regenerate the hair follicle, the sebaceous gland and repair wounded epidermis. There are still other mysteries. Questions such as how is the homeostasis of unwounded epidermis maintained? How is the differentiation of other important skin appendages such as sebaceous and sweats glands specified and maintained? Do they also have their own stem cell population? There is more and more evidence that interfollicular epidermis has its own population of stem cells. Progress in our lab has shown that the sebaceous gland has its own progenitor cells (Horsley et al, 2006; for a review see Horsley and Fuchs, 2008).

The characterization of skin stem cells triggers additional exciting questions: could they be used to repair skin and regenerate hair in humans? Could they be used as a means to deliver corrective gene therapy for skin disorders and maybe even non skin disorders? Would these stem cells have the plasticity to differentiate into non skin cell types? The answer to these exciting questions will most probably be revealed in the coming years. ■

Acknowledgments

My thanks to Bob Marks for correcting the manuscript and to my colleagues: Valerie Horsley for critical reading and for valuable suggestions, Valentina Greco and Geraldine Guasch for providing immunofluorescence pictures.

References

Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E., Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell.* 2004 Sep 3;118(5):635-48.

Cotsarelis G, Sun TT, Lavker RM., Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell.* 1990 Jun 29;61(7):1329-37.

Fuchs E, Horsley V., More than one way to skin . . ., *Genes Dev.* 2008 Apr 15;22(8):976-85.

Horsley V, Aliprantis AO, Polak L, Glimcher LH, Fuchs E., NFATc1 balances quiescence and proliferation of skin stem cells. *Cell.* 2008 Jan 25;132(2):299-310.

Horsley V, O'Carroll D, Tooze R, Ohinata Y, Saitou M, Obukhanych T, Nussenzweig M, Tarakhovsky A, Fuchs E. Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. *Cell.* 2006 Aug 11;126(3):597-609.

Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G., Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol.* 2004 Apr;22(4):411-7. Epub 2004 Mar 14.

Rhee H, Polak L, Fuchs E., Lhx2 maintains stem cell character in hair follicles. *Science.* 2006 Jun 30;312(5782):1946-9.

Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E., Defining the epithelial stem cell niche in skin. *Science.* 2004 Jan 16;303(5656):359-63.

Side-By-Side Comparison? Difficult When Our Coaters Stand Alone.

High Resolution Sputter Coater 208HR for FE-SEM

Superior Features:

- High Resolution Fine Coating
- Wide Choice of Coating Materials
- High Resolution Thickness Control
- Multiple Sample Stage Movements
- Wide Range of Operating Pressures
- Compact, Modern, Benchtop Design

Find out about our complete line of sample coaters.

TED PELLA, INC.
Microscopy Products for Science and Industry

4595 Mountain Lakes Blvd., Redding, CA 96003-1448
Phone: 530-243-2200 or 800-237-3526 (USA) FAX: 530-243-3761
Email: sales@tedpella.com Web Site: www.tedpella.com