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Elaine Fuchs' Lecture Part 2: Tapping the Potential of Adult Stem Cells

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1. Keywords and Terms

Adult stem cells, multipotent, unipotent, skin, epidermis, hair follicle, sebaceous gland, label-retaining cells, Tet-off system, Histone-GFP, transgenic mice, bulge, hair cycle, grafting, Wnt signaling.

2. Review Questions

1. What is the function of the epidermis and its appendages (the hair follicles and sebaceous glands)?
2. Where are unipotent adult skin stem cells located? What do they give rise to?
3. Where are multipotent adult skin stem cells located? What do they give rise to?

4. What keratins do proliferative skin cells express?
5. What is a stem cell niche?
6. What techniques were used to purify label-retaining cells from the epidermis and then analyze their mRNA expression profiles?
7. Name two signaling pathways that keep hair follicle bulge cells quiescent.
8. Name a signaling pathway important for activating hair follicle bulge cells?
9. The β -catenin protein has two distinct functions in the cell. What are they?
10. How do we measure activity of the Wnt signaling pathway within cells?
11. Why should we be cautious about using the Wnt signaling pathway as a clinical treatment for inducing hair growth?

3. Answers to Review Questions

1. The epidermis acts as a barrier against the outside world, keeping bodily fluids in and external pathogens out. Also, along with the hairs, the epidermis provides warmth. Sebaceous glands secrete a fluid to keep the hair and skin lubricated.
2. They are found in the basal layer of the epidermis and give rise to the stratified epidermal epithelium.
3. They are found in the bulge region of the hair follicles and can give rise to the hair follicles, sebaceous glands, and the stratified epidermal epithelium.
4. Keratin 5 and Keratin 14. The promoters of either of these keratins can be used to specifically express transgenes in proliferative epidermal cells.
5. It is the place that stem cells reside within a tissue (usually deep within the tissue where the cells are well protected). It provides an environment where the stem cells are kept in a growth- and differentiation-inhibited state. Stem cells leave the niche when they are activated.

6. Fluorescent Activated Cell Sorting (FACS) to sort the cells, and microarray analysis to look at their mRNA.
7. TGF β and BMP signaling pathways.
8. The Wnt signaling pathway.
9. β -catenin is involved in cell-cell adhesion as a component of adherens junctions (along with E-cadherin and β -catenin). β -catenin is also activated by the Wnt signaling pathway to enter the nucleus and serve as a transcriptional co-activator with members of the Lef/Tcf family of transcription factors.
10. Using a reported transgene such as TOPGAL (Tcf/Lef optimal promoter). The reporter consists of a series of DNA binding sites for Lef/Tcf transcription factors that will drive expression of a reporter gene such as β -galactosidase. The presence of nuclear β -catenin is also sometimes used as an indicator of Wnt signaling activity.
11. Because unchecked Wnt signaling activity can cause tumor formation, such as hair tumors called pilomatricomas.

4. Discussion Questions

1. How are skin stem cells used to treat burns victims? What are the limitations of this treatment and why?
2. How did the Fuchs lab perform a pulse-chase experiment to identify where slowly-dividing stem cells reside in the epidermis?
3. In the chase phase of the experiment, tetracycline is added, which binds to Tet-Vps16 and prevents it from binding the TRE. GFP-Histone expression is shut off (the system is called 'Tet-Off') and the nuclear green fluorescence label is gradually diluted away as cells divide and non-fluorescent histone is incorporated into the chromatin instead.

4. In the pulse-chase experiment performed by the Fuchs lab to identify label-retaining cells corresponding to multipotent hair follicle stem cells, why did the unipotent stem cells located in the basal layer of the epidermis not retain the GFP-histone label?
5. How did the Fuchs lab show that the label retaining cells present in the hair follicle bulge were bona fide multipotent stem cells?
6. What happens when stabilized β -catenin is overexpressed in mouse hair follicles?
7. What happens when β -catenin is removed (knocked-out) from the mouse epidermis? Why do we need to make a conditional, inducible mouse to perform this experiment?

5. Answers to Discussion Questions

1. Skin stem cells taken from the patient can be cultured and expanded in vitro. The cells can then be grafted back onto the burned regions of the patient. However, these stem cells are unipotent and can not contribute to hair formation, so the skin grafts lack hair. And there is still some degree of scarring, because scar tissue arises from dermal cells, which are from a separate lineage to the epidermis.
2. Firstly, the lab constructed two transgenes in mice. The first consisted of a gene fusion called Tet-Vps16, driven by the keratin 5 promoter. The second consisted of a GFP-Histone fusion under the control of a Tet Responsive Element (TRE) DNA sequence. The K5 promoter expresses Tet-Vps16 specifically in the proliferative epidermis. In the absence of a small molecule called tetracycline, Tet-Vps16 is a transcriptional activator which binds to the TRE and drives expression of GFP-Histone, labeling the chromatin of all proliferative epidermal cells. This is the 'pulse'.
3. Stem cells divide relatively infrequently so the fluorescent label is not diluted as rapidly. Label-retaining cells can then be identified in the mouse epidermis after several weeks of feeding the mice with tetracycline.
4. The experiment was based on the idea that hair follicle stem cells divide infrequently, due to the length of the mammalian hair cycle. Hair follicle stem cells will therefore retain Histone-GFP fluorescence for a long time after expression of the fluorescent label is shut off, as they divide slowly and therefore don't dilute out the

label. The 'chase' phase of the experiment was therefore performed over 4 weeks. Unipotent basal cells divide much more rapidly, as the entire epidermis turns over every 2 weeks. Therefore, over the 4 week chase period, these basal cells undergo cell division several times and therefore lose the GFP-Histone label.

5. By tracing the GFP label, the lab could see that these cells exited the bulge to replenish the epidermis following wounding. Similarly, GFP-positive cells contributed to the growth of a new hair follicle at the beginning of the hair cycle. These cells are therefore involved in homeostasis and wound repair – two characteristics of adult stem cells. Culturing the cells in vitro showed that they could continuously produce large, self-renewable colonies of cells, another stem cell characteristic. Finally, the lab took a clonal colony, grown from a single label-retaining cell, and grafted it onto the back of a nude mouse. This mouse developed GFP-labeled epidermis, hair follicles, and sebaceous glands, showing that a single bulge cell can contribute to all of these lineages and is therefore multipotent.
6. When Wnt signaling is not active, cytoplasmic β -catenin (i.e. β -catenin not involved in cell-cell adhesion) is rapidly degraded. Overexpression of a truncated form of β -catenin that lacks the degradative signal results in the stabilization and accumulation of β -catenin. This leads to an increase in Wnt signaling activity when stabilized β -catenin enters the nucleus. Transgenic mice overexpressing stabilized β -catenin in their epidermis precociously grow new hair follicles as they enter the growth phase of the hair cycle too soon. This leads to super furry animals. However, the uncontrolled Wnt signaling produced by stabilized β -catenin can also lead to the formation of hair tumors called pilomatricomas. In humans, these tumors can indeed be caused by mutations in β -catenin.
7. The removal of β -catenin using a conditional, inducible approach causes the mouse to lose all of its stem cells and thence to lose all its hair. This approach was used because mice that completely lack β -catenin in all of their tissues die very early in embryogenesis. β -catenin must therefore only be removed from the mouse epidermis (this is called a conditional knockout approach). Furthermore, conditional β -catenin knockout mice that lose epidermal β -catenin expression in the fetus never form hair, so the effect of losing β -catenin on hair follicle stem cells can't be followed. Loss of β -catenin is therefore induced in the adult mouse using the inducible CreER system (see suggested assignments).

6. Explain or Teach These Concepts to a Friend

1. Explain the structure of mammalian skin, including its different layers and appendages.
2. How does one make a transgenic mouse?
3. Explain the basic Wnt signaling pathway.

7. Research the Literature on Your Own

1. What are different stages of the hair cycle? Explain some of the signals known to be involved in controlling the cycle.
2. What role does the dermal papilla play in regulating hair growth?
3. How does the CreER mouse genetic system work to produce conditional, inducible knockout mice?

8. Papers for Journal Club

1. Tumber, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W.E., Rendl, M. and Fuchs, E. Defining the epithelial stem cell niche in skin. *Science*. 2004. 303: 359-63.

This is the original paper detailing the pulse-chase method and the identification of label-retaining cells in the hair follicle bulge.

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

This paper shows the clonal analysis and grafting experiment demonstrating that the hair follicle bulge cells are multipotent stem cells.

3. Lowry, WE, Blanpain, C, Nowak, JA, Guasch, G, Lewis, L and Fuchs E. Defining the impact of β -catenin/Tcf transactivation on epithelial stem cells. *Genes Dev.* 2005. 19: 1596-611.

This paper dissects the role of Wnt signaling in epithelial stem cells, using both loss and gain of function studies on β -catenin.

4. Yi, R, Poy, MN, Stoffel, M and Fuchs, E. A skin microRNA promotes differentiation by repressing 'stemness'. *Nature.* 2008. 452: 225-9.

Demonstrates how microRNAs can regulate the activation and subsequent differentiation of stem cells.

5. Nowak, JA, Polak, L, Pasolli, HA, and Fuchs, E. Hair follicle stem cells are specified and function in early skin morphogenesis. *Cell Stem Cell.* 2008. 3: 33-43.

Where do adult hair follicle stem cells come from? This paper reveals how they are specified early in embryonic development.

6. Ezhkova, E, Pasolli, HA, Parker, JS, Stokes, N, Su, IH, Hannon, G, Tarakhovsky, A, and Fuchs, E. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell.* 2009. 136: 1122-35.

This paper demonstrates the role that epigenetic modifications – specifically histone methylation – play in regulating skin stem cell differentiation.