

**“Efforts to Characterize Tissue Stem Cells and
Cancer Stem Cells in the CCR”**

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Cancer Stem Cells

Cancer stem cells (CaSC) may represent a subset of cancer cells that have the stem cell properties of self-renewal and unlimited replicative potential for long-term tissue repopulation.

CaSC may generate additional cancer stem cells and differentiate into phenotypically diverse cancer cells with only limited proliferative potential.

In some human cancers, cell surface markers have been identified that distinguish CaSC from other cancer cells with more limited proliferative potential.

Mutations that target normal tissue stem cells and expand the tissue stem cell population may be the first step in cancer formation. These mutations may inappropriately activate pathways that promote the self-renewal of normal stem cells resulting in increased stem cell numbers.

Cancer Stem Cells

Cancer stem cells (CaSC) may represent critical therapeutic targets for treating epithelial carcinomas.

To better characterize CaSC and demonstrate their existence in epithelial cancer, we need to first accomplish two goals:

a. Identify unique panels of cell surface markers on CaSC to provide a “handle” so that these cells can be manipulated and characterized.

b. Develop in vivo assays to determine if putative human CaSC are able to reconstitute cancer and exhibit properties associated with stem cells

Unlike hematopoietic stem cells, well characterized and unique cell surface markers for either normal stem cells or CaSC in epithelial tissues are not known, and good in vivo assays for many human epithelial cancers do not currently exist.

CCR: Tissue and Cancer Stem Cells Studies

Several CCR investigators are actively involved in characterizing tissue stem cells of liver, breast and skin in order to better understand the relationship between normal tissue stem cells and cancer stem cells in these tissues.

1. Dr. Snorri Thorgeirsson has a long-standing interest in the biology of hepatocellular carcinoma (HCC) and has used transgenic animal models to understand how the activation and differentiation of hepatic progenitor cells may not be correctly regulated in murine models of HCC.

CCR: Tissue and Cancer Stem Cells Studies

Recently, Dr. Thorgeirsson has compared the global gene expression patterns or “signatures” of distinctive human HCC phenotypes, with variable prognosis, to gene expression patterns of liver progenitor cells (hepatoblasts) and adult hepatocytes of rat and mouse models in order to identify the cellular origin of human HCC. The hypothesis is that HCC cancer cells will retain gene expression patterns that are characteristic of their cellular origins.

The results show that HCC of a poor prognosis phenotype shared gene expression patterns with fetal rat hepatoblasts, suggesting that this HCC subtype may arise from hepatic progenitor cells.

Nature Medicine, 2006, in press.

CCR: Tissue and Cancer Stem Cells Studies

- 2. Dr. Gilbert Smith has studied mammary gland tumorigenesis and mammary epithelial stem cells with the hypothesis that mammary carcinomas arise as clonal populations of transformed tissue-specific stem cells and their differentiating progeny. His contributions to mammary gland biology include:**
 - a. Helping to establish the mammary fat pad transplantation technique as a valuable in vivo stem cell assay**
 - b. Assessing the ability of different mammary epithelial cell populations to reconstitute mammary glands and/or recapitulate mammary carcinoma; and assessing the frequency of mammary stem cells by limiting dilution and serial transfer studies**
 - c. Understanding lineage relationships and contributions of mammary epithelial progenitor cells to the ductal and lobular (secretory) lineages. Distinct epithelial progenitors include: lobular (secretory) epithelial progenitors; ductal epithelial progenitors; as well as multipotent epithelial progenitors able to produce both lobular and ductal progenitors.**

CCR: Tissue and Cancer Stem Cells Studies

- 3. Drs. Barbara Vonderhaar and Michael Gottesman are establishing in vivo assays for human breast cancer, using the murine mammary fat pad assay.**
- 4. Dr. Michael Dean has been identifying and characterizing MDR genes of the ATP-binding cassette (ABC) family of transporters. ABC genes may be expressed by tissue stem cells such as side population (SP) cells that have a dye-low phenotype, and he is interested in these genes as a therapeutic target for chemotherapeutic agents.**

CCR: Tissue and Cancer Stem Cells Studies

5. In the skin, our major focus has been to develop an “infrastructure” to identify, isolate, and characterize keratinocyte stem cells (KSC) and their progeny.

Our hypothesis is that the knowledge and experimental approaches derived from these KSC studies will provide a roadmap and help us to identify and characterize the role of cancer stem cells in non-melanoma skin cancers such as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC).

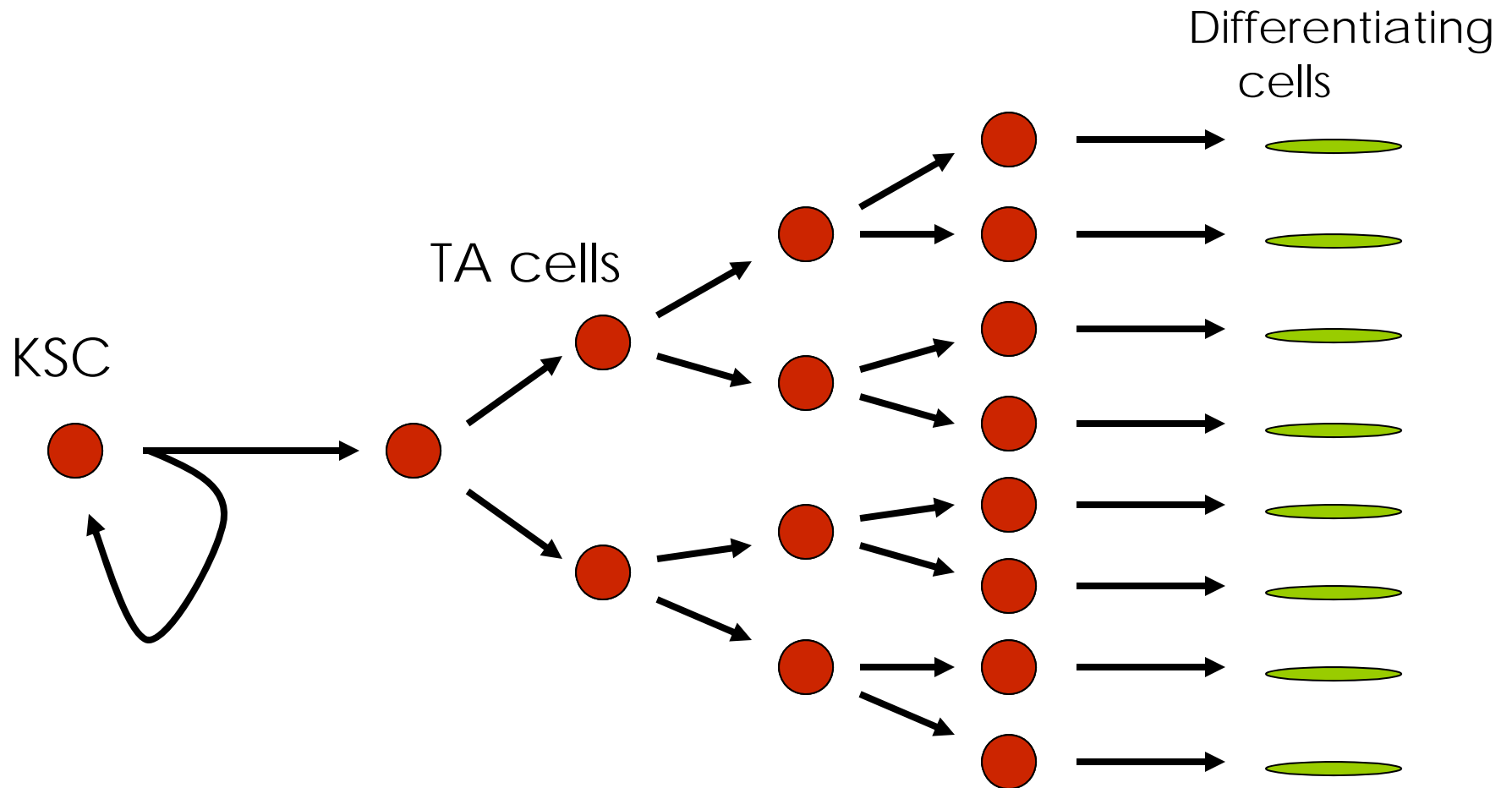
Epithelial stem cells can be identified as label-retaining cells (LRC)

In renewable tissues like the epidermis, mammary gland, and prostate, epithelial stem cells are believed to divide infrequently, and these stem cells can be identified by their ability to retain a nucleotide (BrdU) label and are referred to as label-retaining cells or LRC.

In order to be detected, the LRC need to be fixed and made permeable to antibodies against the BrdU nucleotide label. Consequently, biological studies, including assays to assess their stem cell behavior, cannot be performed.

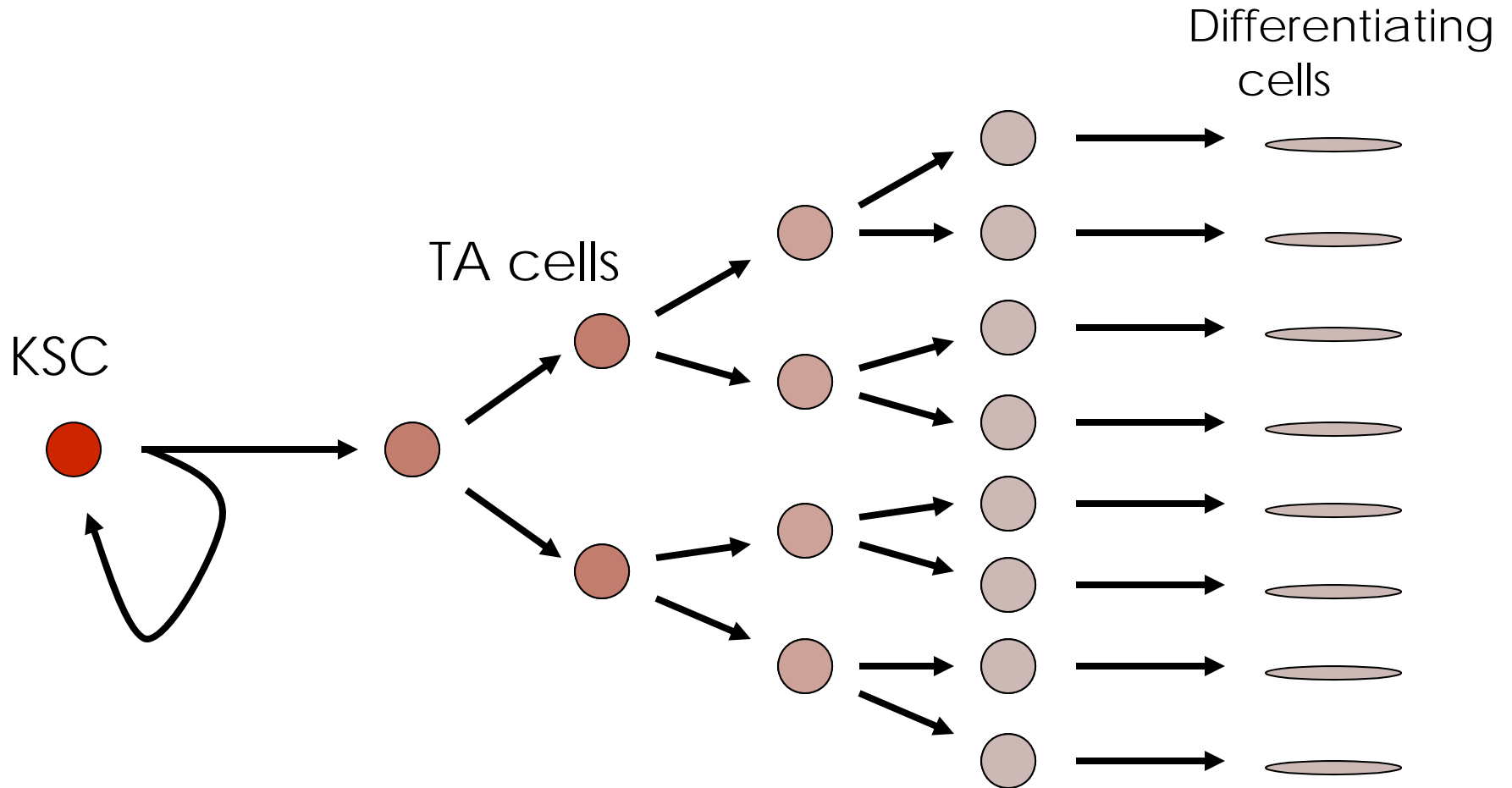
Slowly cycling keratinocyte stem cells (KSC) and rapidly dividing transit amplifying (TA) cells

Labeling with BrdU

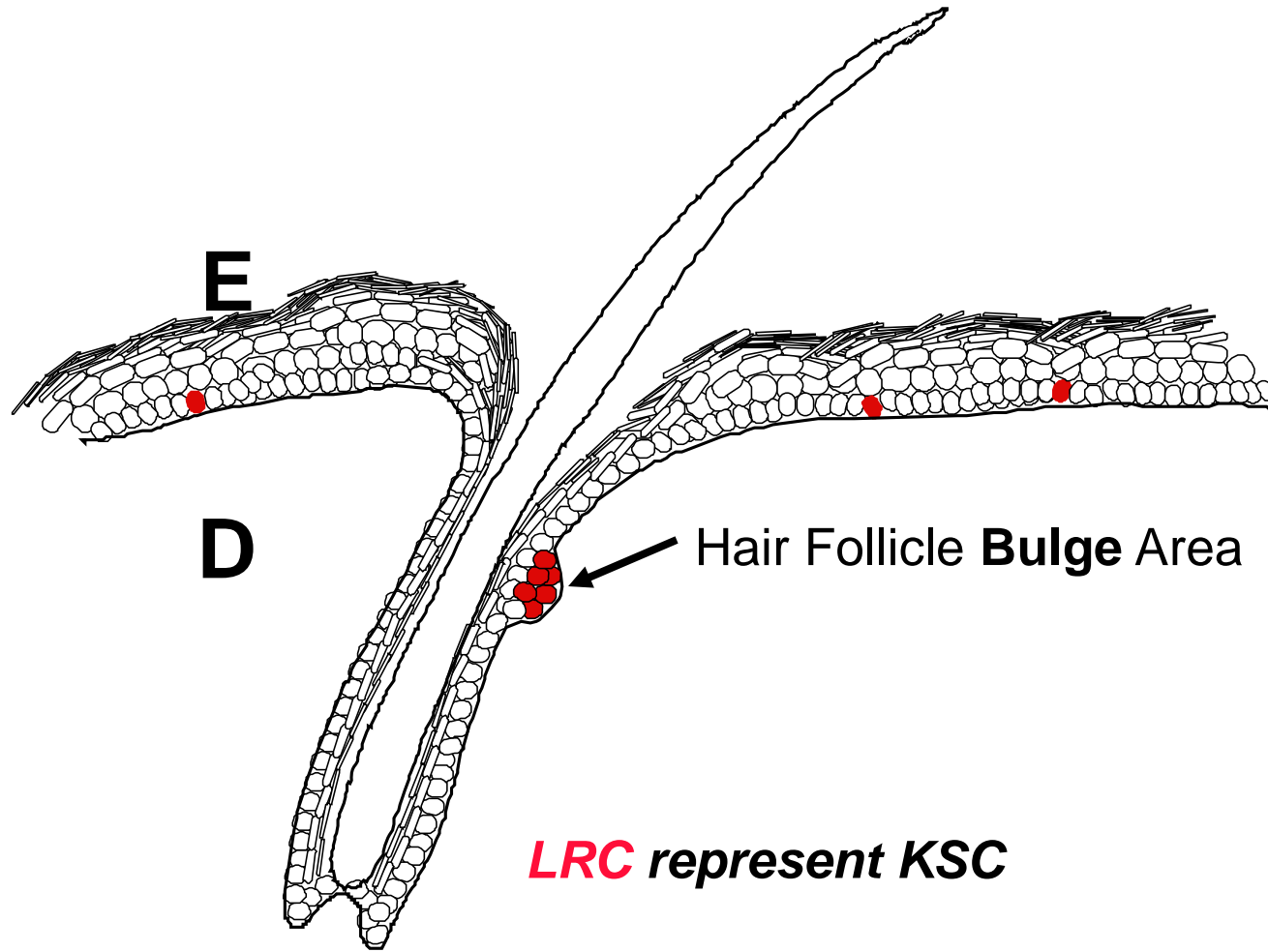


Slowly cycling keratinocyte stem cells (KSC) and rapidly dividing transit amplifying (TA) cells

Removal of BrdU followed by a “washout” period



Label-Retaining Keratinocytes (LRC) in Murine Skin Containing Hair Follicles



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Characterizing Tissue Stem Cells

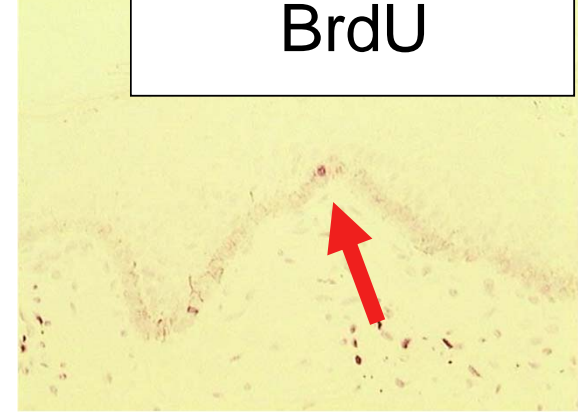
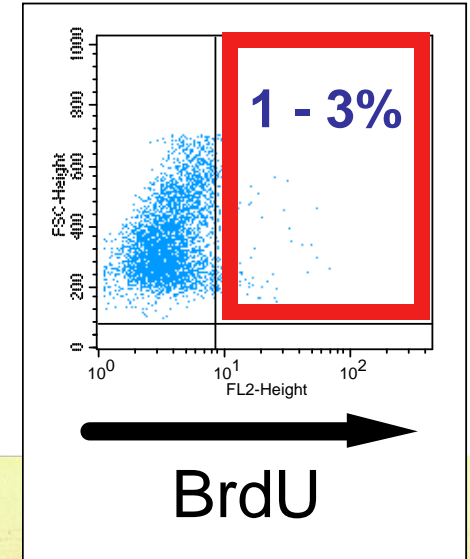
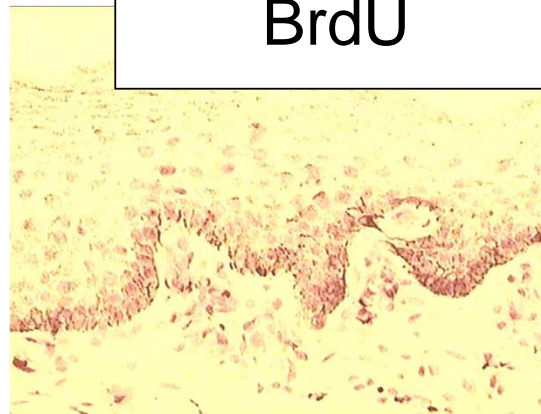
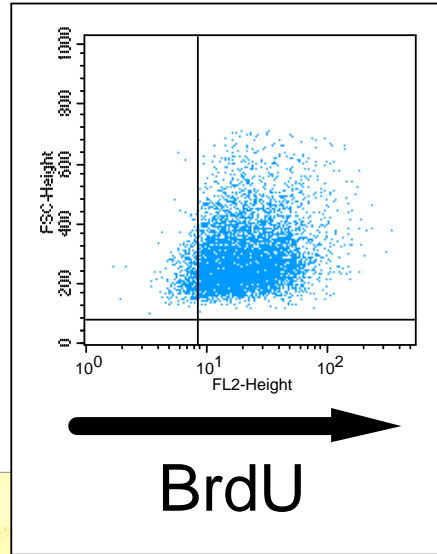
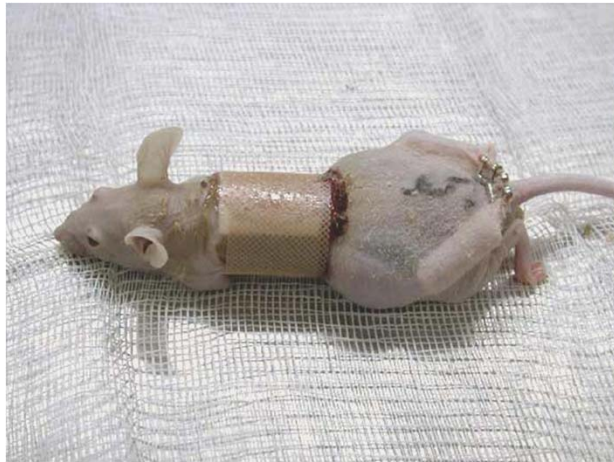
To identify, isolate, and characterize living keratinocyte stem cells (KSC), we first need to identify a unique panel of cell surface markers on human LRC:

- 1. Laser capture microdissection of LRC from the bulge area of human hair follicles, followed by microarray analysis**
- 2. High-throughput mass spectrometry (MS) analysis of membrane proteins on FACS-sorted LRC**

Develop in vivo assays to confirm the stem cell behaviors of self-renewal and long-term repopulating ability of candidate KSC

Characterize cancer stem cells (CaSC) in the non-melanoma skin cancers squamous cell carcinoma (SCC) and basal cell carcinoma (BCC)

Detecting LRC in human skin graft



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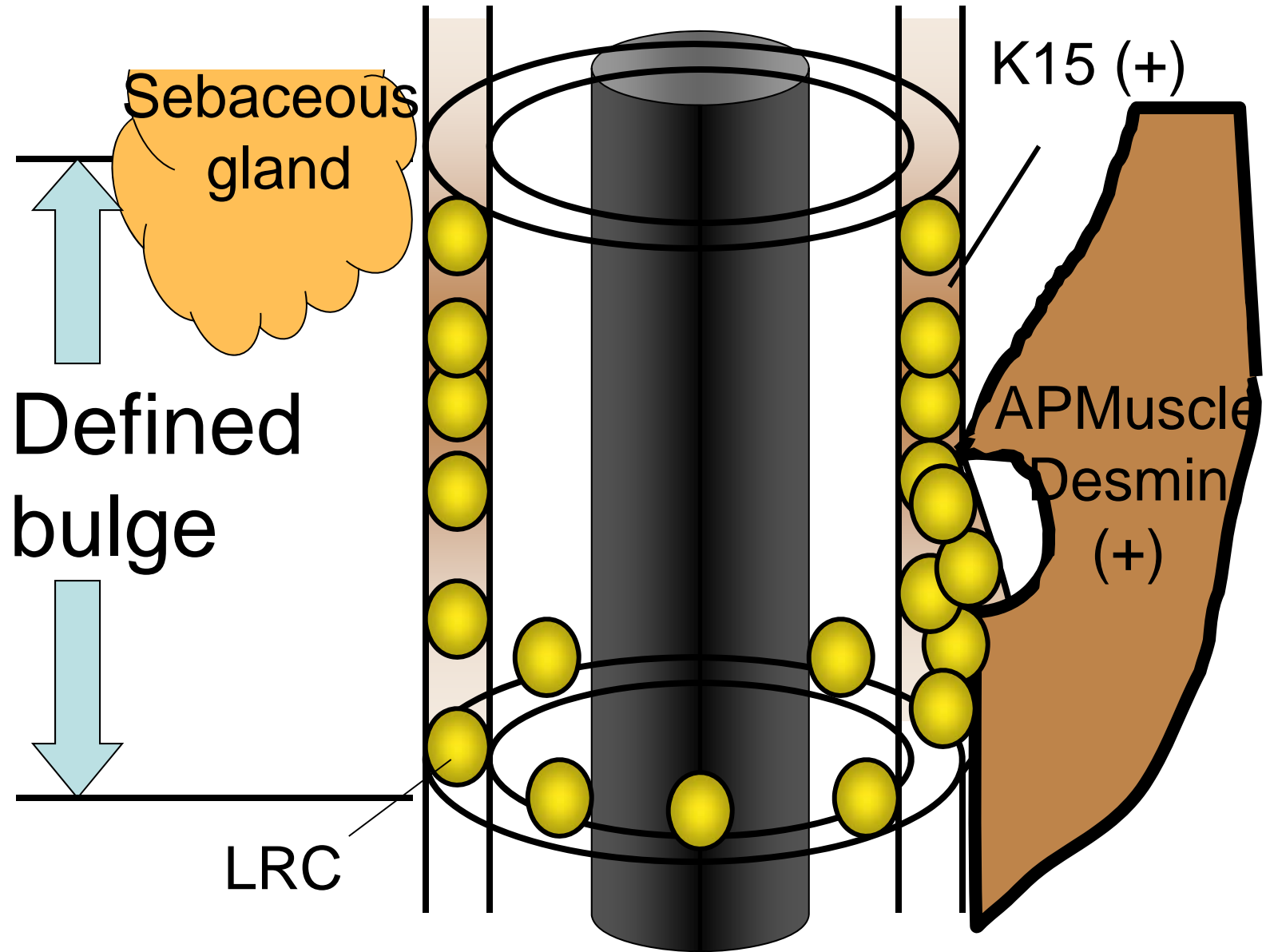
6 weeks

14 weeks

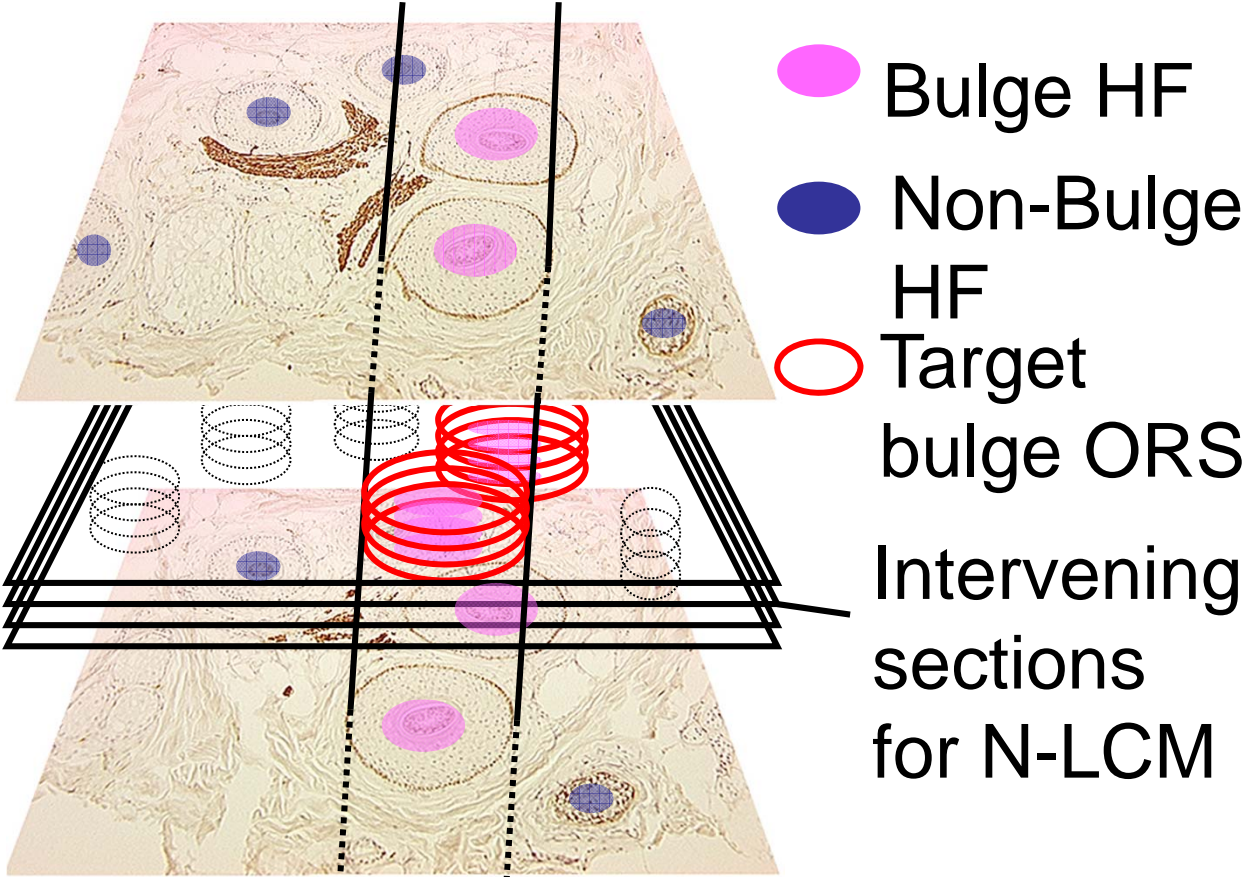
BrdU ON

BrdU OFF

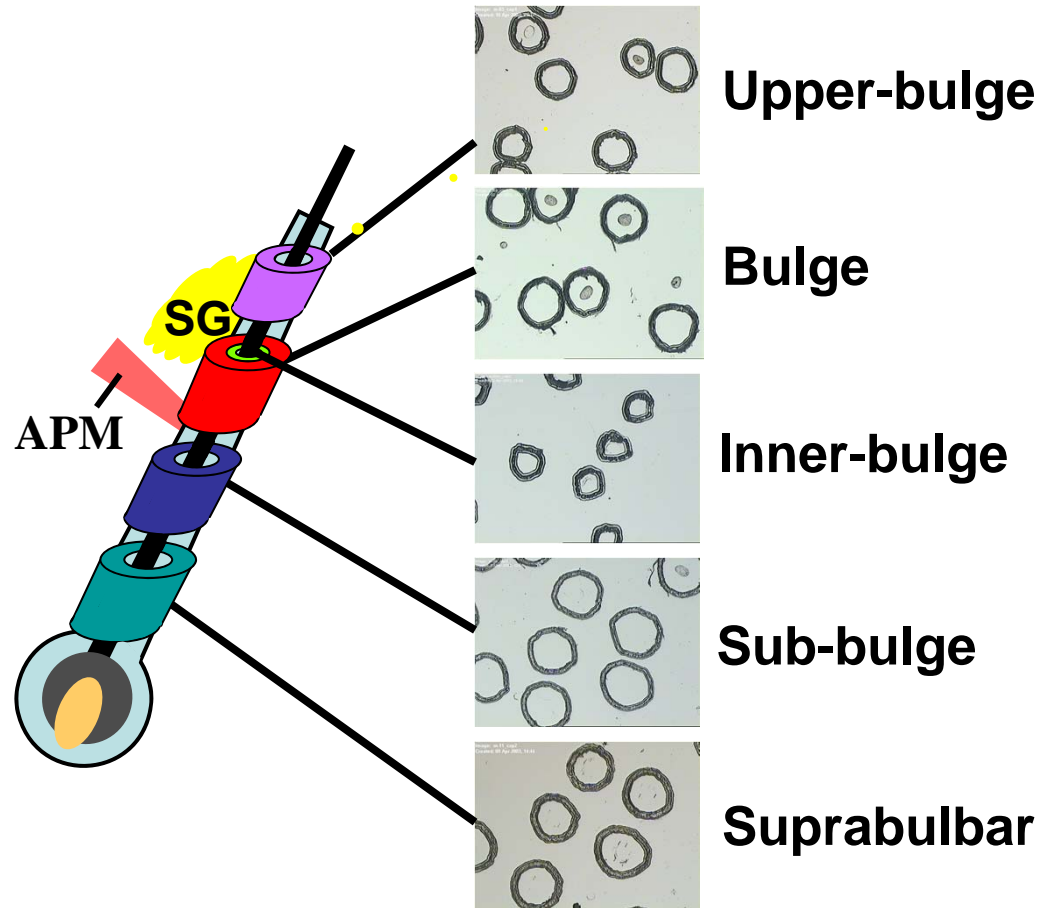
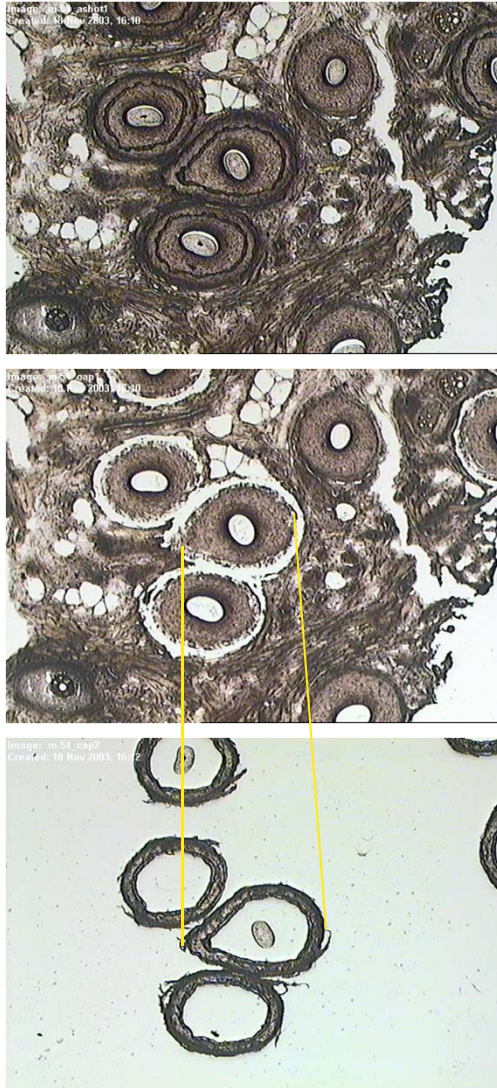
Bulge area of an individual human hair follicle



Navigated-Laser Capture Microdissection of the defined HF bulge and other HF regions.

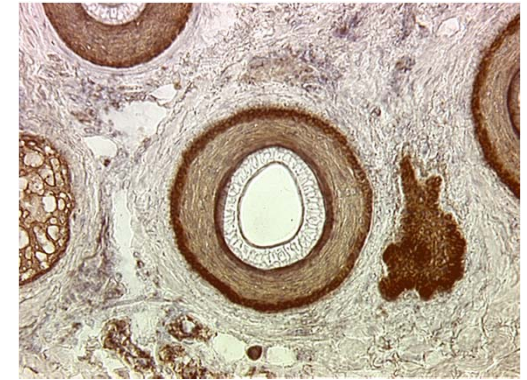
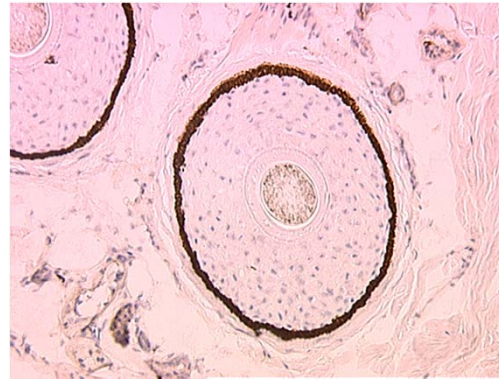
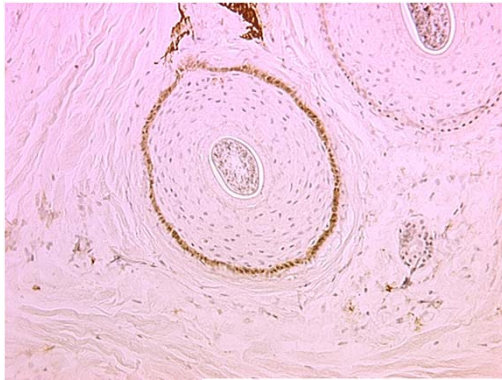


Human hair follicle (HF) bulge and other ORS subsets are isolated by Navigated Laser Capture Microdissection (N-LCM)

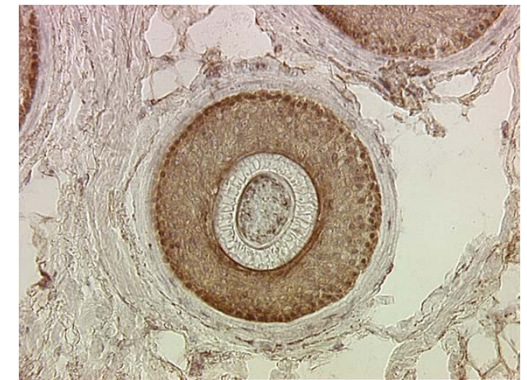
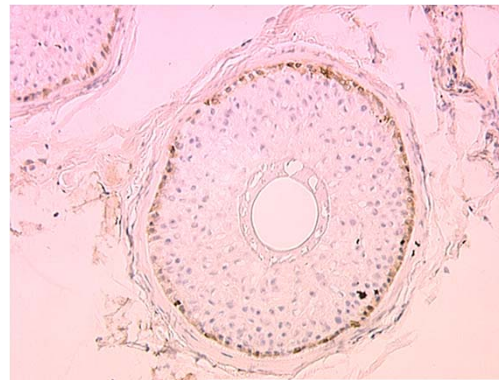
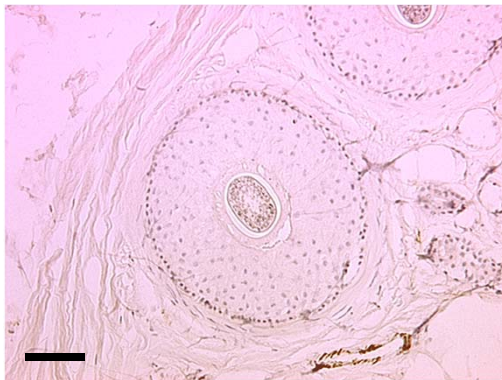


N-LCM-microarray analysis identifies markers for human HF bulge cells

Bulge



Sub-bulge

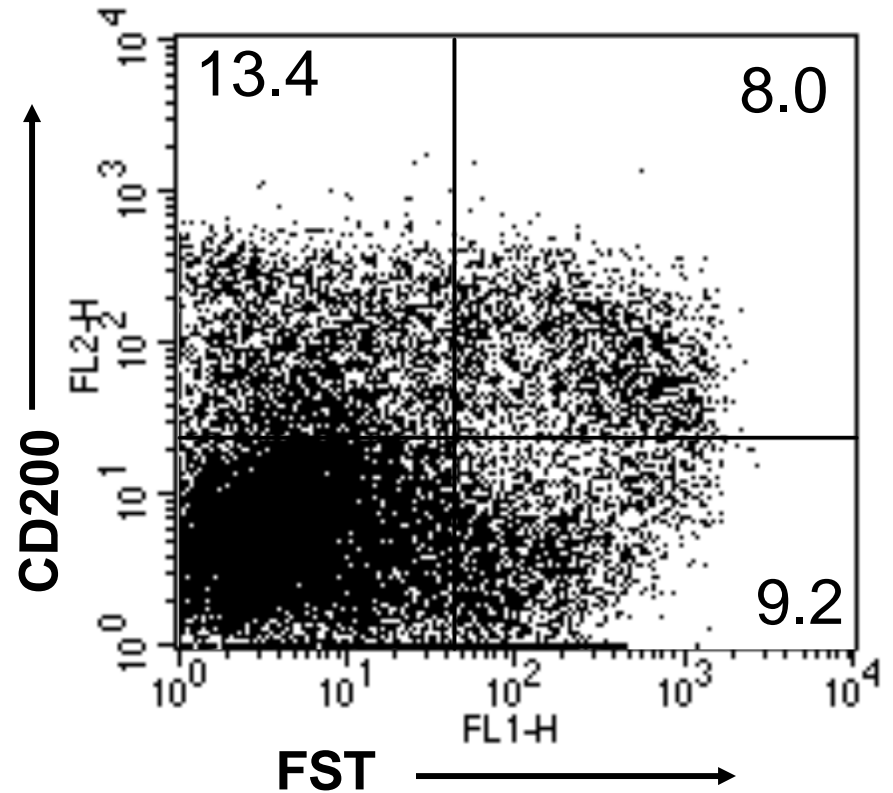
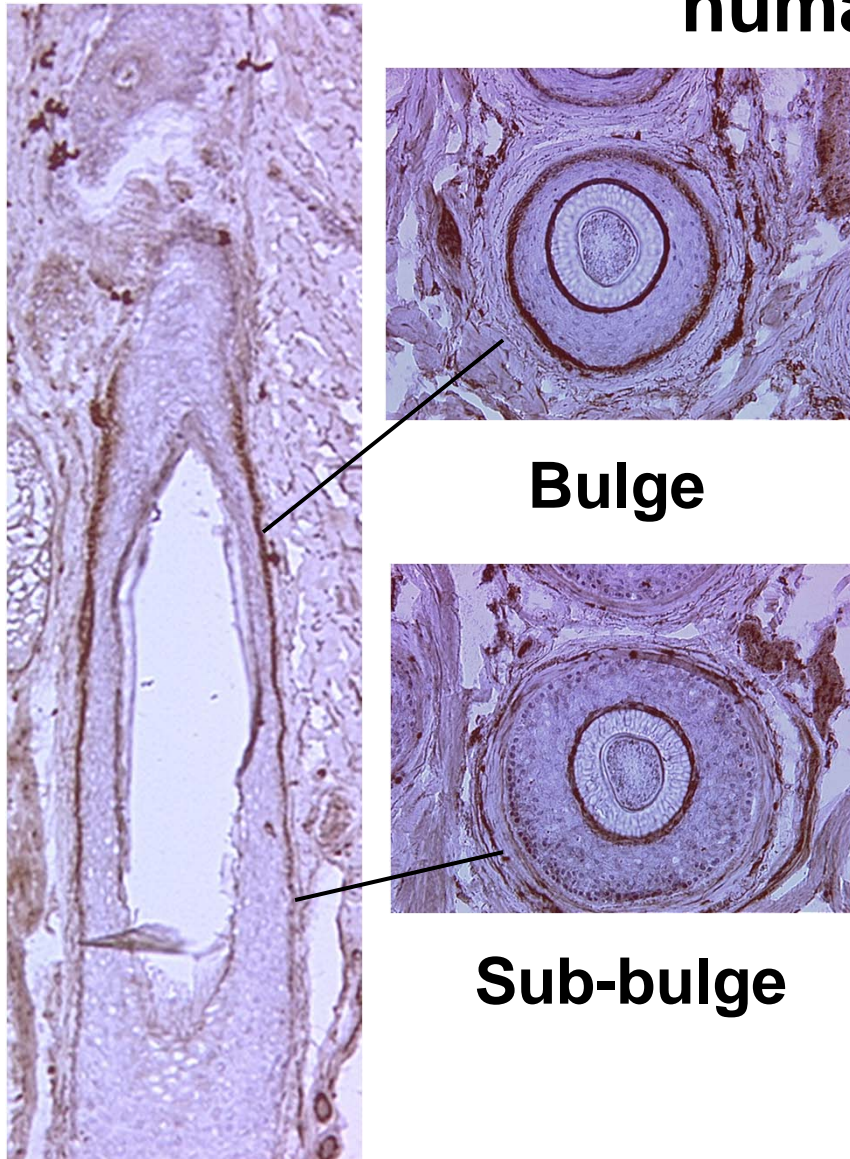


KRT15

FST

FZD1

CD200 is a positive marker for human HF bulge

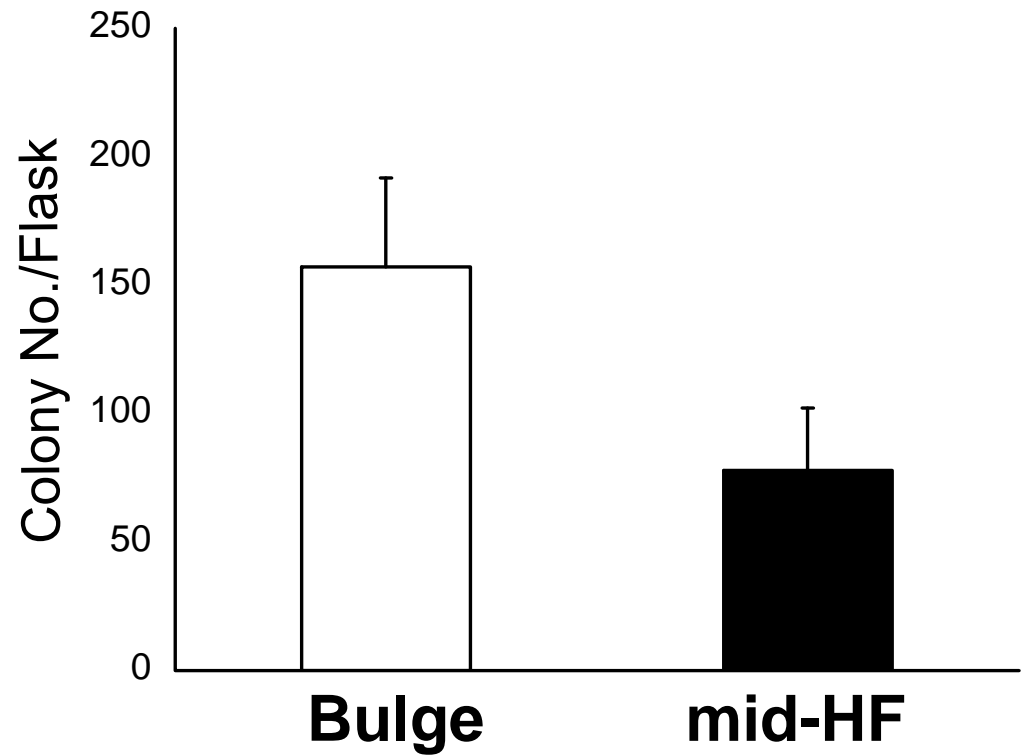


CD200^{hi} bulge cells have greater colony-forming ability than mid-HF cells



Bulge

mid-HF



J. Clinical Invest. 116(1):249-260, 2006

Our Goals:

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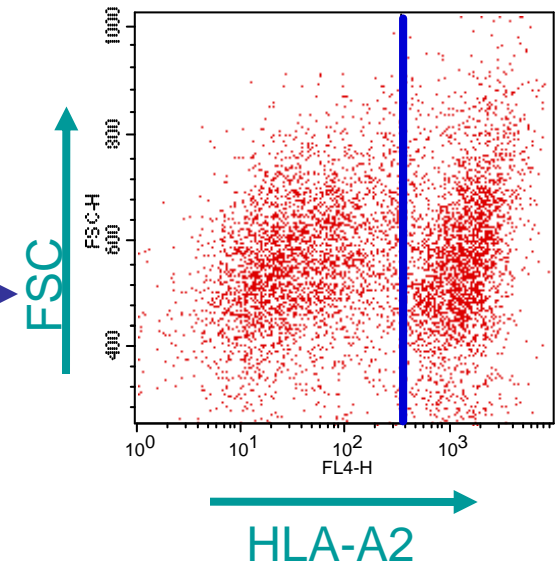
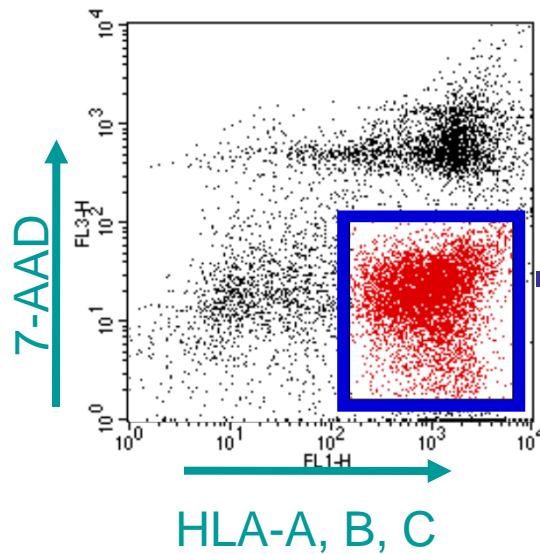
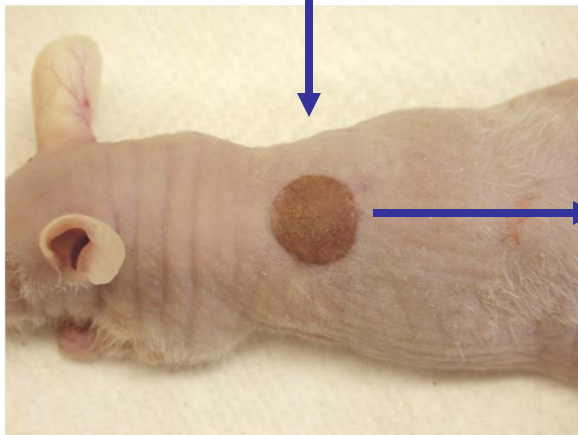
In vivo competitive assay using raft culture-grafting system



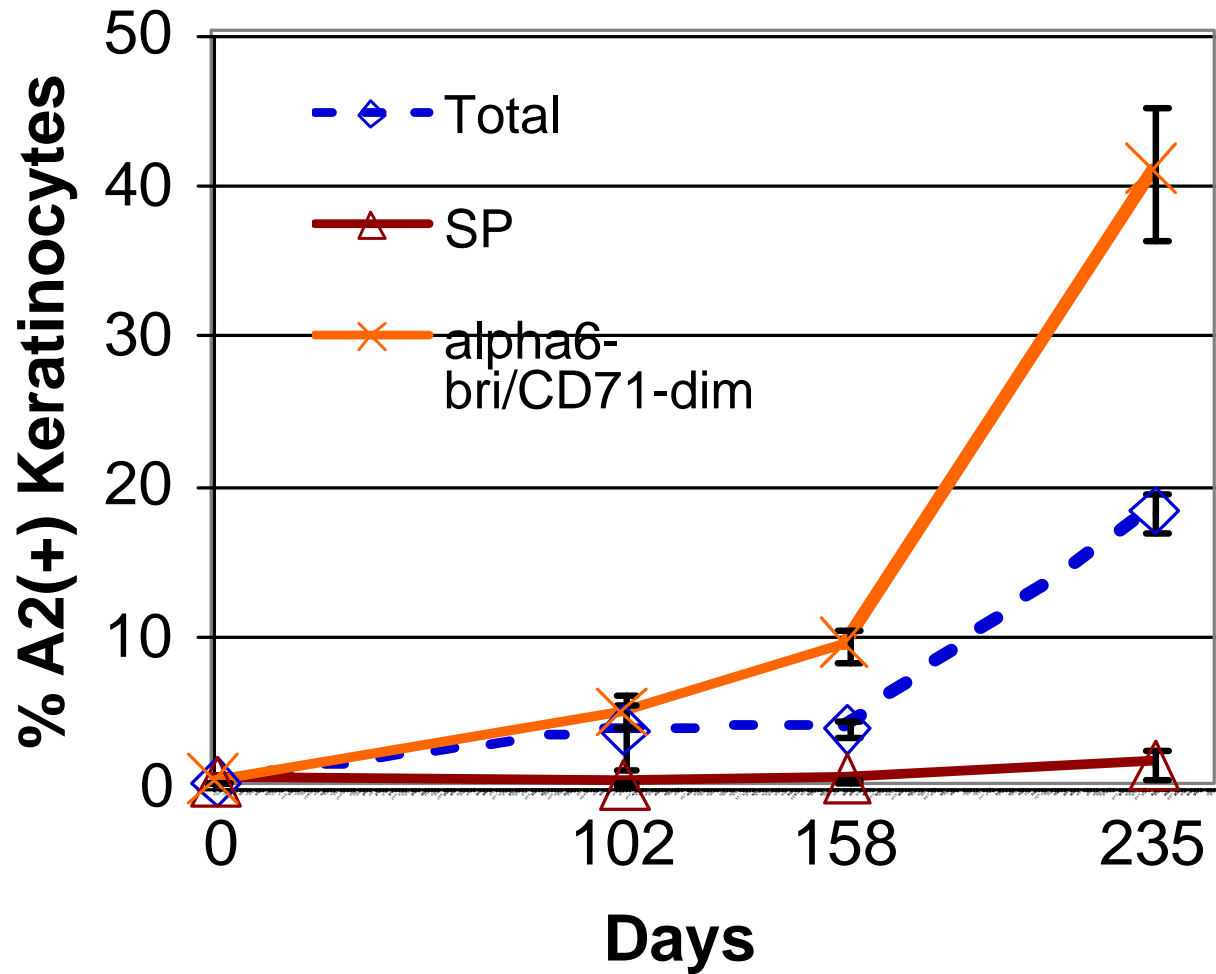
Test population
HLA-A2 (+) KSC

Control population
HLA-A2 (-) non-KSC

Mix together in the raft cultures



In vivo assay was used to determine if alpha6-integrin and side population (SP) keratinocytes possess self-renewal and long-term repopulating ability.



Future Directions: Identifying and characterizing cancer stem cells (CaSC)

In order to characterize the biological behavior of CaSC:

1. Develop in vivo mouse model assays for human SCC and BCC that are able to assess the ability of putative CaSC to recapitulate the cancer in vivo

-3-dimensional raft cultures and nanofibrous scaffold supports

2. Determine if CaSC exist within SCC and develop methods to identify and purify them

-use cell surface markers identified for KSC

-identify sub-populations of SCC with increased self-renewal signaling

Conclusion

Within the CCR, there is considerable expertise and a growing interest in characterizing epithelial stem cells and using this knowledge to understand the role of cancer stem cells in the initiation and maintenance of epithelial carcinomas. Key goals are to:

- a. identify cell surface markers that will serve as handles to isolate and manipulate cancer stem cells,**
- b. develop in vivo models that can assess the ability of putative cancer stem cells to recapitulate human epithelial cancer.**

Acknowledgments

Vogel Lab

Manabu Ohyama
Atsushi Terunuma
Girish Patel
Jianjun Zhou
Carole Yee



Biomedical Proteomics Program

Timothy Veenstra
Thomas Conrads
Josip Blonder

Laboratory of Cellular Oncology,

Michael Radonovich
Cindy Masison
John N. Brady

Dermatology branch

Mark Udey
Maria Turner
Steven Katz

Medicine Branch

William Telford
Veena Kapoor



Laboratory of Pathology, CCR

Michael Emmert-Buck

