Montagna Symposium on the Biology of Skin

Montagna Symposium 2015: Harnessing Stem Cells to Reveal Novel Skin Biology and Disease Treatments

Valerie Horsley[1,2], Molly Kulesz-Martin[3] and Xiao-Jing Wang[4]

Journal of Investigative Dermatology (2016) **136,** 893–896; doi:10.1016/j.jid.2016.01.022

The 64th annual Montagna Symposium on the Biology of the Skin, "Harnessing Stem Cells to Reveal Novel Skin Biology and Disease Treatments," was held from 15–19 October 2015 in Gleneden Beach, Oregon. The meeting brought together scientists in academia and industry working on mechanisms of skin homeostasis, regeneration, and tumorigenesis and trainees who wanted to learn more about stem cells in the skin. The Symposium was chaired by Xiao-Jing Wang and Valerie Horsley with Session Chairs Mayumi Ito, Rui Yi, and John McGrath.

The meeting opened with a Keynote presentation by Haifan Lin, Director of Stem Cell Center at Yale University, who unfolded the history of the discovery of RNAs that regulate gene expression and control stem cell activity. In particular, Dr. Lin discussed the intergenic regions within eukaryotic genomes that contain multiple DNA sequences that are not transcribed into protein-coding genes, including transposons, pseudogenes, long noncoding RNAs (lncRNAs), and P-element induced wimpy testis-interacting RNAs (piRNAs). Data from Dr. Lin's laboratory have revealed that piRNAs can be derived from transposons and pseudogenes and play a major role in degrading mRNAs and lncRNAs in spermatocytes in mice. These data place piRNAs in a core RNA regulatory pathway modulating other RNA species to influence gene expression during meiosis. These exciting new paradigms provide new avenues for exploration of gene regulatory networks in development and stem cell biology in the skin.

"Stem Cells in Development and Diseases" Session Chair Mayumi Ito discussed how melanocyte stem cells function in the hair follicle. Her laboratory showed that Wnt signaling is essential for melanocyte stem cell differentiation; however, lineage tracing of Wnt-active differentiated melanocyte stem cell progeny shows that they can revert back to their undifferentiated state. This plasticity occurs after multiple differentiation stimuli, including UVB irradiation. In aged mice, defective Wnt signaling leads to ectopic differentiation and failure to revert, resulting in melanocyte stem cell loss in the tissue. This novel step in melanocyte self-renewal may be related to melanoma progression, because melanocyte stem cells can generate melanoma in a Wnt-dependent manner.

Marcus Bosenberg continued the discussion of melanoma by describing the current thoughts on cancer stem cells (CSCs) in melanoma. Whether melanoma contains a population of cells that display stem cell properties has been debated in the literature. Dr. Bosenberg's laboratory has generated several mouse models that develop aggressive melanoma and has begun characterizing heterogeneous populations of cells within these tumors to define how intratumor heterogeneity can influence tumor progression.

Tudorita Tumbar described her work on characterizing the dynamic behavior of epithelial stem cells in the skin. She used a transgenic mouse model in which pulse and chase with a histone, H2B–green fluorescent protein (GFP), effectively labeled epithelial cells based on divisional frequency. She showed lineage tracing data testing the classical stem cell model, in which infrequently dividing cells are stem cells and frequently dividing cells are transit amplifying cells. She presented models of dynamic behavior of epithelial stem cells during adult homeostasis and discussed her work on the molecular markers and mechanisms regulating the behavior of epithelial lineages in the skin.

The molecular mechanisms that regulate epidermal differentiation and self-renewal were explained by George Sen's talk on the role of RNA helicase DDX6 in the epidermis. Careful molecular analysis of DDX6's function in keratinocytes defined its role in preventing differentiation through binding to stem loops of mRNAs involved in proliferation and self-renewal to facilitate translation. DDX6 also degrades mRNA transcripts for genes involved in epidermal differentiation, including *KLF4*. These functions reveal novel regulation of translational control in stem cells in the epidermis.

The "Genetic and Epigenetic Regulation of Stem Cells" session began with Session Chair Rui Yi presenting analyses of the mechanisms that regulate self-renewal and proliferation in hair

[1]*Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA;* [2]*Department of Dermatology, Yale University, New Haven, Connecticut, USA;* [3]*Departments of Dermatology and Cell, Developmental & Cancer Biology, Oregon Health & Science University, Portland, Oregon, USA; and* [4]*Departments of Pathology, Otolaryngology, Dermatology & Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, Colorado USA*

Correspondence: Valerie Horsley, Department of Molecular, Cellular and Developmental Biology, Yale University, 219 Prospect Street, Box 208103, New Haven, CT 06520. E-mail: valerie.horsley@yale.edu

Abbreviations: CSC, cancer stem cell; iPS, induced pluripotent cells; Kitl, Kit ligand; lncRNA, long noncoding RNA; piRNA, PIWI-interacting RNA; PRC, polycomb repressive complex; RDEB, recessive dystrophic epidermolysis bullosa; TGF, transforming growth factor

follicle stem cells. Using modern genetic and molecular analyses for transcription factor binding within genomes and mathematical modeling of mRNA profiling data, Dr. Yi's laboratory has identified an adaptive transcription factor network that coordinately controls quiescence in hair follicle stem cells, revealing pathways that can be modulated to regulate hair follicle growth.

Yali Dou's talk initiated a discussion of histone methyltransferase regulation of stem cell biology. Her laboratory has developed a small molecule inhibitor of mixed lineage leukemia protein 1, a lysine 4 (K4) histone methyltransferase. Using this mixed lineage leukemia protein 1 inhibitor, Dr. Dou's laboratory identified the importance of histone methylation at distinct stages of development in embryonic stem cells. Future experiments analyzing how this pharmacological inhibitor alters skin development and regeneration may reveal the importance of K4 methylation in skin biology.

William Lowry presented work on the regulation of tumorigenesis in the skin by stem cells in the hair follicle. Lineage tracing of cell populations in the hair follicle has revealed the capacity of follicular bulge stem cells to form squamous cell carcinomas upon genetic activation of KRAS, and shown how stem cell quiescence protects these cells from contributing to squamous tumors. Dr. Lowry's group is now defining novel mechanisms that regulate squamous cell carcinoma initiation and determining how tumor cell heterogeneity arises in response to different cell origins, genetic alterations, and molecular changes during tumorigenesis.

The regulation of histone methylation in skin progenitor cells continued with the talk of Elena Ezhkova, who analyzed the function of the polycomb repressive complex (PRC) 2, which generates trimethylation of histone H3K27, leading to chromatin compaction. Deletion of components of the PRC2 complex showed that polycomb is not required for generation of epidermal specification. However, mice lacking PRC2 display increased numbers of Merkel cells, the sensory cells involved in the tactile response of skin. Molecular and genetic analysis of keratinocytes lacking PRC2 showed that polycomb is required for activation of transcription factors essential for Merkel cell differentiation. These studies indicate that Merkel cell lineage specification requires chromatin regulation by the polycomb complex.

Session Chair Valerie Horsley introduced the "Microenvironment of Stem Cells in the Skin" session by discussing how dermal adipocytes are regulated during hair cycling and wound healing. Her laboratory previously defined a role of these cells in regulating the hair follicle cycle and fibroblast function during skin wound healing. Her recent data implicate platelet-derived growth factor signaling in the regulation of the dermal adipocyte niche and identified a novel interaction between dermal adipocytes and monocyte-derived cells after skin injury.

Fiona Watt continued the discussion of mesenchymal cells and epidermal cells during skin homeostasis. Her laboratory has studied heterogeneity in the dermal fibroblasts and currently is analyzing the role of signaling pathways that differentially regulate these populations of cells. In addition, her laboratory has continued to explore the heterogeneity of epidermal stem cell populations using lineage tracing in mouse models.

An intriguing presentation by Melissa Wong focused on a unique role of macrophages in controlling CSC properties. Using mouse models of melanoma (as well as colorectal and mammary cancer), Dr. Wong's laboratory has demonstrated that macrophages can fuse with CSCs to form macrophage-tumor cell fusion hybrids that harbor properties of the parental macrophage cell, effectively providing metastatic behaviors to the cancer cell, including the ability to survive as a circulating tumor cell (in humans and mice) and seed distant metastatic sites. Her group's data provide a new paradigm for understanding how cancer cells acquire metastatic traits and have implications for novel approaches to therapeutic targeting of this unique tumor population.

Matthew Rodeheffer presented data on the regulation of adipocyte precursor cells in response to diet. Dr. Rodeheffer's laboratory identified and characterized a population of adipocyte precursor cells in adipose tissue in the mouse that respond rapidly to high-fat diet and contribute to increased fat mass. His talk provided potential targets for obesity that regulate adipocyte precursor cells.

In the Banquet Keynote address, George Cotsarelis outlined his contributions to the hair follicle stem cell field since localizing these cells to the mouse hair follicle bulge in 1990 and the human bulge in 1996. His *Nature Biotechnology* paper in 2004 described the isolation, lineage tracing, molecular characterization, and multipotency of the bulge cells (Morris et al., 2004). These discoveries ignited the field of hair follicle stem cell biology, leading to a multitude of studies from his and other laboratories to solidify the importance of these cells in hair follicle cycling and to identify mechanisms that control their activity. His laboratory also described the preservation of the bulge cells in androgenetic alopecia and the role of prostaglandin D2 in inhibiting stem cells in this disorder.

The "Stem Cell-Based Therapies and Innovative Reprogramming Technologies" session was introduced by John McGrath, who presented the urgent need for therapies for patients with an inherited blistering skin disease, recessive dystrophic epidermolysis bullosa (RDEB). His work has identified the ability of mesenchymal stromal cells to be recruited to skin-damaged areas. Clinical trials using bone marrow transplantation and injection of allogeneic fibroblasts in patients with RDEB have shown some clinical benefits, including reductions in inflammation, pain, and itching. Future cell therapies from other sources were discussed.

This clinical theme was continued by Jakub Tolar, who has performed clinical trials for patients with RDEB after hematopoietic cell transplantation. Previous work from Dr. Tolar's laboratory suggested that hematopoietic cell transplantation increased expression of collagen VII in patients. Dr. Tolar expanded this study, suggesting that modulation of transplanted cells can improve collagen VII expression and reduce blistering, while emphasizing the need for future studies and analysis of cell populations that are contributing, so as to meet the dire clinical need for new treatments.

Angela Christiano presented fascinating progress in the development of three-dimensional skin equivalents