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Adult hair follicle stem cell compartment changes in androgenetic alopecia demonstrate maintenance of progenitor stem cells with loss of descendant CD200high A6 integrinhigh expressing cells

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The status of adult stem cell compartments in tissue specific disease has not been thoroughly addressed. We tested the hypothesis that hair follicle stem cells might be depleted in androgenetic alopecia (AGA), which is characterized by drastic miniaturization of the hair follicle. To compare hair follicle stem cell numbers between paired haired and bald scalp samples from the same individuals, we used flow cytometry to quantitate cell cycle, cell size, and expression of CYTOKERATIN 15 (KRT15), FOLLISTATIN (FST), CD200 and alpha-6 integrin. We found a gradient of stem cell characteristics, as defined by a high degree of KRT15 and FST expression, cellular quiescence and small cell size. This gradient is not grossly altered between haired and bald scalp, and stem cells are maintained in bald scalp. However, a specific CD200 high alpha-6-integrin high population, which has characteristics of early stem cell progeny, is lost in bald scalp. Consistent with the loss of the immunosuppressive CD200 protein, array based expression profiling demonstrates significant increases in inflammation associated genes in androgenetic alopecia. Previous reports of CD200 loss leading to alopecia in mouse models suggest that AGA may be exacerbated or caused by CD200 downregulation in the human hair follicle stem cell compartment.

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Keratinocyte-specific expression of c-myb is dependent on growth and differentiation state

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Many genes with a role first defined in hematopoiesis have recently been shown to define stem cell niches and regulate essential cellular processes in the skin. In hematopoietic cells, c-myb expression is required for proliferation but prohibitive of differentiation. Viral transduction of truncated c-myb causes leukemia in chickens, and murine c-myb null mutation is lethal at e15, due to the failure of hematopoiesis. Here, we examined the expression pattern of the c-myb transcription factor in keratinocytes to determine if it might also regulate cell growth and differentiation in the skin. Upon release from serum starvation, absolute quantification of c-myb RNA levels demonstrated cell cycle-regulated expression in cultured human keratinocytes. Temporal studies of c-myb RNA levels during calcium-induced differentiation defined downregulation of c-myb expression as a late marker of keratinocyte differentiation. Both results are consistent with the expression pattern of c-myb in hematopoietic cell lines. Furthermore, immunohistochemistry on normal murine skin revealed that c-myb protein is present not only in the proliferating basal layer of the interfollicular epidermis, but also in the suprabasal layer. In the hair follicle, c-myb is expressed in a hair cycle-dependent manner. During late anagen, c-myb is expressed in the hair matrix and the outer root sheath and is absent from the inner root sheath, cortex and dermal papillae. This expression pattern extends into early catagen, but c-myb nuclear staining is not detected in telogen. The temporo-spatial expression pattern of c-myb suggests that it is a novel regulator of keratinocyte proliferation and differentiation in the epidermis and hair follicle. Conditional ablation in the epidermis is ongoing, and is expected to reveal *in vivo* functions as well as insights into downstream transcriptional target genes.

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BMP activity defines refractory and competent hair follicle populations during the propagation of regenerative waves

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Hair cycle can be regulated by molecular oscillation within the follicle (autonomously) or by dynamic changes in the inter-follicular environment (non-autonomously). While human hair follicles largely cycle autonomously, cycling of mouse hair follicles is affected by the status of adjacent follicles. Thus hair cycle progression can advance like a transverse wave on the surface of the skin. Observations of the dynamic hair cycle domains on the skin of wild type and Msx2-/- mice show that each hair cycle domain is made of an initiation site, a propagating wave, and boundaries. Analyses of patterns of wave propagation and boundary formation led us to identify a novel stage in telogen. In the first stage of telogen, hair follicles are refractory to the anagen spreading wave, thus forming a sharp domain boundary. Refractory telogen is characterized by multiple site expression of Bmp4/Bmp2 intra-follicularly and inter-follicularly. These Bmps sustain BMP reporter activity within follicles throughout refractory stage of telogen. Telogen follicles become competent to respond to anagen initiation signals after Bmp 2/4 loss. Hair plucking can induce anagen in competent follicles, but not refractory ones. In KRT14-NOG mice there is drastic shortening of the refractory telogen, and fast anagen re-entry. Individual follicle cycles faster and the wave dynamics of follicle populations are altered. Transplantation of a piece of KRT14-NOG skin to normal skin microenvironment results in restoration of Bmp signaling activity and refractory telogen. Our data identifies two new stages of telogen, refractory and competent. They are defined by the ability of telogen follicles to re-enter anagen, and based on the on-and-off switches of Bmp2/4 expression within and surround the follicle. This study presents a novel population approach to the study of hair cycling, and gives a more systematic and integrative explanation to the hair follicle behaviors observed in classical literatures.

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A role of CYLD in hair cycling mouse

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CYLD is a tumor suppressor gene mutated in familial cylindromatosis, an autosomal-dominant condition that predisposes to multiple skin tumors. The gene product, CYLD, is a deubiquitinating enzyme recently implicated in modulation of the NF-κB pathway. Recent report by Massoumi R. et al (2006) described that CYLD protein is prominently expressed in the inner root sheath of normal human scalp hair follicles. Thereupon we considered CYLD might have some effect in normal hair cycle thru NF-κB signaling. To investigate a role of CYLD during hair cycling, we adopted C57BL/6 hair cycling model mouse, and characterized changes in their expression by semiquantitative RT-PCR and western immunoblot and immunohistochemistry for protein. We found that CYLD at mRNA level increased from anagen stage to catagen stage, as well as protein level in immunobolt. In immunohistochemistry, CYLD protein is expressed in surrounding bulge areas. Moreover, we assessed whether CYLD regulate hair growth using in vitro hair culture. Recombinant CYLD inhibited hair growth. In conclusion, our results indicate that CYLD function on conversion from anagen stage to catagen stage.

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Reactive oxygen species (ROS)-mediated androgen-inducible TGF-β1 regulation in dermal papilla cells

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Intracellular signaling roles of reactive oxygen species (ROS) generated in response to androgen hormone in hair follicle dermal papilla cells are not well defined. To assess the correlation of ROS (hydrogen peroxide) known to increase in aging process to hair loss, the rat vibrissae dermal papilla cell line (DP-6) overexpressed with AR was investigated to evaluate the role of ROS on androgen-induced increase of TGF-β1 secretion. The AR stably-transfected DP-6 cells were incubated with synthetic androgen, R1881. Intracellular production of ROS markedly increased with R1881 treatment in DP-6 cells, which was measured by flow cytometry and laser scanning confocal microscopy. Moreover, androgen-inducible TGF-β1 was significantly suppressed by several species of ROS scavenger or inhibitors. Luciferase reporter assays showed suppression of TGF-β1 promoter signaling by ROS scavengers. In conclusion, our study shows for the first time that androgen-induced TGF-β1 regulation would be mediated by ROS and prevented by antioxidants or ROS inhibitors in hair follicle dermal papilla cells. We suggest that antioxidants could be one of the candidates to control androgen-mediated pattern hair loss.

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Global microarray analysis of the follicular and glandular types of epidermal differentiation: a role for BMP signaling in suppressing trans-differrentiation of the foot pad epidermis towards folliculogenesis

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During skin development, epithelium and mesenchyme interact with each other and give raise the formation of epidermis and a variety of appendages (hairs, nails, glands). To characterize global gene expression profiles associated with distinct types of epidermal differentiation (follicular versus glandular), epithelial buds of the hair follicles (HFs) and sweat glands (SWGs), as well as the matrix and epithelium of fully-developed HFs and SWGs were obtained from embryonic and adult mouse foot pads and ventral skin using laser capture microdissection. Epithelial buds of the HFs and SWGs showed expression of the bud-specific adhesion molecules, signaling/transcription components, as well as appendage-specific markers such as HF-specific keratins (Krt1-c29, Krt2-6g) or SWG-specific ion channels (Clcn3, Bcng-3a). Fully developed SWGs were characterized by strong downregulation of over 4000 genes compared to the epidermis and by upregulation of SWG-specific genes involved in regulation of the ion exchange/water metabolism, while HFs showed less prominent differences in gene expression versus the epidermis. In addition, SWG buds and fully developed SWGs showed strongly reduced expression of Bmp7 and Smad1/4 compared to the epidermis and HFs. Interestingly, K14-Noggin and Bmpr-Ib knockout mice showed ectopic formation of the HFs in foot pads accompanied by decreased expression of Engrailed 1, a potent repressor of the dorsal phenotype in ventral epidermis. These data suggest that HFs and SWGs are characterized by markedly different gene expression profiles, which may underlie fundamental differences in their functions, and that BMP signaling suppresses trans-differentiation of the foot pad epidermis towards folliculogenesis at least in part via stimulating the Engrailed 1 expression.