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Follicle stem cell compartments differ in responsiveness to oncogenic Hedgehog signaling

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Deregulated Hedgehog (Hh) pathway activation contributes to tumorigenesis in multiple tissues and organs. The exact cell populations in skin which are capable of responding to oncogenic Hh signals and give rise to tumors are not yet well defined. We have begun to address this question by driving expression of an activated form of GLI2 (GLI2*) in hair follicle stem cells of the bulge and secondary hair germ (SHG), a unique site of low-level, endogenous Hh signaling in resting (telogen) hair follicles. We generated mice combining Cre-inducible and doxycycline-regulated gene switch technologies to achieve exceptionally tight control of transgene expression in K15 positive hair follicle stem cells and their progeny. Activation of GLI2* expression for two weeks during the resting phase of the hair cycle yielded occasional microscopic tumors arising from hair follicles in dorsal skin. In striking contrast, induction of anagen by depilation yielded massive tumors affecting nearly all hair follicles during the same two-week time-frame, revealing the potent effect of stem cell activation on GLI2*-driven skin tumorigenesis. Examination of non-depilated mice four weeks after GLI2* induction revealed spontaneous macroscopic tumor development on paws, whisker pads, and tails. Histology of early lesions suggests that tumors are derived from the telogen SHG, even though GLI2* transgene was detected by immunostaining in cells in the bulge and SHG. Moreover, in situ hybridization revealed expression of Hh target genes Gli1 and Ptch1 in both stem cell compartments. These data reveal that hair follicle stem cells in distinct niches can respond differently to oncogenic Hh pathway activation. In contrast to 'primed' Hh-responsive stem cells located in the SHG, stem cells in the hair follicle bulge appear resistant to oncogenic Hh signaling during the resting phase of the hair cycle.

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The living wave: Self-organizing regenerative behavior of stem cells revealed in the cycling of large hair follicle populations

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Stem cells cycle through a number of activated states. Among large stem cell populations, stem cells may cycle randomly, synchronously or as a coordinated unit. Regenerative behavior of stem cells at this level has not been addressed. Using the experimentally tractable hair cycling paradigm, we develop the concept that stem cells are modulated by a combination of micro-environmental factors and macro-environmental factors to regulate regenerative behavior (Plikus MV, et al., 2008. Nature. 451:340-344). We now combine experimental and computational approaches to further study how these regenerative behaviors are coordinated. We found that activation of stem cells in individual follicles requires the integration of inputs from an intrinsic clock and extrinsic environmental signals. The wave patterns in the same animal of different physiological status, in transgenic mutants of the same species and in different species vary. They range from random (adult human scalp), slow waves (normal mouse), fast trasversing waves (K14 noggin mouse) to fractal-like waves (rabbit) and can be reset and restarted (pregnant mouse). We develop computer simulations using a Cellular Automata model in which each hair follicle is simulated as one automaton. We demonstrate that underlying self-organizing principles show wave patterns with stochastic, scale-invariant, and non-autonomous properties, conferring the hair regenerative system with robust adaptability. New experimental data show more cellular and molecular mechanisms involved in follicle coupling, and the experimental manipulation of parameters led to wave patterns predicted by model simulations. We developed a diagram which uses simple parameters to integrate the diverse wave patterns examined in mice, rabbits and humans.

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Generation of induced pluripotent stem cells by reprogramming human dermal papilla cells

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The dermal papilla (DP) is a unique adult stem cell population located at the base of the hair follicle, which we and others have shown to be capable of directed differentiation into other cell types such as muscle, bone and neurons. Due to its multipotency, we sought to analyze the efficiency of the DP, an easily accessible cell type, for reprogramming into induced pluripotent stem cells (iPS). We isolated and established cultures of DP and dermal skin fibroblasts (DF) from hair follicles taken from the occipital scalp region of human donors. At passage 3 in culture, both DP and DF express Klf4 and cMyc, two of the four iPS-inducing factors. However, unlike mouse dermal papilla, human cells do not express Sox2, *in vivo* or *in vitro*. We therefore introduced Oct4, cMyc, Klf4 and Sox2 into both DP and DF cultures using retroviral transduction. Colonies appeared at the same time, after 21 days in DP and DF cultures, and we were able to establish multiple iPS cell lines from the DP and DF cultures that contained all 4 transduced factors. We detected the expression of undifferentiated pluripotent markers in these cell lines, including SSEA3, Lin28 and nanog, using both RT-PCR and immunocytochemistry. Both human DP-iPS, and DF-iPS were able to differentiate into all three germ layers after embryoid body formation. We are currently analyzing the iPS forming efficiency of human DP and DF cultures using just two factors, Oct4 and Sox2 to complement the endogenous factors, Klf4 and cMyc, that are expressed in the DP. These studies provide insight into the accessibility and efficiency of DP cells as a source of iPS cells in humans, which could be useful in clinical applications due to their inherent plasticity and ease of access.

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Wnt/β-catenin- and depilation-induced activation of hair follicle stem cells requires chromatin regulatory protein Pygo2

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The Wnt/β-catenin signaling pathway is known to be important for hair follicle development and regeneration. Pygopus 2 (Pygo2), recently shown to associate with histone-binding and modifying activities, is a PHD finger-containing protein of the Pygopus family implicated as coactivators of β-catenin/TCF-dependent transcription. However, whether Pygo2 regulates Wnt/β-catenin signaling and hair follicle stem cell development and homeostasis remains to be elucidated. Here we show that Pygo2 is dynamically expressed in both the mesenchymal and epithelial components, including the stem cell compartment, of developing and adult hair follicles. To elucidate the *in vivo* function of Pygo2, we generated and analyzed skin epithelia-specific Pygo2 knockout mice (SSKO), as well as compound mutant mice that are deficient for Pygo2 and overexpressing an undegradable form of β-catenin (ΔN-β-catenin) mimicking activated Wnt signaling. Loss of epithelial Pygo2 does not affect normal hair follicle morphogenesis and cycling. In contrast, Pygo2 is required for ΔN-β-catenin overexpression-induced premature entry into the anagen phase of hair cycle by regulating hair germ cell activation. Furthermore, Pygo2 deficiency abolishes trichofolliculoma formation induced by ΔN-β-catenin overexpression. Our findings uncover an important role for Pygo2 in Wnt-signaling induced stem cell activation and tumorigenesis. Current experiments address the involvement of Pygo2 in depilation-induced hair follicle regeneration, and results of such study will be presented.

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Defining BMP functions in hair follicle stem cells homeostasis by conditional ablation or activation of BMP receptor 1A

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During the hair cycle, the behavior of hair follicle stem cells (hf SCs) is tightly governed by an intricate balance of signaling pathways which induce bouts of SC quiescence and activation, resulting in new hair. We have previously shown that bone morphogenetic protein (BMP) signaling is essential for hf SC homeostasis. However, precisely how BMP signaling functions in hf SCs at the molecular level still remains to be determined. Since the stem cell marker CD34, the only available marker for the isolation of hf SCs, is lost upon BMPR1A deletion we addressed this problem by employing, a Keratin 15 (K15)-driven system to simultaneously label and specifically target BMPR1A ablation within the hf SCs during the second postnatal telogen. We were able to isolate hf SCs marked by eYFP from control and BMPR1A knockout (KO) mice by fluorescence activated cell sorting (FACS) before morphological changes in hair cycle were observed. Additionally, we also utilize an inducible, gain of function system to investigate the consequences of active BMP signaling in hf SCs. Microarray analysis revealed that, following inhibition of BMP signaling in the bulge, BMPR1A KO hf SCs showed down-regulation of approximately 25% of common up-regulated hf SCs signature genes (Greco et al., 2009 and Blanpain et al., 2004). Furthermore, we also revealed that 30% of BMPR1A KO genes overlapped with the previously characterized hair germ signature (Greco et al., 2009). Here we employ both loss and gain of function approaches to address the role of BMP signaling in regulating hf SC homeostasis. Our findings suggest a model where balancing BMP signaling in hf SCs is essential in maintaining their homeostasis and its inhibition could be important to switch them from their quiescence to activation towards hair germ.

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Epigenetic organ regeneration: Development of a model using amputated mouse vibrissae follicles

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Mammalian organ regeneration is the ultimate goal of regenerative biology and medicine. The most dramatic example of organ replacement is known as 'epimorphic regeneration' and occurs mainly in amphibians, in which phenotypically committed cells are reprogrammed at the amputation plane toward a stem cell phenotype. The result is the complete regrowth of an amputated structure from an anatomically complex stump. Although mammals cannot regenerate amputated limbs, the regeneration of the hair follicle end bulb following amputation results in regeneration of the dermal papilla (DP) and the entire organ structure of the hair follicle, and can serve as a model to interrogate this process. To analyze the molecular events underlying this phenomenon, we adapted a mouse model, from previous work carried out in rat and human HFs. The mystacial pads of adult mice were incised to expose vibrissa follicles and end bulbs were excised. DP and end bulb regeneration was examined at several time points up to 21 days by histology and immunohistochemistry. We followed the formation of the new DP using molecular markers, which appears to be reprogrammed from the remaining dermal sheath after amputation. We observed pronounced cellular invasion of the mesenchyme around the amputated end bulb. This preceded the restoration of the DP which coincided with positive Prom-1 expression. Following expansion of epithelial cells down the hair shaft to below the level of the cut, regression and consolidation of a P-cadherin labeled basal epithelium above the newly formed basement membrane preceded differentiation of a new matrix. Similar to the molecular mechanisms involved in epimorphic regeneration in lower vertebrates, dedifferentiation of dermal sheath cells to progenitor/stem cells and their reprogramming to repopulate the DP may occur via the epigenetic reactivation of developmental regulatory genes that function during embryogenesis.