Lymphatic endothelium-derived chemokines CXCL10 and 11 promote expansion of human melanoma stem cells

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There is increasing evidence that many malignant tumors, including cutaneous melanoma, are driven by a subpopulation of multi-potent cells named cancer stem cells (CSCs) that have been implicated in primary tumorigenesis, metastasis, chemo-resistance and cancer recurrence. CSCs receive maintenance signals from a microenvironmental niche which includes vascular and lymphatic endothelial cells. The precise molecular mechanisms of these interactions are unclear. We aimed to investigate the interaction of CSCs and endothelial cells, and to identify molecules which mediate CSC maintenance. Co-culture of blood vascular or lymphatic endothelial cells (LECs) with melanoma stem cells revealed that LECs promoted the self-renewal of melanoma stem cells, as evidenced by increased sphere formation capacity. Using RT-PCR-based transcriptional profiling, we found that the chemokines CXCL9, CXCL10 and CXCL11 were strongly expressed by LECs - but not by other cells such as dermal fibroblasts - when co-cultured with CSCs. These chemokines act via the chemokine receptor CXCR3. Addition of recombinant CXCL10 or CXCL11 promoted sphere formation of melanoma stem cells, and sphere formation by melanoma stem cells was significantly inhibited by a CXCR3 neutralizing antibody. We found that the expression of CXCR3 was strongly increased in sphere-forming melanoma cells compared to melanoma cells in adherent cultures and that CXCR3 is also expressed by a subpopulation of tumor cells in human malignant melanomas of the skin. Similar results were found in a model of breast CSCs. Together, these findings indicate an important role of CXCR3 activation in the maintenance of CSCs and suggest that CXCR3 blockade, likely used in combination therapy, might represent a novel therapeutic strategy to specifically target melanoma stem cells.

Hypoxia levels are increased in photo-aged epidermis and might be associated with decreased regenerative potential of epidermal stem cells

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It has been proposed that human epidermis might be subjected to hypoxic conditions. We aimed to characterize the effects of hypoxia on the functional behavior of epidermal stem cells and to identify the most sensitive hypoxia marker genes in normal human keratinocytes. We also investigated the degree of hypoxia in intrinsically and photo-aged human skin. Normal human epidermal keratinocytes were cultured under normal or hypoxic conditions (≈ 2% O2) for 24 hours and the clonogenic potential of stem-like and transit amplifying keratinocytes prepared by time-dependent adhesion on type IV collagen was investigated. To identify the most sensitive hypoxia marker, normal human epidermal keratinocytes were cultured under hypoxic (≈ 1%, 2%, 5% O2) or normoxic (20% O2) conditions for 24 hours and gene expression levels of hypoxia marker genes, including GLUT-1, carbonic anhydrase-9 (CA9) and HIF-1-alpha were analyzed in stem-like and transit amplifying keratinocytes. Sections of young and aged, photo-protected and photo-exposed skin (n=10 each) were stained for hypoxia markers. The clonogenic potential of distinct keratinocyte populations was reduced by hypoxia, and the effects were more prominent in stem-like keratinocytes than in transit amplifying keratinocytes. The increased expression levels of the hypoxia markers CA 9 and GLUT-1 were inversely correlated with decreased oxygen levels, with CA 9 being the most sensitive gene to hypoxic changes. Stem-like keratinocytes were more sensitive to the changes of oxygen levels than transit amplifying cells. Immunohistochemical staining of skin sections for CA 9 showed that its expression is upregulated in photodamaged aged skin. Together, these findings indicate that increased hypoxia levels in photo-aged skin might be associated with a reduced regenerative potential of epidermal stem cells.

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β1-integrin signaling regulates maintenance and differentiation of adult human epithelial progenitor cells *in situ*

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β1-integrin connects epithelial cells with the extracellular matrix (ECM), thus regulating multiple cell functions. While β1-integrin-mediated signaling in murine epithelial stem cells is well-studied, its role in adult epithelial progenitor cells (ePCs) of human hair follicles (HFs) remains unknown. This was explored by β1-integrin silencing, by specific β1-integrin RGD motif-binding antibodies (ABs) and by pharmacological inhibition of integrin-linked kinase (ILK), a key component of the integrin-induced signaling cascade. β1-integrin knock-down in full-length human scalp HFs initially enhanced expression of the ePC markers keratin 15 (K15) and CD200, but reduced K15 and K6 expression as well as the proliferation of outer root sheath keratinocytes (ORSKs) subsequently. Embedding of HF epithelium into ECM, that mimics HF mesenchyme, significantly enhanced proliferation and emigration of ORSKs, while K15 and CD200 gene and protein expression were inhibited. Application of ECM-embedded β1-integrin-activating or -inhibiting ABs identified differentially responsive of adult human ePC subpopulations, located in distinct HF compartments. β1-integrin inhibitory AB significantly reduced β1-integrin expression and selectively enhanced the proliferation of bulge ePCs, while β1-stimulating AB decreased hair matrix keratinocyte apoptosis and enhanced CD71 immunoreactivity. Pharmacological ILK inhibition in ECM-embedded HF epithelium significantly reduced ORSK migration and proliferation, and induced massive ORSK apoptosis. These findings suggest that maintaining adult ePCs and their progeny in human HFs requires β1-integrin mediated signaling. These insights may be exploited for cell-based regenerative medicine strategies that employ human HF-derived ePCs.

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Lrig1 is involved in the inhibition of the hysalurosome in the pluripotent stem cells of the mouse epidermis

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The interaction between extracellular matrix and keratinocytes are crucial for epidermal homeostasis. We have previously shown that main hyaluronate (HA) receptor, CD44, is one of the essential components of hyalurosome located in keratinocyte filopodia, a cell structure both involved in epidermal growth factor (EGF) response and HA secretion. This hyalurosome, likely constituted of CD44, HA synthase 3 (HAS3), EGF receptor (EGFR), Heparin-binding EGF (HB-EGF) and anchored on F-actin modulates the epidermal homeostasis. Lrig1, a known marker of epidermal pluripotent stem cells in the infundibulum of mice epidermis, was also described as an inhibitor of EGFR signaling and may consequently be an inhibitor of the hyalurosome. In agreement with this hypothesis, we found in mice epidermis that there is no epidermal HA detected in the Lrig1 niche when performing double staining of HA and Lrig1. Similarly, no HA was detected in the human epidermal compartment of pluripotent cells. We further studied Lrig1 pattern in CD44KO mice, where the hyalurosome is defective, and observed an atrophic Lrig1 reservoir and a defective stem cell migration from hair germ in newborns. Activating, the hyalurosome with defined size HA fragments (HAFi) or inhibiting it with clobetasol propionate (CP) also had antagonist effects on the Lrig1 reservoir in mice. Finally, we confirmed functionally in vitro this Lrig1 role on the environment of human keratinocytes as inhibiting Lrig1 expression with SiRNA induced filopodia and HA secretion or in other words the hyalurosome, while overexpressing a GFP-tagged Lrig1 protein rather inhibited the hyalurosome. Our results suggest that the stem cell marker Lrig1 modulates its ECM environment via the hyalurosome, while inversely ECM or the cell niche also regulates stem cells.

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Lgr5+ and Lgr6+ epidermal stem cells in mouse and man

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Stem cells drive the life long renewal of the epidermis, and their long residency makes them prime targets for accumulating mutations from which cancer initiating cells may arise. Goal of this exploratory study was to establish whether newly identified epidermal stem cells (Lgr5+ and Lgr6+) are present in hairless mouse and human epidermis. Lgr5-EGFP and Lgr6-EGFP mouse strains (haired) were available and crossbred with hairless mice to investigate expression in skin samples by confocal microscopy. Expression in human skin compartments was assessed by QRT-PCR. Earlier reported expression of Lgr5 in hair follicles of haired mouse was confirmed, as well as a more scanty expression of Lgr6. However, in the skin of hairless mice we could not detect any Lgr5 expression, except in some deep-seated cysts. Lgr6 expression was observed in hair follicles in cross sections of hairless mouse skin (detected by anti-EGFP antibody), but also in clear clusters of cells in epidermal sheets (detected by EGFP fluorescence). QRT-PCR showed a clear expression of LGR5 in plucked human hairs, but hardly any LGR6 expression. In long-lived human skin equivalents (no hair follicles) expression was reversed, with a clear expression of LGR6 and no expression of LGR5. We were however unable to detect protein expression by using commercially available LGR5 and LGR6 antibodies (both in human and mouse skin). Like expression of the bulge stem cell marker CD34, we found the expression of Lgr5 to differ between haired and hairless mouse skin, most likely owing to the hair cycle defect in hairless mice. Lgr6+ cells were present in hairless mouse epidermis, and could be the major stem cells there. First indications are that the expression of LGR5 and LGR6 in human skin is very similar to that in mice.

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Unraveling the epithelial stem cell niche: miRs-103/107 maintain E-cadherin-mediated cell-cell contacts

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To understand the roles that microRNAs (miRNAs) play in regulating epithelial stem cells (SC) and their transient amplifying (TA) progeny, we compared miRNA expression patterns in the SC-enriched limbal epithelium (n=3) versus the TA cell-enriched corneal epithelium (n=3) using laser capture microdissection and qRT-PCR arrays (Exiqon). Throughout limbal and corneal epithelial development (PN day 1-28), miRs-103/107 were preferentially expressed in the SC-enriched limbal epithelium. Such limbal-preferred expression of miRs-103/107 was confirmed by in situ hybridization. To address the roles of these miRNAs in epithelial SC-enriched locations, primary cultures of human limbal keratinocytes (HLEKs) and foreskin keratinocytes (HEKs) were treated with antagomirs to either miR-103 or miR-107, leading to their down-regulation as assessed by Northern blot analysis. Within 3h of miR-103/107 antagomir treatment, cell-cell contacts were disrupted in association with a marked reduction in E-cadherin (E-cad) immunoreactivity at residual borders. Accordingly, the expression levels of E-cad and p120 catenin, a cytoplasmic binding partner essential for cell surface stabilization of E-cad, were decreased 2-fold. NEDD9, a non-catalytic scaffolding protein that negatively regulates localization of E-cad and promotes its degradation, was shown to be a direct target of miRs-103/107 using luciferase reporter assays in Hela cells and keratinocytes. Loss of miRs-103/107 in HLEKs and HEKs resulted in an increase in NEDD9 protein as well as cofilin, a major factor in actin turnover. Consistent with an increase in cofilin, phospho-cofilin (inactive) was decreased (2-fold) following antagomir treatment. Active cofilin is associated with adherens junction remodeling, which requires a high level of actin dynamics. The identification of miRs-103/107 as key regulators of E-cad-mediated epithelial cell-cell contacts, via the down-regulation of NEDD9 and cofilin, suggests a novel epigenetic mechanism that enables communication between stem cells and TA cells required for SC niche homeostasis.

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The spatio-temporal regulation of melanocyte stem cells determines their stress resistance

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Mammalian adult stem cells are long-lived immature cells with self-renewing capabilities for sustained tissue homeostasis. While many renewing stem cell systems (e.g. bone marrow, intestine and skin) have high regenerating capabilities and resistant with various kinds of stress, they are rather sensitive to ionizing radiation (IR) and the underlying cellular mechanisms remain largely unknown. We focused on hair graying to determine the radio-sensitivity of melanocyte stem cells (McSCs) which supply melanocytes for hair pigmentation. We succeeded in the stable visualization of both quiescent and activated McSCs located within the bulge-subbulge area of hair follicles using Dct-H2B-GFP transgenic mice and found that quiescent McSCs are radiosensitive but become highly radio-resistant upon their activation for cell cycle entry. McSCs located in the subbulge area (secondary hair germ) self-renew but mainly function as short-term stem cells to directly provide mature melanocytes for hair pigmentation. In contrast, McSCs located at the bulge area function as long-term stem cells alternating between two different states: an MCM2- quiescent radio-sensitive state and an MCM2+ activated radio-resistant state. The quiescent McSCs prematurely commit to differentiation after exposure to IR within the niche upon their cell cycle reentry. Collectively with the resistance of quiescent McSCs to chemotherapeutic drugs such as 5-FU, our data indicate that the stress-resistance of stem cell pools against different types of genomic stress is enabled by the differential spatio-temporal regulation of the stem cell pools and their differential activation states.

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Cellular and signaling mechanisms that regulate hair follicle stem cells by live imaging

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Stem cells and their niches are critical for tissue development and regeneration. Yet, we still lack knowledge of the sequential steps stem cells undertake to promote regeneration. Outstanding questions include 1) which signals control stem cell behaviors and 2) what is the functional role of the niche during physiological regeneration. We have recently established an in vivo strategy to visualize the components of the hair follicle stem cell niche, track them over time and manipulate them by two-photon microscopy in live mice. With these techniques, we have established that hair follicle growth relies on both spatial organization of cell divisions as well as directional cell movements within the stem cell/progeny compartments. In order to address which cells and which behaviors within the stem cell/progeny compartments are controlled by hair follicle regenerative signaling pathways, we have utilized genetic approaches to stabilize the Wnt pathway in a subset of cells coupled with live imaging techniques. Our data suggest that Wnt signaling controls specific stem cell/progeny behaviors depending on the position of these cells within their compartments. Furthermore, we previously devised a two-photon based cell type specific ablation approach to show that the niche is required for hair follicle tissue regeneration. We are currently applying this technique to determine if the effect of Wnt activation on specific subsets of cells relies on the presence of the niche. Collectively, our in vivo approach has led to the discovery of unpredicted cellular mechanisms of growth regulation, enabling us to precisely investigate functional requirements of stem cell niche components along with key signaling pathways during the process of physiological regeneration.

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MicroRNA-184 triggers epithelial stem cell differentiation by repressing the stem cell marker cytokeratin 15

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microRNAs (miRs) play a role in various physiological processes including embryogenesis, tissue regeneration and diseases. Many attempts have been made to identify factors that are specifically expressed by epidermal and limbal stem/progenitor cells related to tissue homeostasis. Here, we report that miR-184 plays a general role in regulating epithelial stem cells differentiation. In situ hybridization analysis revealed that miR-184 is not present in putative epidermal and corneal stem cell compartments that are hallmarked by cytokeratin 15 (K15), while its expression increased during epithelial differentiation in vivo and in vitro. We found that miR-184 directly represses K15 production and accelerates early cell differentiation. Moreover, we observed an increase of miR-184 expression coupled with a decrease in K15 production in pannus (abnormal corneal vascularization) of patients with limbal stem cell deficiency. Accordingly, a point mutation in the seed sequence of miR-184 has been recently found in corneal and lens diseases. Given that over expression of miR-184 in epithelial stem cell-enriched culture resulted in a dramatic decrease in clonogenic potential, our data indicate that miR-184 expression induces an escape from stemness state and suggest that abnormal expression of miR-184 may lead to a decline in stem cell pool. In addition, miR-184 expression displayed a major inhibitory effect on both the expression of stratification marker and the stratification ability of epithelial cells in organotypic culture. Given that miR-184 is not expressed in the most upper layers of the epidermis and corneal epithelium, we propose that the decrease in miR-184 allows terminal differentiation. Taken together, we propose that miR-184 is the guardian of epithelial progenitor cells; on one hand, its elevation promotes stem cell commitment into transit amplifying progenitors, while its further decline modulates terminal differentiation.

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The interfollicular epidermis is maintained by a hierarchy defined by stem cells within the non-cycling portion of hair follicles and committed progenitors

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The maintenance of the interfollicular epidermis (IFE) remains a controversial subject. Conflicting studies support the existence a stem cell hierarchy in the IFE or a committed progenitor model of random fate choice between self renewal and differentiation without hierarchy. To allow fate tracking of rare stem cell populations, we performed a high density multicolour labelling of all basal keratinocytes at P21 and followed their fate over time. Epidermal clones within the IFE of dorsal skin grew in size from 3 weeks to 6 months ($p<0.001$) in accordance with the committed progenitor. However, when considering the anatomical distribution of clones, those that were connected to hair follicles were significantly larger ($p<0.05$) and grew more steadily over time ($p<0.0001$). Further investigations showed that clones in areas of active hair cycling were larger compared to clones not attached to HF ($p<0.05$) and that proliferation in the HF infundibulum occurred mainly in anagen ($p<0.0001$) suggesting that this hair cycle phase promoted the growth of IFE clones via a HF contribution. A mathematical model where stem cells in HF generated CPs that further behaved with random fate decision was tested against a CP model with no stem cell in hair follicles. Only computer simulations of the model with stem cells, integrating 6 colour staining and animal growth over time, predicted clone sizes that closely matched our observations. In conclusions, our results support a hierarchical contribution of upper hair follicle stem cells to dorsal IFE by generating committed progenitors. Our findings and our proposed model reconcile recent and previous studies showing the importance of the upper portion of the HFs for IFE homeostasis in murine dorsal skin.

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Suppression of MicroRNA 135b increased the proliferative potential of normal human keratinocytes

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The epidermis is continually renewing tissue consisting of keratinocytes and these cells can be divided into stem cells, transit amplifying cells, and postmitotic differentiating cells. Cell fate of these cells is regulated by activation and repression of lineage specific genes. MicroRNAs (miRNAs) are a class of posttranslational regulators of gene expression with critical function in health and disease. Furthermore, morphogenesis of skin is governed by discrete sets of differentially expressed miRNAs. Thus, differentiation of epidermal stem cell can be regulated by miRNAs. Psoriasis is a hyperproliferative skin disorder and profiling study has identified numerous differentially regulated miRNAs. Especially, it is reported that stratified epidermis express miR-135b. Then, it was hypothesized that miR-135b is closely related with epidermal proliferation. The effect of miRNA-135b was tested in keratinocytes by transfection and proliferation assay. MiR-135b inhibitor transfected cells could proliferate well than mock-transfected cells. Western blotting disclosed that the levels of p63 (4A4) were higher in miR-135b inhibitor transfected cells than mock transfected cells. These findings suggest that inhibition of miR-135b can prolong the proliferative potential of epidermal cells. In addition, the effect of miR-135b was tested in three dimensional skin models. Results showed that expression of α6 integrin and β1 integrin were increased in miR-135b inhibitor models. In addition, the numbers of p63 and PCNA positive cells are much increased. These results are well consistent with those by western blot analysis. In conclusion, our results showed that suppression of miR-135b effectively prolong the proliferative potential of interfollicular epidermal stem cells in the skin.

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Efficient keratinocytes differentiation from transgene-free human induced pluripotent stem cell line: Implication for therapeutic application

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The incuced pluripotent stem cell (iPSC) lines from human origin have a great potential for therapeutics because customized cells or organs would be induced from those iPSC lines. The assessment of the residual reprogramming factors after generation of hiPSC lines is required but an ideal system has been lacking. Here, we have generated hiPSC lines from normal human dermal fibroblasts with piggyBac transposon bearing reprogramming transgenes followed by removal of the transposon by the transposase. In this situation, we can rigorously compare phenotypes of transgene-residual and –free hiPSCs. Although phenotype of transgene-residual and –free hiPSCs without differentiation was similar, keratinocytes from transgene-free but not –residual hiPSC lines seemed to be morphologically and functionally more similar to normal human keratinocytes. The differences could be explained by partial reactivation residual transgenes upon induction of keratinocytes differentiation. These results suggest that transgene-free hiPSC line should be chosen for therapeutic purpose.

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Desmoglein3 balances quiescence versus activation and repair in the hair follicle bulge stem cell niche

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In Pemphigus vulgaris (PV), autoantibodies against the adhesion molecule desmoglein1/3 (Dsg1/3) induce blisters in skin and mucous membranes. In addition, Dsg3 null mice and adult mice injected with the pathogenic monoclonal Dsg3 antibody AK23 exhibit decreased cohesion between hair follicle bulge stem cells (HFSC) and prominent hair loss during synchronized telogen. Alopecia was also described in human PV patients but is less prominent due to unsynchronized hair cycles. Here we addressed the consequences of altered Dsg3 adhesion for HFSC homeostasis and function. Upon AK23 injection into eight-week-old C57Bl/6J mice, adhesion of HFSC was disrupted between the inner K6pos bulge layer and the outer CD34pos cell layer. Time course studies revealed prominent SC activation after AK23 injection, characterized by progressive loss of label retaining cells in K5-tTA;tetO-H2BGFP mice and increased SC proliferation without apoptosis but with increased upward migration shown in Krt1-15-Cre/PGR1;R26R mice. Concomitant downregulation of the quiescence marker NFATc1, reduced CD34 expression and downregulation of the bulge "stemness signature" further indicated profound alterations in SC homeostasis. However, although clonal efficiency was transiently reduced, SC were not lost in the long-term. In contrast, AK23 injected mice were able to enter anagen upon hair plucking and keratinocytes isolated from AK23-treated CAG-EGFP mice were able to replenish all skin lineages in single cell grafting. Mechanistically, BMP2/4 mRNA levels decreased after AK23 injection similarly to actively proliferating SC during normal anagen induction. In contrast, Shh activation, a prerequisite for HF regeneration, was suppressed. In summary we demonstrate in a mouse model of PV that HFSC injury related to loss of Dsg3 adhesion results in SC activation and allows for niche repair by uncoupling proliferation from de novo hair follicle generation. These findings uncover protective measures of the injured HFSC niche to prevent the loss of multipotent SC.

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The cell polarity protein aPKCl controls mammalian epidermal stem cell dynamics and tissue homeostasis through the regulation of cell fate and division orientation

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The atypical protein kinase C (aPKC) is a key regulator of cell polarity and shown to regulate cell fate in lower organisms. However, the role of mammalian aPKC isoforms in the in vivo maintenance of tissue homeostasis and stem cell regulation is not known. Here we show that loss of aPKCl in a self-renewing epithelium, the epidermis, strongly disturbs epidermal homeostasis, stem cell maintenance, hair follicle cycling and lineage differentiation causing progressive morphological changes in different epidermal compartments, e.g. hair follicle and sebaceous glands. This is accompanied by a gradual loss of quiescent bulge stem cells and a temporary increase in different proliferating progenitors. This ultimately leads to loss of proliferative potential, stem cell exhaustion, increased differentiation, complete alopecia and premature aging. Unexpectedly, inactivation of aPKCl increased asymmetric divisions, known to promote differentiation, in all compartments of the epidermis, including the bulge stem cell compartment and the junctional zone, where more committed progenitors reside. Using Lrg5-CreER we are now tracing how loss of aPKCl in stem cells affects their dynamic behavior. Initial data suggest that inactivation of aPKCl in stem cells recapitulates the main phenotypes. Thus, aPKCl is crucial for homeostasis of self-renewing stratifying epithelia, regulation of differentiation and maintenance of epidermal bulge stem cells likely through its role in balancing symmetric and asymmetric division in different compartments of the epidermis.

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Deletion of mTOR inhibitor REDD1 protects CD34+ follicular epithelial stem cells and prevents development of steroid-induced cutaneous atrophy

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One of the major adverse effects of topical glucocorticoid therapy is cutaneous atrophy. To elucidate the mechanisms of the epidermal hypoplasia, we chronically treated mouse skin topically with the glucocorticoid fluocinolone acetonide (FA), which resulted in marked epidermal thinning, decreased keratinocyte size as well as number. In addition, we found that FA inhibited the expression of CD34, the keratinocyte stem cell marker. As a result, in mouse skin chronically treated with FA, the CD34+ stem cell population in the bulge of hair follicles was completely eliminated. We observed that FA strongly induced expression of regulated in development and DNA damage response (REDD1) mRNA and protein in mouse and human epidermis. REDD1 is a nutritional sensor and stress-inducible inhibitor of mTOR complex 1, a master regulator of protein synthesis, cell growth and survival. Accordingly, we noted a marked decrease in phosphorylation of mTOR at S2448 in the epidermis after exposure to FA. Overexpression of REDD1 in 3PC non-transformed mouse keratinocytes resulted in inhibition of mTOR activity, cell growth and decreased cell size. We used REDD1 KO mice to further corroborate the role of the REDD1/mTOR axis in skin. CD34+ follicular epithelial cells in REDD1 KO mice remained intact during chronic FA treatment, and consequently, REDD1 KO animals were much more resistant to FA-induced epidermal atrophy. Expression profiling of REDD1 KO skin revealed that the induction of many primary GR target genes was diminished, suggestive that REDD1 is critically important for optimal glucocorticoid response. Overall, our findings reveal a novel mechanism of glucocorticoid-induced skin atrophy and indicate an important role of negative cross-talk between GR and mTOR signaling in the regulation of bulge stem cells and skin maintenance. In addition, our findings are relevant to the development of safer treatment regiments with topical glucocorticoids when they are used in combination with mTOR modulators.

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Defining label retaining cells (LRCs) from nails as putative new skin stem cells

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Adult stem cells are remarkably flexible in their ability to reconstitute different tissue lineages leading to great interest in assessing their therapeutic potential. Hair follicle stem cells and recently sweat glands have been demonstrated to possess slow cycling LRCs. Although these stem cells have been successfully isolated and characterized using a H2B-GFP label retaining system, still very little is known about other stem cells in different skin appendages like nails and if they share the same LRCs characteristic. So far, there has been no report describing successful isolation and characterization of nail LRCs. In this study, we used the H2B-GFP system to detect and isolate infrequently dividing cells in nails. LRCs were found underneath the proximal fold of the nail and 3-dimensional (3D) visualization was performed. Immunofluorescence staining with basal layer markers localized these LRCs to the basal layer of the nail. Furthermore, these live nail LRCs were isolated by FACS which allowed us to perform gene expression study. Microdissected strips of cells enriched in H2B-GFP marked nail LRCs were transplanted underneath the proximal fold of an immunocompromised mouse. About 2 weeks after transplantation, H2B-GFP labeled cells contributing to the nail structure were observed. Finally, we demonstrate that BMP signaling is required for proper nail morphogenesis and differentiation similar to the hair follicle skin appendage. Supported by NIH, NIAMS, R03 AR061028-01.

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Generation of induced pluripotent stem cells from revertant mosaic keratinocytes: A novel strategy for natural gene therapy for epidermolysis bullosa

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Revertant mosaicism (RM) is a naturally occurring phenomenon involving spontaneous correction of a pathogenic mutation in a somatic cell. It has been observed in several genetic diseases, including epidermolysis bullosa (EB), a group of inherited skin disorders characterized by blistering and scarring. The therapeutic potential of induced pluripotent stem cells (iPSCs) has recently been demonstrated from patients with various diseases. Despite efforts toward developing gene correction of iPSCs including homologous recombination, there remain technical difficulties including low efficiency and excisional footprints after gene correction. Here, we developed a novel strategy of "natural gene therapy" by generating patient specific revertant mosaic iPSCs (RM-iPSCs) in a subset of EB patients with revertant mosaic patches of skin. First, RM skin was identified in a clinically and immunohistochemically healthy area in a junctional EB patient with compound heterozygous COL17A1 mutations. RM-iPSCs were generated from keratinocytes cultured from this region and confirmed the authenticity of RM-iPSCs by RT-PCR and immunostaining for stem cell markers. Sequence analysis of RM-iPSCs showed the paternal COL17A1 mutation in exon 51 (p.R1226X), but the maternal mutation in exon 18 (c.1706delA) was corrected by mitotic gene conversion. Importantly, keratinocytes derived from RM-iPSCs expressed type XVII collagen (COL17) by immunostaining. We also generated in vitro 3D skin equivalents using keratinocytes derived from RM-iPSCs, in which COL17 was strongly expressed in the basal layer. We have demonstrated the feasibility of utilizing revertant keratinocytes as a source of spontaneously corrected cells for developing RM-iPSC-based therapeutic approaches in EB, for both topical and systemic administration.

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Postnatal hair follicle differentiation but not stem cell quiescence can be maintained by Smad8 alone after Smad1 and Smad5 ablation

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Hair follicles (HFs) offer a highly informative model system to study the regulatory mechanisms of stem cell homeostasis and differentiation. Bone Morphogenetic Protein (BMP) signaling is key player in both of these processes. However, the specific role of canonical or non-canonical pathways of BMP signaling in these processes remains unresolved. Here, we conditionally targeted the specific ablation of downstream components of canonical BMP signaling, Smad1 and Smad5 during hair morphogenesis and postnatal cycling in mouse skin. Deletion of Smad1 and Smad5 (dKO) during morphogenesis resulted in neonatal lethality and severe phenotypic abnormalities including open-eyes, lack of visible whiskers, paw deformities, delayed palatal fusion and perturbed tongue differentiation. Interestingly, during morphogenesis distinct activation patterns of phospho-Smad (pSmad) were detected within the interfollicular epidermis (IFE) and HFs. Engraftment of dKO skin revealed retarded hair morphogenesis and failure to differentiate into visible hair shafts. Moreover, formation of the pre-bulge and bulge reservoir for quiescent hair follicle Stem Cells (hfSCs) was precluded in dKO HFs which persisted in a prolonged anagen-like state. Surprisingly, in adult postnatal HFs pSmad8 expression was not longer restricted to IFE, but was also present in dKO bulge hfSCs and matrix progenitors. However, pSmad8 expression in dKO hfSCs did not prevent precocious anagen activation, in contrast, pSmad8 activity alone in matrix progenitors could sustain postnatal HFs differentiation. Together, our data demonstrate a pivotal role for canonical BMP signaling in distinct skin compartments during morphogenesis and postnatal hair cycling and differentiation. Supported by NIH, NIAMS, R01-AR061552.

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Genome organizer Satb1 regulates differentiation-associated silencing of the Nanog locus by modulating its nuclear positioning in human embryonic stem cells

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During development, gene expression programmes in multi-potent stem cells are re-organized at several levels including higher-order chromatin remodelling and nuclear compartmentalization of the genomic loci and transcription machinery. We have analyzed changes in three-dimensional organization of the Nanog gene locus during differentiation of the human embryonic stem cell line H14 in culture. Stem cell differentiation was monitored by downregulation of expression of stem cell markers SSEA-3 and TRA-1-60 in cultured cells. By microarray and RT-PCR analyses, expression of the Nanog gene was strongly decreased in differentiating SSEA-3 negative cells, compared to SSEA-3 positive stem cells. These changes were accompanied by significant changes in the intranuclear Nanog gene positioning, as determined by 3D-FISH analyses and confocal microscopy. In differentiating cells, the Nanog gene locus was located more peripherally relatively to the chromosome territory 12, compared to undifferentiated stem cells, in which the Nanog locus was located more internally. Genome organizer Satb1 protein showed higher expression in differentiating cells, compared to undifferentiated stem cells. Furthermore, shRNA-mediated Satb1 knockdown resulted in the retention of the Nanog gene in the nuclear interior, associated with increase of its transcriptional activity, compared to controls. Thus, these data demonstrate that Satb1 regulates differentiation-associated silencing and relocation of the Nanog locus from the nuclear interior towards nuclear periphery and suggest that higher-order chromatin remodelling is a key regulatory mechanism required for a proper control of cell differentiation process in multi-potent stem cells.

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Special AT-rich binding protein Satb1 controls re-organization of lineage-specific differentiation programs during keratinocyte reprogramming towards the induced pluripotent state

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Reprogramming of adult somatic cells towards the induced pluripotent state (iPS) is associated with marked changes in the genome organization and gene expression programs. Genome organiser and special AT-rich binding protein Satb1 plays an important role in the control of higher-order chromatin remodelling in a number of lineage-specific gene loci including keratinocyte-specific genes, which is required for maintenance of their transcriptionally active status. Here, we show that during reprogramming of primary epidermal keratinocytes isolated from transgenic mice expressing the Dox-inducible cassette of four mouse pluripotency genes (Oct4, Sox2, Klf4 and c-myc) under Col1a1 promoter, the expressions of Satb1 and keratinocyte-specific genes that constitute keratin type I and type II loci, as well as Epidermal Differentiation Complex locus, are markedly downregulated, while expression of the pluripotency gene Nanog is upregulated. ChIP analyses revealed Oct4 binding to regulatory regions of the Satb1 gene indicating that pluripotency factors might directly repress Satb1 expression during reprogramming. Furthermore, treatment of keratinocytes with lentiviruses expressing Satb1 shRNA significantly accelerated appearance of Nanog+ cells and iPS colonies compared to the controls. Acceleration of iPS colony formation under shRNA-mediated Satb1 knockdown was accompanied by more rapid decrease in the expression of keratinocyte-specific genes, compared to controls. Thus, these data demonstrate that Oct4-mediated downregulation of Satb1 serves as an essential step in switching-off keratinocyte-specific gene expression programs during their reprogramming towards the induced pluripotent state.

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The touch dome defines a new niche in the skin epidermis

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In mammalian skin, Merkel cells are mechanoreceptor cells that mediate the perception of light touch stimuli. Recent evidence indicates that mature Merkel cells descend from the proliferative layer of skin epidermis; however, the identification and characterization of the stem cell niche for Merkel cell homeostasis as well as the kinetics of mature Merkel cell turnover have not been reported. To address these issues, we generated a BAC transgenic mouse model that expresses a tamoxifen-inducible Cre recombinase (CreERT2) to selectively targets these touch dome keratinocytes in the interfollicular epidermis, based on their exclusive expression of cytokeratin Krt17. Using this mouse model in combination with other mutant alleles, we provide the first genetic evidence for maintenance of mature Merkel cells during homeostasis by Krt17+ keratinocytes located in touch domes of hairy skin and in the tips of the rete ridges of glabrous skin. Lineage tracing analysis also indicated that the entire pool of mature Merkel cells is turned over every 7-8 weeks in adult epidermis. Finally, selective genetic ablation of Krt17+ touch dome keratinocytes was not recovered by intact stem cell pools outside of the touch dome, and also demonstrated that touch dome keratinocytes, and not mature Merkel cells, are primarily responsible for maintaining innervation of the Merkel cell-neurite complex. Collectively, these findings identify the touch dome as a novel, self-replenishing stem cell niche responsible for Merkel cell turnover during homeostasis, which may have important implications for age-related decline in Merkel cell numbers and pathological skin conditions such as Merkel cell carcinoma.

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Wnt7b regulates hair follicle stem cell homeostasis and hair follicle cycling

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Hair follicles facilitate the study of stem cells (SCs) behavior because SCs in progressive activation stages, ordered within the follicle architecture, are capable of cyclic regeneration. Previously, we revealed how the underlying molecular mechanisms of Bone morphogenetic protein (BMP) signaling governs the homeostasis of hair follicle stem cells (hfSCs) in vivo. We demonstrated that BMP-inactivated hfSCs exhibit molecular profiles resembling those of hair germs, but retain multipotentiality after transplantation in vivo. In addition, BMP-inhibited hfSCs display altered BMP and Wnt pathways, exhibiting up-regulation of Wnt7a, Wnt7b ligands and only one Wnt receptor Frizzled (Fzd) 10. We demonstrated that Wnt7a works intrinsically to regulate ligand–receptor-dependent cross talk between BMP and Wnt signaling in hfSCs homeostasis. These results were recently published and highlight a previously unknown intra-stem cell antagonistic competition, between BMP and Wnt signaling, to balance stem cell activity. In this study, we targeted Wnt7b to determine its role in hfSCs regulation as well as hair morphogenesis and cycling. Since we observed an up-regulation of Wnt7b in hfSCs following BMP inhibition, to determine the significance of Wnt7b in stem cell homeostasis, we specifically deleted Wnt7b in hfSCs in vivo. Wnt7b KO HFs displayed a significant delay in telogen-anagen transition and displayed perturbed differentiation with a shortened hair cycle growth phase. Thus, these findings indicate that Wnt7b is an important intrinsic regulator of hfSCs homeostasis and hair follicle cycling. These findings support our previous discoveries and the existence of one more hierarchical layer regulating stem cell homeostasis in addition to the stem cell–dermal papilla and the hair follicle–adipocyte interaction layers. Supported by NIH, NIAMS, R01-AR061552.

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"Tonic" cannabinoid receptor 1 signaling regulates human hair follicle epithelial progenitor cell survival *in situ*

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The endocannabinoid system (ECS) is now appreciated as a major player in human skin physiology. Previously, we had shown that human hair follicles (HFs) not only contain key endocannabinoids and express functional cannabinoid receptor 1 (CB1), but also that CB1 stimulation inhibits hair matrix (HM) keratinocyte proliferation and induces their apoptosis (Telek et al. FASEB J 2007) and prevents excessive mast cell activation and maturation (Sugawara et al. JACI 2012). However, the role of CB1-mediated signaling in human epithelial progenitor cell (EPC) biology remains unknown. Here, we show in organ-cultured human HFs that a CB1-selective agonist significantly increases the number and proliferation of intrafollicular keratin 15+ EPC, while CB1 gene silencing or a CB1 antagonist have opposite effects and stimulate EPC apoptosis. This is mediated at least in part *via* the MEK/ERK pathway. Intriguingly, bulge CB1 expression *in situ* is reduced in patients with scarring alopecia (lichen planopilaris), whose EPCs suffer irreversible damage. Thus, in striking contrast to HM keratinocytes, whose growth is inhibited by CB1-signaling, human EPC requires continuous CB1-stimulation for survival. CB1-stimulation also promotes reepithelialization in wounded, organ-cultured human skin, possibly *via* stimulating EPC emigration into the regenerating epidermis. Therefore, CB1-stimulation by endogenous ligands has diametrically opposed effects on closely related epithelial cell populations in distinct states of differentiation within the same tissue compartment of human skin. "Tonic" CB1-mediated signaling can be essential for human skin EPC function and survival *in situ*.