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Loss of nuclear membrane protein SUN2 leads to transient alopecia and hair follicle structure defects

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The goal of this study is to investigate the role of nuclear membrane proteins of the SUN (Sad1p, Unc-84) family in hair follicle development. Cytoskeletal proteins, cell adhesion proteins, and nucleoskeletal proteins (e.g. keratins, cadherins, lamins, respectively) are known to play critical roles in cutaneous and hair follicle integrity, development and homeostasis. These molecules can regulate cell behavior by scaffolding signaling molecules and allowing changes in physical tension throughout the cell to guide organelle position and shape and modulate cell-cell adhesion and migration during skin development and wound healing. The role of the nuclear membrane proteins that link the cytoskeleton and nucleoskeleton (i.e. SUN proteins and nesprins) has not been previously reported in skin. Here we show that transgenic knockout (KO) of SUN2 leads to dramatically altered hair follicle structure and alopecia during murine anagen I. However, the follicles recover normal structure and the alopecia resolves during the second hair cycle. Ultrastructural study of the SUN2 KO hair follicle bulbs demonstrates widen intercellular spaces and altered number of desmosomes between the cells of the inner root sheath (IRS) and the outer root sheath (ORS). Microscopically, the SUN2 KO follicle morphology ranges from distortion to dramatic fragmentation in the suprabulbar region extending to the infundibulum of the follicle. Lineage tracing studies show delayed progenitor cell upward migration in follicles lacking SUN2 suggesting defective hair outgrowth. Upregulation of SUN1 expression during anagen II leads to functional hair growth recovery, suggesting compensation by SUN isoforms. This study demonstrates an essential role for SUN proteins in hair follicle structural integrity and growth, and suggests an important role of physical force transduction between cell surface and nucleus in hair structure and function.

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Apical cues induce asymmetric division of epidermal basal cells through PIP3-PDK1 pathway

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Tissues with different cell types are generated from common progenitors through asymmetric cell division (ACD). The differentiation of the epidermis begins with the basal progenitor cells, and ACD in a perpendicular orientation relative to the basement membrane promotes cell differentiation and organizes the stratified epithelium. However, both the molecular cues that trigger organization of the apical complex during ACD and the signaling pathways that drive activation of apical complex components remain to be defined. Phosphoinositide dependent kinase 1 (PDK1) is a serine/threonine kinase, and its activity depends on phosphatidyl inositol 3-kinase (PI-3 kinase) signaling pathways from growth factor receptors and adhesion molecules. We generated mice lacking PDK1 in keratinocytes (PDK1CKO), which display severe defects in epithelial differentiation and stratification resulting in perinatal lethality. Thus, PDK1 is essential for the development of stratified epidermis. The aim of this study is to address the mechanism of PDK1-dependent development of epidermis and identify its cues. ACD in basal cells in E17.5 epidermis from PDK1CKO was significantly decreased compared to wild-type epidermis ($P = 0.0003$) while symmetric cell division was not affected. Cell-cell contact stimuli induce production of PIP3 at the apical side of basal cells. In wild-type epidermis PDK1 asymmetrically localizes to the apical side of dividing basal cells ($P = 1.5E-13$). PDK1 recruits and activates atypical protein kinase C (aPKC), and nucleates formation of the apical complex. PDK1CKO keratinocytes do not undergo calcium-induced activation of aPKC or differentiation in vitro or in vivo. Thus PDK1 regulates both activation and spatial organization of key signaling pathways in response to apical cues acting on basal progenitor cells in developing epidermis.

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β-catenin activation regulates epithelial growth non-cell autonomously within the hair follicle stem cell niche

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Wnt/β-catenin signaling is critical for tissue regeneration and cancer. However, it is unclear how Wnt/β-catenin controls stem cell behaviors to coordinate tissue growth. Using in vivo time lapse imaging, we show that genetic activation of β-catenin specifically within hair follicle stem cells generates new organized axes of hair growth through oriented cell divisions and coordinated cellular displacement. Additionally, β-catenin activation is sufficient to induce growth within the stem cell pool independently of mesenchymal niche signals previously shown to be required for hair follicle regeneration. Further, we demonstrate that wild type cells contribute to induced hair growths and that β-catenin mutant cells act non-cell autonomously to activate Wnt signaling within wild type cells via Wnt ligand upregulation. Collectively, this study demonstrates a novel mechanism by which Wnt/β-catenin signaling controls stem cell-dependent tissue growth non–cell autonomously and carries broader implications toward understanding mechanisms of tumor growth and regeneration.

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Association between Interleukin 18 polymorphisms and alopecia areata in Koreans

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Alopecia areata(AA) is a common, chronic and inflammatory disease. It is considered to be a tissue-specific autoimmune disease. The exact mechanism causing to the AA is not fully elucidated. But human leukocyte antigen(HLA) molecules and various inflammatory proteins, such as interleukin-1(IL1) and autoimmune regulator, are considered to be involved in the pathogenesis of AA. Interleukin 18(interferon-gamma-inducing factor) (IL18) is an important proinflammatory cytokine belonging to the IL-1 family that is produced by a wide range of immune cells, such as monocytes, activated macrophages and Kupffer cells. This study investigated whether IL18 single nucleotide polymorphisms(SNPs) are associated with the susceptibility to AA in Korean population. We tested 2 SNPs(one promoter SNP : rs187238, -137G/C and exonic SNP rs549908, Ser35Ser) associated with the development of AA, and also assessed the relationship between 2 SNPs of IL18 and the clinicopathologic features using a case-control approach. 233 AA patients and 243 healthy control subjects were recruited. Rs187238 and rs549908 in IL18 showed significant differences between AA and control subjects. In conclusion, our data suggest that the IL18 may be a risk factor for susceptibility to AA in Korean population. And, we assessed the relationship between IL18 SNPs and the clinicopathologic features (onset age, family history, type of AA, involvement of nail, and involvement of body hair) of AA. However, we did not observe significant association with IL18.

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Effect of miR-203 silencing in cultured human keratinocytes and reconstructed epidermis

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Epidermis undergoes a continuous renewal through the proliferation and differentiation of keratinocytes anchored to the dermo-epidermal junction. This multistep process requires a complex and coordinated program of gene expression and inhibition. Recent findings suggest that microRNAs, which are post-transcriptional regulators of gene expression, play an essential role in the renewal of epidermis, particularly by controling the shift between basal proliferation of keratinocytes and suprabasal differentiation. Long non-coding RNAs (LncRNAs) also participate in the structure of the epidermis. Bioinformatics is a powerful tool allowing the modelization of the interactions between expressed genes, microRNAs and LncRNAs. We established a bioinformatics model of keratinocyte differentiation in order to understand the interactions between the key players in the regulation of keratinocyte differentiation. MicroRNA-203 (miR-203) is a master regulator of the "exit from stemness" by controlling ΔNp63 expression. With the aim of establishing a reconstructed epidermal test model to study the role of particular microRNAs on epidermal differentiation, we used an anti-miR approach and down-regulated miR-203 expression in keratinocytes, and studied the effect of the silencing in cultured cells, and after epidermal reconstruction.

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Keratin 79 marks a novel population of migratory keratinocytes that mediates the formation and regeneration of the hair follicle canal / infundibulum

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The hair follicle is comprised of multiple layers of concentric epithelial cells surrounding a central hair shaft. The infundibulum (INF) is located at the distal-most aspect of the hair canal and is poorly characterized. Our studies reveal that the INF is multi-layered, biochemically distinct, and maintained by Lrig1-expressing stem cells in the isthmus, but not by bulge stem cells. In particular, we identify a novel keratin, Keratin 79 (K79), as a marker of early differentiating keratinocytes that line the suprabasal layers of the INF. During morphogenesis, K79+ cells are specified in hair germs and migrate distally out into the epidermis. This process is recapitulated during anagen I, when K79+ cells are specified within the reactivated secondary hair germ and stream along the anterior club hair bulge. MMP-9 is localized to migratory streams during development, suggesting that proteolysis may weaken cellular junctions prior to the formation of the hair follicle lumen. We further show that maintenance of the INF relies on Notch signaling, and that disruption of Notch in Lrig1+ stem cells induces cyst formation. These cysts resemble those observed in human acne comedones. In summary, we have identified a novel marker of a previously uncharacterized population of cells, whose outward movements likely mediate formation of the hair follicle canal.

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Interest of adenosine triphosphate in chronic hair loss treatment

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Chronic hair loss in men and in women is caused by shortening of anagen duration, the active growing phase of hair growth cycle. As anagen phase duration reduced, the frequency of the hair growth cycles accelerates and the capacity of hair follicles (HFs) to generate new hair shafts rapidly runs out. Adenosine triphosphate (ATP) is a universal endogenous molecule known to constitute an essential energy source for cells. The aim of this study was to evaluate the effect of ATP on hair follicles survival ex vivo and on anagen key factors expression in HFs. Microdissected human scalp HFs were incubated with ATP for 6 or 48 hours (gene expression analysis using qRT-PCR) and up to 28 days (morphometrically assessment of HFs apoptosis). Human dermal papilla cells (DPCs) were incubated with ATP for 24 hours and Keratinocyte Growth Factor (KGF) expression level was measured in cell culture supernatants using ELISA analysis. We found that ATP inhibited hair bulb degeneration with 25% of survival improvement after 28 days of culture. qRT-PCR analysis revealed that ATP increased KGF and fibronectin mRNA levels in HFs up to respectively 251% and 183% compared to control HFs. KGF production by cultured DPCs was also increased by ATP with a mean of 37% of stimulation. In conclusion, ATP delayed spontaneous HFs regression and therefore could prolonged anagen phase duration. KGF being a key growth factor for HFs development during anagen phase, one of the mechanisms that could explain this result is the enhancement of KGF production by DPCs after ATP incubation. Fibronectin, as a major glycoprotein of HFs dermal sheath, is required for HFs anchorage and enhances DPCs aggregation. The results of this study indicate that ATP is a good candidate for chronic hair loss treatment as it would help to maintain HFs in anagen phase and would also reinforce HFs anchorage.

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3D sebocyte spheroids induce terminal differentiation markers and lipogenesis offering an improved model for drug testing

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Acne and related skin disorders arise predominately as a result of dysfunction of the pilosebaceous unit and include abnormal sebum production and inflammation. Although there has been significant progress in defining the causative factors and molecular mechanisms involved in acne and related oily/dry skin disorders, there remains a need for safe and efficacious treatments for these diseases. This will require the development of well characterized and validated human cell based models for basic research that are compatible with high throughput systems for drug screening. Using adult human primary sebocytes and novel immortalized sebocyte cell lines we have developed a high throughput 3D human sebocyte model suitable for in vitro screening. Unlike traditional monolayer cultures human sebocytes grown in 3D have a higher expression of terminal differentiation markers (MC5R, MUC1/EMA,), genes involved in lipid synthesis (FASN, SREBP1, PPARG, LCN2) and lower levels of the early sebocyte marker KRT7. In addition increases in lipogenic genes were observed following treatment with the LXR agonist T0901317 and insulin indicating that 3D sebocytes are functionally responsive to known regulators of lipogenesis. Taken together these results suggest that culturing sebocytes in 3D platforms may provide a more biologically relevant model for sebocyte differentiation and for identifying novel compounds for acne and other skin related disorders.

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ATP-dependent chromatin remodeler Brg1 regulates the establishment of the topological lineage-specific interactome for the Loricrin gene in keratinocytes during epidermal development

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During development, execution of distinct cell differentiation programs is accompanied by establishment of specific topological arrangements between the genes and their distal regulatory elements in the nucleus. Here, we show that during epidermal development, ATP-dependent chromatin remodeler Brg1, serving as a direct target for p63 transcription factor, regulates relocation of the Epidermal Differentiation Complex (EDC) locus and its constituent Loricrin gene towards the nuclear interior into a compartment enriched in SC35-positive speckles. ChIP-seq data show that Brg1 binds to distinct domains within the EDC locus and regulates expression of Loricrin gene in keratinocytes. Chromatin conformation capture (4C) analyses of epidermal keratinocytes reveal that nuclear neighbourhood of the Loricrin gene is enriched by actively transcribed genes located on chromosome 3 (cis-interactions), as well as on other chromosomes (trans-interactions). Loricrin is not expressed in thymocytes, and its 4C interactomes in thymocytes and keratinocytes showed only 8% of common genes, demonstrating a marked lineage-specificity in the nuclear neighbourhood for Loricrin gene underlying its active (keratinocytes) or inactive (thymocytes) status. ChIP-seq data revealed enrichment of the 4C Loricirin interactome in keratinocytes by the enhancer-specific histone modifications, as well as by the Satb1, Ctcf and cohesin-binding sites that partially overlap with Brg1-binding sites. Thus, Brg1 plays an essential role in remodelling of the higher-order chromatin structure of the EDC locus and, together with other chromatin regulators, contributes to the establishment of lineage-specific nuclear neighbourhood for Loricrin gene required for is efficient expression in epidermal keratinocytes during skin development and differentiation.

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Transcriptional modulation by a hair growth promoting formulation in inter-follicular- and hair follicle-associated human keratinocytes: Comparative analysis by genome-wide expression profiling

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In vitro cultured human keratinocytes are an important tool to investigate general aspects of skin physiology. In the epidermal skin layer the normal human epidermal keratinocytes (NHEK) generate the multi-layered skin barrier by a distinct differentiation process. Within the hair follicle the hair follicle associated keratinocytes (HHFK) build up the major part of the follicle and form as result of another differentiation program the hair-shaft. While general aspects of keratinocyte physiology can be investigated in both cell-types the analyses of hair-specific processes require the use of HHFKs. We established cultivation of keratinocytes in a minimal growth medium (MGM) as an in vitro model for undersupplied keratinocytes, mimicking the situation of diminished hair growth for analyzing the effect of a hair growth promoting formulation, Panto(vi)gar. To investigate the genes that are modulated during Panto(vi)gar treatment we performed an Agilent whole genome array expression analysis using HHFK and NHEK cultivated either in a minimal growth medium (MGM) alone or supplemented with a Panto(vi)gar in vitro correlate (P-IC). From the approximately 1700 P-IC modulated sequences 479 sequences are detected in both cell types whereas 373 sequences appeared to be affected only in HHFK. In accordance with the P-IC induced cellular phenotype, genes that are involved in cell cycle, proliferation and metabolic processes are modulated by P-IC in NHEK and HHFK. Furthermore P-IC appears to regulate genes associated with cell death, extracellular matrix, stress responses and hair follicle physiology. Further analysis of the established pool of P-IC regulated genes in NHEK and HHFK allows us to differentiate in more detail between positive effects of Panto(vi)gar on keratinocytes in general and on hair follicle associated keratinocytes in particular.

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Cell interaction and growth in a 3-D heterotypic hair-follicle spheroid model mimicking diffuse hair-loss

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Cells in a three-dimensional (3-D) environment receive characteristic biophysical and biochemical signals that are essential for obtaining organ specific cell functions. Associated processes like differentiation or morphogenesis can be simulated in vitro by using 3-D culture models. We were interested in a 3-D culture system mimicking more closely the in vivo situation of the human hair follicle. Such a model would help to understand the mode of action of hair-growth promoting formulations as well as to test drug candidates for their ability to improve hair growth. We used an in vitro heterotypic hair follicle spheroid model comprising of human dermal papilla cells (DP) and human hair-follicle associated keratinocytes (FK). Using automated live cell fluorescence microscopy we analyzed the specific migration pattern of DP and FK during the initial spheroid formation process. After spheroid formation the cell type specific distribution in the 3-D spheroid model was examined by light sheet-based fluorescence microscopy. To address the beneficial effect of the hair growth promoting formulation Panto(vi)gar, we mimicked the situation of diffuse hair loss in vitro by a specific minimal growth medium (MGM) allowing to analyze the positive properties of Panto(vi)gar ingredients by MGM supplementation. The formation process of the heterotypic spheroid was impaired in MGM whereas the MGM supplementation with a Panto(vi)gar in vitro correlate restored the spheroid formation process. Taken together, we investigated 3-D heterotypic spheroids as a simple hair follicle model system and analyzed the impact of the hair-growth formulation Panto(vi)gar on spheroid formation in real-time by conventional and light sheet-based fluorescence microscopy. Further studies will help to gain a better understanding of the mode of action of well-established hair growth promoting formulations like Panto(vi)gar and enable us to identify new compounds for hair growth promotion.

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Integration of microRNA-mRNAeExpression profiles reveals regulatory networks controlling hair cycle progression

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Hair cycle-associated tissue remodeling is governed by tightly regulated gene expression programs. MicroRNAs (miRs) act as one of the essential components of the gene expression regulatory machinery. To define co-regulatory miR-mRNA networks, inversely correlated changes in the miRs (n=618) and mRNA (n=1895) expression profiles during hair cycle in mouse skin were integrated with sequence-based miR target prediction algorithm Targetscan. Overall, 2673 putative connections between 318 miRs and 871 mRNAs have been identified. Functional analysis of the network signatures highlighted enrichment of the genes involved in cell cycle, apoptosis, transcriptional regulation, chromatin remodeling, Wnt, EGF and FGF signaling pathways. Network analyses revealed that the majority of miRs showed higher expression levels in telogen versus anagen skin, while their mRNA targets were predominantly expressed in anagen. Investigation of the network topology revealed its scale-free structure, in which miR-mRNA interactomes were organized into subnetworks of highly connected miR and mRNA hubs. The miR-29 family was identified as a major component of the miR modular structure and showed the strongest associations with 150 predicted target genes during the hair cycle. qPCR confirmed that miR-29s are highly expressed in telogen versus anagen skin. Validation of miR-29 sub-networks using transfection of primary keratinocytes with miR-29a mimic confirmed that miR-29a significantly decreases expression of epigenetic regulators such as Cbx2, Bahd1, Dcp2, Dip2b, Tet2, and Tet3. Interestingly, Tet3 enzyme involved in DNA hydroxymethylation is highly expressed in anagen skin, and has also been identified as the most connected gene in the miR-mRNA hair cycle network, which can be targeted by 34 miRs. Thus, this study provides an important platform for further analyses of the miR-mRNA co-regulatory networks during the hair cycle, and identified the miR-29 family as one of the important regulators of epigenetic machinery contributing to the hair cycle progression.