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Anti-aging effects of retinoid hydroxypinacolone retinoate on skin models

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Tretinoin, also known as all-*trans* retinoic acid (ATRA), is well-known for its anti-aging effects on skin. However, skin irritation, photochemical instability, and concerns about toxicity have hindered the use of ATRA in cosmetic products. Therefore, it is desirable to find new molecules that have increased retinoic acidlike activity without the negative side effects. Hydroxypinacolone retinoate (HPR) is a cosmetic grade ester of ATRA that has been shown to have innate retinoic acid activity without causing skin irritation. Here, we compared levels of gene transcription by HPR, ATRA, retinol (ROL), retinaldehyde (RAL), and retinyl palmitate (RP) in DNA using a retinoic acid response element (RARE) reporter assay. In addition, we compared the anti-aging properties of HPR to ATRA by testing the effects on collagen levels and skin irritation in organotypic skin models. Skin models were treated for 5 days with HPR and ATRA, basal media was collected for ELISA analysis, and skins were stained with Massons trichrome (for collagen). RARE assay results showed that HPR had greater levels of gene transcription than ROL, RAL, and RP at the same concentrations, and was less cytotoxic to cells at a 10 times higher concentration; however, HPR did not achieve gene transcription levels of ATRA. Skins treated with HPR significantly increased pro-collagen production as compared with untreated control skins, and was comparable to ATRA. Histological staining of skins for collagen corroborated these results, with the highest dose of HPR out-performing ATRA. IL-1α ELISA analysis showed that HPR did not induce more (or less) inflammatory response than either ATRA or the vehicle control. Together these data suggest that HPR is an effective alternative to ATRA and other less potent retinoids in the treatment of aging skin without the detrimental side effects.

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Efficacy of topical tofacitinib in promoting hair growth in non-scarring alopecia

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Tofacitinib is a janus kinase 3 (JAK3) inhibitor that promotes hair growth; however, the efficacy and mechanism of this effect are not yet understood. This study aimed to evaluate the efficacy and mechanism of topical tofacitinib on hair growth in mice. Eight-week-old male C57BL/6 mice were divided equally into four groups and treated topically with tofacitinib, minoxidil, or vehicle once daily for 21 days. Weekly photographs were taken to determine the area and rate of hair growth, and tissue samples were collected for histopathological evaluation. mRNA and protein expression of anagen-maintaining growth factors, including vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1), were determined via RT-PCR and ELISA, respectively. Tofacitinib-treated mice exhibited more hair regrowth than either minoxidil-treated or control mice did between days 7 and 21 ($P<0.05$). Topical tofacitinib also promoted more rapid hair growth rate than topical minoxidil or control did ($P<0.001$). Histopathology showed a distinct increase in the number of hair follicles, mostly in the anagen phase, in the tofacitinib-treated group. Hair follicles in the minoxidil- and vehicle-treated groups were more often classified as catagen and anagen. VEGF mRNA and protein expression in the tofacitinib-treated group was significantly greater than those in the other groups ($P<0.05$). IGF-1 mRNA expression was not upregulated in tofacitinib-treated mice. Topical tofacitinib is effective in promoting hair growth, and the possible mechanism involves increased VEGF levels and lowered inflammation. This study will help develop a new therapeutic option for non-scarring alopecia.

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Nrf2 activation enhances the healing of cutaneous wounds through the activation of hair follicle stem cells

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The transcription factor Nrf2 is a key regulator of the cellular stress response through the regulation of antioxidant enzymes, cytoprotective proteins and various transporters. Strong genetic activation of Nrf2 in keratinocytes leads to a pilosebaceous phenotype, characterized by hyperplasia of the sebaceous glands and infundibula, hyperkeratosis, and seborrhea, implicating Nrf2 in several other key processes in the epidermis. Here we show that Nrf2 activation in keratinocytes promotes the proliferation and expansion of the junctional zone (JZ) and upper isthmus (UI) hair follicle stem cells, while bulge stem cells are only mildly affected. This was observed to be functionally important for wound repair, since Nrf2 activation also led to the faster closure of excisional wounds through the formation of a longer and larger wound epithelium. This however, was neither due to changes in proliferation or apoptosis of keratinocytes in the wound epithelium, nor their migration as measured *in vitro*. Instead, an increased number and proliferation of follicular JZ and UI stem cells were observed peripheral to the wound. An enhancement of wound healing in Nrf2 transgenic mice following tape stripping of the epidermis revealed a functional link between the Nrf2-mediated expansion of hair follicles stem cells and accelerated re-epithelialization. The effect of Nrf2 activation on JZ and UI stem cells resulted from the Nrf2-mediated up-regulation of the EGF family member Epigen and subsequent EGF receptor activation. These results suggest pharmacological Nrf2 activation as a promising approach for the enhancement of wound healing through expansion of hair follicle stem cell pools.

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***Fzd2* controls multiple aspects of epidermal development through distinct signaling mechanisms**

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Wnt ligands bind to Frizzled (FZD) receptors to activate both β-catenin-dependent (canonical) and β-catenin-independent (non-canonical) signaling. Non-canonical signaling includes planar cell polarity (PCP) and Wnt/calcium pathways. In the skin, canonical Wnt signaling is required for hair follicle development and regenerative growth, while PCP signaling controls hair follicle orientation, and in vivo roles for Wnt/calcium signaling have not been described. Limited information is available regarding the functions of individual FZD receptors in these processes. Here, using constitutive and inducible epidermal deletion mouse models, we show that the FZD family member FZD2 is required for hair follicle placode formation and normal postnatal hair growth, suggesting that it mediates canonical signaling in hair follicles. In addition, we find that early deletion of *Fzd2* in embryonic epidermis unexpectedly causes defective stratification, cornification and barrier formation, a phenotype that has not been described previously upon loss of either canonical or PCP signaling. *Fzd2* mutant epidermis displays a striking reduction in expression of the desmosomal component plakophilin 1 (PKP1). Loss of function mutations of *PKP1* in humans and mice cause ectodermal dysplasia and skin fragility, phenotypes that overlap with those observed in epidermal *Fzd2* mutants but do not include defective epidermal stratification. These data indicate that FZD2 plays multiple roles in skin epithelial development and homeostasis: mediating canonical Wnt signaling in hair follicles; controlling expression of PKP1; and a novel early function in regulating epidermal stratification, cornification and barrier formation independent of PKP1.

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Advanced age impairs self-renewal and biases fate choice of hair follicle dermal stem cells

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The mammalian hair follicle (HF) regeneration cycle is uniquely dependent on the timely activation of its resident epithelial and mesenchymal progenitor cells. In aged mice, HF regeneration is severely impaired and many HFs progressively degenerate- reminiscent of hair loss in the aging human population. We hypothesized that age-related HF dysfunction is associated with the loss of endogenous HF dermal stem cell (hfDSC) function. To test this, we performed long-term lineage tracing of hfDSCs over 24 months. We observed significant declines in both the number of hfDSCs and their differentiated mesenchymal progeny with advanced age. Additionally, aged hfDSCs consistently displayed propensities to acquire a definitive dermal papilla fate a phenomenon rarely observed in young hfDSCs. We then performed *in vivo* clonal analysis of hfDSCs in 2mo vs. 18mo old αSMACreER^{T2}:ROSA^{Confetti} mice. This revealed that aged hfDSCs exhibit significant impairment in self-renewal capacity and preferentially differentiate into dermal sheath cells. Finally, hfDSCs were prospectively isolated from 2mo vs. 18mo old mice and assessed for *in vitro* colony formation. Aged hfDSCs showed a marked reduction in colony number and size, confirming their diminished self-renewal ability and highlighting the intrinsic nature of hfDSC dysfunction. Our findings suggest the ability of hfDSCs to properly maintain the aging HF mesenchyme is compromised with advanced age, ultimately contributing to progressive hair loss. Ongoing work is examining the transcriptional changes in 2mo vs. 18mo hfDSCs to identify key genes underlying age-related hfDSC dysfunction.

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An Integrated model of alopecia areata biomarkers highlights both Th1/Th2 upregulation, with stronger correlations between Th2 activation and disease severity

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Alopecia Areata is a non-scarring hair loss disease that occurs in ~2% of the population. Various studies attempted to profile the cytokine pathways involved in the pathogenesis of AA, with recent shift in focus from only Th1/IFN-γ axis activation to also include Th2 skewing. Our study evaluated both serum cytokines and scalp expressions of lesional and non-lesional cytokines in moderate-to-severe AA patients (n=30) compared to age-matched controls (n=10), to define an integrated model of circulating and tissue biomarkers that also correlate with AA severity (Severity of Alopecia Tool/SALT). We found significantly elevated levels of T-cell activation (IL-15), Th1/IFN (IFN-γ, CCL2, CCL3, CXCL10), and Th2 (IL-13, CCL13, CCL17, CCL22, CCL26) related markers in AA serum ($P<0.05$). Only the T-cell activation (IL-15) and Th2 related (CCL11) significantly correlated with clinical severity/SALT ($P<0.05$). Serum levels of key Th2 markers (IL-13, r=0.54; CCL17 r=0.69; $P<0.05$) were highly correlated with respective mRNA expressions in nonlesional scalp. Scalp mRNAs of Th2-related markers (CCL13, r= 0.71, P=0.0031; CCL18, r=0.51, P=0.051) and IL-12/23p40 (r=0.53, P=0.041) had significant positive correlations, while hair keratins were negatively correlated with AA severity. Our data supports AA as a systemic disease, with immune activation already present in nonlesional skin. This study expands the current paradigm on AA cytokine profile in skin and serum, showing Th1 and Th2 marker up-regulations in both compartments, but stronger correlations between Th2-related measures and disease severity.