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Periodic patterning stem cells and induction of skin appendages: p-ERK-dependent mesenchymal condensation is coupled with Turing mechanism to convert stripes to spots

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A fundamental problem in biology is to understand the mechanisms driving periodic pattern formation of skin appendages. Recent works show that Turing model is involved in the initial induction of hair and feather primordia (Maini, PK, Baker, RE, Chuong, CM., 2006. The Turing model comes of molecular age. Science. 314:1397-1398). Reaction diffusion mechanism converts stem cells from the basal homogeneous status to chemical patterns, reflecting activator and inhibitor activities. How the Turing pattern is then translated to discrete cellular patterns remains unknown. Using the feather model, here we show ERK activity-dependent mesenchymal cell chemotaxis toward initial peaks is essential for completing pattern formation. Adding ERK inhibitors produced a spectrum of placode patterns, ranging from broad stripes (early addition) to spots (late addition). Stripe regions exhibit broadly Shh-positive epithelium and NCAM-positive mesenchyme, implying a status intermediate to the basal and feather bud states. We propose that periodic patterning occurs in a restrictive mode; first a reaction-diffusion mechanism converts the homogeneous stem cell field into patterned regions of pre-primordia. Then cells respond to FGF and other signals by moving toward pre-primordia and also shape the patterning process, until large discrete dermal condensations are established. We developed a mathematical model integrating experimental data and simulating this patterning process.

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Human hair follicles are direct targets for thyroid hormones: involvement in anagen prolongation, hair matrix proliferation, hair pigmentation and metabolism

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Both insufficient and excess levels of thyroid hormones (THs:T3, T4) can result in altered hair/skin structure and function (e.g. effluvium). However, it is still unclear whether THs exert any direct effects on human hair follicles (HFs). Studying microdissected, organ-cultured human scalp HFs, we show here that T4 up-regulates the proliferation of hair matrix keratinocytes, while their apoptosis is downregulated by THs. The latter also prolong anagen duration *in vitro* possibly due to the down-regulation of the key anagen inhibitory factor, TGFβ2. Human HFs transcribe deiodinases and should thus be capable of converting T4 to T3 and of inactivating T3. Immunoreactivity (IR) for the thyroid hormone-responsive keratins CK6 and CK14 is significantly modulated by THs (CK6: enhanced, CK14: down-regulated) and significantly stimulate intrafollicular melanin synthesis. Since THs can stimulate erythropoietin (EPO) expression and human scalp HFs are an extrarenal EPO source, we also assessed whether THs increase intrafollicular EPO expression. Both erythropoietin mRNA expression and immunreactivity were significantly increased by THs. Furthermore, THs upregulate intraepithelial HF IR for a key respiratory chain enzyme, cytochrome- c- oxidase I, suggesting the possible involvement of thyroid hormones in HF metabolism. We identify further thyroid hormone target in the HF connective tissue sheath: thyroid hormones activate (increase number and degranulation) mast cells. Thus, human HFs are direct targets of thyroid hormone, which modulate multiple different HF functions, ranging from HF cycling to pigmentation.

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Reversal of epidermal aging by NF-κB blockade

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Aging is associated with subtle yet widespread alterations in gene expression, but the mechanisms controlling these alterations are not well understood. By identifying combinations of cis-regulatory elements that are predictive of expression patterns in individuals of different ages, we predicted that the transcription factor NF-κB is a major regulator of gene expression changes associated with aging in many human and murine tissues. Inducible genetic blockade of NF-κB in the epidermis of chronologically aged mice substantially reverted the global gene expression program and tissue characteristics to those of young mice. We further show that a classic mouse mutant of premature aging is due to excessive NF-κB activation. These results suggest that NF-κB activity is required for enforcing many characteristics of aging, especially in skin.

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Wnt modulates the differentiation status of interfollicular epidermal melanocytes arising from melanocyte stem cells in the hair follicle bulge after wounding or UVB irradiation

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Melanocyte stem cells residing in the hair follicle bulge area are ultimately responsible for pigmentation of the hair. However, it is unknown whether bulge melanocytes contribute to pigmentation of the interfollicular epidermis. To study whether melanocyte stem cells located in the bulge migrate to the interfollicular epidermis after injury or UVB irradiation, we utilized Trp2-lacZ transgenic reporter mice that express LacZ in melanocytes. We found that melanocytes originally located in the hair follicle bulge migrated to the infundibulum and then epidermis where they remained for at least 4 months. The bulge- derived melanocytes in the epidermis were well-differentiated and produced melanin. Using transgenic mice (tet-O-dkk1/K5-rtTA) in which Dkk1, a potent inhibitor of Wnt signaling, was over-expressed in the basal epidermis, we found that Wnt signaling regulated melanocyte stem cell differentiation during their migration to the epidermis. Dkk1 over- expression in the epidermis during reepithelialization resulted in a dramatic down- regulation of melanocyte differentiation markers such as tyrosinase, although it did not significantly change the number of melanocytes in the wound epidermis. Cessation of Dkk1 induction after reepithelialization permitted hair follicle neogenesis and the bulge-derived melanocytes then exhibited the capacity to regenerate the de novo pigmentary unit in the new hair follicles. These findings indicate that melanocyte stem cells residing in the hair follicle bulge have the ability to give rise to durable epidermal melanocytes and that wnt signaling modulates melanocyte stem cell differentiation status.

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Laminin-511 is an early epithelial message promoting dermal papilla development and function during early hair morphogenesis

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Hair morphogenesis takes place through reciprocal epithelial and mesenchymal signaling, however the mechanisms controlling signal exchange are poorly understood. Laminins are extracellular matrix proteins that play critical roles in both adhesion and signaling. We have previously shown that laminin-511 is required for hair morphogenesis and here we elucidate the underlying mechanism. Although we showed that endogenous laminin-511 in hair morphogenesis was produced by epithelium, surprisingly we found that it was dermal papillae (DP), in the absence of laminin-511 (in lama5-/- skin), which displayed several key developmental defects by E16.5 including a failure to maintain expression of the key morphogen noggin. This maintenance was critical as it coincided with an abrupt arrest of hair follicle downgrowth ultimately resulting in complete hair involution of lama5-/- skin. Exogenous introduction of purified laminin-511, noggin or the downstream noggin effector Sonic hedgehog (Shh), was sufficient to restore hair follicle development in lama5-/- skin. Laminin-511 with a deleted heparin binding domain supported noggin production and hair follicle development after application to lama5-/- skin, however laminin-511 lacking the integrin binding site or wild type laminin-111 showed no hair promoting effects. Previous studies have shown that Shh signaling requires microtubule based signaling organelles termed primary cilia. Interestingly, mutant lama5-/- DP showed a striking decrease in primary cilia length and structure, both *in vitro* and *in vivo*. Laminin-511, but not laminin-111, restored primary cilia formation in lama5-/- DP and dramatically triggered noggin expression in E16.5 mouse dermal mesenchyme in an Shh and PDGF dependent manner. These studies show that epithelial-derived laminin-511 is a critical early signal which directs DP ciliary function and maintenance as a requirement for hair follicle development.

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Canonical Wnt signaling activity is required for Hedgehog pathway-induced epithelial bud and follicular hamartoma development

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Aberrant Hedgehog (Hh) signaling plays a critical role in the development of several undifferentiated tumors arising in skin, including basal cell carcinoma (BCC) and basaloid follicular hamartoma. Although these tumors are considered to be of follicular origin, the identity and location (follicular versus interfollicular epidermis) of tumor progenitor cells have not been firmly established. Superficial BCCs frequently exhibit buds of proliferating basal cells growing directly from the epidermis into the underlying dermis, strongly resembling embryonic hair germs which are initiated by canonical Wnt/β-catenin signaling. We investigated the potential involvement of canonical Wnt signaling in epithelial bud development in early human BCCs and a mouse model of deregulated Hh signaling, driven by an activated Hh pathway effector, SMO*. Similar to embryonic hair germs, human BCC buds exhibited elevated levels of nuclear and cytoplasmic β-catenin, a marker of canonical Wnt signaling, and expressed early hair follicle lineage markers, including Sox9, K17, and CDP. Analogous changes were detected in BCC-like epithelial buds and follicular hamartomas arising in SMO* mice, along with expression of multiple *Wnt* genes, *Tcf4*, the Wnt target gene *Axin2*, and non-phosphorylated (active) β-catenin. Furthermore, by selectively blocking canonical Wnt signaling with Dkk1, we dramatically inhibited epithelial bud and hamartoma development in SMO* mice, despite continued, uncontrolled Hh signaling. Our findings reveal an essential function for Wnt signaling downstream of pathologic Hh signaling in skin, help elucidate how 'follicular' tumors can arise from interfollicular epidermis, and provide a molecular explanation for the morphological and biochemical similarity between BCC buds and hair germs.