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Review

Wnt signaling in skin organogenesis

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Abbreviations: AKT, thymoma in AKR mouse; AP1, activating protein 1; APC, adenomotous polyposis coli; β-cat, β-catenin; cad, cadherin; DAG, Diacyl glyceraldehyde; DiI, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; DKK, Dikkopf; Dvl, disheveled; Fz, frizzled; GSK-3β, glycogen synthase kinase-3 β; HR, hairless protein; *Hr*, *hairless* gene; Hex, haematopoietically expressed homeobox; Igfbp, insulin-like growth factor binding protein; JNK, C-Jun N-terminal kinase; Lef, lymphocyte enhancer factor; LRP, low density lipoprotein receptor-related protein; MITF, microthalmia-associated transcription factor; mmp, matrix metalloproteinase; PKC, protein kinase C; PP2A, protein phosphatase 2A; Prh, proline-rich homeodomain; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SFRPs, soluble frizzled related proteins; Tcf, T cell factor; TOPGAL, β-catenin inducible β-galactosidase plasmid; Ub, polyubiquitin; WIF, Wnt inhibitory factor; WISE, Wnt modulator in surface ectoderm; Wnt, wingless/Int

Key words: morphogenesis, hair, feathers, tracts, epithelium-mesenchyme interactions, Wnt signaling pathway

While serving as the interface between an organism and its environment, the skin also can elaborate a wide range of skin appendages to service specific purposes in a region-specific fashion. As in other organs, Wnt signaling plays a key role in regulating the proliferation, differentiation and motility of skin cells during their morphogenesis. Here I will review some of the recent work that has been done on skin organogenesis. I will cover dermis formation, the development of skin appendages, cycling of appendages in the adult, stem cell regulation, patterning, orientation, regional specificity and modulation by sex hormone nuclear receptors. I will also cover their roles in wound healing, hair regeneration and skin related diseases. It appears that Wnt signaling plays essential but distinct roles in different hierarchical levels of morphogenesis and organogenesis. Many of these areas have not yet been fully explored but are certainly promising areas of future research.

The integument forms the interface between an organism and its environment.[1,2] As such it protects against dehydration, infection, temperature extremes, etc while providing a means for display, camouflage and other functions.[3] The skin can elaborate remarkable structural diversity producing specialized functions in a region-specific fashion to provide organisms with a selective advantage. For example, the development of feathers led to the acquisition of flight in birds and the formation of mammary glands enabled mammals to nurse their young.[4] The advantage of these evolutionary developments can be seen by the number of birds and mammals present today.

Skin appendages, such as skin, hairs, feathers, scales, glands and teeth grow from the epithelium as a result of epithelial-mesenchymal interactions,[5] largely in response to common molecular signals with slight variations in their placement and timing during tissue morphogenesis.[6] Theoretically, stem cells are totipotent and progressively can be guided toward their specific fates by exposure to specific regulatory signals. The juxtaposition of molecular signals or lack thereof may have a tremendous impact on cell fate decisions. Hence, the difference between skin appendages is due to the topological arrangement of the epithelia during developmental processes. These are presumably regulated by adhesion molecules whose expression is controlled by signaling molecules as well as by physical constraints.

Hairs and feathers are attractive model systems for experimental research because of their ability for seasonal or periodic renewal. Obviously not all hairs or feathers are replaced at one time or birds would lose all of their feathers at once and fall from the sky in mid-flight; rather hairs and feathers are replaced over a period of time in a wave-like pattern.[7] Yet this cycling behavior enables thousands of entirely new organs to be regenerated again and again throughout these animal's lives. Hairs and feathers demonstrate an incredible diversity of forms arising in different locations over the body surface. For instance, hairs on the scalp, face and body differ in size, coarseness, color, etc. This regional specificity indicates that in each cycle skin stem cells are directed to form distinct structures through a series of molecular and cellular interactions.

Wnt Signaling Pathways

One of the critical signaling pathways that play an important role in skin organogenesis and morphogenesis is the Wnt signaling pathway. In fact, Wnts play decisive roles at multiple steps during the development of the skin. Wnts are a large family of secreted signaling molecules with homology to the fly Wingless protein. There are 19 known family members in humans and mice. Signaling is mediated by binding to transmembrane receptors, the Frizzled (Fz) proteins.[8] The Fz proteins have ten family members. There are also a large number of secreted Wnt inhibitory molecules (SFRPs, WIF, DKKs).[9] The consequences of Wnt signaling are proliferation, differentiation,

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cell migration, changes in cell polarity and cell adhesion. The best characterized of the Wnt signaling pathways is termed the canonical pathway. In the absence of Wnt signaling β-catenin is phosphorylated by a degradation complex containing APC, axin and GSK-3β, marking it for proteasomal degradation.[10] When Wnts bind to the Fz receptors and to low density lipoprotein receptor-related protein (LRP) 5/6,[11] intracellular Dishevelled inhibits the β-catenin degradation complex.[12] This allows β-catenin to accumulate within the cell, translocate to the nucleus and induce transcription in conjunction with its transcriptional coactivators, lymphocyte enhancer factors (Lefs) and/or T-Cell Factors (Tcfs).[13] Many downstream target genes have been identified and found to play roles in proliferation, differentiation, tissue remodeling, etc. β-catenin also collects at the intracellular side of the plasma membrane where it may regulate cadherin-mediated calcium-dependent adhesion.[14] It also binds to the actin cytoskeleton via fascin and may help to coordinate cell motility via the actin cytoskeleton[15] (Fig. 1).

The noncanonical Wnt signaling pathways function independently of β-catenin. One of the noncanonical Wnt signaling pathways can also induce planar cell polarity convergent extension in vertebrates (analogous to Drosophila). Here Wnts signaling through Dishevelled stimulate rho GTPase to stimulate C-Jun N-terminal kinase (JNK) which regulates the alignment of cytoskeletal elements[16] (Fig. 1). There is some evidence that suggests that specific Wnts and Fzs favor the canonical versus the planar cell polarity/convergent extension pathways.[17] However, the downstream signaling response may in fact be due to the cellular context in which the signaling takes place. In this paper I will review the functions of Wnts during skin organogenesis primarily using data derived from chicken and mouse models.

Formation of the Dorsal Dermis

The dermis has to be specified prior to skin formation. Molecular signals from neighboring tissues help to pattern the somites. The identification of these cells' origin largely has been carried out in the developing chick. In short, the dorsal dermis arises from the dermomyotome and the ventral dermis arises from the somatopleure,[18] while dermis within the head and neck come from cranial neural crest cells.[19]

How does the dermomyotome arise? The ventral half of the somite gives rise to the sclerotome, the source of the vertebral column and ribs. The dorsal region of the somite can become the dermomyotome, the source of muscle and dermis. Wnt signaling helps to distinguish the fate of cells within the dorsal, medial and lateral dermomyotome. Wnt-1 and Wnt-3a secreted from the neural tube induce the expression of Wnt-11 in the dorsomedial lip of the dermomyotome. Wnt-11 then inhibits the epithelial expression of Wnt-6 but maintains the epithelial character of the dermatomal dorsomedial lip and enables the expansion of the medial dermomyotome.[20,21] This promotes the further compartmentalization of the somites. Wnts-4, -6 and -7a from the surface ectoderm establish the lateral dermomyotome.[20] Blocking Wnt signaling by expressing ectopic DKK produced a thin dermis[22] demonstrating the functional importance of Wnt signaling in dorsal dermis formation.

Using quail-chick transplantation experiments, cells from the medial dermomyotome were found exclusively to form dorsal dermis.[23] More recently, using DiI cell tracing, cells from the medial and lateral dermomyotome migrated to the dorsal dermis.[24] These conflicting results suggest that the origin of the dorsal dermis still may require further study. The formation of the compartments within the somite and subsequent dermis formation is nicely reviewed in greater detail by Scaal and Christ.[25]

The migrating cells of the dermomyotome express N-cadherin but its expression is subsequently downregulated in the dermis and sustained in the myotome. Electroporatation of a truncated form of cadherin, lacking most of its extracellular binding domain, disrupted epithelial cell adhesion fostering a delamination of the basement membrane and a loss of myogenic cells; rather, all dermomyotomal cells migrated to the dermis.[26] Expression of a form of N-cadherin lacking its β-catenin binding domain showed a similar phenotype.[26] A diagram summarizing the role of Wnt signaling in dermis formation prior to hair and feather induction is shown in Figure 2.

Skin Appendage Formation

Feathers and hairs are each induced through a series of epithelial-mesenchymal interactions. They begin to form when the skin consists of a single layer of epithelium overlying a thin dermis. Several molecular and cellular aspects of their development are similar. Here I will review the formation of the first hair and feather follicles. After the initial structures are formed they undergo cycles of loss and regrowth which will be discussed in a later section.

Hairs

Hairs first form as an epithelial placode (thickening) overlying a dermal condensation at about embryonic day 14.5 (E14.5) in response to a series of epithelial-mesenchymal interactions. In hairs the epithelium invaginates into the underlying dermis and joins the dermal condensation to form the hair germ at E15.5. The epithelium continues to grow into the mesenchyme and surrounds the dermal papilla, a specialized mesenchymal signaling center, to form the hair peg at E16.5-E17.5. The inner root sheath, an epithelial structure, forms at the bulbous hair peg stage beginning at E18.5. It then becomes surrounded by a cylinder of epithelial cells called the outer root sheath. The hair shaft grows from these structures.[27]

Members of the canonical Wnt signaling pathway (β-catenin and Lef-1) are present in the skin prior to hair formation and are elevated in the forming placodes as they form. Wnts 10a and 10b are found in the epithelial placodes. Wnt 10b stimulates the growth of shafts cultured mouse vibrissae shafts via enhanced nuclear β-catenin localization within the dermal papilla.[28] Wnt signaling is mediated through the Frizzled (Fz) receptors. Fz 1 and 10 are also expressed in the epithelial placodes. In addition, Fz 10 is found in the dermal condensation.[29] The involvement of Wnt signaling was further demonstrated by misexpressing DKK1 in the basal epithelium of transgenic mice. These mice failed to form hairs, vibrissae, teeth and mammary glands at a very early stage of development.[30] Hence Wnt signaling is an essential early step for all skin appendage development. Interestingly, the DKK1 expressing skin did differentiate as evinced by their expression of keratin, filaggrin and involucrin. Ectopic DKK1 expression suppressed the downstream upregulation of Lef1, Wnt10b, β-catenin and Edar normally associated with hair follicle formation.[30]

In the absence of Wnt signaling, a degradation complex consisting of adenomatous polyposis coli (APC), glycogen synthase kinase-3 (GSK-3), Axin and protein phosphatase 2A (PP2A) phosphorylates

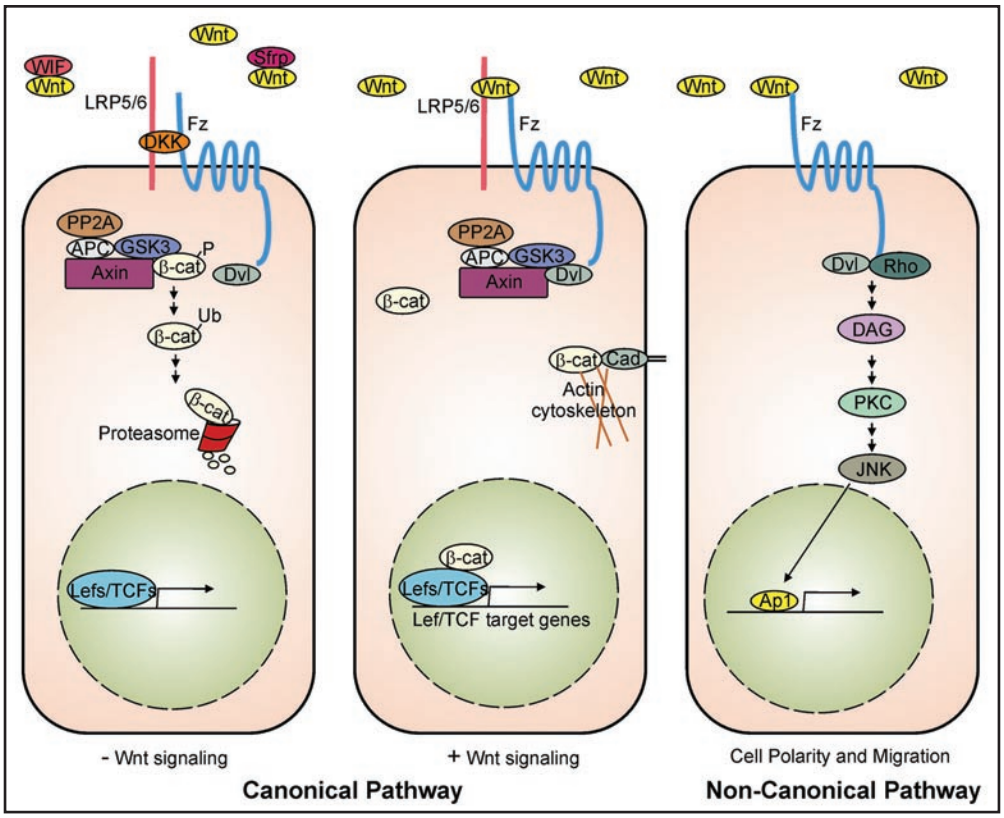

Figure 1. Schematic diagrams depicting the Wnt signaling pathways. The canonical Wnt signaling pathway (left 2) and planar cell polarity pathway (right) are shown. Wnts, represented by yellow circles represent a large family of signaling molecules. In general, Wnts 1, 3a, 8 and 8b induce the canonical Wnt signaling pathway while Wnts 5a and 11 favor the non-canonical pathway; however, significant cross talk between these pathways has been observed. There are a number of other Wnt signaling pathways that have not been described here as their role in skin appendages is yet to be established. More detailed views are presented elsewhere.[87,88]

β-catenin on amino terminal serines and threonines, targeting it for ubiquitin-dependent, proteasome-mediated degradation[31] (Fig. 1). Impairment of the β-catenin degradation complex can cause nuclear β-catenin accumulation leading to tumor formation in a number of target organs. To investigate the effect of inhibiting degradation on skin development, a mouse with a conditional knockout of APC directed to the basal keratinocytes was created. These mice die shortly after birth and have several embryonic anomalies. Embryonic hair development was delayed but finally emerged as tightly clustered hair follicles whose shafts were misoriented. Their whiskers were short and curly. Their foot pads grew hair in contrast to the hairless foot pads of their wild type siblings. These APC knockout mice also showed the abortive growth of multiple tooth buds initiating at the normal site of a single tooth. In addition they had a small thymus with squamous metaplasia.[32]

In the canonical Wnt signaling pathway, Wnt binding to the Frizzled-LRP co-receptors activates disheveled which leads to an inactivation of the degradation complex and hence to the accumulation of intracellular β-catenin. Therefore, suppression of β-catenin should inhibit hair formation. This was found using mice with a conditional knockout of β-catenin in basal keratinocytes. In fact this study showed that β-catenin is required for initial hair placode formation and then later in the catagen phase of subsequent hair cycles.[33] In another approach, investigators expressed a stable, dominant negative form of β-catenin lacking both the amino terminal degradation box and the carboxy terminal activation domain in the basal keratinocytes of transgenic mice. Expression of this construct which has the armadillo repeat region that can bind to Lefs/Tcfs but cannot activate transcription, blocked hair formation and created epidermal cysts; whereas the interfollicular epithelium was induced to form hair germs.[34] While the specific mechanism through which this differential response is mediated is not known, it suggests that differences in the interactions between β-catenin and its partners may influence its effects in different cell types.

Another mechanism to regulate β-catenin levels has also been found. SMAD7 normally inhibits SMAD phosphorylation and recruits SMAD ubiquitination-related factor (Smurf 2) to reduce SMAD signaling.[35] SMAD7 can bind directly to β-catenin as shown by co-immunoprecipitation and lead to the ubiquitination of β-catenin by Smurf 2 and subsequent turnover. This resulted in reduced β-catenin activity which enhanced sebaceous glands but inhibited hair follicle development within the skin.[36]

In the normal canonical Wnt signaling pathway, if β-catenin is not degraded, it can accumulate within the cell and be translocated to the nucleus, where it associates with Lefs/Tcfs in order to induce the transcription of a number of target genes. Targeted disruption of Lef-1 expression in transgenic mice led to lethality shortly after birth. Embryonic studies revealed a reduction in the number of hair follicles and mammary glands. Teeth did not develop beyond the bud stage. Whisker development was totally absent. Other skin appendages, such as nails and sweat glands developed normally.[37] Expression of a truncated form of Lef-1 which lacks the β-catenin

binding domain resulted in the formation of cysts and the progressive loss of hair follicles.[38] These findings are consistent with a requirement for Wnt signaling in hair and other skin appendage formation and suggest that the levels of β-catenin expression may regulate whether progenitor cells form hair or interfollicular epidermis.

Since the Lefs/Tcfs play crucial roles in mediating canonical Wnt signaling, understanding their regulation is of key importance. BMPs are known to be required early in hair development, but maintenance of its expression suppresses follicle growth. BMP can be inhibited by the expression of its antagonist, Noggin. Noggin mediated suppression of BMPs was found to be required for Lef-1 expression. This finding links two previously "independent" signaling pathways.[39] BMPs suppression by Noggin was independently found to increase the size of anagen hair follicles.[40] During early mouse skin development, E-cadherin is expressed in the epidermis. P-cadherin is expressed later in hair follicle development. Surprisingly, the interaction of β-catenin and Lef-1 which were thought to co-activate β-catenin downstream genes, combine to repress the expression of E-cadherin. Down regulation of E-cadherin is essential for hair buds to form.[39]

Feathers

The surface of each bird is covered with several different tracts of skin appendages. The feathers forming in each tract show similar characteristics but may show a size gradient. We and others have explored the role of Wnt signaling in tract and feather bud formation. In the dorsal tract of the chicken, feathers begin to form at embryonic day 6.5. The first signal seems to originate from the mesenchyme to induce an epithelial thickening called the placode. This is followed by an epithelial signal that induces a dermal condensation. What is the nature of this signal? Wnt-1, -3a, -5a, -7a, -11 and β-catenin were found to be expressed at moderate levels throughout the skin.[22,41-43] Prior to morphological alterations, Wnt-1, -3a, -7a and β-catenin are expressed at a high level, restricted to the prospective feather placode epithelium, while Wnt-5a is elevated in the interplacodal epithelium. Wnt-11 becomes restricted in the interplacodal dermis.

The functional role of different Wnts in tract and feather development was tested through perturbation studies. Ectopic expression of Wnt-1 reduced the size of the dorsal tract, but enhanced the size of feather buds which grew within it. In contrast, ectopic expression of Wnt-3a expanded the size of the dorsal tract, reduced interbud spacing and made large buds which did not show the normal tapering of control feather buds.[22] Expression of β-catenin enabled feathers to grow from apteric regions, which are normally devoid of feathers. It also caused feathers to grow at the leading edge of scales.[43] Exogenous Wnt-11 and the expression of a dominant negative Wnt-1 increased feather bud spacing, partially by making very thin feather buds.[22] Misexpression of Wnt-7a produced feather buds with a plateau shape, suggesting a dorsalization of the feather buds.[41] The homeobox protein, Hex/Prh, promotes epithelial cell proliferation.[44] Its expression mirrors that of Wnt-7a.[45] Misexpression of Hex/Prh induced Wnt-7a and β-catenin expression, suggesting that it lies upstream of the Wnt signaling pathway.[46] These functional studies suggest that the same molecules can act at different levels of tissue organization (dermis formation, tract development, feather bud formation) and have different effects in different cellular contexts.

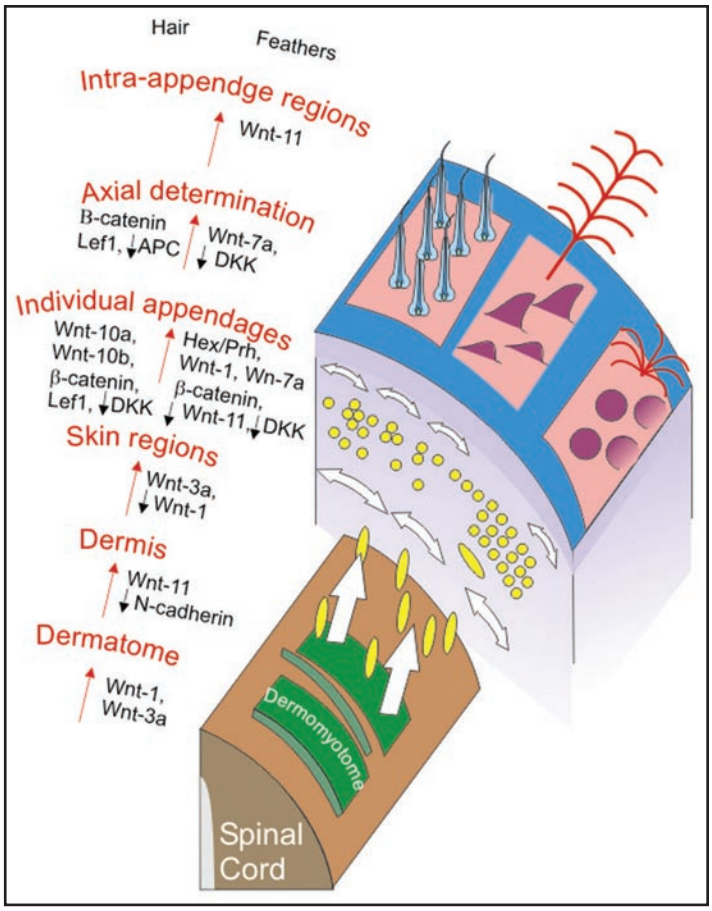

Figure 2. Involvement of Wnt signaling in the specification of dermis. Schematic showing the involvement of Wnt signaling involvement in dermis, tract and skin appendage development as determined in mouse hairs (left of arrows) and chicken feathers (right of red arrows indicating developmental progression). Morphogenetic events that take place in the dermomyotome, dermis, feather tracts and individual feather primordia are shown. Spindles and circles represent dermal cells. Two different tracts with different density and shape of skin appendages are shown. The figure is modified from ref. 22.

As the feather buds form, they are initially radially symmetric. Later, as they elongate by evaginating from the skin surface, they establish morphological anterior-posterior asymmetries. The changes in morphology are preceded by the development of molecular asymmetries (i.e., tenascin is expressed in the anterior epithelium, sonic hedgehog is in the distal epithelium and Wnt-7a is the posterior epithelium). We have found that this asymmetric configuration helps to establish a localized growth zone within the feathers, so they can elongate normally. Proliferation takes place at a higher rate in the early stage, posterior feather bud and then later moves toward the base of elongated, asymmetric feather buds. The sites of proliferation correspond to sites of Wnt expression, but the specific Wnt expressed at different stages of growth changed over time. The ectopic expression of Wnt-6 produced a localized region of expanded proliferation at the site of transduction within the feather.[47] While ectopic Wnt-7a expression posteriorized the buds and prevented normal feather follicle invagination and subsequent elongation, the buds did seem to differentiate normally[41] (Fig. 3).

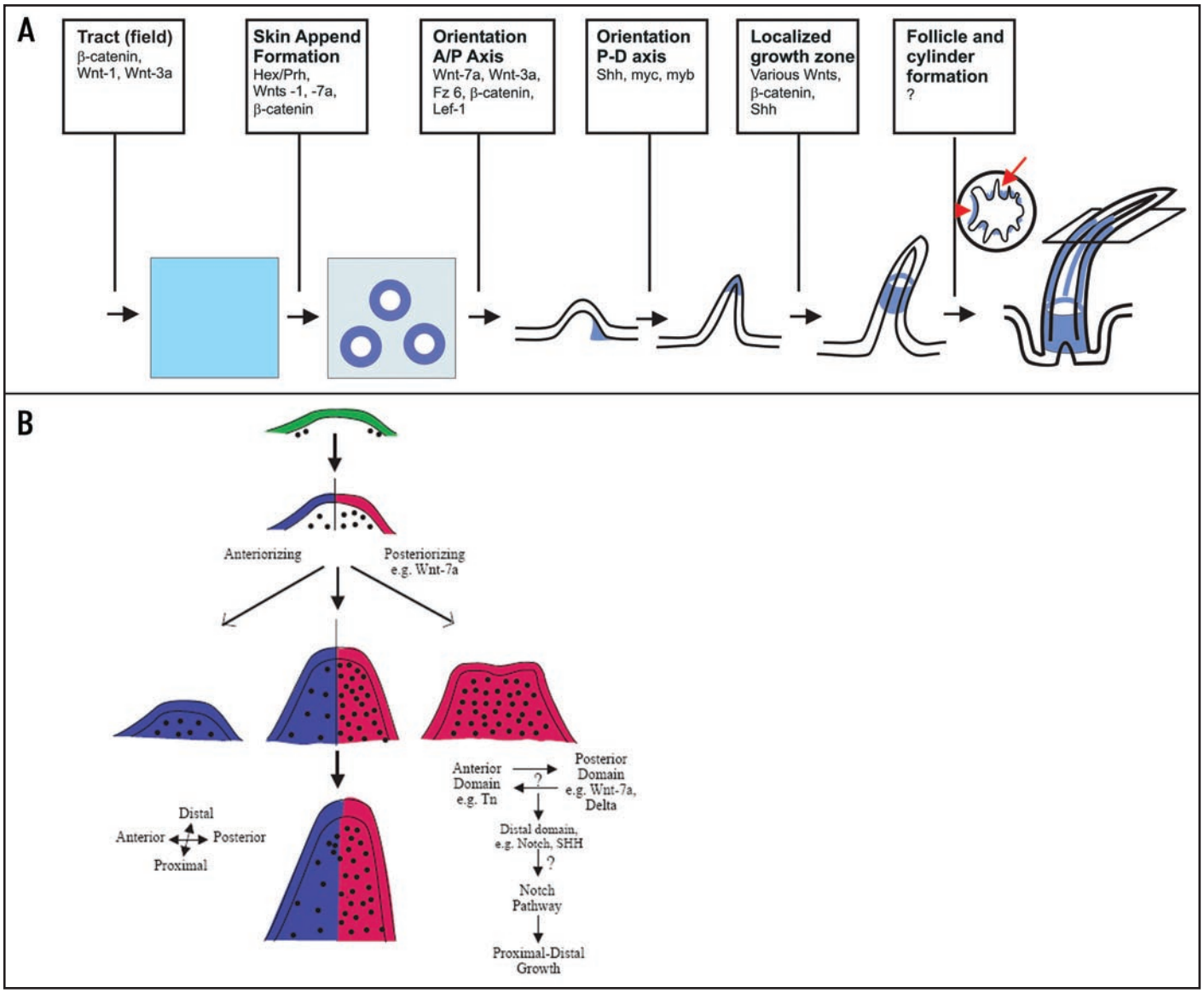

Figure 3. Involvement of Wnt signaling in the embryonic development of skin appendage organs. (A) The involvement of Wnt signaling molecules in the steps of feather development are shown. Proliferative cells are indicated in gray. The darker shades of gray indicate areas with higher proliferation. The plane on the right most feather indicates the site at which the adjacent cross section was taken to show barb ridge (arrow) and rachis (arrowhead) formation. AP-axis: anterior-posterior axis; PD-axis: proximal-distal axis. (B) Molecular asymmetries establish axis determination. The juxtaposition of anterior and posterior compartments form a bud growth zone which enables feather follicles to elongate. Markers of anterior and posterior regions are indicated. (A) is modified (from 47). (B) is modified from (ref. 89).

Skin Appendage Follicle Structure

Hair follicle structure. The hair follicle has a densely packed dermal papilla at its base which interacts with hair stem cells to begin a new hair cycle. The dermal papilla is surrounded by the epithelial hair matrix. Above this lies the precortex and cortex. The hair shaft is surrounded by an epithelial inner root sheath which itself is surrounded by the epithelial outer root sheath. The outer root sheath is continuous with the interfollicular epithelium. Approximately half way up the outer root sheath, below the sebaceous gland at the arrector pili muscle attachment site, is a protrusion called the hair follicle bulge. This site houses the epithelial stem cell niche. Above the follicle bulge are sebaceous glands (Fig. 4).[48]

Feather follicle structure. One of the hallmarks of feathers is their ability to branch, yielding a high diversity of feather types (i.e., downy feathers vs vaned wing feathers). The mechanism producing this multiplicity of feather forms is just coming to light, but it probably couldn't occur without the basic structure of the feather follicle. The base of a feather cycle contains a specialized dense, dermal structure called the dermal papilla. Interactions between epithelial stem cells and the dermal papilla are required to replace feathers once they have molted. Collar epithelium surrounds the dermal papilla. The loosely packed dermis above the dermal papilla is the pulp which helps to support blood vessels. Later, the pulp cells die by apoptosis to allow the feather branches to open. A bulge within the epithelial collar protrudes slightly into the pulp. This is the epithelial stem cell niche of the feather.[49] Above this region lie the epithelial components of the feather proper which initially form as a cylinder surrounded by the epithelial feather sheath. The feather branches are made in the ramogenic zone from epithelial structures

within the feather known as barb ridges (Fig. 4). Each barb ridge is separated by marginal plate epithelium. The marginal plate cells die by apoptosis to form the barbs, otherwise known as the branches commonly associated with feathers. Within the barb ridges are two columns of barbule plate epithelium separated by a single column of axial plate epithelium. The axial plate epithelial cells apoptose to release the feather barbules. The barbules from adjoining barbs can stick together to form pennaceous feather vanes such as those used in flight or stay separated to form downy, plumulaceous feathers used to provide warmth. The lower, unbranched portion of the feather is called the calamus. The major backbone of the feather is called the rachis (Fig. 5).[50]

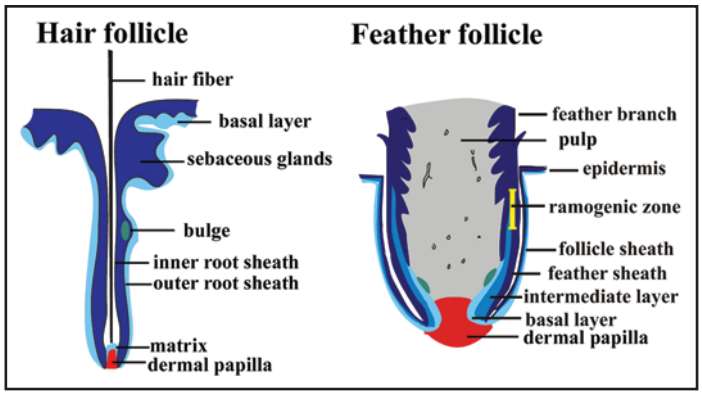

Figure 4. Structure of hairs and feathers. Schematic diagrams showing the layout of the hairs and feathers.

Skin Appendage Cycles

Hairs. After the hair follicle is formed, it goes through a growth cycle in which several components of the hair follicle are replaced. The hair cycle starts with a growth phase (anagen), followed by regression (catagen) largely due to apoptosis of the lower half of the hair follicle and then a resting phase whose length differs in a site and species-specific manner (telogen).[51] Hairs are replaced in subsequent cycles as waves that traverse the surface of the skin. The domains which are replaced in each wave decrease in size as the animal ages. Experimental data are consistent with the idea that the hair cycle waves are regulated through a reaction-diffusion mechanism in which activating and inhibitory signals regulate the spacing of developing hair follicles. Experimentally, the beginning of a new cycle can be induced by plucking large numbers of hairs within an area.

In the mouse skin, Fz 1 and 10 are expressed in the hair follicles. Signaling through these frizzled receptors activate the canonical Wnt pathway. In contrast, Wnts-4 and -6 and Fz 3 and 6 are expressed in the interfollicular epidermis[29,52] and signal through the non-canonical pathway.[53,54] Wnts 5a and 11 are expressed in the interfollicular mesenchyme[54] and also signal through the non-canonical Wnt signaling pathways. Other Fz receptors are expressed later in hair follicle development. Their complex and partially overlapping expression patterns suggest that they may perform redundant functions.

DKK4 expression is transiently expressed in early stage epithelial placodes from hair follicles, vibrissae, dental laminae, mammary glands and ecrine glands. This inhibitor of Wnt signaling is essential for skin appendage formation. In the mouse, the promoter region for DKK4, an inhibitor of the canonical Wnt signaling pathway, contains Lef/Tcf concensus binding sites.[55] The author's proposed that inhibition of canonical Wnt signaling may promote activation of the planar cell polarity pathway at this time to foster cellular rearrangements prior to hair follicle formation.

β-catenin is required for hair formation to commence in mice.[33] BMPs, Shh and Patched which are normally expressed during hair formation were missing in the skin of mice in which K14 was used to direct the early knock out of β-catenin.[33] β-catenin acts as a switch later in life to guide cells toward a hair keratinocyte cell fate. In its absence cells become epidermal keratinocytes. β-catenin may function by activating the downstream Notch signaling pathway.[56]

To further explore the role of canonical Wnt signaling during the hair cycle, a stable, estrogen inducible form of β-catenin that is preferentially expressed in the suprabasal epithelium was made. Expression could be induced in transgenic mice using 4-hydroxytamoxifen. Activation of β-catenin in telogen phase skin induced the start of a new anagen phase.[57] As is true for the formation of the first hair follicle, β-catenin is required to initiate a new hair cycle.

Feathers. Feathers are lost through seasonal molts and regrown throughout the life of birds. The cycle of feather loss and replacement can be described as growth, resting, molting and initiation phases.[50,58] The lower portion of the hair follicle is not lost in the cycling process. During the growth phase, the transient amplifying cells of the feather collar generate a feather vane and calamus. In the resting phase, keratinocytes of the calamus connect feather barbs to the follicle. In the molting phase, the collar epithelium becomes keratinized and flakes off, dislodging the feather shaft. In initiation, skin stem cells from the feather follicle bulge migrate down to make contact with the dermal papilla to begin a new cycle of growth.

Feathers cycle in waves. In the dorsal tract, feathers are lost in a medial to lateral direction and also starting above the forelimbs in both anterior and posterior directions.[50] The length of the feather cycle is tract-dependent. Feather cycles can be experimentally induced by plucking the feather shaft from its follicle.

Patterning. Wnts and DKKs are expressed early in hair follicle formation in the mouse, suggesting that Wnts might serve as activators and DKKs might serve as inhibitors in setting up hair follicle patterning. To test this possibility, Sick[59] investigated transgenic mice expressing DKK2 in the epithelium as an inhibitor in the reaction-diffusion model.[60] These mice had reduced hair density with bundled hair shafts in contrast to the widely distributed hair shafts found in wild type animals. These findings are consistent with mathematical modeling of reaction-diffusion.

Skin appendage patterning is easiest to see in the developing feather follicles because each feather placode is surrounded by a hexagon of adjacent, equidistant feathers. The reaction-diffusion hypothesis proposes that patterns are made through the interactions of activators and inhibitors. Activators promote the formation of structures within the pattern and inhibitors block their formation and also suppress expression of the activators.[61]

During the formation of the first feather follicles, many Wnts and β-catenin are expressed at low levels and then are upregulated either within the feather bud or the interbud region.[22,41,43] This suggests that Wnt signaling is helping to establish the initial placement of the feather buds or perhaps helping to consolidate the feather buds within the feather field Figure 3.

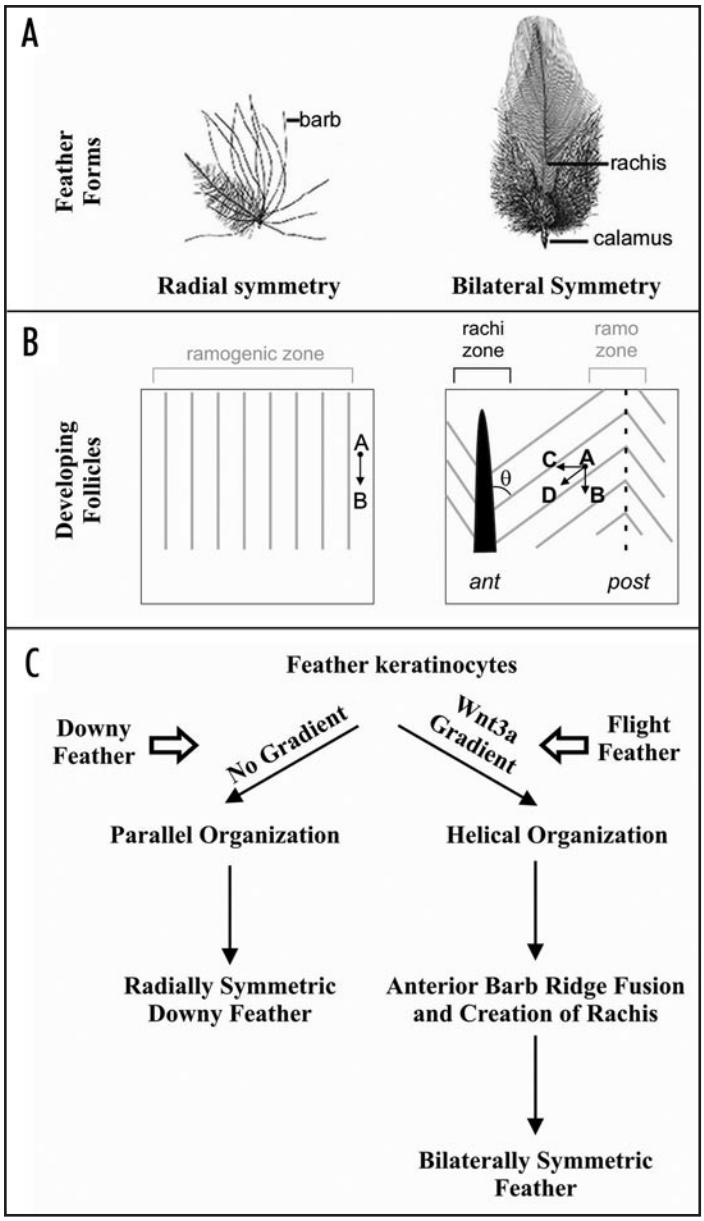

Figure 5. Involvement of Wnt signaling in the cycling of skin appendage organs. Comparison of feathers with radial vs bilateral symmetry. (A) Drawings of feathers are modified from Lucas and Stettenheim, 1972. (B) Schematic of developing follicles shows that in radial feathers, the barbs do not converge on a rachis; whereas in bilaterally symmetric feathers the barbs join the rachis with the angle, θ. The slant of the barb organization is due to the contribution of vertical (growth, vector AB) and horizontal (response to Wnt-3a gradient, vector AC) displacement. This results in feather branches organized along the vector AD. (C) A model summarizes this result. The remaining panels are modified (from 62).

Orientation. One of the non-canonical Wnt signaling pathways identified in Drosophila causes cuticle bristles to grow with a proper orientation. Disruption of this planar cell polarity pathway leads to misorientation of the bristles. In this case, each bristle is a single cell. The planar cell polarity pathway also plays a role in vertebrate organ orientation, whereby several cells in an organ take on a similar orientation. In the feathers, the placement of beads coated with Wnt-3a protein to produce a local Wnt-3a gradient caused barb ridges to reorient toward the highest concentration of Wnt-3a and frequently formed an ectopic rachis. The Wnt-3a gradient appears to be regulated by the dermal papilla.[62]

In mouse hairs it has been found that Fz-6 mediates planar cell polarity. Deletion of Fz-6 causes a disruption of the normal caudal orientation of parallel follicles forming regions with convergent/divergent orientation. This produces whorls on the hind feet, tufts on the head and misoriented hairs on the body.[63]

Another attribute of the hair shaft that has been investigated in terms of orientation is the bending of the hair shaft. A recent paper has found that Wnt signaling may be involved. In this paper, the Wnt signaling inhibitor DKK-1 was expressed in the hair cortex and weakly in the epidermis. Mice expressing high levels of DKK-1 have no hair as was found by Andl,[30] but mice expressing moderate levels show some interesting hair anomalies. These mice have a hairless patch behind the ears, their hairs do not bend like wildtype mice, the hairs are shortened and their footpads lack sweat glands. These characteristics are found in common with ectodysplasin deficient Tabby mice and both show weak expression of *Igfbp5*. Expression of ectodysplasin corrected the DKK-1 induced phenotype, suggesting that ectodysplasin acts downstream of DKK-1. Sonic hedgehog (Shh) was expressed asymmetrically within the hair bulb in response to DKK-1 and ectodysplasin.[64] Their data suggests that Wnt and ectodysplasin act to establish molecular asymmetries in the growing hair.

Regional specificity in skin pigmentation. To investigate regional specificity in human skin Yamaguchi[65] used molecular profiles to explore molecular determinants distinguishing pigment patterns in the hands and feet versus the torso. The hands and feet express high levels of DKK-1 compared to the trunk and have less pigment due to a lower melanocyte density. High levels of DKK-1 expression in the epidermis inhibit melanocyte function and differentiation. Transgenic mice expressing ectopic DKK-1 had reduced pigmentation in the trunk[30] consistent with this notion. The molecular analyses showed that β-catenin was down regulated in fibroblasts from DKK-1 treated skin. Microthalmia-associated transcription factor (MITF), the master regulator of melanocyte differentiation regulates melanocyte proliferation and melanin production. MITF was inhibited in fibroblasts derived from DKK-1 treated skin.[65]

Adult Stem cells

Hair. Adult stem cells should have properties of multipotency, self renewal and infrequent proliferation, yet high proliferative potential. Putative epithelial stem cells were identified in the hair by their ability to proliferate infrequently detected as label retaining cells. Later these cells were found to express CD34 (an antigen expressed by stem cells) and keratin 15 which can be used as identifying markers. CD34 is also expressed by the lower segment of the outer root sheath. The stem cells were found to reside in a specialized niche within the hair follicle called the hair bulge, a swelling from the outer root sheath. Their multipotency was verified by lineage tracing studies. They were found to give rise to all the cells within the hair follicle. In recombination studies they were shown to be able to regenerate hairs, sebaceous glands and interfollicular epidermis.[66] It is believed that stem cells migrate down from the bulge to come in contact with the dermal papilla in order to initiate a new hair cycle.

One member of the Lef/Tcf family, Tcf-3, can act as a repressor of Wnt mediated transcription. It is expressed within the hair follicle bulge and basal layer of the outer root sheath. Ectopic expression of Tcf-3 had no bearing on cell proliferation activity but did induce

keratinocytes to express a molecular profile that resembles that of progenitor cells. Adult mouse epithelial cells that expressed ectopic Tcf-3 did not express molecular markers for epidermis, sebaceous glands or hair follicles. Hence Tcf-3 seems to maintain cells in a precursor state and inhibit progression toward terminal differentiation in a β-catenin-independent manner.[67]

The hairless protein (HR) is a nuclear receptor co-repressor.[68] Mice with a *Hairless* (*Hr*) mutation have a normal hair distribution at birth, but do not replace the hairs as they are shed. The hair follicles go through catagen and telogen, but do not regenerate. Normally the wild type *Hr* gene is found in the nucleus of basal layer keratinocytes within the outer root sheath as hairs enter the anagen phase of the hair cycle. Microarray analysis suggested that the Wnt modulator in surface ectoderm (WISE), a Wnt pathway inhibitor, was inhibited by expression of HR. This was confirmed in mice by the co-localization of axin2, a β-catenin target gene with hair regrowth.[69]

Feathers. The location of chicken feather stem cells was also found by searching for slow cycling cells. This label retaining population resided in a bulge protruding from the epithelial collar into the central pulp. Through transplantation studies these cells were shown to be pluripotential giving rise to cells of the bud, interbud and barb ridges. The position of the stem cells enables them to be retained by the follicle during normal cyclic molting.[49]

Since the cells are in the collar epithelium surrounding the pulp, they form a ring. The ring was parallel to the surface of the skin in radially symmetric feathers but tilted down toward the rachis in bilaterally symmetric feathers.[49] In radially symmetric feathers Wnt-3a had an even distribution from the anterior to the posterior regions of the feather follicle, but in bilaterally symmetric feathers Wnt-3a expression levels were highest at the site of the rachis in the anterior feather follicle and formed an anterior to posterior concentration gradient (Fig. 5). Misexpression of Wnt-3a reoriented barb formation toward the Wnt-3a source. A global suppression of Wnt-3a obscured the gradient in bilaterally symmetric feather buds and transformed the developing feathers from bilateral to radial symmetry.[62]

Roles in wound healing. The protective functions of the skin are compromised by wounding, therefore it is vital to have a strong and rapid means of restoring skin integrity through wound healing. Unfortunately wound healing (repair) is not the same as regeneration and can lead to scarring. The healed tissue does not grow skin appendages. Understanding differences between embryonic skin morphogenesis and wound healing may help us to improve prospects for patients with large wounds.

Canonical Wnt signaling is active in the dermis during cutaneous wound healing. At the same time it acts to inhibit cell migration of keratinocytes through a noncanonical pathway. Wound size, mostly attributed to the dermal component, responded proportionally to β-catenin expression levels in transgenic mice. However, the rate of wound closure was not affected by β-catenin expression. Wound healing induced by TGFβ is regulated, in part, by β-catenin. TGFβ induces the expression of *Mmp-3* and *Mmp-14* during wound healing. This induction requires β-catenin expression.[70] Hence, β-catenin is required to mediate the effects of TGFβ during normal wound healing.

The involvement of Wnts in wound healing was further investigated by examining their expression at various times after wounding. Wnt-1 was not expressed but Wnt-4 was expressed early in the process. Wnt-5a and 11 were expressed later, peaking at a time of wound remodeling. β-catenin activation was assessed using TOPGAL mice. These mice express β-galactosidase where β-catenin/Tcf induced transcription occurs. β-galactosidase activity was increased in hair follicles located adjacent to the wound site. The role of β-catenin in wound repair was tested by suppressing β-catenin degradation. Treated wounds grew hair follicles and sebaceous glands. The role of non-canonical Wnt signaling in wound healing was next examined. Wounds were transduced with retrovirus expressing Wnt-5a which favors non-canonical signaling. These wounds formed epithelial cysts, hairs and sebaceous glands but did not induce β-galactosidase activity in TOPGAL mice.[71] These data suggest that canonical as well as non-canonical Wnt signaling may be involved in directing adult skin progenitor cells toward regeneration.

In another study, hairs were found to regenerate in mice with large, full thickness wounds (1 cm^2) but not in mice with smaller wounds (Fig. 6). The regenerated hair follicles expressed a similar molecular sequence of genes as those formed during embryonic morphogenesis. Morphologically, the regenerated follicles also formed hair germs, hair pegs, hair shafts and sebaceous glands. Using lineage tracing, the new hair follicles were found to originate from stem cells residing in the interfollicular epidermis as well as from the upper portion of the hair follicles but not from the follicle bulge. Ectopic DKK-1 expression blocked hair regeneration. Transgenic mice expressing exogenous Wnt-7a grew twice as many hair follicles as controls.[72] Wnt-7a previously was shown to maintain the ability of cultured dermal papilla cells to induce hair follicles.[73] The expression of Wnt-3a and a stabilized form of β-catenin also could maintain an ability to induce hair formation.[74] Mice with a conditional knockout of β-catenin in the epidermis could not regenerate hairs.[72] All of these data indicate that canonical Wnt signaling in the epidermis is required for hair follicle regeneration and that Wnts can be active at different stages of skin organogenesis and during repair.

Disease. Psoriasis affects the skin and joints. On the skin it appears as red, itchy patches of skin. It is believed to develop from genetic causes which cause an inflammatory response, producing increased cell proliferation. Nuclear β-catenin was detected in the suprabasal layers of psoriatic skin. Transglutaminase 1 is a terminal differentiation marker of the skin whose transcription is regulated by β-catenin. It was expressed in the same region of psoriatic skin as β-catenin. Inhibiting the degradation of β-catenin by blocking GSK-3β activity increased transglutaminase 1 expression. Increasing β-catenin activity also increased transglutaminase 1 expression suggesting that transglutaminase 1 is regulated by β-catenin mediated transcription.[75]

Focal dermal hypoplasia is an X-linked dysplasia of the skin that can be distributed along the lines of Blaschko among other defects affecting the eyes, teeth, skeletal, urinary, gastrointestinal, cardiovascular and central nervous systems. A genetic linkage study involving a family with 6 affected individuals identified this to be caused by mutations in the PORCN gene. PORCN, a member of the porcupine family, is believed to encode an O-acetyltransferase that is required for the palmitoylation and subsequent secretion of Wnts; a necessary step for signaling.[76] In an independent line of research, using a comparative genomic hybridization based approach, a 219 kb region containing seven candidate genes was identified as being lost in one patient with focal dermal hypoplasia. Mutations in

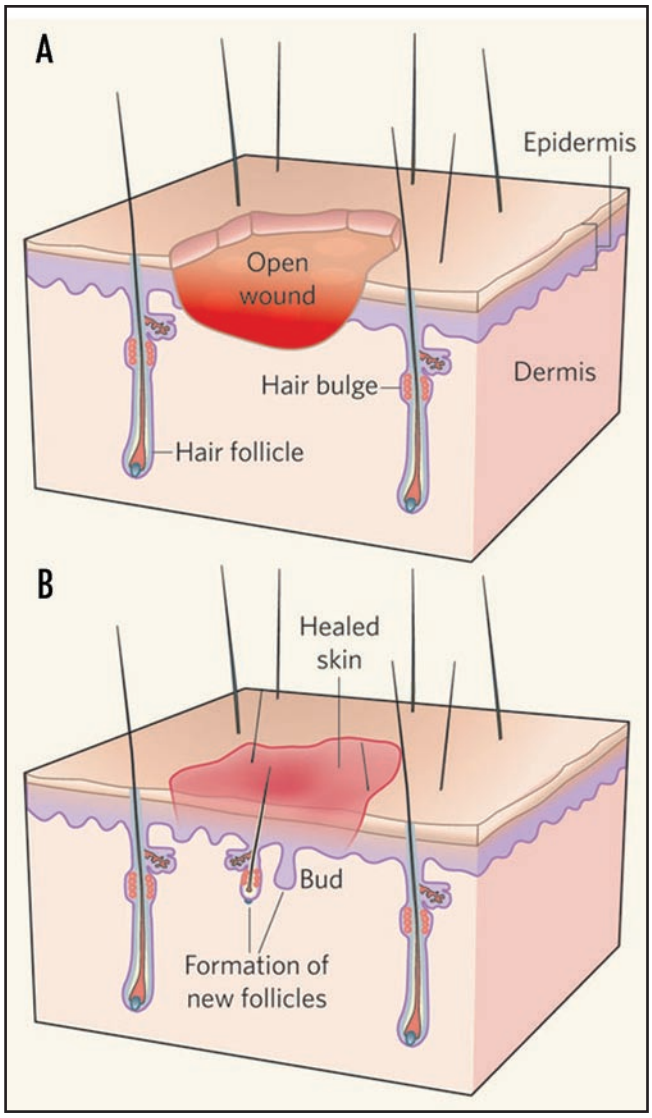

Figure 6. Involvement of Wnt signaling in the de novo regeneration of skin appendage organs. (a) Large open wounds in mouse skin become reepithelialized. (b) Skin appendages are generated from the center of wounds that are 1.0 cm in diameter or larger. Wnt signaling during wound healing increased the number of hair follicles induced in during wound healing. The figure is taken from Chuong.[90]

some of the 7 genes were previously associated with other syndromes. Comparison of the sequences covering this region with other focal dermal hypoplasia patients and their parents helped to identify that mutations in the PORCN gene was associated with focal dermal hypoplasia.[77]

Wnt signaling has been implicated in the carcinogenesis of many different tissues. Pilomatricoma hair tumors are derived from the hair matrix. Expression of a stabilized, truncated β-catenin form induced ectopic hair formation and in some cases pilomatricomas.[78] A high frequency of mutations was found in the amino terminal serines and threonines of β-catenin phosphorylated by GSK-3β in naturally occurring pilomatricomas. The DSG sequence, a ubiquitination target motif, was also mutated. These alterations prevented the ubiquitin dependent turnover of β-catenin.[79] Expression of a Lef-1 lacking the β-catenin binding domain resulted in the formation of tumors with sebaceous and interfollicular epidermal but not hair-like characteristics.[80] Mice expressing the truncated Lef-1 were sensitized to chemical carcinogenesis suggesting it may act as a tumor promoter. They expressed low levels of the tumor suppressor, p53.[80] The fact that tumors appeared in sebaceous glands and interfollicular skin suggests that canonical Wnt signaling is required for hair differentiation.

New findings examining how the Wnt signaling pathway is involved in many diseases (bone disease, cancer, etc.,) have marked tremendous progress over the last five years. This suggests that although we probably still have a lot to discover about Wnt signaling dysfunctions and pathology, this will be a very challenging and exciting field, since Wnt modulators might be useful in the future treatment of diseases.

Interaction of Wnt signaling with sex hormone receptors. Sex hormones have been shown to differentially regulate the growth of skin appendages in a region-specific manner.[81,82] While it is known that the hormones must bind to their receptors to initiate a response, variance in regional specificity suggests that complex molecular interactions modulate cellular response. One way this can be achieved is through the interaction of hormone receptors with other proteins. The androgen receptor is known to bind directly to β-catenin[83] and also to Tcf-4.[84] β-catenin can act as an inducer of androgen receptor mediated transcriptional activity.[85] A number of co-activators have been found to be recruited to nuclear receptors to enhance their transcriptional activity. One of these, CARM1, was found to bind to the β-catenin and with p300 can synergistically activate androgen receptor mediated transcription.[86] Androgens have been shown to have profound effects on hair. They can induce beard formation yet cause balding on the scalp. The role of Wnt signaling in beard growth or male pattern baldness remains to be studied.

Conclusions

Wnt signaling is essential at multiple steps during the complex organogenesis of the skin and its appendages. It is required to induce the formation of the dorsal dermis and regulates the size of the different skin appendage tracts. Later Wnt signaling is required for the very early stages of skin appendage formation as shown through studies involving perturbation of several players in the pathway. Skin appendage distribution, orientation and pigmentation are regulated, in part by Wnt signaling. The pathway is critical for stem cell maintenance, required for the multiple cycles of organogenesis in the adult through the loss and renewal of skin appendages and for the regeneration of lost skin and skin appendages during wound healing. Disruption of the pathway can lead to the formation of skin appendage tumors. The importance of hormone signaling to skin appendage morphogenesis and the direct interactions observed between hormone receptors and Wnt signaling pathway members suggests new and important ways this pathway may effect skin and skin appendage organogenesis.

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References

1. Landmann L. The Skin of Reptiles: Epidermis and Dermis. In: Bereiter-Hahn J, Matoltsy AG, Richards KS. Biology of the Integument, Vol. 2, Vertebrates, New York: Springer-Verlag 1986; 150-87.
2. Wu P, Hou L, Plikus M, Hughes M, Scehnet J, Suksaweang S, Widelitz RB, Chuong CM. Evo-Devo of amniote integuments and appendages. Int J Dev Biol 2004; 48:249-70.
3. Chuong CM, Nickoloff BJ, Elias PM, Goldsmith LA, Macher E, Maderson PA, Sundberg JP, Tagami H, Plonka PM, Thestrup-Pederson K, Bernard BA, Schroder JM, Dotto P, Chang CM, Williams ML, Feingold KR, King LE, Kligman AM, Rees JL, Christophers E. What is the 'true' function of skin? Exp Dermatol 2002; 11:159-87.
4. Widelitz RB, Veltmaat JM, Mayer JA, Foley J, Chuong CM. Mammary glands and feathers: comparing two skin appendages which help define novel classes during vertebrate evolution. Semin Cell Dev Biol 2007; 18:255-66.
5. Sengel P. Morphogenesis of skin. Cambridge: Cambridge University Press, 1976.
6. Lin CM, Jiang TX, Widelitz RB, Chuong CM. Molecular signaling in feather morphogenesis. Curr Opin Cell Biol 2006; 18:730-41.
7. Plikus MV, Chuong CM. Complex Hair Cycle Domain Patterns and Regenerative Hair Waves in Living Rodents. J Invest Dermatol 2007; Advanced On-line Publication.
8. Wang Y, Macke JP, Abella BS, Andreasson K, Worley P, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. A large family of putative transmembrane receptors homologous to the product of the Drosophila tissue polarity gene frizzled. J Biol Chem 1996; 271:4468-76.
9. Kawano Y, Kypta R. Secreted antagonists of the Wnt signaling pathway. J Cell Sci 2003; 116:2627-34.
10. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. Beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J 1997; 16:3797-804.
11. Pinson KI, Brennan J, Monkley S, Avery BJ, Skarnes WC. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature 2000; 407:438-535.
12. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J 1998; 17:1371-84.
13. van de Wetering M, Cavallo R, Dooijes D, van Beest M, van Es J, Loureiro J, Ypma A, Hursh D, Jones T, Bejsovec A, Peifer M, Mortin M, Clevers H. Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 1997; 88:789-99.
14. Choi HJ, Huber AH, Weis WI. Thermodynamics of beta-catenin-ligand interactions: the roles of the N- and C-terminal tails in modulating binding affinity. J Biol Chem 2006; 281:1027-38.
15. Tao YS, Edwards RA, Tubb B, Wang S, Bryan J, McCrea PD. βcatenin associates with the actin-bunding protein fascin in a noncadherin complex. J Cell Biol 1996; 134:1271-81.
16. Strutt D. Frizzled signalling and cell polarisation in Drosophila and vertebrates. Development 2003; 130:4501-13.
17. Yamanaka H, Moriguchi T, Masuyama N, Kusakabe M, Hanafusa H, Takada R, Takada S, Nishida E. JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates. EMBO Rep 2002; 3:69-75.
18. Mauger A. The role of somitic mesoderm in the development of dorsal plumage in chick embryos I. Origin, regulative capacity and determination of the plumage-forming mesoderm. J Embryol Exp Morphol 1972; 28:313-41.
19. Le Lievre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J Embryol Exp Morphol 1975; 34:125-54.
20. Dietrich S, Schubert FR, Lumsden A. Control of dorsoventral pattern in the chick paraxial mesoderm. Development 1997; 124:3895-908.
21. Geetha-Loganathan P, Nimmagadda S, Huang R, Christ B, Scaal M. Regulation of ectodermal Wnt6 expression by the neural tube is transduced by dermomyotomal Wnt11: a mechanism of dermomyotomal lip sustainment. Development 2006; 133:2897-904.
22. Chang CH, Jiang TX, Lin CM, Burrus LW, Chuong CM, Widelitz R. Distinct Wnt members regulate the hierarchical morphogenesis of skin regions (spinal tract) and individual feathers. Mech Dev 2004; 121:157-71.
23. Olivera-Martinez I, Coltey M, Dhouailly D, Pourquie O. Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras. Development 2000; 127:4611-7.
24. Ben-Yair R, Kahane N, Kalcheim C. Coherent development of dermomyotome and dermis from the entire mediolateral extent of the dorsal somite. Development 2003; 130:4325-36.
25. Scaal M, Christ B. Formation and differentiation of the avian dermomyotome. Anat Embryol (Berl) 2004; 208:411-24.
26. Cinnamon Y, Kahane N, Kalcheim C. Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. Development 1999; 126:4305-15.
27. Schmidt-Ullrich R, Paus R. Molecular principles of hair follicle induction and morphogenesis. Bioessays 2005; 27:247-61.
28. Ouji Y, Yoshikawa M, Moriya K, Ishizaka S. Effects of Wnt-10b on hair shaft growth in hair follicle cultures. Biochem Biophys Res Commun 2007; 359:516-22.
29. Reddy ST, Andl T, Lu MM, Morrisey EE, Millar SE. Expression of Frizzled genes in developing and postnatal hair follicles. J Invest Dermatol 2004; 123:275-82.
30. Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. Dev Cell 2002; 2:643-53.
31. Ratcliffe MJ, Itoh K, Sokol SY. A positive role for the PP2A catalytic subunit in Wnt signal transduction. J Biol Chem 2000; 275:35680-3.
32. Kuraguchi M, Wang XP, Bronson RT, Rothenberg R, Ohene-Baah NY, Lund JJ, Kucherlapati M, Maas RL, Kucherlapati R. Adenomatous polyposis coli (APC) is required for normal development of skin and thymus. PLoS Genet 2006; 2:1362-74.
33. Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. Beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 2001; 105:533-45.
34. DasGupta R, Rhee H, Fuchs E. A developmental conundrum: a stabilized form of beta-catenin lacking the transcriptional activation domain triggers features of hair cell fate in epidermal cells and epidermal cell fate in hair follicle cells. J Cell Biol 2002; 158:331-44.
35. Massague J, Seoane J, Wotton D. Smad transcription factors. Genes Dev 2005; 19:2783-810.
36. Han G, Li AG, Liang YY, Owens P, He W, Lu S, Yoshimatsu Y, Wang D, Ten Dijke P, Lin X, Wang XJ. Smad7-induced beta-catenin degradation alters epidermal appendage development. Dev Cell 2006; 11:301-12.
37. van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, Grosschedl R. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes Dev 1994; 8:2691-703.
38. Niemann C, Owens DM, Hulsken J, Birchmeier W, Watt FM. Expression of DeltaNLef1 in mouse epidermis results in differentiation of hair follicles into squamous epidermal cysts and formation of skin tumours. Development 2002; 129:95-109.
39. Jamora C, DasGupta R, Kocieniewski P, Fuchs E. Links between signal transduction, transcription and adhesion in epithelial bud development. Nature 2003; 422:317-22.
40. Sharov AA, Sharova TY, Mardaryev AN, Tommasi di Vignano A, Atoyan R, Weiner L, Yang S, Brissette JL, Dotto GP, Botchkarev VA. Bone morphogenetic protein signaling regulates the size of hair follicles and modulates the expression of cell cycle-associated genes. Proc Natl Acad Sci USA 2006; 103:18166-71.
41. Widelitz RB, Jiang TX, Chen CW, Stott NS, Jung HS, Chuong CM. Wnt-7a in feather morphogenesis: involvement of anterior-posterior asymmetry and proximal-distal elongation demonstrated with an in vitro reconstitution model. Development 1999; 126:2577-87.
42. Noramly S, Freeman A, Morgan BA. Beta-catenin signaling can initiate feather bud development. Development 1999; 126:3509-21.
43. Widelitz RB, Jiang TX, Lu J, Chuong CM. Beta-catenin in epithelial morphogenesis: conversion of part of avian foot scales into feather buds with a mutated beta-catenin. Dev Biol 2000; 219:98-114.
44. Obinata A, Akimoto Y, Omoto Y, Hirano H. Expression of Hex homeobox gene during skin development: Increase in epidermal cell proliferation by transfecting the Hex to the dermis. Dev Growth Differ 2002; 44:281-92.
45. Obinata A, Akimoto Y. Expression of Hex during feather bud development. Int J Dev Biol 2005; 49:885-90.
46. Obinata A, Akimoto Y. Involvement of Hex in the initiation of feather morphogenesis. Int J Dev Biol 2005; 49:953-60.
47. Chodankar R, Chang CH, Yue Z, Jiang TX, Suksaweang S, Burrus L, Chuong CM, Widelitz R. Shift of localized growth zones contributes to skin appendage morphogenesis: role of the Wnt/beta-catenin pathway. J Invest Dermatol 2003; 120:20-6.
48. Sundberg JP, Peters EM, Paus R. Analysis of hair follicles in mutant laboratory mice. J Investig Dermatol Symp Proc 2005; 10:264-70.
49. Yue Z, Jiang TX, Widelitz RB, Chuong CM. Mapping stem cell activities in the feather follicle. Nature 2005; 438:1026-29.
50. Lucas AM, Stettenheim PR. Avian Anatomy—Integument. Agricultural Handbook 362. Washington DC: Agricultural Research Services, US Department of Agriculture, 1972.
51. Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev 2001; 81:449-94.
52. Hung BS, Wang XQ, Cam GR, Rothnagel JA. Characterization of mouse Frizzled-3 expression in hair follicle development and identification of the human homolog in keratinocytes. J Invest Dermatol 2001; 116:940-6.
53. Sheldahl LC, Park M, Malbon CC, Moon RT. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. Curr Biol 1999; 9:695-8.
54. Reddy S, Andl T, Bagasra A, Lu MM, Epstein DJ, Morrisey EE, Millar SE. Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. Mech Dev 2001; 107:69-82.
55. Bazzi H, Fantauzzo KA, Richardson GD, Jahoda CA, Christiano AM. The Wnt inhibitor, Dickkopf 4, is induced by canonical Wnt signaling during ectodermal appendage morphogenesis. Dev Biol 2007; 305:498-507.
56. Estrach S, Ambler CA, Lo Celso C, Hozumi K, Watt FM. Jagged 1 is a beta-catenin target gene required for ectopic hair follicle formation in adult epidermis. Development 2006; 133:4427-38.
57. Van Mater D, Kolligs FT, Dlugosz AA, Fearon ER. Transient activation of beta -catenin signaling in cutaneous keratinocytes is sufficient to trigger the active growth phase of the hair cycle in mice. Genes Dev 2003; 17:1219-24.
58. Yu M, Yue Z, Wu P, Wu DY, Mayer JA, Medina M, Widelitz RB, Jiang TX, Chuong CM. The biology of feather follicles. Int J Dev Biol 2004; 48:181-91.
59. Sick S, Reinker S, Timmer J, Schlake T. WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism. Science 2006; 314:1447-50.
60. Maini PK, Baker RE, Chuong CM. Developmental biology. The Turing model comes of molecular age. Science 2006: 314:1397-8.
61. Turing AM. The chemical basis of morphogenesis. Phil Trans R Soc B 1952; 237:37-72.
62. Yue Z, Jiang TX, Widelitz RB, Chuong CM. Wnt3a gradient converts radial to bilateral feather symmetry via topological arrangement of epithelia. Proc Natl Acad Sci USA 2006; 103:951-5.
63. Guo N, Hawkins C, Nathans J. Frizzled6 controls hair patterning in mice. Proc Natl Acad Sci USA 2004; 101:9277-81.

64. Hammerschmidt B, Schlake T. Localization of Shh expression by Wnt and Eda affects axial polarity and shape of hairs. Dev Biol 2007; 305:246-61.
65. Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. J Biol Chem 2007; 282:27557-61.
66. Cotsarelis G. Epithelial stem cells: a folliculocentric view. J Invest Dermatol 2006 126:1459-68.
67. Nguyen H, Rendl M, Fuchs E. Tcf3 governs stem cell features and represses cell fate determination in skin. Cell 2006; 127:171-83.
68. Potter GB, Beaudoin IIIrd GM, DeRenzo CL, Zarach JM, Chen SH, Thompson CC. The *Hairless* gene mutated in congenital hair loss disorders encodes a novel nuclear receptor corepressor. Genes Dev 2001; 15:2687-701.
69. Beaudoin GM 3rd, Sisk JM, Coulombe PA, Thompson CC. Hairless triggers reactivation of hair growth by promoting Wnt signaling. Proc Natl Acad Sci USA 2005; 102:14653-8.
70. Cheon S, Poon R, Yu C, Khoury M, Shenker R, Fish J, Alman BA. Prolonged beta-catenin stabilization and tcf-dependent transcriptional activation in hyperplastic cutaneous wounds. Lab Invest 2005; 85:416-25.
71. Fathke C, Wilson L, Shah K, Kim B, Hocking A, Moon R, Isik F. Wnt signaling induces epithelial differentiation during cutaneous wound healing. BMC Cell Biol 2006; 7:4.
72. Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature 2007; 447:316-20.
73. Kishimoto J, Burgeson RE, Morgan BA. Wnt signaling maintains the hair-inducing activity of the dermal papilla. Genes Dev 2000; 14:1181-5.
74. Shimizu H, Morgan BA. Wnt signaling through the beta-catenin pathway is sufficient to maintain, but not restore, anagen-phase characteristics of dermal papilla cells. J Invest Dermatol 2004; 122:239-45.
75. Hampton PJ, Ross OK, Reynolds NJ. Increased nuclear beta-catenin in suprabasal involved psoriatic epidermis. Br J Dermatol 2007; In Press.
76. Grzeschik KH, Bornholdt D, Oeffner F, Konig A, del Carmen Boente M, Enders H, Fritz B, Hertl M, Grasshoff U, Hofling K, Oji V, Paradisi M, Schuchardt C, Szalai Z, Tadini G, Traupe H, Happle R. Deficiency of PORCN, a regulator of Wnt signaling, is associated with focal dermal hypoplasia. Nat Genet 2007; 39:833-5.
77. Wang X, Reid Sutton V, Omar Peraza-Llanes J, Yu Z, Rosetta R, Kou YC, Eble TN, Patel A, Thaller C, Fang P, Van den Veyver IB. Mutations in X-linked PORCN, a putative regulator of Wnt signaling, cause focal dermal hypoplasia. Nat Genet 2007; 39:836-8.
78. Gat U, DasGupta R, Degenstein L, Fuchs E. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 1998; 95:605-14.
79. Chan EF, Gat U, McNiff JM, Fuchs E. A common human skin tumour is caused by activating mutations in beta-catenin. Nat Genet 1999; 21:410-3.
80. Niemann C, Owens DM, Schettina P, Watt FM. Dual role of inactivating Lef1 mutations in epidermis: tumor promotion and specification of tumor type. Cancer Res 2007; 67:2916-21.
81. Randall VA. Hormonal regulation of hair follicles exhibits a biological paradox. Semin Cell Dev Biol 2007; 18:274-85.
82. Ohnemus U, Uenalan M, Inzunza J, Gustafsson JA, Paus R. The hair follicle as an estrogen target and source. Endocr Rev 2006; 27:677-706.
83. Mulholland DJ, Cheng H, Reid K, Rennie PS, Nelson CC. The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. J Biol Chem 2002; 277:17933-43.
84. Amir AL, Barua M, McKnight NC, Cheng S, Yuan X, Balk SP. A direct beta-catenin-independent interaction between androgen receptor and T cell factor 4. J Biol Chem 2003; 278:30828-34.
85. Truica CI, Byers S, Gelmann EP. Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. Cancer Res 2000; 60:4709-13.
86. Koh SS, Li H, Lee YH, Widelitz RB, Chuong CM, Stallcup MR. Synergistic coactivator function by coactivator-associated arginine methyltransferase (CARM) 1 and beta-catenin with two different classes of DNA-binding transcriptional activators. J Biol Chem 2002; 277:26031-5.
87. Macdonald BT, Semenov MV, He X. SnapShot: Wnt/beta-catenin signaling. Cell 2007; 131:1204.
88. Semenov MV, Habas R, Macdonald BT, He X. SnapShot: Noncanonical Wnt Signaling Pathways. Cell 2007; 131:1378.
89. Widelitz RB, Jiang TX, Yu M, Shen T, Shen JY, Wu P, Yu Z, Chuong CM. Molecular biology of feather morphogenesis: a testable model for evo-devo research. J Exp Zoolog B Mol Dev Evol 2003; 298:109-22.
90. Chuong, CM. Regenerative biology: New hair from healing wounds. Nature 2007; 447:265-6.