Monstrous attempts at adnexogenesis: regulating hair follicle progenitors through Sonic hedgehog signaling

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Epithelial organs such as the vertebrate hair control periodic self-renewal by regulating the growth of progenitor cells. Recent studies implicate Sonic hedgehog target gene induction in the growth of multipotent hair follicle epithelium and the development of a variety of hair follicle tumors such as basal cell carcinomas. These studies suggest Sonic hedgehog signaling may regulate progenitor cells in other organs.

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Abbreviations

BCC	basal cell carcinoma
BMP	bone morphogenetic proteins
DP	dermal papilla
ePr	epidermal progenitors
hPr	hair follicle progenitors
Ptc1	Patched1
SCC	squamous cell carcinoma
Shh	Sonic hedgehog
Smo	Smoothened

Introduction

"*The great majority of adnexal tumors come under the heading of organoid tumors and may be considered monstrous attempts at adnexogenesis…*" H Pinkus, 1966 [1].

Despite their architectural and functional diversity, epithelial organs, or 'adnexa' such as the vertebrate hair, share common developmental strategies. An intriguing property of many epithelial tissues is periodic self-renewal through the regulation of multipotent progenitor cells [2]. One of the key signals involved in the growth and differentiation of many epithelial organs is the hedgehog (hh) signaling pathway. Recent studies in cutaneous biology have shown that hh signaling has an important role in the growth of hair follicles, as well as in the development of hair follicle tumors such as basal cell carcinomas (BCCs).

Here we review recent evidence implicating hh signaling in the expansion and differentiation of hair follicle progenitor cells, and the consequences of inappropriate hh target-gene induction to tumor formation.

The Shh pathway and follicular growth

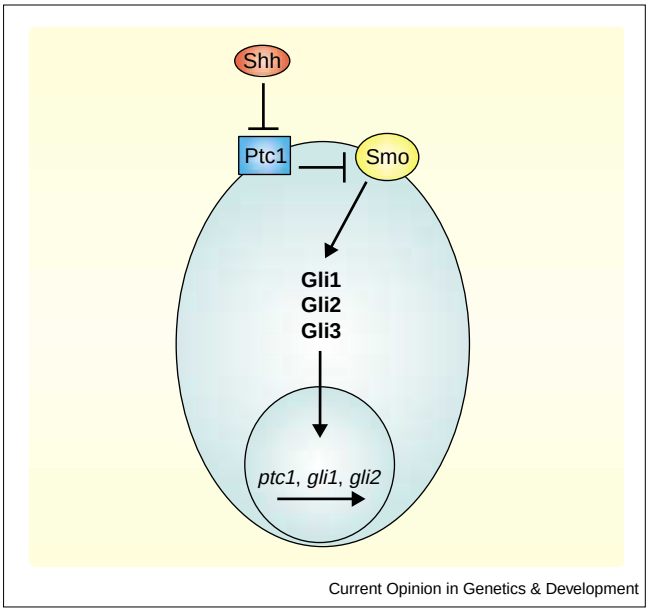
Components of the hh signaling cascade were first identified in *Drosophila melanogaster* and have subsequently been found and extensively studied in many vertebrate and invertebrate organisms [3–5]. Conserved components of the pathway include the secreted, lipid-modified protein hh. In vertebrates, there are three hh genes of which Sonic hedgehog (Shh) is the most extensively studied and the only vertebrate hh expressed in skin. Shh induces transcription of target genes in receiving cells by antagonizing the activity of its transmembrane receptor Patched (Ptc1) (Figure 1). In the absence of Shh, Ptc1 represses Shh target genes such as *ptc1*, *gli1* and *gli2* by inhibiting the activity of the seven-pass transmembrane protein Smoothened (Smo). Smo induces the activity of the Gli/cubitus interruptus zinc-finger family of transcription factors, which are thought to mediate most of the transcriptional effects of hh signaling. Three Gli genes (Gli1, -2 and -3) have been identified in vertebrates and together mediate both activation and repression of Shh target genes [6–8].

Recent studies have extended a working model for hair follicle growth [9]. In this model, a hair follicle niche is created during embryogenesis by reciprocal signaling between the epithelium and aggregates of mesenchymal cells that eventually become the dermal papilla (DP) of the postnatal hair [9] (Figure 2). The inductive signals from the mesenchymal DP cells segregate portions of the epithelium into hair and non-hair epithelium and then induce growth of the follicular epithelium.

Histologically, the growth of postnatal hair epithelium resembles embryonic hair growth. The postnatal hair follicle originates from multipotent progenitors, which are thought to be located in a specialized epithelial compartment of the permanent hair follicle referred to as the 'bulge' [10,11••,12••,13]. Cells within the bulge give rise to progenitors that migrate in one of two directions [11••,12••]. The progenitor cells, or 'ePr' cells, that migrate superficially populate the basal layer of the interfollicular epidermis and form stratified epithelium.

Alternatively, the progenitor cells, or 'hPr' cells, that migrate inward toward the dermis in response to DP signals populate the growing hair epithelium and form the hair follicle. As the hPr cells divide and move further away from the dermal papilla (DP), they stop proliferating and begin a process of hair follicle differentiation, giving rise to the six concentric epithelial layers of the growing hair follicle [14]. Growth and differentiation of the hPr cells continues throughout the growth or 'anagen' phase, after which proliferation ceases and the distal hair follicle regresses ('catagen'). Hair follicle quiescence ('telogen'), hair-shaft shedding ('exogen'), and finally the initiation of a new growth cycle ('anagen') complete hair follicle renewal [15].

Figure 1



A simplified model of the Shh signaling pathway. Conserved components of the vertebrate hh signaling pathway include the secreted protein Shh and its transmembrane receptor Ptc1. In the absence of Shh, Ptc1 functions, at least in part, by inhibiting the activity of Smo. When Shh binds to Ptc1, the repression of Smo is lifted, followed by activation of the Gli transcription factors and induction of Shh target genes, such as *ptc1*, *gli1* and *gli2*. For a complete review of the pathway, see [3–5].

Recent studies suggest that Shh signaling has a central role in hair follicle growth by regulating both epithelial and mesenchymal components of the hair follicle. During embryonic folliculogenesis, *shh* transcripts are expressed in invaginating epithelial cells of the proliferating hair follicle. Shh expression seems to influence both proliferating hPr cells and adjacent, aggregating dermal cells, as indicated by their expression of the Shh target genes *ptc1* and *gli1* [16–19].

The first evidence that Shh signaling is required in folliculogenesis came from analyses of Shh knockout mice. Mice lacking Shh function show normal follicle spacing, but have arrested development of embryonic hair follicles. The arrested hair follicles demonstrate both a decrease in epithelial proliferation and a failure of the underlying mesenchyme to aggregate and form a mature DP. Although complete folliculogenesis does not occur, many markers of follicular differentiation are expressed [17,18].

A similar role for Shh signaling has been identified during the adult anagen hair cycle. *Shh* expression is restricted to cells at the distal portion of the growing hair follicle with its target genes *ptc1* and *gli1* expressed in both the proliferating hPr cells and the adjacent DP ([20]; A Oro, unpublished data). While mice treated with anti-Shh antibodies show impaired epithelial growth at sites of folliculogenesis [21•]. As seen in *shh* mutants, the hair follicle epithelium that does form is capable of some follicular differentiation. Although only one study has been carried out so far [21•], the similarity of this phenotype to that of *shh* mutants suggests that additional studies will confirm the role of Shh in postnatal hair morphogenesis. Because defects are seen in the DP and epithelium, both of which are required for continuous growth of hair follicles, it is unclear whether Shh signaling is required in the epithelium, in the DP, or in both. Heterotopic recombination experiments using epidermis and DP from different genetic backgrounds or conditional knockout mutants are required to delineate these cell autonomy issues.

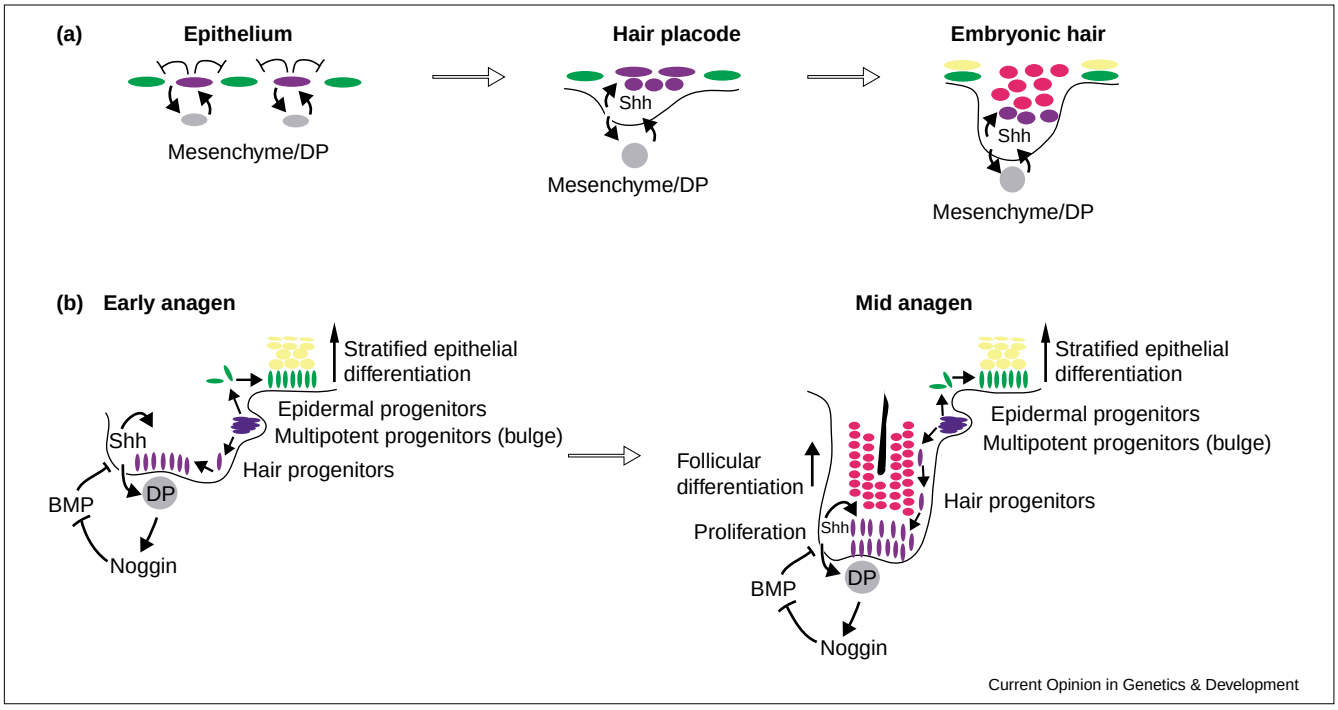
Follicular tumors and induction of epithelial Shh target genes

Although *shh* mutants have shown that there is a requirement for Shh in normal follicular growth, recent studies have suggested that unregulated induction of epithelial Shh target genes promotes the formation of hair follicle tumors through its proliferative influences on hPr-like cells (Figure 3). If Shh target-gene induction is sufficient for the proliferation of hPr-like cells, then expression of Shh target genes should generate ectopic epithelium with follicular differentiation.

Consistent with this notion, forced activation of Shh target genes in epithelium induces follicular tumors, the most clinically significant of which are BCCs. These carcinomas are composed of cells that are ultrastructurally and immunophenotypically similar to hPr cells and, thus, are the least differentiated of tumors derived from hair follicles [1,22,23]. Since the first studies that associated mutations in *ptc1* with Gorlin Syndrome, an inherited susceptibility to BCC formation, analyses of sporadic BCCs have verified a link between activation of the Shh pathway, target-gene induction and BCC formation [24–26].

Additional human genetic and animal model studies indicate that the ectopic expression of Shh target genes is sufficient to produce many other follicular tumors. Transgenic mice overexpressing either Shh [19], a gain-of-function mutation for Smo [27], Gli1 [28] or Gli2 [29] in skin epithelium develop tumors displaying various stages of follicular differentiation. For example, mice overexpressing Shh initially develop undifferentiated, BCC-like tumors that eventually differentiate into hair follicle structures [19]. Mice overexpressing Gli1 develop tumors resembling both poorly differentiated follicular neoplasms (BCCs and trichoblastomas) and more differentiated hair follicle tumors (trichoepitheliomas and cylindromas) [28]. The fact that these tumors are generally diploid [30], arise in skin without apparent precursor lesions [31], and generally lack mutations that are causally associated with other non-melanoma skin cancer [28], suggests that induction of Shh target genes is sufficient for initiating follicular tumors. Shh's role in promoting the proliferation of hPr cells during folliculogenesis, combined with the fact that ectopic target-gene induction promotes follicular tumors, suggests that Shh target genes are sufficient to stimulate follicular tumor production through the expansion of hPr-like cells.

Figure 2



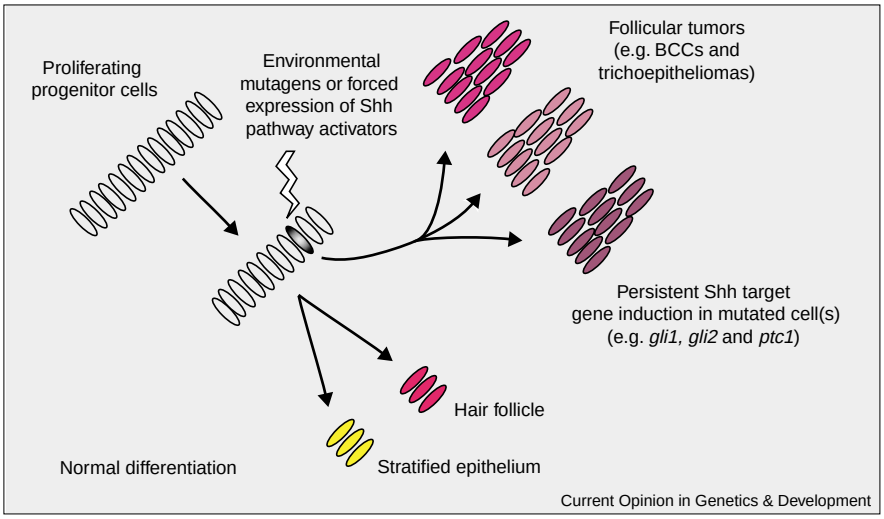
Shh controls the growth of both embryonic and postnatal hair follicle progenitor cells. (a) During embryogenesis, a sequence of reciprocal signals is passed between the epithelium and mesenchyme to induce properly spaced epithelial placodes and juxtaposed mesenchymal condensations. Epithelial Shh is required for the continued growth and morphogenesis of the hair follicle epithelium as well as maturation of the dermal papilla. (b) A similar role for Shh signaling has been identified during the adult anagen hair cycle, in which epithelial Shh acts on the follicular epithelium to promote proliferation and down-growth of the anagen hair follicle. The regulation of BMP activity by Noggin appears to regulate Shh expression and hPr proliferation. Blue cells represent multipotent progenitors, purple cells are hair progenitors that will differentiate into the hair follicle (red). Green cells represent stratified epithelial progenitors that will differentiate into stratified epithelium (yellow).

Effects of Shh target genes on progenitors of stratified epithelium

The induction of epithelial Shh target genes regulates the growth of hPr-like cells, but does it have a role in the proliferation or differentiation of progenitor cells that give rise to the stratified epithelium, that is, ePr cells? Initial studies suggest that Shh does not have a major role in these processes. First, normal Shh expression and the induction of target genes seem to be restricted to the anagen hair follicle [20,21•]. Second, *shh* mutants and animals

Figure 3

Unregulated epithelial Shh target-gene induction promotes follicular tumors. Activating mutations of the Shh signaling pathway, (i.e. loss-of-function mutations in *ptc1*or gain-of-function mutations in *smo*) or forced *gli1* or *gli2* expression in the epithelium leads to unregulated transcriptional activation of Shh target genes in progenitor cells (represented by the black gradient labeled cell). Persistent Shh target-gene induction in mutant cells stimulates the growth of epithelium with follicular differentiation (various shades of purple). White cells represent hair or stratified epithelial progenitors and the red or yellow cells represent differentiated hair or stratified epithelial cells, respectively.



injected with anti-Shh antibodies display a wild-type pattern of differentiation of stratified epithelium [17,18,21•]. Last, tumors of ePr cells such as squamous cell carcinomas (SCCs) do not express high levels of Shh target genes [32••,33]. Shh signaling may, however, have a minor role in the growth of SCCs. These tumors are found at slightly higher frequency in irradiated *ptc1*/+ mice, and mutations in the human *ptc1* gene are detected at low frequency in sporadic SCCs [34,35].

Accumulating evidence indicates that ectopic induction of Shh target genes can re-specify ePr cell fates and form follicular tumors, even though Shh is not directly involved in specifying ePr differentiation. The competence for ePr cells to differentiate into follicular structures has been demonstrated previously by exposing interfollicular epidermis to DP cells or key regulatory signals [20,36]. If ectopic Shh target genes can alter progenitor cell fates, then one would expect follicular tumors to develop in interfollicular regions of the skin. Indeed, three independent studies using ectopic Shh expression suggest that Shh target-gene induction can promote follicular tumors at interfollicular locations [19,37,38]. Moreover, in adult *ptc1*/+ mice irradiated with ultraviolet light, BCCs and trichoblastomas develop in the interfollicular epidermis owing to ectopic induction of Shh target genes [32••]. These data suggest that ePr cells are competent to respond to Shh target genes and that ectopic Shh target-gene induction can change the fate of ePr cells to that of hPr-like cells.

Does the induction of Shh target genes control only the proliferation of hPr-like cells, or does it also continue to specify different cell fates within the follicular differentiation program? The production of histologically distinct follicular tumors from mice, either within a single transgenic line or expressing different Shh pathway members, could support a role for Shh target-gene induction in specifying differentiation. Subtle differences in levels of Shh target genes might explain the differing types of follicular tumors that occur with forced expression of Shh pathway activators. Further evidence to support this hypothesis comes from studies showing that trichoepitheliomas have higher levels of the *ptc1* gene product than BCCs [39]. Alternatively, varying follicular differentiation might come from the expansion of multipotent hPr cells that are already committed to different differentiation pathways. Studies that carefully address the degree of follicular differentiation relative to levels of Shh target-gene induction in tumors and the normal hair might distinguish between these possibilities.

Mechanisms regulating Shh target-gene induction in a multipotent epithelium

As the ectopic induction of Shh target genes can redirect ePr cell fates, there must be a critical need for spatial and temporal restriction of Shh target-gene induction for controlled hair follicle growth and for preventing follicular tumorigenesis. How then does the adult hair normally regulate the induction of Shh target genes in progenitor cells? The hair follicle niche, which is created by inductive signals during embryogenesis, continues to control the spatial and temporal expression of Shh in the adult.

Regulation by Noggin and the BMPs

Key regulators of the developing niche include the fibroblast growth factors, which promote Shh expression in the hair placode, and the bone morphogenetic proteins (BMPs), which antagonize Shh expression [9]. As in embryonic hair development, the domain of epithelial Shh expression in the postnatal hair seems to be limited by BMP activity in the differentiating hair progenitor cells. Noggin, an inhibitor of BMPs, is secreted from the underlying DP cells to antagonize BMP activity, allowing Shh expression to promote hair follicle growth. Loss of Noggin function results in increased BMP activity and a decrease in the number and size of hair follicles [40••].

Overexpression of Noggin in the hair matrix cells, using the Msx2 promoter, results in a broadening of Shh expression, which is associated with an increased domain of proliferating hPr cells and a decreased number of differentiation markers [41•]. Interestingly, no follicular tumors are seen despite the overexpression of Shh, suggesting that differentiation cues within the hair follicle niche suppress the overgrowth of multipotent hPr cells and thus prevent tumorigenesis.

Shh self-regulation

Another key factor in the regulation of Shh expression may be Shh itself. Forced expression of Shh throughout mouse skin using an adenovirus vector results in the premature initiation of anagen in resting telogen hairs [42••]. Although the ectopic expression of Shh throughout the skin in this study limited the interpretation of where Shh is functioning, the results do indicate that Shh can initiate premature anagen if applied a few days before initiation of the normal hair growth cycle.

Given that Shh regulates the follicular mesenchyme, Shh may be acting on the DP in postnatal hair follicles by promoting mesenchymal signals that are required for anagen initiation. Further studies using cell-specific loss-of-function mutants are needed to separate epithelial from dermal Shh functions.

Temporal regulation

In addition to spatial regulation of Shh production, evidence is emerging that Shh target-gene induction is temporally regulated in the epithelium. Temporal regulation of target-gene induction might act to coordinate Shh-dependent growth of progenitors with other developmental regulators. As mentioned above, ectopic Gli1 and Gli2 expression in epithelium is sufficient to induce tumorigenesis. But although transgene-derived ectopic expression of Gli1 or Gli2 begins early in embryogenesis, the phenotypes from these transgenes are not manifest until at least 3 weeks after birth [28,29].

Temporal restrictions on hh signaling are also seen in other model systems. For example, injections of *Xenopus* hh (vhh) into the frog neural tube reveal a spatial and temporal restriction of vhh target-gene induction [43]. Similarly, studies in chick feather-bud epithelium using a Shh retrovirus reveal that Shh induction at various locations and times yields markedly different results [38].

The chick studies reveal the presence of developmental windows within which the epithelium is competent to develop large feather bud tumors when Shh target genes are induced. Although these studies do not address the cellular or molecular mechanism(s) through which temporal regulation occurs, their paradigms may hold clues towards unraveling the mechanisms of epithelial growth regulation.

Conclusions

Studies of epithelial growth and development are establishing a role for Shh signaling in the expansion of progenitor cells during organ development and regeneration. Recently, analyses of the fly ovary, as well as vertebrate cerebellum and mesoderm [44–46] also show that the induction of hh or Shh target genes is normally associated with the expansion of multipotent progenitor cells. Similar to the skin, ectopic Shh target-gene induction in the cerebellum or mesoderm can yield progenitor cell tumors such as medulloblastomas or rhabdomyosarcomas, respectively [47,48].

Future studies addressing how local niches normally regulate the timing and location of Shh signaling will give us a better understanding of epithelial organogenesis and tumorigenesis. This in turn may lead to novel treatments for common organoid tumors.

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