1 *Review*

Roles of the Hedgehog Signaling Pathway in Skin Development, Homeostasis and Cancer

Yoshinori Abe [1] and Nobuyuki Tanaka [1],*

[1] Department of Molecular Oncology, Institute for Advanced Medical Sciences, Nippon Medical School. 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211-8533, Japan; yoshiabe@nms.ac.jp (Y.A.), nobuta@nms.ac.jp (N.T.)

* Correspondence: nobuta@nms.ac.jp; Tel.: +81-44-733-1860

Abstract: The epidermis is the outermost layer of skin and provides a protective barrier against environmental insults. It is a rapidly renewing tissue undergoing constant regeneration, maintained by several types of stem cells. Hedgehog (HH) ligands activate one of the fundamental signaling pathways that contribute to epidermal development, homeostasis and repair. The HH pathway interacts with other signal transduction pathways such as those activated by Wnt and bone morphogenetic protein. Furthermore, aberrant activation of HH signaling is associated with various tumors, including basal cell carcinoma. Therefore, an understanding of the regulatory mechanisms of the HH signaling pathway is important to elucidate fundamental mechanisms underlying both organogenesis and carcinogenesis. In this review, we discuss the role of the HH signaling pathway in skin development, homeostasis and basal cell carcinoma formation, providing an update of current knowledge in this field.

Keywords: Hedgehog signaling; skin; epidermis; hair follicle; development; homeostasis; basal cell carcinoma; signaling crosstalk

1. Introduction

Although the thickness of the skin is as little as 2 mm on average, it is a complex stratified organ consisting of an outer epidermis and appendages (for example, hair follicles, sebaceous glands and sweat glands) separated from the underlying dermis by a basement membrane. It is the largest organ in the human body, by area and weight. Skin is the primary barrier that protects the body from major environmental stresses such as dehydration, scratches, wounds and microbial infection [1]. To repair damaged tissue and replace old cells, different stem cell pools resident in the skin contribute to the maintenance and repair of the various epidermal tissues, including interfollicular epidermis, hair follicles and sebaceous glands [2,3]. The stem cells residing in the epidermis are maintained by various signaling pathways such as those activated by Wnt, transforming growth factor β (TGF-β), Notch and Hedgehog (HH) [3]. These signaling pathways also have important roles in embryonic skin development [4]. However, the dysregulation of signaling pathways related to skin development and homeostasis induces epigenetic reprograming of skin cells, resulting in cancer development. In particular, aberrant activation of HH signaling is the major cause of basal cell carcinoma (BCC) [5]. In this review, we provide a current understanding of the role of the HH signaling pathway in embryonic skin development, skin homeostasis and skin cancer.

2. The HH Signaling Pathway

The HH pathway is one of the most important signal transduction pathways in tissue development, homeostasis and repair. It regulates the morphogenesis of various organs during embryogenesis [6]. We show a schematic diagram of the HH signaling pathway in Figure 1. However, HH signaling is complex, and several comprehensive reviews have been published describing the molecular mechanisms in detail [7–10]. In the canonical pathway, HH signaling is

45   initiated by one of three spatiotemporally confined ligands: Sonic Hedgehog (SHH), Indian
46   Hedgehog (IHH) and Desert Hedgehog (DHH). HH ligands control numerous developmental
47   outcomes in a concentration- and duration-dependent manner. Each HH ligand has distinct spatial
48   and temporal expression patterns, but all activate HH signaling by binding to Patched (PTCH) 1 or
49   2, which are 12-pass transmembrane-spanning receptors. PTCH1 is considered to be the primary
50   receptor for HH ligands. In the absence of HH, PTCH1 is localized in primary cilia and constitutively
51   suppresses the activity of Smoothened (SMO), a 7-pass transmembrane-spanning protein that is a
52   member of the G-protein-coupled receptor superfamily [11]. Suppressor of fused (SUFU) is a key
53   negative regulator of the HH signaling pathway [12]. In the absence of HH ligands, SUFU inhibits
54   signaling by sequestration of GLI proteins in the cytoplasm (Figure 1A). In addition to PTCH, other
55   HH-binding cell surface proteins have been identified, such as CAM-related/down-regulated by
56   oncogenes (CDO), brother of Cdo (BOC) and growth-arrest-specific 1 (GAS1) [13,14]. These
57   molecules function as HH coreceptors to facilitate HH signal reception. Following the binding of a
58   HH ligand to PTCH, SMO accumulates at a specialized compartment in the primary cilium called
59   the EvC zone, mediated by the Ellis van Creveld Syndrome (EVC)–EVC2 complex [15,16]. From
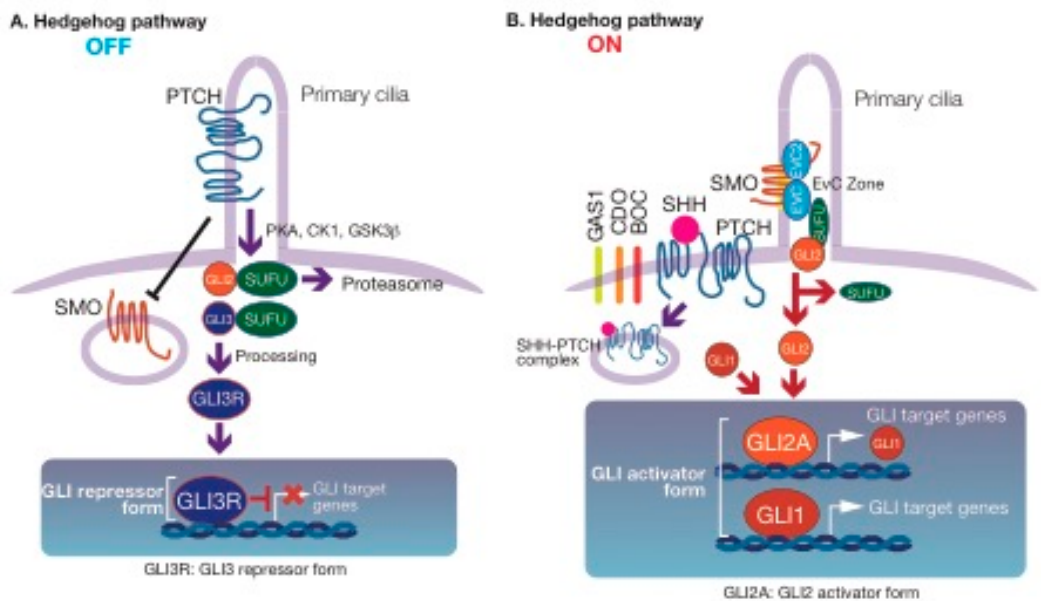60   here, SMO transmits signals that activate GLI (Figure 1B) [17,18].

61   **Figure 1.** The Hedgehog signaling pathway. (A) In the absence of Hedgehog (HH) ligand, Patched
62   (PTCH) blocks ciliary localization of Smoothened (SMO), and a repressor form of GLI (mainly
63   GLI3R) suppresses the induction of GLI target genes. (B) Signaling is activated in the presence of HH
64   ligand. The binding of HH ligand to PTCH prevents PTCH inhibition of SMO, and SMO then
65   interacts with the Ellis van Creveld Syndrome (EVC)–EVC2 complex and translocates into primary
66   cilia to be fully activated. SMO then activates GLI family transcription factors, mainly GLI2. GLI2
67   upregulates expression of GLI1 as well as GLI target genes. GLI1 is also activated downstream of
68   SMO. Activated GLI2 (GLI2A) and GLI1 further upregulate the expression of various GLI target
69   genes. This figure is adapted from a my previous work [19].

70   Graded levels of HH signaling trigger the expression of different sets of response genes,
71   depending on the ratio of Gli activator (GLI-A) to repressor (GLI-R) forms. In vertebrates, the GLI
72   family consists of three proteins, GLI1, GLI2 and GLI3 [9]. All GLI proteins contain an activator
73   domain at their C-terminus; GLI2 and GLI3 also have an N-terminal repressor domain [20]. Studies
74   in mutant mice suggest that GLI2 is the major activator of HH signaling [21], whereas GLI3 is the
75   major repressor [22,23]. GLI1 most likely serves as a signal amplifier downstream of GLI2 [21,24].
76   When HH signaling is activated, SUFU–GLI2 complexes dissociate and GLI2 is activated [25]. *Gli2*

77 knockout mice die at birth, whereas *Gli1* knockout mice show normal development, unless one copy
78 of *Gli2* is also defective [26]. Interestingly, experiments in mutant mice further suggest that Gli2 can
79 rescue Gli1 protein function, whereas *Gli1* knock-in into the *Gli2* allele can rescue the *Gli2*-null
80 phenotype [27].

81 Constitutive activation of HH signaling has been observed in many cancers [28] and promotes
82 cancer cell proliferation, metastasis and cancer stem cell maintenance. For example, somatic
83 mutations of *PTCH1* and *SMO* have been identified in patients with BCC and medulloblastoma [29–
84 32]. Other mutations in genes encoding HH pathway components have been reported, including
85 *SUFU* mutations in BCC and medulloblastoma [33]. Furthermore, multiple mechanisms of HH
86 pathway activation in cancer have been proposed, and reviewed in detail elsewhere [10,34,35].

87 HH signaling exhibits crosstalk with other signaling pathways, including Wnt [36–39], TGF-β
88 [40], epidermal growth factor receptor (EGFR) [41], rat sarcoma viral oncogene homolog GTPase
89 (RAS)–extracellular signal-regulated kinase (ERK) [42,43], phosphatidylinositol 3-kinase (PI3K)–
90 Akt–mechanistic target of rapamycin (mTOR) [44] and Notch [45]. Interestingly, the crosstalk
91 between HH signaling and other pathways seem to be different between developmental,
92 homeostatic and carcinogenic processes.

93 **3. Overview of Epidermal and Hair Follicle Development**

94 *3.1. Development of the Epidermis*

95 During embryonic development, the epidermis is derived from the ectoderm. In the mouse,
96 after gastrulation, the embryo surface consists of a single layer of neuroectoderm, which will form
97 the nervous system and skin epithelium from embryonic day 9.5 (E9.5) to E12.5. Mesenchymal cells
98 from the underlying layer transmit signals that instruct the stratification of the epidermis and dictate
99 the positioning of downgrowths that mark the initiation of hair follicle morphogenesis [1]. In
100 alliance with the mesenchyme, the inner-most, or basal layer of the stratifying epidermis produces
101 and organizes an underlying basement membrane that is rich in extracellular matrix proteins and
102 growth factors. The epidermis adheres to this basement membrane, which serves not only as a
103 growth-promoting platform but also as a physical boundary between the epithelium and the dermis
104 [46]. During the initial steps of stratification (E12.5–E15.5), cell proliferation is almost completely
105 confined to the basal layer, and these cells differentiate immediately. In this period, the single layer
106 of epidermal cells is covered by a transient protective layer called the periderm. Although there is
107 regional variation, the stratification program is largely complete around E17.5 [47].

108 *3.2. Hair Follicle Development*

109 The development of hair follicles is intrinsically related to the stratification of the embryonic
110 epidermis. Hair follicle development is the result of epithelial–mesenchymal interactions. Although
111 hair follicle development occurs in eight morphologically distinct stages, it can more simply be
112 divided into three phases: induction, organogenesis and cytodifferentiation [48]. In the induction
113 phase, which is the initiation of hair follicle development (E13), signaling crosstalk between the
114 epidermis and dermis is induced, and epidermal keratinocytes form clusters that become enlarged
115 and elongated to form hair placodes (E14). Hair follicle development then enters the organogenesis
116 phase. A cluster of specialized fibroblasts, the precursors of the dermal papilla, is formed just under
117 the placode, and the signaling crosstalk between these specialized fibroblasts and the epithelial
118 placode leads to cell proliferation in both structures. The enhanced cell proliferation leads to a
119 downward growth of the epidermal component, shaping the dermal papilla. The resulting structure,
120 called the hair germ, is typically observed at E15.5. Keratinocyte downgrowth continues, whereby
121 the most proximally located keratinocytes begin to wrap around the dermal papilla (E16.5–E17.5).
122 As follicles enter the cytodifferentiation phase, the inner root sheath is formed, triggering the
123 terminal differentiation of keratinocytes above the dermal papilla to generate the hair shaft.

124   Simultaneously, the outer root sheath starts to form a cylinder around the inner root sheath to create
125   a bulbous peg structure (E18.5). Additional appendages such as sebaceous glands and touch domes
126   also develop during this phase. These timelines are summarized in Figure 2. For a more detailed
127   discussion of hair follicle development, please refer to several published reviews [2,4,49].
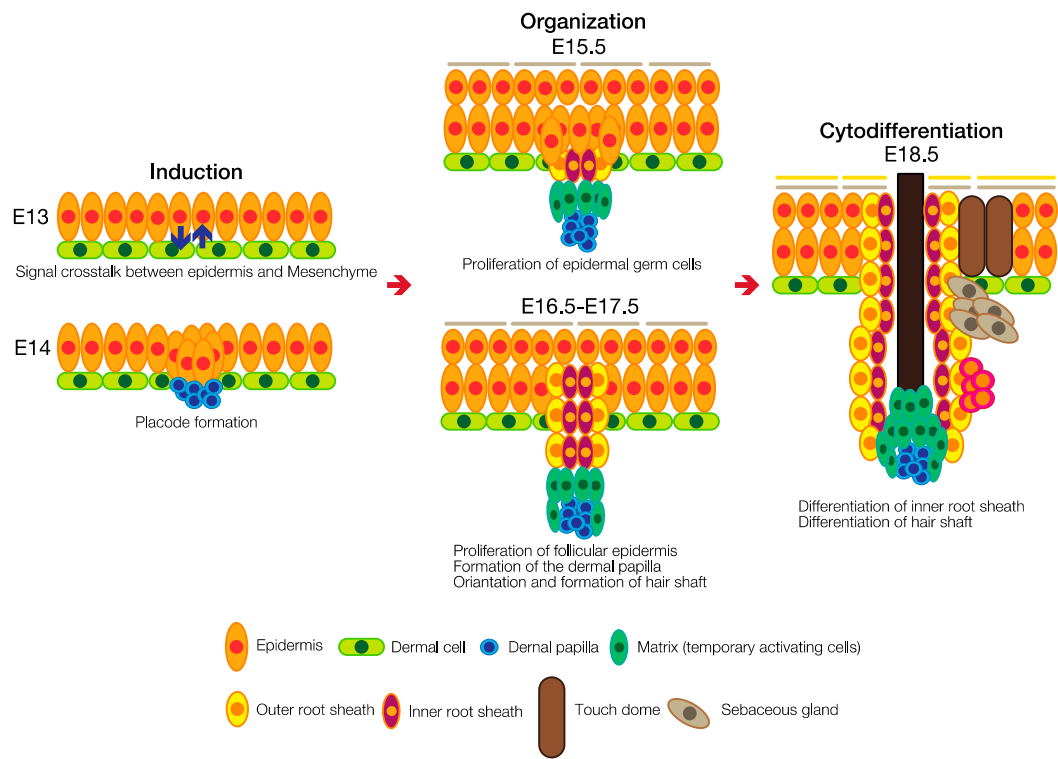

128

129   **Figure 2.** At the beginning of hair follicle development, gradients of activators and inhibitors
130   generate a well-circumscribed inductive field (E13). These gradients induce epithelial and dermal
131   cell proliferation to develop a placode and a dermal papilla (E14). The dermal papilla sends growth
132   signals back to the epithelial cells to form the hair follicle (E15.5–E17.5). In the cytodifferentiation
133   phase, epithelial cells differentiate into inner and outer root sheath, which generates the hair shaft. In
134   addition, melanocytes and hematopoietic cells migrate into the hair follicle, and touch domes and
135   sebaceous glands are formed.

136   **4. The Roles of HH Signaling in Hair Follicle Development**

137   In mouse skin at E15.5, at the beginning of the organogenesis phase of hair follicle development,
138   *Ptch1*, *Smo*, *Gli1* and *Gli2* are expressed in both the epithelial and dermal components of the early
139   hair follicle, although higher levels of *Ptch1* and *Gli1* are observed in the follicular dermal
140   mesenchyme. In contrast, *Ptch2* expression is restricted to epithelial cells of the hair bud where it is
141   co-expressed with *Shh* [47,50]. Therefore, the HH signaling pathway functions in both the epithelium
142   and the mesenchyme. In mice lacking *Shh*, hair follicle formation is initiated and specialized
143   fibroblasts are condensed, indicating that the initial events of hair follicle development are
144   independent of HH signaling. However, in the hair follicle maturation phase, the loss of Shh
145   selectively reduces keratinocyte proliferation in the developing follicle epithelium, so that the
146   downgrowth of epithelial cells fails. Therefore, the number of hair follicles is reduced in $Shh^{-/-}$ mice.
147   Thus, HH signaling is necessary for the transition from the induction to the organogenesis phase,
148   and for the downgrowth of epithelial cells after the induction phase. Furthermore, the maturation of
149   the dermal papilla also depends on HH signaling, since $Shh^{-/-}$ mice have smaller dermal papillae
150   [50,51]. It is believed that GLI2 is the primary transducer of HH signaling, whereas GLI1, the
151   expression of which is transcriptionally regulated by GLI2, plays a secondary role in potentiating the
152   HH response [9]. Mill *et al.* demonstrated that hair follicle maturation fails in $Gli2^{-/-}$ skin because of

153 suppressed epithelial cell proliferation, similar to *Shh*−/− skin [52]. Development of the dermal papilla
154 is also severely affected by the failure to respond to SHH in *Gli2*−/− skin. In addition, *Shh*−/− as well as
155 *Gli2*−/− skin fails to induce Gli1. Together, these results show that the SHH–GLI2 axis is required for
156 the proliferation and subsequent downgrowth of the follicular epithelium, and for the maturation of
157 the dermal papilla.

158 As described above, the expression of *Ptch1*, *Smo*, *Gli1* and *Gli2* is seen in the dermal papilla
159 [50], indicating that HH signaling also functions in dermal cells. RNAi-mediated SMO knockdown
160 in dermal cells results in the loss of the dermal papilla precursor, the dermal cell condensate, and in
161 hair follicle arrest at the organogenesis phase, similar to *Shh*−/− skin. However, HH signaling in
162 dermal cells does not affect cell survival or epithelial proliferation. Rather, dermal HH signaling
163 regulates the expression of a subset of dermal papilla-specific signature genes. These results suggest
164 that dermal HH signaling regulates genes required to maintain the maturation of the papilla [53].
165 Sex determining region Y-box 2 (SOX2) is expressed only in the dermal papilla and determines hair
166 follicle type [54,55]. Recently, Driskel *et al.* demonstrated that dermal papilla cells are heterogenous
167 and SOX2+/CD133+ dermal papilla cells are associated with guard, awl and auchene hair follicle
168 formation whereas SOX2−/CD133+ dermal papilla cells are involved in the formation of zigzag hair
169 follicles. Moreover, they demonstrated the HH signaling pathway is activated in SOX2−/CD133+
170 dermal papilla cells, suggesting that HH signaling regulates hair type fate determination through
171 the maintenance of dermal papilla cell heterogeneity [55]. The roles of the HH signaling pathway in
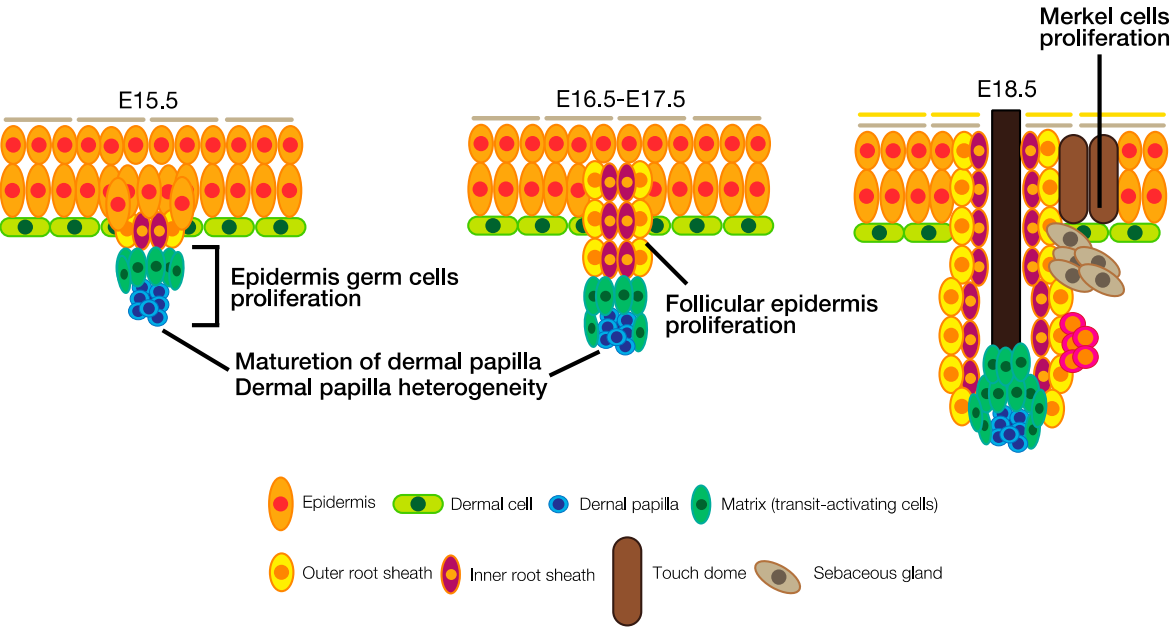172 hair follicle development are summarized in Figure 3.

173 **Figure 3.** The roles of the HH signaling pathway in embryonic hair follicle development. In
174 epidermal cells, HH signaling is involved in germ cell and follicular cell proliferation. HH signaling
175 is also involved in the maturation of the dermal papilla, as well as dermal papilla heterogeneity.
176 Furthermore, HH signaling is essential for Merkel cell proliferation and touch dome development.

177 On the other hand, negative regulators of HH signaling are also important for hair follicle
178 development. PTCH1 and 2 are known to be negative regulators of HH signaling, and *Ptch1* is a GLI
179 target gene [47,56]. Basal cell-specific *Ptch1*-null skin exhibits hyperplasia and skin neoplasia
180 formation, although *Ptch2*-null skin is undistinguishable from wild-type skin with respect to
181 embryonic hair follicle development [57,58]. However, the skin of both basal cell-specific *Ptch1*-null
182 and *Ptch2*-null mice exhibits excessive proliferation of epithelial cells, suggesting that epidermal
183 stem and progenitor cells enter a hyperproliferative state. In addition, progenitor cells fail to
184 differentiate, resulting in the loss of epidermal lineage specification. Therefore, PTCH1- and

185   2-mediated HH signaling suppression is important for regulating epidermal differentiation [59].
186   Another study reported that increased numbers of immature hair follicles in the induction phase are
187   seen in *Shh⁻/⁻/Gli3⁻/⁻* skin, in which the repressor form of GLI3 (GLI3R), another negative regulator of
188   HH signaling, is lost. Furthermore, keratinocyte differentiation fails in *Shh⁻/⁻/Gli3⁻/⁻* skin, resulting in
189   the failure of hair follicle maturation [60]. Recently, Adolphe *et al.* discovered that there is a HH
190   signaling gradient along the proximodistal axis of developing hair follicles [61]. At E17.5, the peak of
191   HH signaling activity is in follicular cells, which are a source of SHH, and HH signaling is
192   endogenously low in basal cells. This HH signaling gradient is established by PTCH1 and PTCH2.
193   These results suggest that HH signaling has the capacity for progenitor cell maintenance, and the
194   intensity of HH signaling is important for both epithelial and mesenchymal cell fate determination
195   during hair follicle development.

5. Signaling Crosstalk between HH and Other Pathways in Hair Follicle Development

197   The hair follicle develops as a result of complex reciprocal signaling between epithelial and
198   mesenchymal cells. Various signaling pathways such as those activated by Wnt and bone
199   morphogenetic protein (BMP) are involved in hair follicle development, and exhibit signaling
200   crosstalk with the HH pathway. In this section, we discuss the crosstalk between HH and other
201   signaling pathways in hair follicle development.

5.1. Wnt signaling

203   The Wnt signaling pathway has been implicated in many developmental processes [62].
204   Canonical Wnt signaling is mediated by a transcriptionally active complex between stabilized
205   β-catenin and members of the T-cell factor (TCF)/Lymphoid enhancer-binding factor 1 (LEF-1)
206   family of DNA-binding proteins [63]. One of the first, essential events required to form a follicle
207   placode is the activation of Wnt signaling in the epidermis. Wnt signaling also transmits subsequent
208   signals for hair follicle development and patterning. Wnt ligands are expressed in interfollicular
209   epidermis as well as the hair follicle during all stages of follicle development, suggesting that Wnt
210   signaling is a master regulator of follicle development [36,64,65].
211   The Wnt and HH signaling pathways are often associated. During hair follicle development in
212   mouse embryos, the canonical Wnt signaling pathway is upstream of HH signaling, since β-catenin
213   activation induces *Shh* expression in epidermis during skin development [36,37]. On the other hand,
214   Reddy *et al.* showed that dermal Wnt5a disappears in *Shh⁻/⁻* skin, indicating that Wnt5a is
215   downstream or even a direct target of HH signaling [66]. Therefore, SHH-induced dermal Wnt5a as
216   well as GLI1 expression is important for dermal papilla maturation. Wnt5a suppresses expression of
217   LEF-1, a well-recognized Wnt/β-catenin signaling target in human dermal papilla cells, indicating
218   suppression of canonical Wnt signaling [67]. Therefore, the HH–Wnt5a axis terminates cell
219   proliferation and induces differentiation to promote mature hair follicle formation. Since PTCH1
220   and 2, repressors of HH signaling, are also involved in the induction of cell differentiation,
221   cooperation between Wnt5a and negative regulators of HH signaling may be important for cell
222   differentiation during hair follicle development.

5.2. BMP signaling

224   BMPs are members of TGF-β family. BMP signaling has been suggested to regulate hair follicle
225   induction and the patterning of follicles within the skin by repressing the placode fate [68,69].
226   Suzuki *et al.* demonstrated crosstalk among the Wnt, HH and BMP signaling pathways during hair
227   follicle development [37]. Expression of constitutively active β-catenin induces aberrant keratinocyte
228   proliferation and differentiation in mouse skin during hair follicle development; the aberrant cell
229   differentiation is suppressed by BMP signaling inhibition, and the aberrant cell proliferation is
230   suppressed by HH signaling inhibition. These results demonstrate that Wnt/β-catenin signaling
231   relayed through HH and BMP signals is the principal regulatory mechanism underlying changes in
232   hair follicle cell fate. Furthermore, activation of the HH signaling pathway induces expression of

233   BMP2 in the organogenesis phase of hair follicle development. A possible mechanism for the
234   regulation of hair follicle development involves cell fate being controlled by the difference in
235   diffusion area between promoters such as SHH and suppressors such as BMP [70,71]. Therefore, the
236   presence of signaling crosstalk involving the Wnt, HH and BMP pathways would regulate the
237   diffusion areas of HH and BMP ligands to determine hair follicle cell fate.

238   The HH signaling pathway also affects dermal cells during hair follicle development. Noggin is
239   known as an antagonist of BMPs, and Noggin signaling is a dermal papilla-specific pathway. In
240   *noggin⁻/⁻* mice, hair follicles arrest at the placode formation stage of embryonic development [68,72].
241   Oro *et al.* showed that interaction between HH signaling and Noggin regulates dermal papilla
242   maturation during hair follicle development [53]. Dermal cell-specific RNAi-mediated SMO
243   knockdown does not affect dermal cell proliferation in mice. However, dermal cell condensation
244   following placode formation fails in these mice. These authors found that dermal HH signaling
245   induces dermal papilla signature genes such as *Sox2* [54,55], *Sox18* [73,74] and *noggin*. Among these
246   genes, *noggin* partially rescues dermal SMO knockdown-associated hair regeneration defects by
247   increasing epithelial *Shh* expression. These results suggest that dermal HH signaling regulates
248   specific dermal papilla gene expression signatures to maintain dermal papilla maturation while
249   maintaining a reciprocal HH–Noggin signaling loop to drive embryonic hair follicle development.

250   *5.3. The EdaA1–EdaR–NF-κB axis*

251   Ectodysplasin A1 (EdaA1) and ectodysplasin receptor (EdaR) respectively belong to the tumor
252   necrosis factor (TNF) multigene family of ligands and receptors, and are able to activate the nuclear
253   factor-κB (NF-κB) and Jun amino-terminal kinase (JNK)/activator protein 1 (AP1) pathway *in vitro*
254   [75–78]. The EdaA1–EdaR pathway is activated at placode formation only in primary guard hair
255   follicles. It has been reported that the EdaA1–EdaR–NF-κB axis is involved in placode formation,
256   since NF-κB is directly activated by EdaA1–EdaR signaling via the IκB kinase (IKK) complex.
257   Schmidt-Ullrich *et al.* demonstrated an interaction between the EdaA1–EdaR–NF-κB axis and the
258   HH signaling pathway [79]. They found that EdaA1–EdaR signaling-mediated NF-κB activation is
259   essential for the induction of SHH expression and subsequent placode downgrowth. However,
260   NF-κB induces cyclin D1 indirectly, suggesting direct induction by HH (or Wnt) signaling. These
261   results taken together suggest that, during hair follicle development, NF-κB transmits EdaA1–EdaR
262   signaling to activate the HH signaling pathway via the direct NF-κB-mediated induction of SHH.

263   **6. Epidermal Homeostasis**

264   The adult epidermis consists of several layers, each with its own characteristics. Cells in the
265   basal layer proliferate to provide new cells that replace those shed from the cornified exterior of the
266   tissue [80]. This renewal process is called homeostasis. Epidermal homeostasis requires a balance
267   between keratinocyte proliferation and differentiation [81,82]. In the skin, multiple populations of
268   epidermal stem cells have a crucial role in maintaining tissue homeostasis, providing new cells to
269   replace those that are constantly lost during tissue turnover or following injury [83]. The epidermis
270   is composed of the interfollicular epidermis, hair follicles, sebaceous glands and sweat glands, and
271   each of these tissues has its resident stem cells. The permanent, lower part of the hair follicle, known
272   as the bulge, is one reservoir of stem cells. Bulge stem cells were originally identified as slow cycling
273   cells [84,85]. A large number of markers have been described for these cells. CD34 cell surface
274   glycoprotein [86,87] and Leu-rich repeat-containing G protein-coupled receptor 5 (LGR5) are
275   considered to be markers of bulge stem cells [88,89]. Recent studies have reported that HH signaling
276   is involved in the maintenance of one type of bulge stem cells. We discuss the role of the HH
277   signaling pathway in bulge stem cell maintenance below.

278

7. Roles of the HH Signaling Pathway in Epidermal Homeostasis

Treatment of adult mice with an anti-SHH antibody blocks the active phase of the hair growth cycle (anagen) and blocks hair regrowth [90], indicating that SHH is required for the regenerative function of adult bulge stem cells. Conversely, exogenously administered SHH triggers anagen onset in follicles in the resting phase of the hair cycle (telogen), and stimulates hair growth [91]. These reports suggest that HH signaling is involved in hair follicle maintenance.

In adult skin, stem cells in the hair follicle bulge cyclically regenerate the follicle, whereas a distinct stem cell population maintains the epidermis. In telogen hair follicles, GLI2 and GLI3 continue to be broadly expressed in both the follicle and the surrounding dermis. Throughout the hair cycle, GLI1 expression is restricted to two distinct epidermal stem cell domains [92]. One population of GLI1-expressing (GLI1+) stem cells co-expresses LGR5 [88,89]. Stem cells expressing both GLI1 and LGR5 (GLI1+/LGR5+) exhibit greater proliferative properties and are localized to the lower portion of the bulge, close to the telogen hair germ. In this phase of the hair cycle, GLI1+/LGR5+ stem cells only partially overlap with other bulge stem cells markers such as CD34 and keratin 15. However, in anagen hair follicles, GLI1+/LGR5+ stem cells are localized to the lower part of the outer root sheath of the reforming hair follicle, where they lose expression of bulge stem cell markers [93]. This localization change during the hair cycle suggests that GLI1+/LGR5+ stem cells may be also necessary for hair follicle growth in anagen. Another population of GLI1+ stem cells in the dermal papilla is involved in dermal papilla maintenance. These results show that, during anagen, the induction of HH target genes, including *Gli1*, occurs in both epidermal and dermal papilla cells adjacent to SHH-expressing cells, such as those in the follicle matrix.

Another population of GLI1+ stem cells that does not express LGR5, CD34 or keratin 15 exists in the upper fringe of the bulge. GLI1+ upper bulge stem cells are induced by bulge-associated nerve cells releasing SHH. GLI1+ stem cells from the upper bulge stably remain in a wounded area of the skin long after it has been repaired [92]. Therefore, upper bulge cells might constitute a long-term reservoir of stem cells poised to contribute to the repair of the interfollicular epidermis in a permanent manner. Interestingly, the HH signaling pathway is essential for the contribution of upper bulge stem cells to wound repair, but not for hair follicle cycling. This emphasizes the relevance of the niche for the function of different populations of hair follicle stem cells [92]. Matrix cells are known to be a source of SHH, and secreted SHH regulates epithelial cell proliferation and dermal papilla maturation. Moreover, SHH governs dermal adipocyte precursors in adult hair cycling. In turn, dermal adipocytes and adipocyte precursors influence hair cycle progression [94–96]. In addition, dermal adipocytes secrete antimicrobial peptides to protect the skin from bacterial infection [97]. The roles of HH signaling pathway in skin homeostasis are summarized in Figure 4.
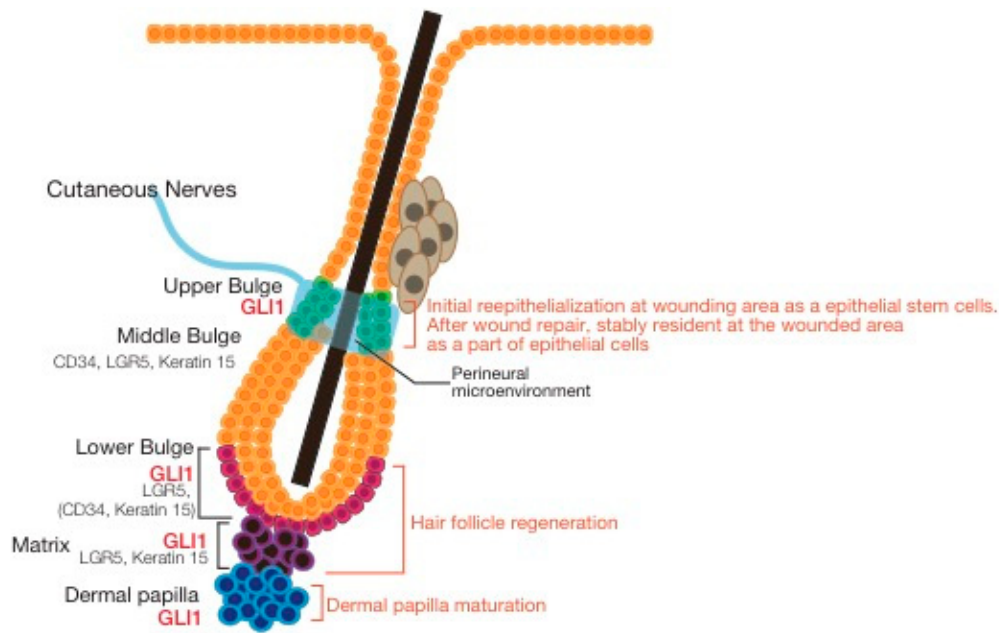
313 **Figure 4.** GLI1-expressing hair follicle stem cells and their roles. The expression of GLI1 and other
314 markers defines molecularly distinct zones in the telogen hair follicle, including regionalization of
315 the bulge into the upper, middle and lower bulge. GLI1-expressing cells in the upper bulge receive a
316 Hedgehog signal from hair follicle-associated nerve cells, and are functionally distinct from cells in
317 the middle bulge, lower bulge and matrix in their ability to become epidermal stem cells during
318 wound repair. The roles of each GLI1+ bulge stem cell population are described in orange text on the
319 right.

320 **8. Signaling Crosstalk between HH and Other Signaling pathways in Skin Homeostasis**

321 *8.1. Wnt signaling*

322   The Wnt signaling pathway is also important for the maintenance of hair follicles in adult skin.
323 As described above, activation of canonical Wnt signaling induces SHH expression, indicating HH
324 signaling activation during hair follicle development in mouse embryos. Previous studies have
325 demonstrated that the Wnt–HH signaling axis is also essential for maintaining the hair cycle.
326 Epidermal basal cell-specific β-catenin activation in adult mouse skin also induces SHH expression,
327 resulting in HH signaling activation [38,39], and HH signaling is required for β-catenin-mediated
328 hair follicle formation [39].

329 *8.2. The Hippo–YAP signaling pathway*

330   The Hippo signaling pathway is a master regulator of cell proliferation and organ size. Hippo
331 pathway molecules such as macrophage-stimulating 1/2 (MST1/2) and large tumor suppressor 1/2
332 (LATS1/2) regulate a transcriptional coactivator, Yes-associated protein (YAP) [98,99]. YAP controls
333 stem or progenitor cell proliferation in the mouse postnatal epidermis [100]. Furthermore, YAP
334 functions in balancing growth and differentiation in the skin [101,102].

335   Akladios *et al.* proposed a novel interplay among HH, YAP, Rho-associated protein kinase
336 (ROCK) and β-catenin in the control of skin homeostasis [103]. HH signaling induces YAP
337 activation, and activated YAP in turn induces β-catenin activation. HH, YAP and β-catenin together
338 induce GLI2 activation to regulate epidermis homeostasis. At the same time, HH signaling activates
339 ROCK in the epidermis. HH signaling and activated ROCK regulate cell proliferation and actin
340 remodeling in the dermis.

341 *8.3. p53/p63*

342 The tumor suppressor, p53 is expressed in the epidermal basal layer and also in the outer root
343 sheath and matrix of the hair follicles. Its expression is increased during the transition from anagen
344 to the regression phase of the hair cycle (catagen), suggesting its role in hair follicle involution [104].
345 In addition, we previously demonstrated that HH signaling enhances the ubiquitin-mediated
346 degradation of p53 through GLI1 [105]. Therefore, it is possible that HH signaling regulates hair
347 cycling through regulating p53 protein.

348 A homolog of p53, p63, has an essential role in development. Mice lacking *p63* show extensive
349 defects in epidermal development and die at birth [106,107]. In epidermis, an antiapoptotic protein
350 and GLI target gene product, B-cell lymphoma 2 (BCL2) [108,109], is expressed in basal keratinocytes
351 and the outer root sheath cells of the hair follicle. Its expression is associated with undifferentiated
352 cells having proliferative potential. On the other hand, suprabasal keratinocytes do not express
353 BCL2 [110]. These results suggest that BCL2 has an important role in regulating keratinocyte
354 proliferation for skin homeostasis. Chari *et al.* demonstrated crosstalk between ΔNp63α, an isoform
355 of p63 that is a crucial regulator of epidermal development, and HH signaling in keratinocytes [111].
356 HH signaling activation inhibits keratinocyte differentiation via GLI1-mediated upregulation of
357 BCL2. At the same time, HH signaling activation results in ΔNp63α expression. Upregulated p63
358 then activates transcription in keratinocytes of a negative regulator of HH signaling, SUFU. *In vivo*
359 analysis indicates that SUFU is also a negative regulator of the Wnt signaling pathway [112]. Overall,
360 these results suggest that the HH–ΔNp63α axis regulates keratinocyte fate determination
361 (proliferation or differentiation) for skin homeostasis.

362 **9. Aberrant HH Signaling Pathway Activation and BCC**

363 BCCs are so named because of their histological resemblance to basal cells of the interfollicular
364 epidermis and epidermal appendages, that is, the keratinocytes adjacent to the stroma [113,114].
365 Activation of the HH signaling pathway through various mechanisms has been observed in BCCs
366 [5]. Below, we introduce the mechanisms of BCC formation via the HH signaling pathway.

367 Although in general, BCCs seem to have relatively stable genomes, 67% of BCCs exhibit loss of
368 *PTCH1*, 5% exhibit loss of *SUFU* and 10% have activating mutations of *SMO* [32,115,116]. However,
369 GLI mutations (*GLI1*, *GLI2* and *GLI3*) are not observed in BCC patients, although GLI
370 overexpression is observed. In addition, *NRAS*, *KRAS*, *HRAS*, *BRAF* and *CTNNB1* are not known to
371 carry mutations in BCC patients [115].

372 Based on the molecular genetics analysis of human BCCs, several studies have been performed
373 using genetically modified mice in which the HH signaling pathway is constitutively activated.
374 Constitutive or conditional overexpression of either GLI1 [117] or GLI2 [118] in keratinocytes can
375 produce BCC-like lesions in the skin. Similarly, expression of mutant SMO carrying the activating
376 mutations identified in human BCCs [32] also can produce murine BCCs. Furthermore, *Ptch1⁺/⁻* mice
377 develop BCCs, and those BCCs often exhibit a deletion of the wild-type copy of *Ptch1* as well as
378 upregulation of HH signaling. Similarly, mice carrying one inactivated, mutant allele of the HH
379 suppressor, *SUFU* are also susceptible to BCC development [119].

380 Although these results show that the HH signaling pathway has important roles in BCC
381 formation, the actual cell of origin remains to be elucidated. X-ray-induced BCCs in *Ptch1⁺/⁻* mice
382 arise predominantly, and likely exclusively, from hair follicle bulge stem cells expressing keratin 15
383 (a bulge cell marker). Furthermore, upregulation of SMO expression is necessary for BCC formation
384 in X-irradiated *Ptch1⁺/⁻* mice [120]. Using an inducible mouse model enabling expression of a
385 constitutively active SMO mutant (SMO-M2) [32] in the adult epidermis, HH pathway activation
386 was found to reprogram adult interfollicular cells to an embryonic hair follicle progenitor fate.

387 Furthermore, HH pathway activation induces the activation of Wnt/β-catenin signaling. Deletion of
388 β-catenin in adult SMO-M2-expressing cells prevents reprogramming to an embryonic hair follicle
389 progenitor fate and also prevents tumor initiation [121]. Recently, deletion of *Ptch1* in different
390 cellular compartments revealed that tumors can arise from multiple hair follicle stem cells and from
391 innervated GLI1-expressing progenitor cells in the sensory structure known as the touch dome. In
392 contrast, tumors do not arise from interfollicular epidermal stem cells [122].

393   BCC-specific signaling crosstalk between the HH and other signaling pathways has been
394 reported. Eberl *et al.* reported crosstalk between the HH and EGFR signaling pathways in BCCs [41].
395 Skin-specific SMO-M2-exppressing transgenic mice develop BCC-like lesions. However,
396 skin-specific knockout of EGFR in SMO-M2-expressing mice reduces the number and size of BCCs.
397 Furthermore, cooperation of HH and EGFR signaling induces specific GLI target genes such as
398 *FGF19*, *TGFA*, *CXCR4*, *SOX9* and *SOX2*. These genes are not induced by activation of the canonical
399 HH signaling pathway alone. These results suggest that cooperation between HH and EGFR
400 signaling is important for BCC development, which is mediated by HH–EGFR
401 cooperation-responsive GLI target genes.

402   Findings from studies of HH signaling activation-mediated tumorigenesis have recently
403 culminated in the USA Food and Drug Administration's approval of GDC-0449 (vismodegib) [123],
404 an oral inhibitor of SMO, as a therapeutic for treating advanced BCC. However, some studies have
405 reported the emergence of SMO inhibitor-resistant tumors [124,125]. It will be important to
406 understand the mechanisms by which SMO inhibitor-resistant cancer cells emerge, and to find novel
407 therapeutic targets for these tumors. Atwood *et al.* reported that atypical protein kinase Ci/λ
408 (aPKC-i/λ) is a novel GLI1 target gene product, and a novel therapeutic target for SMO
409 inhibitor-resistant BCC [126]. aPKC-i/λ functions downstream of SMO to phosphorylate and activate
410 GLI1, resulting in maximal DNA binding and transcriptional activation. Activated aPKC-i/λ is
411 upregulated in SMO inhibitor-resistant tumors, and targeting aPKC-i/λ suppresses GLI1 activation
412 and the growth of SMO inhibitor-resistant BCC cell lines. These results suggest that aPKC-i/λ is a
413 novel therapeutic target for SMO inhibitor-resistant BCC.

414   Deregulated HH signaling is also involved in other skin cancers, including melanoma,
415 squamous cell carcinoma and Merkel cell tumors. For the roles of the HH signaling pathway in these
416 tumors, please refer to a published review [127].

417 **10. Conclusions**

418   The HH signaling pathway is one of the most fundamental in epidermal development and
419 epidermal stem cell maintenance. However, deregulated HH signaling induces BCC. Hence, it is
420 important that the intensity of the HH signal is strictly regulated during skin generation and
421 homeostasis. To achieve precise regulation of HH signaling intensity, negative regulators of HH
422 signaling are necessary, as well as crosstalk between the HH and other signal transduction pathways
423 such as those activated by Wnt and BMP. Recently, it has been reported that low HH signaling
424 activity is important for lung homeostasis [128]. Therefore, it is possible that fine-tuning the intensity
425 of a HH signal may be involved in the homeostasis of multiple organs, including the skin.

426   As described above, aberrant activation of HH signaling causes BCC formation. A tumor
427 microenvironment would exhibit not only the HH pathway regulatory mechanisms that are
428 common with skin development and homeostasis, but also cancer cell-specific regulatory
429 mechanisms. Therefore, understanding the precise mechanisms of HH pathway regulation in the
430 tumor microenvironment is important to advance new therapeutic strategies for SMO
431 inhibitor-resistant BCC. Moreover, such knowledge may also help to provide new therapeutic
432 strategies for other HH pathway-activated cancers.

435 **Author Contributions:** Yoshinori Abe and Nobuyuki Tanaka contributed equally to this work.

436 **Conflicts of Interest:** The authors declare no conflict of interest.

References

1.   Koster, M. I.; Roop, D. R. Mechanisms Regulating Epithelial Stratification. *Annual Review of Cell and Developmental Biology* **2007**, *23*, 93–113, doi:10.1146/annurev.cellbio.23.090506.123357.

2.   Fuchs, E. Scratching the surface of skin development. *Nature* **2007**, *445*, 834–842, doi:10.1038/nature05659.

3.   Blanpain, C.; Fuchs, E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nature reviews. Molecular cell biology* **2009**, *10*, 207–17, doi:10.1038/nrm2636.

4.   Schmidt-Ullrich, R.; Paus, R. Molecular principles of hair follicle induction and morphogenesis. *BioEssays* **2005**, *27*, 247–261, doi:10.1002/bies.20184.

5.   Epstein, E. H. Basal cell carcinomas: attack of the hedgehog. *Nature reviews. Cancer* **2008**, *8*, 743–54, doi:10.1038/nrc2503.

6.   Thayer, S. P.; di Magliano, M. P.; Heiser, P. W.; Nielsen, C. M.; Roberts, D. J.; Lauwers, G. Y.; Qi, Y. P.; Gysin, S.; Fernández-del Castillo, C.; Yajnik, V.; Antoniu, B.; McMahon, M.; Warshaw, A. L.; Hebrok, M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* **2003**, *425*, 851–6, doi:10.1038/nature02009.

7.   Ng, J. M. Y.; Curran, T. The Hedgehog's tale: developing strategies for targeting cancer. *Nature Reviews Cancer* **2011**, *11*, 493–501, doi:10.1038/nrc3079.

8.   Brechbiel, J.; Miller-Moslin, K.; Adjei, A. a Crosstalk between hedgehog and other signaling pathways as a basis for combination therapies in cancer. *Cancer treatment reviews* **2014**, *40*, 750–9, doi:10.1016/j.ctrv.2014.02.003.

9.   Aberger, F.; Ruiz i Altaba, A. Context-dependent signal integration by the GLI code: The oncogenic load, pathways, modifiers and implications for cancer therapy. *Seminars in Cell & Developmental Biology* **2014**, *33*, 93–104, doi:10.1016/j.semcdb.2014.05.003.

10.   Pak, E.; Segal, R. A. Hedgehog Signal Transduction: Key Players, Oncogenic Drivers, and Cancer Therapy. *Developmental cell* **2016**, *38*, 333–44, doi:10.1016/j.devcel.2016.07.026.

11.   Rohatgi, R.; Milenkovic, L.; Scott, M. P. Patched1 Regulates Hedgehog Signaling at the Primary Cilium. *Science* **2007**, *317*, 372–376, doi:10.1126/science.1139740.

12.   Lee, Y.; Kawagoe, R.; Sasai, K.; Li, Y.; Russell, H. R.; Curran, T.; McKinnon, P. J. Loss of suppressor-of-fused function promotes tumorigenesis. *Oncogene* **2007**, *26*, 6442–7, doi:10.1038/sj.onc.1210467.

13.   Allen, B. L.; Tenzen, T.; McMahon, A. P. The Hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development. *Genes and Development* **2007**, *21*, 1244–1257, doi:10.1101/gad.1543607.

470 14.   Okada, A.; Charron, F.; Morin, S.; Shin, D. S.; Wong, K.; Fabre, P. J.; Tessier-Lavigne, M.;
471   McConnell, S. K. Boc is a receptor for sonic hedgehog in the guidance of commissural axons.
472   *Nature* **2006**, *444*, 369–373, doi:10.1038/nature05246.

473 15.   Yang, C.; Chen, W.; Chen, Y.; Jiang, J. Smoothened transduces Hedgehog signal by forming a
474   complex with Evc/Evc2. *Cell Research* **2012**, *22*, 1593–1604, doi:10.1038/cr.2012.134.

475 16.   Dorn, K. V.; Hughes, C. E.; Rohatgi, R. A Smoothened-Evc2 Complex Transduces the
476   Hedgehog Signal at Primary Cilia. *Developmental Cell* **2012**, *23*, 823–835,
477   doi:10.1016/j.devcel.2012.07.004.

478 17.   Corbit, K. C.; Aanstad, P.; Singla, V.; Norman, A. R.; Stainier, D. Y. R.; Reiter, J. F. Vertebrate
479   Smoothened functions at the primary cilium. *Nature* **2005**, *437*, 1018–21,
480   doi:10.1038/nature04117.

481 18.   Haycraft, C. J.; Banizs, B.; Aydin-Son, Y.; Zhang, Q.; Michaud, E. J.; Yoder, B. K. Gli2 and Gli3
482   localize to cilia and require the intraflagellar transport protein polaris for processing and
483   function. *PLoS genetics* **2005**, *1*, e53, doi:10.1371/journal.pgen.0010053.

484 19.   Abe, Y.; Tanaka, N. The Hedgehog Signaling Networks in Lung Cancer: The Mechanisms
485   and Roles in Tumor Progression and Implications for Cancer Therapy. *BioMed Research*
486   *International* **2016**, *2016*, 1–11, doi:10.1155/2016/7969286.

487 20.   Sasaki, H.; Nishizaki, Y.; Hui, C.; Nakafuku, M.; Kondoh, H. Regulation of Gli2 and Gli3
488   activities by an amino-terminal repression domain: implication of Gli2 and Gli3 as primary
489   mediators of Shh signaling. *Development (Cambridge, England)* **1999**, *126*, 3915–24.

490 21.   Bai, C. B.; Auerbach, W.; Lee, J. S.; Stephen, D.; Joyner, A. L. Gli2, but not Gli1, is required for
491   initial Shh signaling and ectopic activation of the Shh pathway. *Development (Cambridge,*
492   *England)* **2002**, *129*, 4753–61.

493 22.   Masuya, H.; Sagai, T.; Moriwaki, K.; Shiroishi, T. Multigenic control of the localization of the
494   zone of polarizing activity in limb morphogenesis in the mouse. *Developmental biology* **1997**,
495   *182*, 42–51, doi:10.1006/dbio.1996.8457.

496 23.   Büscher, D.; Bosse, B.; Heymer, J.; Rüther, U. Evidence for genetic control of Sonic hedgehog
497   by Gli3 in mouse limb development. *Mechanisms of development* **1997**, *62*, 175–82,
498   doi:10.1016/S0925-4773(97)00656-4.

499 24.   Ding, Q.; Motoyama, J.; Gasca, S.; Mo, R.; Sasaki, H.; Rossant, J.; Hui, C. C. Diminished Sonic
500   hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. *Development*
501   *(Cambridge, England)* **1998**, *125*, 2533–43, doi:9636069.

502 25.   Tukachinsky, H.; Lopez, L. V.; Salic, A. A mechanism for vertebrate Hedgehog signaling:
503   Recruitment to cilia and dissociation of SuFu-Gli protein complexes. *Journal of Cell Biology*
504   **2010**, *191*, 415–428, doi:10.1083/jcb.201004108.

505   26.   Park, H. L.; Bai, C.; Platt, K. A.; Matise, M. P.; Beeghly, A.; Hui, C. c; Nakashima, M.; Joyner,
506   A. L. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a
507   Gli2 mutation. *Development (Cambridge, England)* **2000**, *127*, 1593–605,
508   doi:10.1101/gad.10.16.2003.

509   27.   Bai, C. B.; Joyner,   a L. Gli1 can rescue the in vivo function of Gli2. *Development (Cambridge,*
510   *England)* **2001**, *128*, 5161–72.

511   28.   Rubin, L. L.; de Sauvage, F. J. Targeting the Hedgehog pathway in cancer. *Nature reviews.*
512   *Drug discovery* **2006**, *5*, 1026–33, doi:10.1038/nrd2086.

513   29.   Raffel, C.; Jenkins, R. B.; Frederick, L.; Hebrink, D.; Alderete, B.; Fults, D. W.; James, C. D.
514   Sporadic medulloblastomas contain PTCH mutations. *Cancer research* **1997**, *57*, 842–5.

515   30.   Wolter, M.; Reifenberger, J.; Sommer, C.; Ruzicka, T.; Reifenberger, G. Mutations in the
516   human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic
517   basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central
518   nervous system. *Cancer research* **1997**, *57*, 2581–5.

519   31.   Reifenberger, J.; Wolter, M.; Weber, R. G.; Megahed, M.; Ruzicka, T.; Lichter, P.; Reifenberger,
520   G. Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive
521   neuroectodermal tumors of the central nervous system. *Cancer research* **1998**, *58*, 1798–803.

522   32.   Xie, J.; Murone, M.; Luoh, S. M.; Ryan, A.; Gu, Q.; Zhang, C.; Bonifas, J. M.; Lam, C. W.;
523   Hynes, M.; Goddard, A.; Rosenthal, A.; Epstein, E. H.; de Sauvage, F. J. Activating
524   Smoothened mutations in sporadic basal-cell carcinoma. *Nature* **1998**, *391*, 90–2,
525   doi:10.1038/34201.

526   33.   Taylor, M. D.; Liu, L.; Raffel, C.; Hui, C.; Mainprize, T. G.; Zhang, X.; Agatep, R.; Chiappa, S.;
527   Gao, L.; Lowrance, A.; Hao, A.; Goldstein, A. M.; Stavrou, T.; Scherer, S. W.; Dura, W. T.;
528   Wainwright, B.; Squire, J. a; Rutka, J. T.; Hogg, D. Mutations in SUFU predispose to
529   medulloblastoma. *Nature genetics* **2002**, *31*, 306–10, doi:10.1038/ng916.

530   34.   Pandolfi, S.; Stecca, B. Cooperative integration between HEDGEHOG-GLI signalling and
531   other oncogenic pathways: implications for cancer therapy. *Expert reviews in molecular*
532   *medicine* **2015**, *17*, e5, doi:10.1017/erm.2015.3.

533   35.   Aberger, F.; Ruiz i Altaba, A. Context-dependent signal integration by the GLI code: The
534   oncogenic load, pathways, modifiers and implications for cancer therapy. *Seminars in Cell and*
535   *Developmental Biology* **2014**, *33*, 93–104, doi:10.1016/j.semcdb.2014.05.003.

536   36.   Huelsken, J.; Vogel, R.; Erdmann, B.; Cotsarelis, G.; Birchmeier, W. β-Catenin Controls Hair
537   Follicle Morphogenesis and Stem Cell Differentiation in the Skin. *Cell* **2001**, *105*, 533–545,
538   doi:10.1016/S0092-8674(01)00336-1.

539   37.   Suzuki, K.; Yamaguchi, Y.; Villacorte, M.; Mihara, K.; Akiyama, M.; Shimizu, H.; Taketo, M.
540   M.; Nakagata, N.; Tsukiyama, T.; Yamaguchi, T. P.; Birchmeier, W.; Kato, S.; Yamada, G.
541   Embryonic hair follicle fate change by augmented -catenin through Shh and Bmp signaling.
542   *Development* **2009**, *136*, 367–372, doi:10.1242/dev.021295.

543   38.   Gat, U.; DasGupta, R.; Degenstein, L.; Fuchs, E. De Novo hair follicle morphogenesis and hair
544   tumors in mice expressing a truncated beta-catenin in skin. *Cell* **1998**, *95*, 605–14,
545   doi:10.1016/S0092-8674(00)81631-1.

546   39.   Silva-Vargas, V.; Lo Celso, C.; Giangreco, A.; Ofstad, T.; Prowse, D. M.; Braun, K. M.; Watt, F.
547   M. β-Catenin and Hedgehog Signal Strength Can Specify Number and Location of Hair
548   Follicles in Adult Epidermis without Recruitment of Bulge Stem Cells. *Developmental Cell*
549   **2005**, *9*, 121–131, doi:10.1016/j.devcel.2005.04.013.

550   40.   Javelaud, D.; Pierrat, M.-J.; Mauviel, A. Crosstalk between TGF-β and hedgehog signaling in
551   cancer. *FEBS letters* **2012**, *586*, 2016–25, doi:10.1016/j.febslet.2012.05.011.

552   41.   Eberl, M.; Klingler, S.; Mangelberger, D.; Loipetzberger, A.; Damhofer, H.; Zoidl, K.;
553   Schnidar, H.; Hache, H.; Bauer, H.-C.; Solca, F.; Hauser-Kronberger, C.; Ermilov, A. N.;
554   Verhaegen, M. E.; Bichakjian, C. K.; Dlugosz, A. a; Nietfeld, W.; Sibilia, M.; Lehrach, H.;
555   Wierling, C.; Aberger, F. Hedgehog-EGFR cooperation response genes determine the
556   oncogenic phenotype of basal cell carcinoma and tumour-initiating pancreatic cancer cells.
557   *EMBO Molecular Medicine* **2012**, *4*, 218–233, doi:10.1002/emmm.201100201.

558   42.   Nolan-Stevaux, O.; Lau, J.; Truitt, M. L.; Chu, G. C.; Hebrok, M.; Fernández-Zapico, M. E.;
559   Hanahan, D. GLI1 is regulated through Smoothened-independent mechanisms in neoplastic
560   pancreatic ducts and mediates PDAC cell survival and transformation. *Genes & development*
561   **2009**, *23*, 24–36, doi:10.1101/gad.1753809.

562   43.   Mazumdar, T.; DeVecchio, J.; Agyeman, A.; Shi, T.; Houghton, J. A. The GLI genes as the
563   molecular switch in disrupting Hedgehog signaling in colon cancer. *Oncotarget* **2011**, *2*, 638–
564   45.

565   44.   Wang, Y.; Ding, Q.; Yen, C.-J.; Xia, W.; Izzo, J. G.; Lang, J.-Y.; Li, C.-W.; Hsu, J. L.; Miller, S. A.;
566   Wang, X.; Lee, D.-F.; Hsu, J.-M.; Huo, L.; Labaff, A. M.; Liu, D.; Huang, T.-H.; Lai, C.-C.; Tsai,
567   F.-J.; Chang, W.-C.; Chen, C.-H.; Wu, T.-T.; Buttar, N. S.; Wang, K. K.; Wu, Y.; Wang, H.;
568   Ajani, J.; Hung, M.-C. The crosstalk of mTOR/S6K1 and Hedgehog pathways. *Cancer cell* **2012**,
569   *21*, 374–87, doi:10.1016/j.ccr.2011.12.028.

570   45.   Androutsellis-Theotokis, A.; Leker, R. R.; Soldner, F.; Hoeppner, D. J.; Ravin, R.; Poser, S. W.;
571   Rueger, M. a; Bae, S.-K.; Kittappa, R.; McKay, R. D. G. Notch signalling regulates stem cell
572   numbers in vitro and in vivo. *Nature* **2006**, *442*, 823–6, doi:10.1038/nature04940.

573   46.   Mikkola, M. L. Genetic basis of skin appendage development. *Seminars in Cell and*
574   *Developmental Biology* 2007, *18*, 225–236.

575 47. Motoyama, J.; Takabatake, T.; Takeshima, K.; Hui, C. Ptch2, a second mouse Patched gene is
576   co-expressed with Sonic hedgehog. *Nature genetics* **1998**, *18*, 104–6, doi:10.1038/ng0298-104.

577 48. Paus, R.; Müller-Röver, S.; Van Der Veen, C.; Maurer, M.; Eichmüller, S.; Ling, G.; Hofmann,
578   U.; Foitzik, K.; Mecklenburg, L.; Handjiski, B. A comprehensive guide for the recognition and
579   classification of distinct stages of hair follicle morphogenesis. *Journal of Investigative*
580   *Dermatology* 1999, *113*, 523–532.

581 49. Forni, M. F.; Trombetta-Lima, M.; Sogayar, M. C. Stem cells in embryonic skin development.
582   *Biological research* **2012**, *45*, 215–22, doi:10.4067/S0716-97602012000300003.

583 50. St-Jacques, B.; Dassule, H. R.; Karavanova, I.; Botchkarev, V. A.; Li, J.; Danielian, P. S.;
584   McMahon, J. A.; Lewis, P. M.; Paus, R.; McMahon, A. P. Sonic hedgehog signaling is essential
585   for hair development. *Current Biology* **1998**, *8*, 1058–68, doi:10.1038/ng0298-104.

586 51. Chiang, C.; Swan, R. Z.; Grachtchouk, M.; Bolinger, M.; Litingtung, Y.; Robertson, E. K.;
587   Cooper, M. K.; Gaffield, W.; Westphal, H.; Beachy, P. a; Dlugosz, a a Essential role for Sonic
588   hedgehog during hair follicle morphogenesis. *Developmental biology* **1999**, *205*, 1–9,
589   doi:10.1006/dbio.1998.9103.

590 52. Mill, P. Sonic hedgehog-dependent activation of Gli2 is essential for embryonic hair follicle
591   development. *Genes & Development* **2003**, *17*, 282–294, doi:10.1101/gad.1038103.

592 53. Woo, W.-M.; Zhen, H. H.; Oro, A. E. Shh maintains dermal papilla identity and hair
593   morphogenesis via a Noggin-Shh regulatory loop. *Genes & development* **2012**, *26*, 1235–46,
594   doi:10.1101/gad.187401.112.

595 54. Biernaskie, J.; Paris, M.; Morozova, O.; Fagan, B. M.; Marra, M.; Pevny, L.; Miller, F. D. SKPs
596   Derive from Hair Follicle Precursors and Exhibit Properties of Adult Dermal Stem Cells. *Cell*
597   *Stem Cell* **2009**, *5*, 610–623, doi:10.1016/j.stem.2009.10.019.

598 55. Driskell, R. R.; Giangreco, A.; Jensen, K. B.; Mulder, K. W.; Watt, F. M. Sox2-positive dermal
599   papilla cells specify hair follicle type in mammalian epidermis. *Development (Cambridge,*
600   *England)* **2009**, *136*, 2815–23, doi:10.1242/dev.038620.

601 56. Chen, Y.; Struhl, G. Dual roles for patched in sequestering and transducing Hedgehog. *Cell*
602   **1996**, *87*, 553–563, doi:10.1016/S0092-8674(00)81374-4.

603 57. Adolphe, C.; Hetherington, R.; Ellis, T.; Wainwright, B. Patched1 functions as a gatekeeper by
604   promoting cell cycle progression. *Cancer research* **2006**, *66*, 2081–8,
605   doi:10.1158/0008-5472.CAN-05-2146.

606 58. Nieuwenhuis, E.; Motoyama, J.; Barnfield, P. C.; Yoshikawa, Y.; Zhang, X.; Mo, R.;
607   Crackower, M. A.; Hui, C. -c. Mice with a Targeted Mutation of Patched2 Are Viable but
608   Develop Alopecia and Epidermal Hyperplasia. *Molecular and Cellular Biology* **2006**, *26*, 6609–
609   6622, doi:10.1128/MCB.00295-06.

610 59. Adolphe, C.; Nieuwenhuis, E.; Villani, R.; Li, Z. J.; Kaur, P.; Hui, C.; Wainwright, B. J. Patched
611   1 and Patched 2 Redundancy Has a Key Role in Regulating Epidermal Differentiation. *Journal*
612   *of Investigative Dermatology* **2014**, *134*, 1981–1990, doi:10.1038/jid.2014.63.

613 60. Mill, P.; Mo, R.; Hu, M. C.; Dagnino, L.; Rosenblum, N. D.; Hui, C.-C. Shh controls epithelial
614   proliferation via independent pathways that converge on N-Myc. *Developmental cell* **2005**, *9*,
615   293–303, doi:10.1016/j.devcel.2005.05.009.

616 61. Adolphe, C.; Junker, J. P.; Lyubimova, A.; van Oudenaarden, A.; Wainwright, B. Patched
617   Receptors Sense, Interpret, and Establish an Epidermal Hedgehog Signaling Gradient. *Journal*
618   *of Investigative Dermatology* **2017**, *137*, 179–186, doi:10.1016/j.jid.2016.06.632.

619 62. Clevers, H. Wnt/beta-catenin signaling in development and disease. *Cell* **2006**, *127*, 469–80,
620   doi:10.1016/j.cell.2006.10.018.

621 63. Van Camp, J. K.; Beckers, S.; Zegers, D.; Van Hul, W. Wnt Signaling and the Control of
622   Human Stem Cell Fate. *Stem Cell Reviews and Reports* 2014, *10*, 207–229.

623 64. Fuchs, E.; Merrill, B. J.; Jamora, C.; Dasgupta, R. At the Roots of a Never-Ending Cycle.
624   *Developmental Cell* 2001, *1*, 13–25.

625 65. Widelitz, R. B. Wnt signaling in skin organogenesis. *Organogenesis* **2008**, *4*, 123–133,
626   doi:10.4161/org.4.2.5859.

627 66. Reddy, S.; Andl, T.; Bagasra, A.; Lu, M. M.; Epstein, D. J.; Morrisey, E. E.; Millar, S. E.
628   Characterization of Wnt gene expression in developing and postnatal hair follicles and
629   identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis.
630   *Mechanisms of Development* **2001**, *107*, 69–82, doi:10.1016/S0925-4773(01)00452-X.

631 67. Kwack, M. H.; Kim, M. K.; Kim, J. C.; Sung, Y. K. Wnt5a attenuates Wnt/β-catenin signalling
632   in human dermal papilla cells. *Experimental Dermatology* **2013**, *22*, 229–231,
633   doi:10.1111/exd.12101.

634 68. Jamora, C.; DasGupta, R.; Kocieniewski, P.; Fuchs, E. Links between signal transduction,
635   transcription and adhesion in epithelial bud development. *Nature* **2003**, *422*, 317–322,
636   doi:10.1038/nature01458.

637 69. Rendl, M.; Polak, L.; Fuchs, E. BMP signaling in dermal papilla cells is required for their hair
638   follicle-inductive properties. *Genes and Development* **2008**, *22*, 543–557,
639   doi:10.1101/gad.1614408.

640 70. Millar, S. E. Molecular mechanisms regulating hair follicle development. *The Journal of*
641   *investigative dermatology* **2002**, *118*, 216–25, doi:10.1046/j.0022-202x.2001.01670.x.

642 71. Mikkola, M. L.; Millar, S. E. The mammary bud as a skin appendage: Unique and shared
643   aspects of development. *Journal of Mammary Gland Biology and Neoplasia* **2006**, *11*, 187–203,
644   doi:10.1007/s10911-006-9029-x.

645   72.   McMahon, A. P.; Paus, R. Noggin is a mesenchymally derived stimulator of hair-follicle
646   induction. *Nature cell biology* **1999**, *1*, 158–164, doi:10.1038/11078.

647   73.   Irrthum, A.; Devriendt, K.; Chitayat, D.; Matthijs, G.; Glade, C.; Steijlen, P. M.; Fryns, J.-P.;
648   Van Steensel, M. A. M.; Vikkula, M. Mutations in the Transcription Factor Gene SOX18
649   Underlie Recessive and Dominant Forms of Hypotrichosis-Lymphedema-Telangiectasia. *The*
650   *American Journal of Human Genetics* **2003**, *72*, 1470–1478, doi:10.1086/375614.

651   74.   Pennisi, D.; Bowles, J.; Nagy,   a; Muscat, G.; Koopman, P. Mice null for sox18 are viable and
652   display a mild coat defect. *Molecular and cellular biology* **2000**, *20*, 9331–9336,
653   doi:10.1128/MCB.20.24.9331-9336.2000.Updated.

654   75.   Headon, D. J.; Overbeek, P. a Involvement of a novel Tnf receptor homologue in hair follicle
655   induction. *Nature genetics* **1999**, *22*, 370–4, doi:10.1038/11943.

656   76.   Aggarwal, B. B. Signalling pathways of the TNF superfamily: a double-edged sword. *Nature*
657   *Reviews Immunology* **2003**, *3*, 745–756, doi:10.1038/nri1184.

658   77.   Kumar, A.; Eby, M. T.; Sinha, S.; Jasmin, A.; Chaudhary, P. M. The ectodermal dysplasia
659   receptor activates the nuclear factor-kappaB, JNK, and cell death pathways and binds to
660   ectodysplasin A. *The Journal of biological chemistry* **2001**, *276*, 2668–77,
661   doi:10.1074/jbc.M008356200.

662   78.   Mikkola, M. L.; Pispa, J.; Pekkanen, M.; Paulin, L.; Nieminen, P.; Kere, J.; Thesleff, I.
663   Ectodysplasin, a protein required for epithelial morphogenesis, is a novel TNF homologue
664   and promotes cell-matrix adhesion. *Mechanisms of Development* **1999**, *88*, 133–146,
665   doi:10.1016/S0925-4773(99)00180-X.

666   79.   Schmidt-Ullrich, R.; Tobin, D. J.; Lenhard, D.; Schneider, P.; Paus, R.; Scheidereit, C.
667   NF-kappaB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and
668   controls post-initiation hair placode down growth. *Development (Cambridge, England)* **2006**,
669   *133*, 1045–57, doi:10.1242/dev.02278.

670   80.   Dai, X.; Segre, J. A. Transcriptional control of epidermal specification and differentiation.
671   *Current Opinion in Genetics and Development* 2004, *14*, 485–491.

672   81.   Fuchs, E.; Byrne, C. The epidermis: rising to the surface. *Current Opinion in Genetics and*
673   *Development* **1994**, *4*, 725–736, doi:10.1016/0959-437X(94)90140-X.

674   82.   Simpson, C. L.; Patel, D. M.; Green, K. J. Deconstructing the skin: cytoarchitectural
675   determinants of epidermal morphogenesis. *Nature Reviews Molecular Cell Biology* **2011**, *12*,
676   565–580, doi:10.1038/nrm3175.

677   83.   Blanpain, C.; Horsley, V.; Fuchs, E. Epithelial Stem Cells: Turning over New Leaves. *Cell*
678   2007, *128*, 445–458.

679   84.   Cotsarelis, G.; Sun, T. T.; Lavker, R. M. Label-retaining cells reside in the bulge area of
680   pilosebaceous unit: Implications for follicular stem cells, hair cycle, and skin carcinogenesis.
681   *Cell* **1990**, *61*, 1329–1337, doi:10.1016/0092-8674(90)90696-C.

682   85.   Braun, K. M.; Niemann, C.; Jensen, U. B.; Sundberg, J. P.; Silva-Vargas, V.; Watt, F. M.
683   Manipulation of stem cell proliferation and lineage commitment: visualisation of
684   label-retaining cells in wholemounts of mouse epidermis. *Development (Cambridge, England)*
685   **2003**, *130*, 5241–5255, doi:10.1242/dev.00703.

686   86.   Blanpain, C.; Lowry, W. E.; Geoghegan, A.; Polak, L.; Fuchs, E. Self-renewal, multipotency,
687   and the existence of two cell populations within an epithelial stem cell niche. *Cell* **2004**, *118*,
688   635–648, doi:10.1016/j.cell.2004.08.012.

689   87.   Trempus, C. S.; Morris, R. J.; Bortner, C. D.; Cotsarelis, G.; Faircloth, R. S.; Reece, J. M.;
690   Tennant, R. W. Enrichment for living murine keratinocytes from the hair follicle bulge with
691   the cell surface marker CD34. *Journal of Investigative Dermatology* **2003**, *120*, 501–511,
692   doi:10.1046/j.1523-1747.2003.12088.x.

693   88.   Panteleyev,   a a; Jahoda, C. a; Christiano,   a M. Hair follicle predetermination. *Journal of cell*
694   *science* **2001**, *114*, 3419–31.

695   89.   Barker, N.; van Es, J. H.; Jaks, V.; Kasper, M.; Snippert, H.; Toftgård, R.; Clevers, H. Very
696   long-term self-renewal of small intestine, colon, and hair follicles from cycling Lgr5+ve stem
697   cells. *Cold Spring Harbor symposia on quantitative biology* **2008**, *73*, 351–6,
698   doi:10.1101/sqb.2008.72.003.

699   90.   Wang, L. C.; Liu, Z. Y.; Gambardella, L.; Delacour, A.; Shapiro, R.; Yang, J.; Sizing, I.;
700   Rayhorn, P.; Garber, E. A.; Benjamin, C. D.; Williams, K. P.; Taylor, F. R.; Barrandon, Y.; Ling,
701   L.; Burkly, L. C. Conditional disruption of hedgehog signaling pathway defines its critical
702   role in hair development and regeneration. *Journal of Investigative Dermatology* **2000**, *114*, 901–
703   908, doi:10.1046/j.1523-1747.2000.00951.x.

704   91.   Sato, N.; Leopold, P. L.; Crystal, R. G. Induction of the hair growth phase in postnatal mice by
705   localized transient expression of Sonic hedgehog. *Journal of Clinical Investigation* **1999**, *104*,
706   855–864, doi:10.1172/JCI7691.

707   92.   Brownell, I.; Guevara, E.; Bai, C. B.; Loomis, C. A.; Joyner, A. L. Nerve-derived sonic
708   hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem
709   cells. *Cell Stem Cell* **2011**, *8*, 552–565, doi:10.1016/j.stem.2011.02.021.

710   93.   Hsu, Y. C.; Pasolli, H. A.; Fuchs, E. Dynamics between stem cells, niche, and progeny in the
711   hair follicle. *Cell* **2011**, *144*, 92–105, doi:10.1016/j.cell.2010.11.049.

712   94.   Plikus, M. V.; Mayer, J. A.; de la Cruz, D.; Baker, R. E.; Maini, P. K.; Maxson, R.; Chuong,
713   C.-M. Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration.
714   *Nature* **2008**, *451*, 340–344, doi:10.1038/nature06457.

715   95.   Festa, E.; Fretz, J.; Berry, R.; Schmidt, B.; Rodeheffer, M.; Horowitz, M.; Horsley, V. Adipocyte
716   lineage cells contribute to the skin stem cell niche to drive hair cycling. *Cell* **2011**, *146*, 761–
717   771, doi:10.1016/j.cell.2011.07.019.

718   96.   Keyes, B. E.; Segal, J. P.; Heller, E.; Lien, W.-H.; Chang, C.-Y.; Guo, X.; Oristian, D. S.; Zheng,
719   D.; Fuchs, E. Nfatc1 orchestrates aging in hair follicle stem cells. *Proceedings of the National*
720   *Academy of Sciences of the United States of America* **2013**, *110*, E4950-9,
721   doi:10.1073/pnas.1320301110.

722   97.   Zhang, L. -j.; Guerrero-Juarez, C. F.; Hata, T.; Bapat, S. P.; Ramos, R.; Plikus, M. V.; Gallo, R. L.
723   Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. *Science*
724   **2015**, *347*, 67–71, doi:10.1126/science.1260972.

725   98.   Halder, G.; Johnson, R. L. Hippo signaling: growth control and beyond. *Development*
726   *(Cambridge, England)* **2011**, *138*, 9–22, doi:10.1242/dev.045500.

727   99.   Zhao Li, L., Lei, Q. and Guan, K. L., B. The Hippo-YAP pathway in organ size control and
728   tumorgenesis: an updated version. *Genes & Development* **2010**, *24*, 862–874,
729   doi:10.1101/gad.1909210.The.

730   100.   Akladios, B.; Mendoza-Reinoso, V.; Samuel, M. S.; Hardeman, E. C.; Khosrotehrani, K.; Key,
731   B.; Beverdam, A. Epidermal YAP2-5SA-ΔC Drives β-Catenin Activation to Promote
732   Keratinocyte Proliferation in Mouse Skin In Vivo. *Journal of Investigative Dermatology* **2017**,
733   *137*, 716–726, doi:10.1016/j.jid.2016.10.029.

734   101.   Schlegelmilch, K.; Mohseni, M.; Kirak, O.; Pruszak, J.; Rodriguez, J. R.; Zhou, D.; Kreger, B. T.;
735   Vasioukhin, V.; Avruch, J.; Brummelkamp, T. R.; Camargo, F. D. Yap1 acts downstream of
736   α-catenin to control epidermal proliferation. *Cell* **2011**, *144*, 782–95,
737   doi:10.1016/j.cell.2011.02.031.

738   102.   Zhang, H.; Pasolli, H. A.; Fuchs, E. Yes-associated protein (YAP) transcriptional coactivator
739   functions in balancing growth and differentiation in skin. *Proceedings of the National Academy*
740   *of Sciences* **2011**, *108*, 2270–2275, doi:10.1073/pnas.1019603108.

741   103.   Akladios, B.; Mendoza Reinoso, V.; Cain, J. E.; Wang, T.; Lambie, D. L.; Watkins, D. N.;
742   Beverdam, A. Positive regulatory interactions between YAP and Hedgehog signalling in skin
743   homeostasis and BCC development in mouse skin in vivo. *PloS one* **2017**, *12*, e0183178,
744   doi:10.1371/journal.pone.0183178.

745   104.   Botchkarev, V. A.; Flores, E. R. p53/p63/p73 in the epidermis in health and disease. *Cold*
746   *Spring Harbor perspectives in medicine* **2014**, *4*, 466–472, doi:10.1101/cshperspect.a015248.

747   105.   Abe, Y.; Oda-Sato, E.; Tobiume, K.; Kawauchi, K.; Taya, Y.; Okamoto, K.; Oren, M.; Tanaka,
748   N. Hedgehog signaling overrides p53-mediated tumor suppression by activating Mdm2.
749   *Proceedings of the National Academy of Sciences of the United States of America* **2008**, *105*, 4838–43,
750   doi:10.1073/pnas.0712216105.

751   106.   Mills, A. A.; Zheng, B.; Wang, X. J.; Vogel, H.; Roop, D. R.; Bradley, A. P63 Is a P53
752   Homologue Required for Limb and Epidermal Morphogenesis. *Nature* **1999**, *398*, 708–713,
753   doi:10.1038/19531.

754   107.   Yang, A.; Schweitzer, R.; Sun, D.; Kaghad, M.; Walker, N.; Bronson, R. T.; Tabin, C.; Sharpe,
755   A.; Caput, D.; Crum, C.; McKeon, F. p63 is essential for regenerative proliferation in limb,
756   craniofacial and epithelial development. *Nature* **1999**, *398*, 714–718, doi:10.1038/19539.

757   108.   Bigelow, R. L. H.; Chari, N. S.; Undén, A. B.; Spurgers, K. B.; Lee, S.; Roop, D. R.; Toftgård, R.;
758   McDonnell, T. J. Transcriptional Regulation of bcl-2 Mediated by the Sonic Hedgehog
759   Signaling Pathway through gli-1. *Journal of Biological Chemistry* **2004**, *279*, 1197–1205,
760   doi:10.1074/jbc.M310589200.

761   109.   Regl, G.; Kasper, M.; Schnidar, H.; Eichberger, T.; Neill, G. W.; Philpott, M. P.; Esterbauer, H.;
762   Hauser-Kronberger, C.; Frischauf, A.-M.; Aberger, F. Activation of the BCL2 promoter in
763   response to Hedgehog/GLI signal transduction is predominantly mediated by GLI2. *Cancer*
764   *research* **2004**, *64*, 7724–31, doi:10.1158/0008-5472.CAN-04-1085.

765   110.   Polakowska, R. R.; Piacentini, M.; Bartlett, R.; Goldsmith, L. A.; Haake, A. R. Apoptosis in
766   human skin development: Morphogenesis, periderm, and stem cells. *Developmental Dynamics*
767   **1994**, *199*, 176–188, doi:10.1002/aja.1001990303.

768   111.   Chari, N. S.; Romano, R. A.; Koster, M. I.; Jaks, V.; Roop, D.; Flores, E. R.; Teglund, S.; Sinha,
769   S.; Gruber, W.; Aberger, F.; Medeiros, L. J.; Toftgard, R.; McDonnell, T. J. Interaction between
770   the TP63 and SHH pathways is an important determinant of epidermal homeostasis. *Cell*
771   *Death and Differentiation* **2013**, *20*, 1080–1088, doi:10.1038/cdd.2013.41.

772   112.   Taylor, M. D.; Zhang, X.; Liu, L.; Hui, C.-C.; Mainprize, T. G.; Scherer, S. W.; Wainwright, B.;
773   Hogg, D.; Rutka, J. T. Failure of a medulloblastoma-derived mutant of SUFU to suppress
774   WNT signaling. *Oncogene* **2004**, *23*, 4577–83, doi:10.1038/sj.onc.1207605.

775   113.   Rubin, A. I.; Chen, E. H.; Ratner, D. Basal-Cell Carcinoma. *New England Journal of Medicine*
776   **2005**, *353*, 2262–2269, doi:10.1056/NEJMra044151.

777   114.   Donovan, J. Review of the hair follicle origin hypothesis for basal cell Carcinoma.
778   *Dermatologic Surgery* 2009, *35*, 1311–1323.

779   115.   Reifenberger, J.; Wolter, M.; Knobbe, C. B.; K??hler, B.; Sch??nicke, A.; Scharw??chter, C.;
780   Kumar, K.; Blaschke, B.; Ruzicka, T.; Reifenberger, G. Somatic mutations in the PTCH,
781   SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas. *British Journal of*
782   *Dermatology* **2005**, *152*, 43–51, doi:10.1111/j.1365-2133.2005.06353.x.

783   116.   Khavari, P. a Modelling cancer in human skin tissue. *Nature Reviews Cancer* **2006**, *6*, 270–280,
784   doi:10.1038/nrc1838.

785   117.   Nilsson, M.; Undèn, A. B.; Krause, D.; Malmqwist, U.; Raza, K.; Zaphiropoulos, P. G.;
786   Toftgård, R. Induction of basal cell carcinomas and trichoepitheliomas in mice
787   overexpressing GLI-1. *Proceedings of the National Academy of Sciences of the United States of*
788   *America* **2000**, *97*, 3438–43, doi:10.1073/pnas.050467397.

789   118.   Grachtchouk, M.; Mo, R.; Yu, S.; Zhang, X.; Sasaki, H.; Hui, C. C.; Dlugosz,   a a Basal cell
790   carcinomas in mice overexpressing Gli2 in skin. *Nature genetics* **2000**, *24*, 216–7,
791   doi:10.1038/73417.

792   119.   Svärd, J.; Heby-Henricson, K.; Henricson, K. H.; Persson-Lek, M.; Rozell, B.; Lauth, M.;
793   Bergström, A.; Ericson, J.; Toftgård, R.; Teglund, S. Genetic elimination of Suppressor of
794   fused reveals an essential repressor function in the mammalian Hedgehog signaling
795   pathway. *Developmental cell* **2006**, *10*, 187–97, doi:10.1016/j.devcel.2005.12.013.

796   120.   Wang, G. Y.; Wang, J.; Mancianti, M. L.; Epstein, E. H. Basal cell carcinomas arise from hair
797   follicle stem cells in Ptch1+/- mice. *Cancer Cell* **2011**, *19*, 114–124, doi:10.1016/j.ccr.2010.11.007.

798   121.   Youssef, K. K.; Lapouge, G.; Bouvrée, K.; Rorive, S.; Brohée, S.; Appelstein, O.; Larsimont,
799   J.-C.; Sukumaran, V.; Van de Sande, B.; Pucci, D.; Dekoninck, S.; Berthe, J.-V.; Aerts, S.;
800   Salmon, I.; del Marmol, V.; Blanpain, C. Adult interfollicular tumour-initiating cells are
801   reprogrammed into an embryonic hair follicle progenitor-like fate during basal cell
802   carcinoma initiation. *Nature cell biology* **2012**, *14*, 1282–94, doi:10.1038/ncb2628.

803   122.   Peterson, S. C.; Eberl, M.; Vagnozzi, A. N.; Belkadi, A.; Veniaminova, N. A.; Verhaegen, M. E.;
804   Bichakjian, C. K.; Ward, N. L.; Dlugosz, A. A.; Wong, S. Y. Basal cell carcinoma preferentially
805   arises from stem cells within hair follicle and mechanosensory niches. *Cell Stem Cell* **2015**, *16*,
806   400–412, doi:10.1016/j.stem.2015.02.006.

807   123.   Robarge, K. D.; Brunton, S. A.; Castanedo, G. M.; Cui, Y.; Dina, M. S.; Goldsmith, R.; Gould, S.
808   E.; Guichert, O.; Gunzner, J. L.; Halladay, J.; Jia, W.; Khojasteh, C.; Koehler, M. F. T.; Kotkow,
809   K.; La, H.; LaLonde, R. L.; Lau, K.; Lee, L.; Marshall, D.; Marsters, J. C.; Murray, L. J.; Qian, C.;
810   Rubin, L. L.; Salphati, L.; Stanley, M. S.; Stibbard, J. H. A.; Sutherlin, D. P.; Ubhayaker, S.;
811   Wang, S.; Wong, S.; Xie, M. GDC-0449-A potent inhibitor of the hedgehog pathway.
812   *Bioorganic and Medicinal Chemistry Letters* **2009**, *19*, 5576–5581, doi:10.1016/j.bmcl.2009.08.049.

813   124.   Sharpe, H. J.; Pau, G.; Dijkgraaf, G. J.; Basset-Seguin, N.; Modrusan, Z.; Januario, T.; Tsui, V.;
814   Durham, A. B.; Dlugosz, A. A.; Haverty, P. M.; Bourgon, R.; Tang, J. Y.; Sarin, K. Y.; Dirix, L.;
815   Fisher, D. C.; Rudin, C. M.; Sofen, H.; Migden, M. R.; Yauch, R. L.; de Sauvage, F. J. Genomic
816   Analysis of Smoothened Inhibitor Resistance in Basal Cell Carcinoma. *Cancer Cell* **2015**, *27*,
817   327–341, doi:10.1016/j.ccell.2015.02.001.

818   125.   Atwood, S. X.; Sarin, K. Y.; Li, J. R.; Yao, C. Y.; Urman, N. M.; Chang, A. L. S.; Tang, J. Y.; Oro,
819   A. E. Rolling the Genetic Dice: Neutral and Deleterious Smoothened Mutations in
820   Drug-Resistant Basal Cell Carcinoma. *The Journal of investigative dermatology* **2015**, *135*, 2138–
821   41, doi:10.1038/jid.2015.115.

822 126. Atwood, S. X.; Li, M.; Lee, A.; Tang, J. Y.; Oro, A. E. GLI activation by atypical protein kinase
823   C ι/λ regulates the growth of basal cell carcinomas. *Nature* **2013**, *494*, 484–8,
824   doi:10.1038/nature11889.

825 127. Li, C.; Chi, S.; Xie, J. Hedgehog signaling in skin cancers. *Cellular Signalling* **2011**, *23*, 1235–
826   1243, doi:10.1016/j.cellsig.2011.03.002.

827 128. Peng, T.; Frank, D. B.; Kadzik, R. S.; Morley, M. P.; Rathi, K. S.; Wang, T.; Zhou, S.; Cheng, L.;
828   Lu, M. M.; Morrisey, E. E. Hedgehog actively maintains adult lung quiescence and regulates
829   repair and regeneration. *Nature* **2015**, *526*, 578–82, doi:10.1038/nature14984.