Odontogenic Keratocysts Arise from Quiescent Epithelial Rests and Are Associated with Deregulated Hedgehog Signaling in Mice and Humans

Marina Grachtchouk,* Jianhong Liu,* Ai Qin Wang,*
Lebing Wei,* Christopher K. Bichakjian,*
Jonathan Garlick,[†] Augusto F. Paulino,[‡]
Thomas Giordano,[‡] and Andrzej A. Dlugosz*

From the Departments of Dermatology* and Pathology,[‡]
University of Michigan Comprehensive Cancer Center, Ann
Arbor, Michigan; and the Department of Oral and Maxillofacial
Pathology,[†] Tufts University School of Dental Medicine,
Boston, Massachusetts

Odontogenic keratocysts in humans are aggressive, noninflammatory jaw cysts that may harbor *PTCH1* mutations, leading to constitutive activity of the embryonic Hedgehog (Hh) signaling pathway. We show here that epithelial expression of the Hh transcriptional effector Gli2 is sufficient for highly penetrant keratocyst development in transgenic mice. Mouse and human keratocysts expressed similar markers, leading to tooth misalignment, bone remodeling, and craniofacial abnormalities. We detected Hh target gene expression in epithelial cells lining keratocysts from both species, implicating deregulated Hh signaling in their development. Most mouse keratocysts arose from rests of Malassez—quiescent, residual embryonic epithelial cells that remain embedded in the periodontal ligament surrounding mature teeth. In *Gli2*-expressing mice, these rests were stimulated to proliferate, stratify, and form a differentiated squamous epithelium. The frequent development of keratocysts in *Gli2*-expressing mice supports the idea that Gli transcription factor activity mediates pathological responses to deregulated Hh signaling in humans. Moreover, *Gli2*-mediated reactivation of quiescent epithelial rests to form keratocysts indicates that these cells retain the capacity to function as progenitor cells on activation by an appropriate developmental signal. (Am J Pathol 2006, 169:806–814; DOI: 10.2353/ajpath.2006.060054)

The discovery of *PTCH1* mutations in patients with nevoid basal cell carcinoma syndrome (NBCCS), also called Gorlin's syndrome or basal cell nevus syndrome,^{1,2} provided the starting point for studies aimed at understanding the molecular pathogenesis of a variety of neoplasms and developmental abnormalities. Ptch1 is a transmembrane receptor for Sonic hedgehog and other Hedgehog (Hh) proteins, which are secreted signaling molecules required for embryonic development of a wide variety of structures.³ Ptch1 normally represses the function of the signaling effector Smoothened (Smo), a seven-pass transmembrane protein with homology to G protein-coupled receptors. Initiation of normal Hh signaling is strictly dependent on the presence of Hh proteins, which are expressed in a spatially and temporally restricted manner. Binding of Hh antagonizes Ptch1, which results in derepression of Smo and, ultimately, the transcriptional activation of Hh target genes. Up-regulation of the Hh target genes *Ptch1* and *Gli1* is thus a reliable measure of Hh signaling activity. In contrast to reversible, ligand-dependent Hh pathway activation, which operates under physiological conditions, Hh signaling is constitutively activated in the common skin tumor basal cell carcinoma (BCC), primarily because of loss of function mutations of *PTCH1* or gain of function mutations involving *SMO*.⁴ Recent studies also implicate unrestrained Hh signaling activity in the pathogenesis of a variety of internal malignancies,^{5,6} supporting the concept that inappropriate reactivation of embryonic signaling pathways can contribute to cancer development after birth.

The combined function of Hh-regulated Gli transcription factors (Gli1, Gli2, and Gli3) controls Hh-mediated alterations in gene expression in responsive cell types

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Address reprint requests to Andrzej A. Dlugosz, Department of Dermatology and Comprehensive Cancer Center, University of Michigan, 3316 CCGC, Box 0932, 1500 E. Medical Center Dr., Ann Arbor, MI 48109-0932. E-mail: dlugosza@umich.edu.

cell.^{7,8} *In vivo*, Gli1 and Gli2 act primarily as transcriptional activators, whereas Gli3 generally functions as a repressor. Despite the fact that *Gli1* is consistently up-regulated when the Hh pathway is stimulated, *Gli1*-null mice are normal.^{9,10} *Gli2*-deficient mice, in contrast, exhibit defects in multiple organs whose development is dependent on Hh signaling,^{10–17} implying that Gli2 is the primary transcriptional activator mediating responses to physiological Hh signaling in the mouse. Particularly relevant to the data in this report, previous studies have established that Hh/Gli signaling is essential for normal tooth development, playing an important role in the regulation of epithelial cell proliferation.^{18–22}

Overexpression of either Gli2 or Gli1 leads to tumor development in transgenic mice,^{23–26} suggesting that one, or both, of these transcription factors contributes to tumorigenesis associated with alterations in upstream components of the Hh pathway leading to constitutive pathway activation. In keeping with this concept, *GLI1* and *GLI2* are highly expressed in nearly all human BCCs examined.^{27–30} The potential role of Gli proteins in the pathogenesis of nonneoplastic disorders linked to abnormal Hh signaling in postnatal epithelia has not been investigated.

Along with BCCs, which arise exclusively in skin, NBCCS patients also have an increased incidence of medulloblastomas and may be predisposed to the development of several other tumors.^{31,32} These individuals commonly exhibit a variety of additional abnormalities, including craniofacial and skeletal defects, pits in the skin of their palms and soles, and jaw cysts (odontogenic keratocysts), which have an incidence as high as 80%. Odontogenic keratocysts, which also arise in the general population, can exhibit aggressive growth leading to extensive bone restructuring and gross deformity.^{33,34} The treatment of choice is surgery, but recurrences are common, occurring in up to ~60% of cases,³⁵ and the biological behavior of odontogenic keratocysts has been compared to that of benign neoplasms.^{36–38} In patients with NBCCS, keratocysts are frequently multiple and represent a major source of morbidity. Although *PTCH1* deficiency has been described in the epithelial lining of human odontogenic keratocysts,^{39–41} and this genetic alteration would be expected to lead to constitutive Hh pathway activity, there are conflicting reports regarding the status of Hh signaling in these lesions.^{42–44}

In this report, we characterize odontogenic keratocysts that develop in transgenic mice overexpressing Gli2 under the control of the keratin 5 (K5) promoter and compare them to human keratocysts. The mouse keratocysts exhibit many of the same gross, microscopic, biochemical, and molecular features that are seen in odontogenic keratocysts arising in humans, which include deregulated activation of the Hh pathway in epithelial cells comprising the cyst wall. Moreover, analysis of early keratocysts in *K5-Gli2* mice reveals that these lesions originate by reactivation of quiescent epithelial rests of Malassez, triggering proliferation, stratification, and terminal differentiation of these normally quiescent cells. The robust ability of Gli2 to induce multiple keratocysts when overexpressed in transgenic mice points to a central role for

GLI transcription factors in the pathogenesis of human odontogenic keratocysts and raises the possibility that agents aimed at blocking GLI function may have therapeutic value in the treatment of keratocysts and other disorders related to unrestrained Hh signaling in humans.

Materials and Methods

Transgenic Mice and Tissue Processing

K5-Gli2 mice were generated by personnel from the University of Michigan Transgenic Model Core by injection into fertilized mouse eggs according to standard protocols. All mice were housed and maintained according to University of Michigan institutional guidelines. Tissue was fixed overnight in either neutral-buffered formalin or Bouin's solution and decalcified either in Cal-Ex II (catalog no. CS511-4D; Fisher, Pittsburgh, PA) at room temperature for 10 days or in acetic acid/formal saline (4% formaldehyde in 0.85% NaCl with 10% acetic acid) at 4°C for 3 weeks. Decalcification solution was refreshed every 2 to 3 days, and vials with tissue were kept on a rotating platform. After decalcification, tissue was transferred to 70% ethanol, processed, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E) or used for immunohistochemistry or *in situ* hybridization. All processing steps for *in situ* hybridization analysis were performed under RNase-free conditions. Archived paraffin blocks containing human keratocyst samples or BCCs were obtained according to institutional review board-approved guidelines and sectioned at 5 μ m for *in situ* hybridization.

Immunostaining and *in Situ* Hybridization

Immunostaining was performed on sections of paraffin-embedded tissue as previously described,⁴⁵ using rabbit polyclonal antibodies recognizing keratins K5 or K10 (Covance, Princeton, NJ) or K17,⁴⁶ generously provided by Pierre Coulombe (John Hopkins School of Medicine, Baltimore, MD). For proliferating cell nuclear antigen (PCNA) staining, sections were blocked with 10% normal goat serum for 30 minutes and incubated at 4°C overnight with rabbit polyclonal antibody (NeoMarkers, Fremont, CA), diluted 1:300 in phosphate-buffered saline containing 2 mg/ml bovine serum albumin. The remainder of the immunostaining protocol was as previously described.

Protocols for *in situ* hybridization using digoxigenin-labeled mouse (*Gli1*, *Ptch1*) and human (*GLI1*, *PTCH1*) riboprobes, as well as the sources for these reagents, are described elsewhere.⁴⁵ Probe for mouse *Gli2*, which detects both endogenous *Gli2* and mRNA produced by transgene, was provided by Chi-Chung Hui (Hospital for Sick Children, Toronto, ON, Canada), and plasmids to make riboprobes against mouse *Cyclin D1* and *Cyclin D2* were provided by Piotr Sicinski (Harvard Medical School, Boston, MA). We designed a *GLI2* riboprobe to detect expression of this gene in human tissue sections. RNA from a human BCC was used as

a template for first strand synthesis using T3 and T7 promoters with the following polymerase chain reaction (PCR) primers from human *GLI2* (forward 5'-CCCGAACTCCTTGACCCTG-3', and reverse 5'-GTTTGCATTCCTTCCTGTCC-3'). The 415-bp PCR product was used for *in vitro* transcription (Roche, Indianapolis, IN) to generate digoxigenin-UTP-labeled *GLI2* riboprobe.

Results

Incisor Defects in K5-Gli2 Transgenic Mice

Rodent incisors, unlike molars, continue to grow and deposit new enamel on their labial surfaces throughout adult life, allowing alterations in enamel formation to be detectable postnatally. The mandibular incisors of *K5-Gli2* mice were frequently chalky white in appearance, compared to the pale yellow color of incisors of control mice (Figure 1, A and B), a finding that has previously been described in mice with defective enamel deposition.^{47–51} Analysis of H&E-stained coronal sections from *K5-Gli2* mice revealed striking morphological abnormalities affecting ameloblasts, the terminally differentiated cells responsible for enamel production (Figure 1, C and D). In contrast to control incisors, which contained ameloblasts with a uniform columnar morphology, an eccentrically placed nucleus, and abundant cytoplasm (Figure 1C), incisors from *K5-Gli2* mice contained masses of undifferentiated appearing cells with a relatively high nuclear-to-cytoplasmic ratio (Figure 1D). These cells in *K5-Gli2* mice were arranged haphazardly and expressed proliferation markers (data not shown) that are normally not seen in mature ameloblasts. These findings were not attributable to a complete absence of ameloblasts in *K5-Gli2* mice because some cells exhibiting morphological features typical for ameloblasts were detected in transgenic mice (Figure 1D; inset, bracket), but overall, hypoplasia of differentiated ameloblasts was seen in affected incisors. Moreover, additional histological findings suggested that enamel was being produced, albeit abnormally, on the incisors of *K5-Gli2* mice. Routine tissue decalcification leads to complete loss of fully mineralized enamel in control teeth, leaving an empty space between the ameloblast layer and dentin (Figure 1C). In contrast, sections from affected incisors of *K5-Gli2* mice frequently contained acellular, eosinophilic material adjacent to the abnormal ameloblast cell layer (Figure 1D), indicating the presence of hypomineralized enamel. Because the K5 promoter is active in ameloblasts and their progenitors, these findings indicate that although deregulated Hh signaling disrupts the orderly program of ameloblast differentiation and enamel deposition, it does not block the formation of this cell type.

K5-Gli2 Mice Develop Jaw Cysts Resembling Human Odontogenic Keratocysts

K5-Gli2 mice occasionally developed grossly misaligned incisors (Figure 2A), and a subset of *K5-Gli2* mice developed obvious protrusions from the mandible, or less commonly, the maxilla (Figure 2A). Nec-

ropsy of these mice revealed large cystic cavities lined by a thin wall (Figure 2B), and these cysts frequently contained an amorphous white substance that consisted of keratinized epithelial cells (see below). As reported in some human jaw cysts, large mouse jaw cysts occasionally contained an impacted tooth (Figure 2B). The smallest lesions were spheroidal and had an opalescent, pearly appearance (Figure 2B, inset). Although multiple cysts were commonly seen in the mandible and maxilla near molar roots, isolated cysts were rarely detected immediately above the hard palate (not shown). Human keratocysts can also arise in atypical locations,⁵² indicating that although the progenitor cells for the majority of keratocysts are likely to be located in close proximity to teeth, other cells are also capable of giving rise to cysts.

H&E-stained sections of heads from *K5-Gli2* mice revealed jaw cysts with an orderly pattern of differentiation from the basal cell layer to keratinized squamous cells that accumulated in the center of the cysts (Figure 2, C and D). In general, keratinized cells in mouse keratocysts were devoid of nuclear remnants (orthokeratotic). Although this pattern of keratinization can be seen in human keratocysts,⁵³ squames with residual nuclei (parakeratotic) are more common (Figure 2E). Extensive bone remodeling, a typical feature of human odontogenic keratocysts, was frequently seen in sections containing mouse keratocysts (Figure 2C). Jaw cysts were detected in four independent *K5-Gli2* transgenic mouse lines, and detailed analysis of two of these lines revealed that the keratocyst phenotype was highly penetrant. In the *K5-Gli2*²⁷ line, 100% of mice older than 6 months of age ($n = 25$) had keratocysts; in *K5-Gli2*⁹⁹⁵ mice, 95% of mice sacrificed after 7 months of age ($n = 21$) had keratocysts. Development of keratocysts was a highly reproducible and stable phenotype because progeny from multiple generations, currently up to N8, continue to produce keratocysts.

Immunostaining of mouse keratocysts revealed the presence of keratins K5, K10, and K17 (Figure 2, F–H), mimicking the keratin expression profile previously reported in human odontogenic keratocysts.^{53,54} Strong expression of K17, as seen in mouse keratocysts (Figure 2G), is frequently observed in human odontogenic keratocysts.⁵⁴ In addition, the restricted expression of K10 to just the most superficial nucleated cell layers of cyst epithelium (Figure 1H) also reflects the expression pattern of K10 reported in human odontogenic keratocysts.⁵³ Proliferation in mouse keratocysts was limited to the basal cell layer, based on immunostaining directed against the proliferation marker PCNA (Figure 2I).

Mouse Keratocysts Are Derived from Reactivated Epithelial Rests of Malassez

Examination of tissue from younger *K5-Gli2* mice, before the appearance of grossly apparent cysts, enabled us to identify cysts at early stages in their devel-

Figure 1. Enamel defect attributable to an ameloblast alteration in incisors of *K5-Gli2* mice. **A** and **B**: Gross appearance of lower incisors of control and *K5-Gli2* transgenic mice. White color of lower incisors in transgenic mice suggests defective enamel formation. **C** and **D**: Histology of lower incisors within mandible after tissue decalcification. **C**: Normal appearance seen in control sections, which includes surrounding alveolar bone, a vascular sinus, supporting stroma, ameloblast cell layer (ambl) with associated stratum intermedium (si) (arrowheads and inset), clear space (previously occupied by fully mineralized enamel), and dentin. Note highly ordered, columnar appearance of normal ameloblasts, with eccentrically placed nucleus. **D**: Severe disruption of normal morphology in maxilla from *K5-Gli2* mouse. The ameloblast and stratum intermedium layers are replaced primarily by cells with an undifferentiated morphology and scant cytoplasm, extending into the stromal compartment (arrowheads). Some cells resembling ameloblasts are present (inset, bracket), together with an amorphous, eosinophilic material (asterisks) that resembles incompletely mineralized enamel (see text).

opment. Although a small minority of cysts appeared to arise directly from oral mucosa (data not shown), the overwhelming majority of microscopic cysts were found within the periodontal ligament, which anchors the molar tooth roots to the surrounding alveolar bone (Figure 3, A–C). This localization is consistent with the proposed origin of keratocysts from the epithelial rests of Malassez, cells that are left behind as much of the epithelial root sheath regresses during late stages of tooth formation.^{55,56} Cysts at various stages of development could sometimes be identified in the same tissue section, with early lesions clearly arising within the periodontal ligament (Figure 3C). Because epithelial rests express K5 protein (Figure 3D),⁵⁷ the *K5-Gli2*

transgene is also likely to be active in these cells. K10 expression, detected in mature keratocysts, was also seen in superficial cell layers in early cysts within the periodontal ligament. Moreover, the proliferation marker PCNA was expressed in enlarged rests and the basal layer of early cysts (Figure 3D) but was not detected in epithelial rests from control mice (Figure 3D). Increased expression of transcripts encoding both *Gli2* (transgene-derived and endogenous) and *Ptch1* were detected in activated rests in *K5-Gli2* mice, but *Gli1* expression was not appreciably altered (data not shown). Taken together, these results suggest that deregulated activation of Hh signaling, via expression of the *Gli2* transgene, leads to reactivation of prolifer-

Figure 2. Keratinizing jaw cysts in *K5-Gli2* mice resemble human odontogenic keratocysts. **A**: Gross appearance of transgenic mouse showing severe abnormalities in orientation of lower incisors associated with mandibular mass (dashed line). **B**: Gross coronal section showing appearance of large cyst (dashed line) containing keratinized material and impacted incisor. Multiple small cysts were detected near molar tooth roots (higher magnification in inset). Similar histology of mouse jaw cysts (**C**, **D**) and human odontogenic keratocyst (**E**). Epithelial cell lining of variable thickness surrounds keratinized, terminally differentiated squamous cells. **F–H**: Keratin expression in mouse keratocysts (keratins 5, 17, and 10) is consistent with what has previously been reported in human keratocysts. **I**: PCNA immunostaining reveals proliferative cells limited to the basal cell layer of mouse keratocysts.

Figure 3. Mouse keratocysts are derived from quiescent epithelial rests of Malassez. **A** and **B**: Histology of control and *K5-Gli2* mice. **A**: Epithelial rests of Malassez are located within the periodontal ligament, which connects the molars (tooth) to the surrounding alveolar bone. In *K5-Gli2* mice (**B**), these rests are frequently severalfold larger than in control mice (**A**). **C**: Section showing epithelial rest of Malassez (**1**) and keratocysts at several stages of development (**2–4**), arising within the periodontal ligament (asterisks). **D**: Immunohistochemistry shows baseline expression of keratins 5 and 17 in control epithelial rests and enlarged rests from *K5-Gli2* mice. Neither the proliferation marker PCNA nor the terminal differentiation marker keratin 10 was detected in control epithelial rests. In contrast, in activated rests and keratocysts at various stages of development, PCNA was present in the basal cell layer, and keratin 10 was expressed in differentiating suprabasal cells.

ation, followed by terminal differentiation, of the normally quiescent epithelial rests of Malassez.

Constitutive Hh Signaling Activity in Mouse and Human Keratocysts

The epithelium of human keratocysts, similar to BCCs arising in skin, reveals loss-of-heterozygosity at the *PTCH1* locus,^{39–41} suggesting a role for pathologically activated Hh signaling in the pathogenesis of both types of lesions due to loss of *PTCH1* function. To further examine this concept, we performed a series of *in situ* hybridization studies to examine the expression of Hh target genes, which are either undetectable or expressed at low levels in the majority of tissues in adult animals. We first focused on keratocysts arising in *K5-Gli2* mice. *Gli2* riboprobe, which detects expression of exogenous (transgene-derived) and endogenous *Gli2*, revealed a strong signal in the basal layer of cyst walls (Figure 4B).

As expected, cells in the basal layer also expressed the Hh target genes *Gli1* and *Ptch1* (Figure 4, A and C), indicating that Hh signaling is activated in these cells. Transcripts encoding the Hh-responsive cyclins D1 and D2^{16,45,58–60} were also detected in the basal layer of cyst walls (Figure 4, D and E) and may play an important role in driving proliferation of keratocysts. Interestingly, although *Gli2* appears to require Hh ligand for full transcriptional activity in some settings,¹⁶ neither *Shh* nor *Indian hedgehog* transcripts were detected in mouse keratocyst epithelium or surrounding stroma (data not shown), suggesting that *Gli2* is capable of signaling in the absence of ligand. Similarly, we have previously reported that *Shh* was not detected in BCCs arising in *K5-Gli2* mice.²⁴ Expression of *Ptch1* (Figure 4F) and other Hh target genes (not shown) was undetectable in quiescent epithelial rests of Malassez in control mice.

Hh target gene expression was also examined in the epithelial lining of human keratocysts and compared to

Figure 4. *In situ* hybridization examining expression of Hh target genes in mouse keratocysts. Expression of the classical Hh target genes, *Gli1* and *Ptch1*, was detected in the basal cell layer of mouse keratocysts (**A** and **C**). **B**: Similarly, *Gli2* expression (transgene-derived and endogenous) was limited to the basal layer of mouse keratocysts. Altered expression of Hh target genes was not seen in stroma surrounding the cysts. **D** and **E**: Transcripts encoding the Hh-responsive G1 cyclins, *Cyclin D1* and *Cyclin D2*, were also expressed in the basal layer of mouse keratocysts. **F**: Expression of *Ptch1* or other Hh target genes (not shown) was not detected in epithelial rests of Malassez in control mice.

levels in BCCs, which consistently exhibit constitutively elevated Hh signaling.^{61–64} *GLI1* and *PTCH1* mRNA were detected in human keratocysts (Figure 5, A and C), indicating that the Hh pathway is activated in these lesions. *GLI2* was also detected in these lesions (Figure 5B), consistent with the hypothesis that *GLI2* plays a key role in the pathogenesis of human keratocysts. Although Hh target genes were detected preferentially in the lower epithelial cell layers of the cyst wall, cells with the strongest *in situ* signal appeared to express Hh target genes at

a level grossly comparable to what was observed in human BCC tumor cells (Figure 5, D–F), with the caveat that *in situ* data are semiquantitative at best.

Discussion

Our findings establish that ectopic activation of Hh signaling in dental epithelium, by expression of the transcription factor *Gli2*, is a potent stimulus for the development of odontogenic keratocysts. Most mouse keratocysts arise from quiescent rests of Malassez, revealing that epithelium-specific Hh signaling can reprogram these cells to proliferate, stratify, and terminally differentiate. In keeping with a pathogenic role for deregulated Hh signaling in jaw cyst development, we demonstrate elevated levels of Hh target genes in the epithelial lining of both mouse and human keratocysts. Together with earlier studies implicating the Hh pathway in the development of keratocysts,^{39,40,42–44,65,66} our findings suggest that agents capable of blocking Hh signaling^{67,68} may provide a novel approach to treating these aggressive lesions, assuming that they arise because of alterations in Hh signaling elements that lie upstream of pharmacological targets.

Jaw cysts have also been described in *Ptch*^{+/-} mice, which harbor the same genetic defect as NBCCS patients. However, these microscopic keratocysts were detected in a minority of *Ptch*^{+/-} mice (25%), and in contrast to human OKCs or the lesions described in this report, none of the lesions arising in *Ptch*^{+/-} mice were detectable either grossly or radiographically.^{69,70} The relatively low incidence of keratocysts in *Ptch*^{+/-} mice may reflect a requirement for inactivation of the remaining *Ptch* allele, as appears to occur in human keratocysts^{39–41} and other jaw cysts.⁶⁵ The nearly complete penetrance of jaw cysts in *K5-Gli2* mice points to a central role for Gli transcriptional activity in keratocyst formation. Moreover, the robust development (in *K5-Gli2* mice) of BCCs and keratocysts, both of which are cardinal features of NBCCS, suggests that pathological consequences of deregulated *PTCH1* function in human epithelia may be mediated primarily by *GLI2*.

By analyzing *K5-Gli2* mice at various ages, we provide direct evidence that mouse keratocysts arise from epithelial rests of Malassez located within the periodontal ligament. Interestingly, although the K5 promoter is active in epithelia located throughout the oral cavity and nasopharynx,⁷¹ extracutaneous pathology in the heads of *K5-Gli2* mice appears to be primarily limited to keratocysts arising in the mandible and maxilla and alterations in ameloblasts, which are strictly dependent on proper Hh signaling during development.^{18,19} Although the mechanism underlying the exquisite responsiveness of epithelial rests to deregulated *Gli2* expression/Hh signaling is not known, recent studies examining Hh-associated cancer development may provide a clue. Studies performed in lung, prostate, and the gastrointestinal tract, suggest that Hh-associated cancers may arise from cells undergoing a regenerative response.⁶ This also appears to be the case in BCCs: skin tumors in *Gli2*-expressing trans-

Figure 5. Elevated Hh pathway activity in epithelial cells of human odontogenic keratocysts. Elevated expression of Hh target genes is detected in lower cell layers of human odontogenic keratocysts (A–C). Expression of one or more Hh target genes was detected in 9 of the 10 human keratocysts that were examined. Hh target gene expression levels are grossly similar to those detected in human BCC tumor cells (D–F), implying comparable levels of Hh pathway activation in the proliferating cells in these two lesions.

genic mice develop exclusively from hair follicles,^{24,26} which undergo repeated cycles of regeneration throughout life.⁷² Although normally quiescent, epithelial rests of Malassez can also be stimulated to proliferate in response to injury in rats,⁷³ and these reactivated rests are morphologically similar to the enlarged rests seen in *K5-Gli2* mice (Figure 3). Further work will be required to ascertain whether ectopic *Gli2* expression, at sufficiently high levels, can cause reactivation of rests and cyst development or whether a regenerative response in rests is a prerequisite for responsiveness to *Gli2* and subsequent cyst development.

It is intriguing that aberrant activation of Hh signaling leads to such vastly different responses in cutaneous versus dental epithelium, further underscoring the importance of cellular context in determining responsiveness to a potentially oncogenic stimulus. In skin of both humans and mice, uncontrolled Hh signaling is associated with the development of BCCs composed of masses of undifferentiated tumor cells. In contrast, deregulated Hh signaling in the ameloblast layer leads to hyperplasia and defective terminal differentiation leading to a deficiency of mature ameloblasts, but tumors arising from cells in this lineage, ameloblastomas, are not observed. Yet another response is seen in the quiescent epithelial rests of Malassez, where Hh pathway activation triggers proliferation, leading to the formation of a cyst that undergoes an orderly program of terminal differentiation and keratinization. The identification of cellular determinants responsible for divergent responses to pathological Hh signaling could provide important insights into Hh pathway-associated disease.

In situ analysis revealed notable differences in the localization of Hh signaling in cells comprising keratocysts versus BCCs. Hh target gene expression was restricted

to the lowermost cell layer both in mouse and human keratocysts, whereas nearly all BCC tumor cells expressed Hh target genes (Figures 4 and 5).²⁴ In the mouse cysts, this could be attributed to the fact that *K5* promoter activity, and therefore *Gli2* and Hh target gene expression, are limited to basal cells in the cyst wall. However, in human keratocyst epithelium deficient in *PTCH*, terminally differentiating cells apparently lose the capacity to respond to constitutive, proximal activation of the Hh pathway. Although physiological Hh signaling is precisely regulated by the activity of various effectors that can either stimulate or repress Hh pathway activity,⁷⁴ further studies are needed to gain insight into how pathological Hh signaling can be modulated in different cellular backgrounds.

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