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Primary cilia can both mediate and suppress Hedgehog pathway–dependent tumorigenesis

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Abstract

Primary cilia are present on most mammalian cells and are implicated in transducing Hedgehog (Hh) signals during development; however, the prevalence of cilia on human tumors remains unclear, and the role of cilia in cancer has not been examined. Here we show that human basal cell carcinomas (BCCs) are frequently ciliated, and we test the role of cilia in BCC by conditionally deleting *Kif3a* (encoding kinesin family member 3A) or *Ift88* (encoding intraflagellar transport protein 88), genes required for ciliogenesis, in two Hh pathway–dependent mouse tumor models. Ciliary ablation strongly inhibited BCC-like tumors induced by an activated form of Smoothened. In contrast, removal of cilia accelerated tumors induced by activated Gli2, a transcriptional effector of Hh signaling. These seemingly paradoxical effects are consistent with a dual role for cilia in mediating both the activation and the repression of the Hh signaling pathway. Our findings demonstrate that cilia function as unique signaling organelles that can either mediate or suppress tumorigenesis depending on the nature of the oncogenic initiating event.

Elevated Hh pathway activity is associated with diverse tumors, including basal cell carcinoma, the most commonly diagnosed cancer in North America^{1,2}. Hh signaling is normally restrained by the tumor suppressor Patched (Ptch1), which inhibits the function of the protooncogene Smoothened (Smo), a central activator of the pathway³. Binding of Hh ligand to Ptch1 relieves its inhibition of Smo, allowing Smo to induce downstream Gli activators and inhibit the formation of Gli repressors. In BCCs, loss of function mutations in *PTCH1*, gain of function mutations in *SMO*, and upregulation of *GLI1* and *GLI2* are frequently observed, suggesting that dysregulated Hh signaling is the underlying cause of this disease^{4–8}.

Recent studies have shown that the primary cilium has a prominent role in modulating mammalian Hh signaling⁹. Ptch1 suppresses the Hh pathway, at least in part, by preventing the trafficking of Smo into the primary cilium¹⁰. Upon binding of Hh ligand to Ptch1, Smo moves to the cilium¹¹. This event seems to be crucial for activating the Hh pathway, as a mutant form of Smo that fails to localize to the cilium cannot fully transduce Hh signals,

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whereas an activated mutant form of Smo isolated from individuals with BCC localizes constitutively to the cilium¹¹.

In addition to its essential role in transducing Hh signals, the cilium also negatively regulates the pathway. Genetic analyses have shown that the cilium is required for the proteolytic processing of Gli3 into a form that represses the Hh transcriptional program (Gli3-R)^{12–15}. Cilium-dependent formation of Gli3-R occurs in the absence of Hh and is suppressed upon Smo movement to the cilium. Thus, the cilium exerts both positive and negative control over the Hh pathway.

Although cilia are present on many vertebrate cells, their involvement in cancer has not been explored. Early ultrastructural studies have indicated that individual cells from human BCCs can be ciliated¹⁶; however, the prevalence of these ciliated cells has remained unclear. To determine whether cancer cells frequently possess cilia, we examined clinical biopsies from eight human BCCs. We observed that five BCCs contained numerous ciliated cells (Fig. 1a and Supplementary Fig. 1). In addition, ciliated cells were present in BCC-like lesions that arose in mouse skin expressing the constitutively active M2 allele of *Smo* (*Smo*M2^{cond})^{4,17} (Fig. 1b). We also observed ciliated cells in normal mouse skin, including the follicular outer root sheath, the mesenchymal dermal papilla and the interfollicular epidermis, as well as in cultured keratinocytes (Fig. 1c,d, data not shown and as recently reported¹⁸).

To test the role of cilia in skin tumorigenesis, we generated mice that harbor both a conditionally-activatable allele of *Smo*M2 and a conditional loss of function allele of *Kif3a* (*Kif3a*^{flox}), which encodes a subunit of kinesin-II, a heterotrimeric motor required for assembly of the primary cilium¹⁹. Deletion of *Kif3a* and induction of *Smo*M2 was mediated by a *Ker14* gene (encoding keratin-14) promoter-driven Cre recombinase (*Ker14-Cre*^{ERT}), which is active only in the presence of tamoxifen and induces recombination throughout the skin²⁰ (Supplementary Fig. 2). Thus, adult skin cells harboring these conditional alleles both activate *Smo*M2 expression and delete the floxed allele of *Kif3a* in response to tamoxifen, allowing spatial and temporal control of tumor initiation and ciliation (Fig. 2a).

We treated *Ker14-Cre*^{ERT};*Smo*M2^{cond} mice that were either *Kif3a*^{flox/+} or *Kif3a*^{flox/-} with tamoxifen at approximately 30 d of age and collected skin biopsies 5, 10 and 20 weeks after induction. We observed that *Ker14-Cre*^{ERT};*Smo*M2^{cond};*Kif3a*^{flox/+} mice developed epidermal hyperplasia beginning 5 weeks after induction (data not shown). By 10–20 weeks, the neoplastic epithelium extended numerous downgrowths into the underlying dermis (Fig. 2b). Neoplastic cells showed high nuclear to cytoplasmic ratios and manifested histopathological features of BCC, such as palisading (Supplementary Fig. 3). Consistent with our previously reported results showing that an oncogenic form of Smo constitutively localizes to cilia *in vitro*¹¹, SmoM2 localized to tumor cilia *in vivo* (Fig. 2c). As expected, ablation of *Kif3a* in *Ker14-Cre*^{ERT};*Kif3a*^{flox/-} mice reduced the number of ciliated cells in the follicular epithelium (Supplementary Fig. 4).

Strikingly, loss of cilia protected against SmoM2-induced tumorigenesis (Fig. 2b,d). The average thickness of the interfollicular epidermis from *Ker14-Cre*^{ERT};*Smo*M2^{cond};*Kif3a*^{flox/-} mice was nearly indistinguishable from that of control nontumorigenic skin of *Ker14-Cre*^{ERT};*Kif3a*^{flox/-} or *Ker14-Cre*^{ERT};*Kif3a*^{flox/+} mice 10 weeks after induction (Fig. 2d). Within 10 weeks after SmoM2 induction, *Kif3a*^{flox/+} mice also developed hyperplastic ear and tail skin, which was markedly alleviated in mice that had deleted *Kif3a* (Fig. 2e–f and Supplementary Figs. 5 and 6).

Genetic analyses in the embryonic neural tube have previously shown that cilia are required for Smo-mediated induction of Hh target genes^{14,15,21}. Consistent with this, *in situ* analyses in skin revealed that SmoM2-induced upregulation of *Gli1* and *Ptch1*, direct targets of Hh

signaling, was blocked by ciliary ablation (Fig. 2g). Furthermore, loss of cilia prevented SmoM2-induced upregulation of Gli2 (Fig. 2h). We confirmed this observation *in vitro* by expressing the mouse homolog of SmoM2 (SmoA1) in transformed mouse embryonic fibroblasts (MEFs). Whereas SmoA1 increased Gli2 protein levels in ciliated transformed MEFs, we did not observe such elevation in unciliated MEFs deficient in either *Kif3a* or *Ift172*, another gene required for ciliogenesis²¹ (Fig. 2i).

As previous studies have shown that Hh signaling promotes proliferation of the follicular epithelium^{22–24}, we examined whether cilia are required for SmoM2-induced proliferation in the skin. Indeed, ciliated *Kif3a*^{flox/+} tumors showed significantly higher proliferation relative to unciliated *Kif3a*^{flox/-} lesions (Fig. 2j). Thus, these results indicate that cilia are essential for Smo-mediated skin tumorigenesis.

In a variety of organs including skin, Hh pathway activity downstream of Smo is mediated primarily by Gli2 (ref. 24). To further define how cilia function in the Hh pathway, we tested the requirement for *Kif3a* in a second skin tumorigenesis model that uses conditional expression of a Myc-tagged, constitutively active human GLI2 lacking its amino-terminal repressor domain *Tg(CAG-loxP-eGFP-loxP-GLI2ΔN-Myc)*, referred to here as *CLEG2*^{cond} (ref. 25). We predicted that if cilia function specifically at the level of Smo for Hh pathway activation, loss of *Kif3a* or cilia should not affect GLI2ΔN-induced skin tumorigenesis.

As in the *SmoM2* experiments, we injected *Ker14-Cre*^{ERT};*CLEG2*^{cond} mice that were either *Kif3a*^{flox/+} or *Kif3a*^{flox/-} with tamoxifen. By 4–5 weeks after induction, GLI2ΔN-driven *Kif3a*^{flox/+} epidermal lesions were mildly hyperplastic and rarely extended into the dermis (Fig. 3a,b). However, in contrast to both our predictions and the effects that we observed in SmoM2-dependent tumorigenesis, loss of *Kif3a* markedly accelerated GLI2ΔN-induced skin neoplasia (Fig. 3a–c). *Ker14-Cre*^{ERT};*CLEG2*^{cond};*Kif3a*^{flox/-} mice showed gross changes in the skin, including hair loss, disorderly pelage hair and scaling, after induction (Fig. 3a). Histological analysis revealed that the majority of these *Ker14-Cre*^{ERT};*CLEG2*^{cond};*Kif3a*^{flox/-} mice developed BCC-like lesions that extended strands and nests of cells into the dermis (Fig. 3b). As expected, lesions that arose in *Kif3a*^{flox/-} mice did not contain ciliated cells (Supplementary Fig. 7).

Further molecular characterization revealed that the number of lesions with high endogenous Gli2 protein expression was significantly expanded in *Kif3a*^{flox/-} mice compared to *Kif3a*^{flox/+} mice (Fig. 3c). In addition, we observed that *Gli1* and *Ptch1* were both upregulated in *Kif3a*^{flox/-} lesions compared to normal skin, although some *Kif3a*^{flox/+} lesions also showed increased Hh signaling (Fig. 3d). Together, these results indicate that cilia suppress GLI2ΔN-induced tumorigenesis in the skin.

As noted above, in addition to being required for full Hh pathway activation, the cilium also negatively regulates the Hh transcriptional program by mediating Gli3 processing into Gli3-R^{12–15}. Transformed MEFs lacking cilia formed less Gli3-R than did ciliated transformed MEFs, confirming the role of cilia in Gli3-R formation (Fig. 3e). To determine whether cilia also mediate Gli3 processing in skin cells, we deleted *Kif3a* and induced expression of *GLI2ΔN* by treating keratinocytes isolated from *Ker14-Cre*^{ERT};*CLEG2*^{cond} mice that were either *Kif3a*^{flox/+} or *Kif3a*^{flox/-} with the tamoxifen derivative 4-hydroxytamoxifen (4-OHT). 4-OHT induced recombination in ~60% of cells (Supplementary Fig. 8), and, consistent with results from MEFs, loss of *Kif3a* decreased Gli3-R formation (Fig. 3f). These findings suggest that loss of cilia may promote GLI2ΔN-mediated tumorigenesis by inhibiting Gli3-R formation.

To elucidate how the cilium restrains GLI2ΔN activity, we examined GLI2ΔN induction of Hh target genes in 4-OHT-treated keratinocytes. 4-OHT increased expression of *GLI2ΔN* to

comparable levels regardless of Kif3a status, indicating that the cilium itself does not affect expression of the transgene (Fig. 3g). On average, GLI2ΔN expression induced ten-, 55- and threefold increases in expression of the Hh target genes *Ptch1*, *Gli1* and *Mycn*, respectively, over that of cells treated with vehicle control (Fig. 3g). As with GLI2ΔN, similar levels of these genes were induced regardless of Kif3a status.

However, in agreement with our immunohistochemical results, GLI2ΔN significantly increased expression of endogenous *Gli2* in *Kif3a*^{flox/-} keratinocytes relative to similarly-treated *Kif3a*^{flox/+} cells (Fig. 3h). Loss of Kif3a by itself was not sufficient to upregulate *Gli2* (Fig. 3h). Expression of *Bcl2*, another Hh target gene, was also increased to a greater extent in GLI2ΔN-induced keratinocytes that had lost Kif3a (Fig. 3h). Thus, loss of Kif3a increases GLI2ΔN-mediated induction of some Hh targets (*Gli2* and *Bcl2*), but not others (*Ptch1*, *Gli1* and *Mycn*).

In light of the increased *Gli2* expression that we observed in GLI2ΔN-induced *Kif3a*^{flox/-} cells, we examined whether endogenous Gli2 protein was also upregulated with an antibody that specifically recognizes mouse Gli2 (Supplementary Fig. 9). We observed that induction of GLI2ΔN, coupled with loss of Kif3a, increased endogenous Gli2 protein levels in keratinocytes (Fig. 3i). As cilia have been reported to be required for platelet-derived growth factor–induced mitogen-activated protein (MAP) kinase signaling²⁶, we also probed keratinocyte lysates for phosphorylated extracellular signal–related kinase-1 and extracellular signal–related kinase-2 (here referred to as ERK1/2). We observed that GLI2ΔN induction consistently increased ERK1/2 phosphorylation to a slightly greater extent in cells that had retained Kif3a, relative to cells that had lost Kif3a (Fig. 3i). As Wnt signaling is involved during folliculogenesis²⁷ and has recently been linked to BCC progression²⁸, we also assessed the expression of four Wnt target genes but found that none differed significantly between GLI2ΔN-expressing *Kif3a*^{flox/+} and *Kif3a*^{flox/-} cells ($P > 0.05$) (Supplementary Fig. 10). Together, these results suggest that augmented expression of some Hh target genes, including *Gli2* and *Bcl2*, but not differences in MAP kinase or Wnt signaling, may contribute to the accelerated progression of GLI2ΔN-induced BCC-like lesions upon loss of cilia.

Because ciliary loss disrupted formation of Gli3-R and increased expression of *Gli2*, we tested whether inhibiting *Gli3* expression in ciliated cells was sufficient to elevate endogenous *Gli2* levels. We derived keratinocytes from *Ker14-Cre*^{ERT};*CLEG2*^{cond} mice that were either wild type for *Gli3* or heterozygous for a null allele of *Gli3* (*Gli3*^{Xt}) and observed that GLI2ΔN-expressing *Gli3*^{Xt/+} keratinocytes showed elevated *Gli2* expression relative to that in GLI2ΔN-expressing *Gli3*^{+/+} cells upon induction with 4-OHT (Fig. 3j). Moreover, further down-regulation of *Gli3* by RNA interference (RNAi) in *Gli3*^{Xt/+} keratinocytes augmented *Gli2* expression (Fig. 3j).

Because Kif3a possesses nonciliary functions^{29,30}, we repeated our studies using conditional deletion of *Ift88*, which encodes an intra-flagellar transport protein required for ciliogenesis³¹. Similarly to the strategies described above, we induced expression of SmoM2 or GLI2ΔN in *Ker14-Cre*^{ERT} mice that were either *Ift88*^{flox/+} or *Ift88*^{flox/-}. As observed previously with *Kif3a*, loss of *Ift88* suppressed SmoM2-induced tumorigenesis (Fig. 4a,b). Also concordant with previous results, loss of *Ift88* accelerated the formation of GLI2ΔN-induced BCCs-like lesions (Fig. 4c,d). These findings confirm that the cilium specifically modulates the Hh-related tumor phenotypes observed in this study.

We have shown here that tumor cells from human BCCs are frequently ciliated and that these cilia possess dual roles in modulating Hh pathway–dependent tumor phenotypes in the skin. Whereas the cilium normally suppresses the Hh pathway by mediating Gli3-R

formation in the absence of Hh, the cilium is also required for Smo-mediated activation of the pathway in the presence of Hh (Fig. 4e). Our data suggest that tumors initiated by Smo depend upon the cilium both for transducing positive signals through Gli activators and for suppressing negative ones such as Gli3-R. For tumors initiated by constitutively active GLI2ΔN, loss of the cilium prevents Gli3-R formation, permitting unopposed activation of downstream signaling (Fig. 4e).

We have also shown here that in Smo-mediated tumorigenesis, upregulation of Gli2 requires cilia, whereas GLI2ΔN-induced elevation of endogenous Gli2 is augmented by loss of cilia, consistent with our hypothesis that ciliary control of Gli3 processing likely modulates gene expression in tumors. Indeed, we also observed that disruption of Gli3 increases *Gli2* expression, concordant with previous findings that Gli3 binds the *Gli2* promoter³². In addition to Gli2, Gli3-R likely represses other genes that promote tumorigenesis, such as *Bcl2*.

In addition to disrupting Gli3-R formation, loss of cilia might potentially inhibit formation of a Gli2 repressor, although evidence for a Gli2 repressor function is currently lacking. In a variety of cells, loss of cilia has also been associated with increased response to Wnt, although basal levels of Wnt signaling are unaffected^{30,33}. As Wnt signaling regulates folliculogenesis²⁷ and has recently been implicated in the pathogenesis of BCC²⁸, we examined the expression of Wnt target genes in keratinocytes. Although we found no evidence that Wnt signaling is affected either by GLI2ΔN induction or by ciliary status, it remains possible that increased Wnt responsiveness may contribute to tumorigenesis after loss of cilia *in vivo*.

Because the majority of human BCCs possess mutations in either *SMO* or *PTCH1*, and loss of a single copy of *PTCH1* underlies basal cell nevus (Gorlin's) syndrome, it is noteworthy that cells from three of eight human BCCs examined appeared poorly ciliated. One explanation for this may be that Hh signaling in a small population of ciliated cells is sufficient for tumor growth. Alternatively, unciliated cells may be supported by signaling pathways functioning independently of cilia and, as our GLI2ΔN studies might suggest, may even show growth advantages upon ciliary loss. Indeed, several reports have described signaling pathways that potentiate Gli transcriptional activity, including KRAS-mediated MAP kinase signaling³⁴, phosphoinositide 3-kinase–Akt signaling³⁵ and transforming growth factor-β signaling^{34,36}. Although it remains unclear whether these pathways are frequently perturbed in BCC, the GLI2ΔN-induced skin tumors provide illustrative support for a tumor-suppressive capacity for cilia during Smo- and ciliary-independent tumorigenesis. As recent studies have reported a lack of correlation between Smo activity and downstream Hh pathway activity in a variety of human cancer cells, it is possible that Smo-independent modulation of the Hh pathway occurs in many different tumor types³⁷.

Our findings advance several general predictions. First, human tumors that rely on upstream activation of Hh signaling, such as many BCCs and medulloblastomas, are predicted to retain cilia. Indeed, we have shown here that both human and mouse skin tumors are ciliated, and others have found that SmoM2-induced medulloblastomas are similarly ciliated and dependent upon ciliary function (Y.-G. Han, H. Kim, A.A.D., D. Ellison, R. Gilbertson and A. Alvarez-Buylla, personal communication). Second, inhibition of ciliogenesis in Smo-dependent tumors is likely to prevent growth. This has major implications for therapy, as the presence of cilia may be a useful biomarker for identifying Hh-dependent cancers responsive to Smo antagonists currently being tested in clinical trials². Third, given that recent studies have suggested a paracrine role for Hh signaling during tumorigenesis^{34,37}, disruption of cilia in the surrounding tumor mesenchyme may also indirectly affect tumor growth. A final prediction from this study is that loss of cilia may favor progression of

tumors initiated by oncogenic events that activate the Hh pathway independently of Smo. Thus, the dual functions of cilia as both a mediator of Smo-dependent tumorigenesis and as a suppressor of Smo-independent tumorigenesis suggest that inhibiting ciliogenesis may be therapeutic in a subset of cancers but may also exacerbate cancer growth in others.

Methods

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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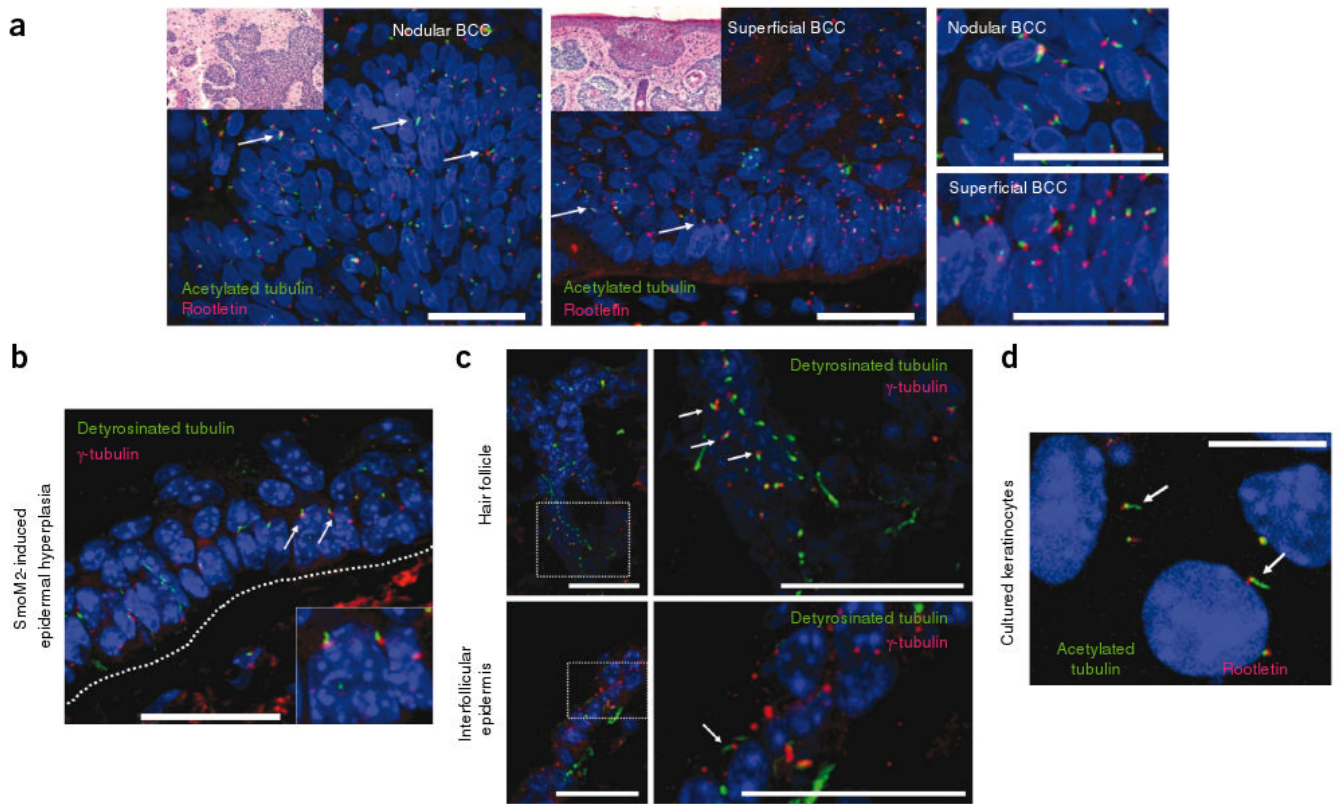
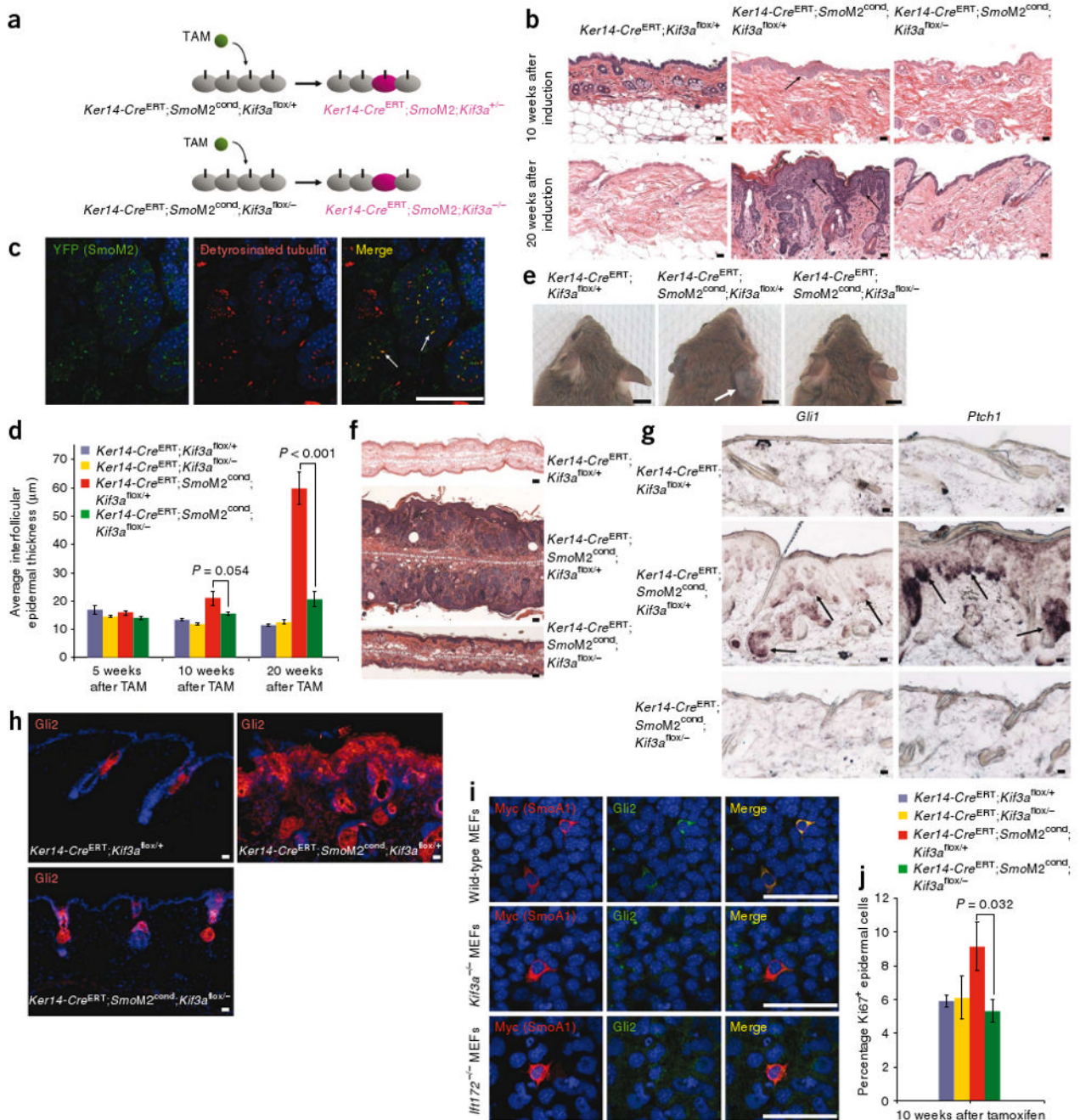


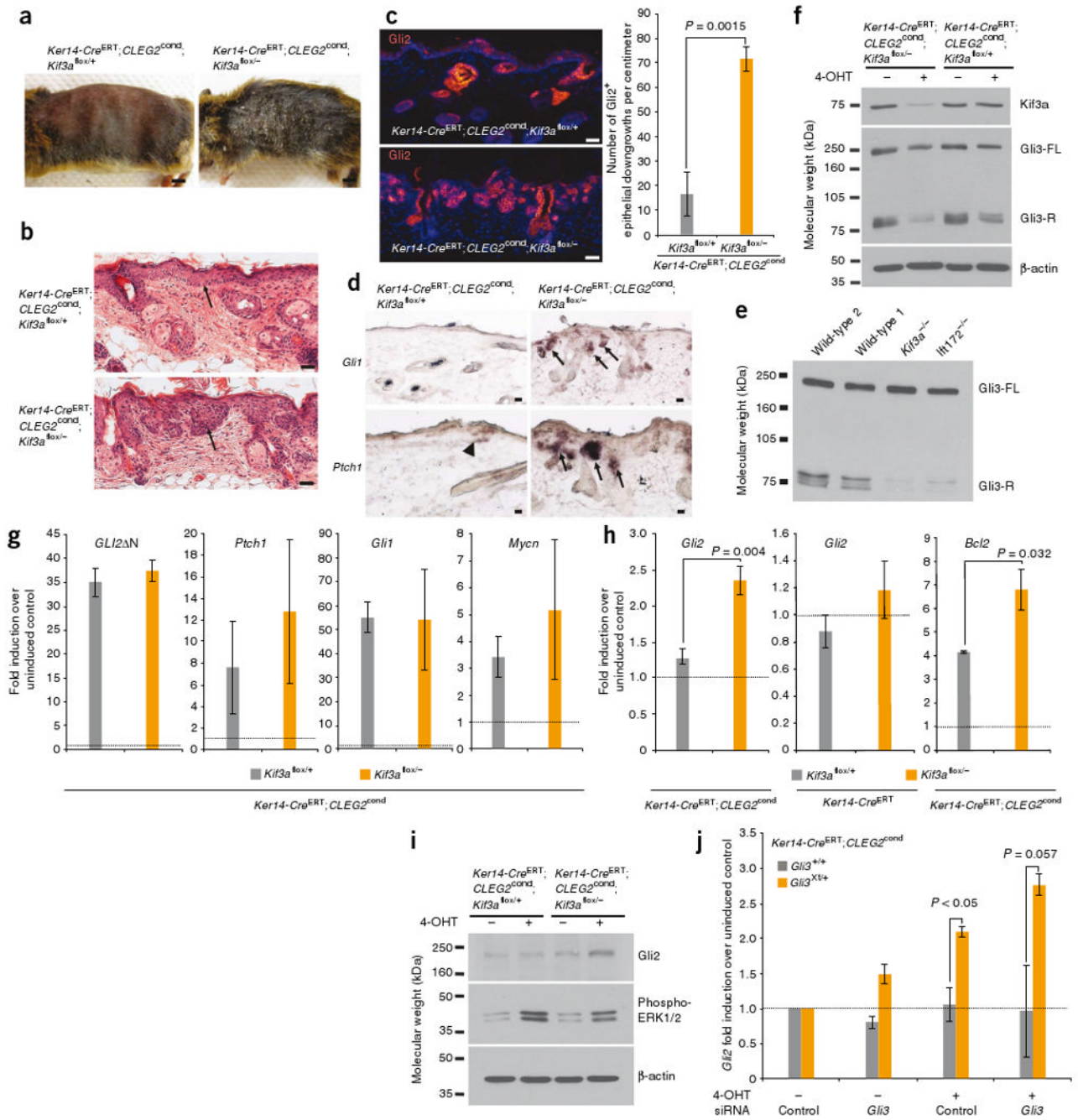
Figure 1.

BCC and normal skin cells possess primary cilia. **(a)** Immunofluorescence imaging of the ciliary marker acetylated tubulin (green) and rootletin (red), a ciliary rootlet component. Five of eight independent human clinical BCCs, including nodular and superficial subtypes, contain numerous ciliated cells (arrows). Insets, H&E staining of adjacent biopsies. Right images are views of the left and middle panels. **(b)** Immunofluorescence imaging of the ciliary marker detyrosinated tubulin (green) and the centrosomal marker γ -tubulin (red) (dotted line, epidermal basal layer; inset, enlarged view). Arrows indicate ciliated cells present in SmoM2-induced regions of mouse epidermal hyperplasia. **(c)** Cilia extending from normal skin cells (arrows). Left images, low magnification; right images, high magnification of boxed areas. **(d)** Cilia extending from cultured keratinocytes (arrows). All scale bars are 50 μ m except that in **d** (10 μ m).

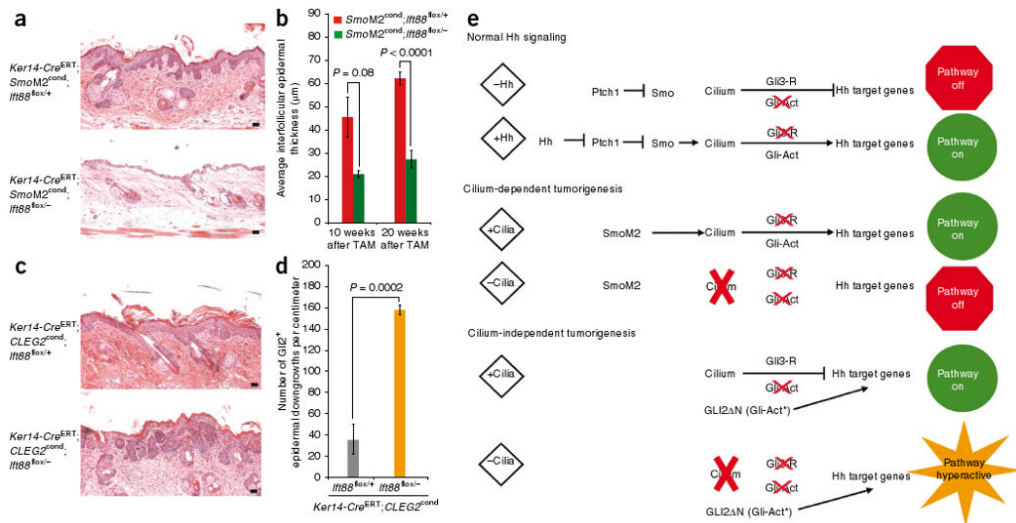
**Figure 2.**

Cilia are essential for SmoM2-induced neoplasia. **(a)** Schematic showing that skin epithelial cells exposed to tamoxifen (TAM) both delete *Kif3a*^{lox} and activate *SmoM2*^{cond} (red cells), whereas unexposed cells retain *Kif3a*^{lox} and do not activate *SmoM2*^{cond} (gray cells). Cilia are lost in cells from *Kif3a*^{lox/-} but not *Kif3a*^{lox/+} mice exposed to tamoxifen. **(b)** H&E staining of dorsal skin biopsies, showing that removal of *Kif3a* (*Kif3a*^{lox/-}) blocks SmoM2-initiated tumorigenesis (arrows). **(c)** Immunofluorescence imaging showing that SmoM2 localizes to cilia (arrows) in neoplastic epidermal downgrowths. **(d)** Quantification of interfollicular epidermis thickness. Seven or eight independent *Ker14-Cre*^{ERT};*SmoM2*^{cond};*Kif3a*^{lox/+} and *Ker14-Cre*^{ERT};*SmoM2*^{cond};*Kif3a*^{lox/-} mice per genotype

were analyzed. Four or five mice were analyzed for all other genotypes. **(e)** Protection against SmoM2-induced ear skin hyperplasia upon loss of Kif3a (arrow). **(f)** H&E staining of representative ear sections. **(g)** *In situ* staining showing that SmoM2-induced skin lesions upregulate Hh target genes *Gli1* and *Ptch1* (arrows). **(h)** Immunofluorescence imaging showing that Gli2 is upregulated in SmoM2-induced tumors that express Kif3a (stainings are representative of the mice analyzed in **d** and **j**). **(i)** Immunofluorescence imaging showing that Gli2 is upregulated upon expression of Myc-tagged SmoA1 in ciliated (wild type), but not unciliated (*Kif3a*^{-/-} and *Ift172*^{-/-}), transformed MEFs. **(j)** Cellular proliferation in skin, as assessed by Ki67 staining. Eight *Ker14-Cre*^{ERT};*SmoM2*^{cond};*Kif3a*^{lox/+} and *Ker14-Cre*^{ERT};*SmoM2*^{cond};*Kif3a*^{lox/-} mice per genotype were analyzed, as well as four or five mice for the other genotypes. All scale bars are 50 μm except that in **e** (5 mm). All error bars shown, ± s.e.m.



Kif3a protein expression is lower and Gli3-R formation is impaired in 4-OHT-treated *Ker14-Cre^{ERT};CLEG2^{cond};Kif3a^{flox/-}* keratinocytes, relative to control cells. **(g)** Expression of constitutively active human *GLI2* (*GLI2ΔN*), *Ptch1*, *Gli1* and *Mycn* in *Kif3a^{flox/+}* and *Kif3a^{flox/-}* keratinocytes treated with 4-OHT. (All expression values expressed relative to those of vehicle-treated control cells, set to a baseline of '1', dotted line.) **(h)** Quantitative RT-PCR assessment of *GLI2ΔN*-mediated induction of endogenous *Gli2* and *Bcl2* in ciliated and unciliated *Kif3a^{flox/-}* keratinocytes. **(i)** Endogenous *Gli2* and phosphorylated ERK1/2 protein levels in 4-OHT-treated *Ker14-Cre^{ERT};CLEG2^{cond}* keratinocytes that are either *Kif3a^{flox/+}* or *Kif3a^{flox/-}*. **(j)** Expression of endogenous *Gli2* in *GLI2ΔN*-induced *Gli3^{Xt/+}* or *Gli3^{+/+}* keratinocytes, treated with siRNA against *Gli3* or control. All expression values are normalized to those of vehicle-treated cells transfected with control siRNAs (baseline set to '1', dotted line). All scale bars are 50 μm except that in **a** (5 mm). All error bars show means ± s.e.m.

**Figure 4.**

Loss of *Ift88* restrains SmoM2-mediated tumorigenesis and promotes GLI2ΔN-induced BCC-like lesions in tamoxifen-treated mice. (a) H&E staining of dorsal skin biopsies, revealing that loss of *Ift88* (*Ift88^{lox/-}*) blocks SmoM2-induced epidermal hyperplasia and tumorigenesis. (b) Quantification of epidermal thickness for **a**. Three to five mice per genotype were analyzed 10 weeks after induction by tamoxifen, and five mice per genotype were assessed 20 weeks after tamoxifen treatment. (c) H&E staining revealing that loss of *Ift88* promotes skin lesions induced by activated Gli2. All scale bars, 50 μm. (d) Quantification of the number of Gli2⁺ downgrowths in the skin. Four mice were analyzed per genotype. (e) Schematic showing that in nonpathological conditions and in the absence of Hh, the cilium mediates the formation of Gli3-R, a repressor of downstream Hh target genes. In the presence of Hh ligand, Ptch1-mediated inhibition of Smo is suppressed, allowing Smo to act through the cilium to inhibit Gli3-R formation and induce Gli activators (Gli-Act), which turn on the Hh transcriptional program. In cilium-dependent oncogenesis (for example, SmoM2-induced tumors), the cilium is essential for Gli-Act formation and, thus, tumorigenesis. In cilium-independent oncogenesis (for example, tumors induced by constitutively active GLI2 (Gli-Act*)), the cilium restrains tumorigenesis through production of counter-balancing Gli3-R. Loss of the cilium inhibits Gli3-R formation, allowing unopposed Gli-Act* to aggressively drive tumorigenesis. All error bars show means ± s.e.m.