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Review Series

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Basal cell carcinoma — molecular biology and potential new therapies

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Basal cell carcinoma (BCC) of the skin, the most common malignancy in individuals of mixed European descent, is increasing in incidence due to an aging population and sun exposure habits. The realization that aberrant activation of Hedgehog signaling is a pathognomonic feature of BCC development has opened the way for exciting progress toward understanding BCC biology and translation of this knowledge to the clinic. Genetic mouse models closely mimicking human BCCs have provided answers about the tumor cell of origin, and inhibition of Hedgehog signaling is emerging as a potentially useful targeted therapy for patients with advanced or multiple BCCs that have hitherto lacked effective treatment.

Introduction

Description of basal cell carcinoma. In 1827 Arthur Jacob termed the skin tumor that we now call basal cell carcinoma (BCC) "Ulcus rodens" (1). In 1900, Krompecher described BCC as a malignant, locally invasive, and destructive cancer and named it "Carcinoma epitheliale adenoides"; he then went on to pioneer the classification of skin tumors using histogenetic principles, three years later coining the term "Basalzellenkrebs" (2, 3), a term indicating that the tumor originated in the basal layer of the epidermis or hair follicle (HF). In contrast, in 1910, Mallory used the term "hair matrix tumor" to specify the follicular origin of BCC (4), illustrating the long-standing controversy and uncertainty about the cellular origin of BCC. The locally aggressive, but overall rather benign, course of the disease, with metastasis being largely absent, was similarly noted early on and spurred the debate as to whether BCC could be considered a truly malignant cancer or a "semi-malignant" tumor. The WHO classification has retained the name "BCC" since 1974 (5). BCC is the most common human cancer and accounts for about two-thirds of all skin cancers in patients of mixed European descent. In the US this corresponds to approximately one million cases per year (6, 7).

Clinical appearance of human BCC and related tumors. The incidence of BCC is strongly associated with exposure to UV radiation; tumors develop primarily on the sun-exposed skin of elderly individuals with fair skin phototypes, are rarely found on palmoplantar surfaces or in children, and never appear on the mucosa. Additional established risk factors include ionizing radiation (IR), arsenic, and immune suppression (8, 9). Clinically, BCCs appear as pearly and telangiectatic papules or nodules with or without ulceration, or as indurated, erythematous, or ulcerated patches with a discrete papular border, and may be pigmented.

Morphologically, BCC encompasses a group of epithelial intradermal tumors characterized by a primary cellular component that resembles the undifferentiated basal cells of the epidermis and its appendages. These basaloid cells are often arranged in palisades at the tumor periphery, are separated from the surrounding stroma by optically empty spaces, and form nodules, bands, or strings, with some continuity with the overlying epithelium in most cases. Visible desmosomal intercellular structures are absent, and the tumor cells have little cytoplasm and show chromatin-rich nuclei with frequent mitoses when compared with normal skin; however, they are often apoptotic, consistent with slow tumor growth (10).

BCCs display different morphological growth patterns: superficial, nodular, micronodular, infiltrating, sclerosing, and fibroepithelial (Figure 1). Nodular BCCs in particular may resemble adnexal tumors or, in some regions, squamous cell carcinoma, as these BCCs demonstrate a variety of types of differentiation including basosquamous or metatypical, cystic, adenoid, pigmented, and infundibulocystic differentiation.

The diversity in the phenotypic appearance of BCCs indicates that the cell of origin may be a stem or progenitor cell. Moreover, these observations raise the question as to whether BCC is a monoclonal tumor or whether it is the result of field cancerization. Studies investigating clonal patterns of X chromosome inactivation suggest that the majority of BCCs do represent monoclonal tumors and that anatomically distinct BCCs may sometimes share the same cellular origin (11, 12).

Genetics of BCC development. Major advances in our understanding of the molecular changes leading to BCC formation have come from studies of patients with a hereditary predisposition to BCC development. As early as 1894, Jarisch and White described patients with features typical of the autosomally inherited syndrome (13, 14) now known as basal cell nevus syndrome (BCNS, also known as Gorlin syndrome), which was later described in detail by Robert Gorlin and others (15, 16). The birth incidence of BCNS in the United Kingdom is 1 in 19,000 (17); BCNS patients typically develop numerous BCCs starting at a young age and are prone to developing other tumors including medulloblastomas. Soon after the cloning of the Hedgehog (Hh) receptor Patched 1 (*PTCH1*) as the BCNS disease gene (18, 19), it became clear that a majority, if not all, of sporadic BCCs show abnormal activation of the Hh pathway (9, 20), ascribing constitutive activation of the Hh signaling pathway (Figure 2 and reviewed in ref. 21) as a prerequisite for the development of a BCC.

Other genetic syndromes such as Bazex-Dupré-Christol syndrome (22), Rombo syndrome (23), cartilage-hair hypoplasia (CHH) (24), and xeroderma pigmentosum (XP) (25) are associated with a high risk for BCC (reviewed in ref. 26), illustrating the

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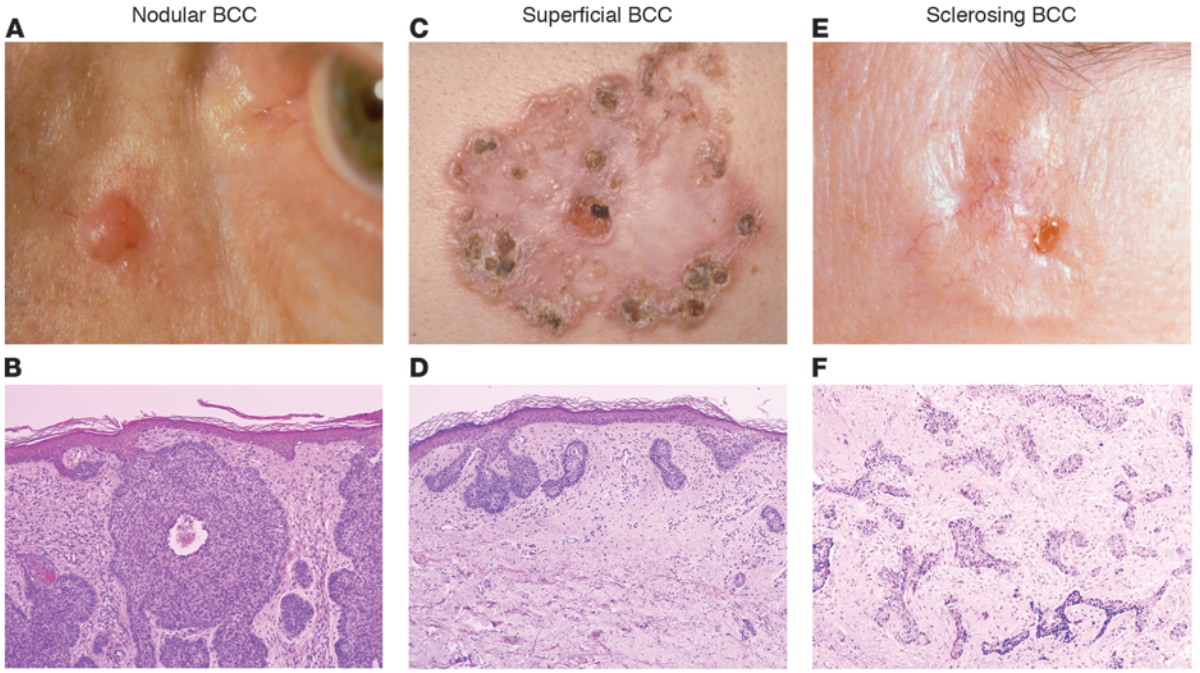

Figure 1
Major subtypes of human BCC. (**A**–**E**) Macroscopic (**A**, **C**, and **E**) and microscopic (**B**, **D**, and **F**) appearance of nodular (**A** and **B**), superficial (**C** and **D**), and sclerosing (**E** and **F**) human BCCs. Original magnification, ×100 (**B**, **D**, and **F**).

involvement of additional genetic factors and pathways such as DNA repair (XP) and telomere maintenance (CHH).

Crosstalk between Hh and other molecular signaling pathways in BCC. The Wnt pathway has a well-established role in normal HF development and cycling, and both human and mouse BCCs have increased levels of β-catenin, a critical mediator of Wnt signaling (27, 28). In accordance with these observations, overexpression of the potent Wnt antagonist, Dkk1, in mouse epidermis resulted in the inhibition of benign Hh-driven hamartomas, showing that active Wnt signaling is required for their growth (29).

In line with its importance in epidermal development, the EGFR/MEK/ERK pathway has been shown to modulate GLI-dependent transcription in human keratinocytes (30) and to synergistically induce oncogenic transformation of human keratinocytes (31). Additionally, the tumor suppressor p53 may influence BCC development. The complete loss of *p53* was shown to result in upregulated expression of the Hh pathway mediator smoothened (Smo) in the interfollicular epidermis (IFE) in mice, thereby making these keratinocytes receptive to BCC induction (32).

Finally, the correct cellular context is important for the persistent growth of BCCs, and epithelial-stromal interactions play a role in creating a favorable microenvironment. Stromal cells, isolated from human BCCs, express high levels of gremlin 1, which antagonizes the pro-differentiation factors BMP2 and BMP4, synthesized in the BCC tumor cells, thereby sustaining tumor growth (33).

Modeling the disease

Genetic mouse models represent a major advance in cancer research as they provide the possibility of studying tumors in the context of the entire organism. The study of BCC tumorigenesis in mice has been hindered by the inexplicable failure of mutagenic chemicals and UV or IR to induce BCCs (34). However, genetically engineered mouse models for BCC provide the option to investigate the molecular mechanisms of BCC formation and progression and the identification of the cells of origin. Due to the obligate dependency of BCCs on activated Hh signaling, all current BCC mouse models target different components of the Hh signal transduction pathway, and these are summarized in Tables 1 and 2. In this context it is important to note that tumors developing in the mouse may not always fully mimic human BCCs but represent various stages in a spectrum of benign to malignant Hh pathway–induced BCC-like tumors, likely reflecting a similar variation in humans, where benign hamartomas and BCCs appear to be driven by different levels of Hh pathway activation (35–38).

Cell of origin and morphological diversity of BCC. Stem and progenitor cells are thought to be the most probable sources of tumor initiation due to their longevity and ability to self-renew (39, 40). In the skin, several populations of cells with stem cell (SC) properties have been discovered; however, defined SC markers are, as yet, limited to the HF (Figure 3; reviewed in refs. 41, 42). A collection of recent publications describes the use of mouse genetics to identify BCC cells of origin using Cre-mediated cell-specific targeting, either by lineage tracing, which involves the genetic labeling of cells, or by the activation of oncogenic Hh signaling in distinct skin cell populations (Figure 3). The identification of tumor cells of origin and, equally importantly, cells that cannot initiate tumorigenesis, will make it possible to pinpoint molecular mechanisms that either predispose or protect a cell from oncogenic transformation.

In the first publication to address the cellular origin of BCC, Youssef et al. used mice conditionally expressing SmoM2 (43), a constitutively active variant of Smo (44). When SmoM2 expression was activated in different cell compartments in the epidermis,

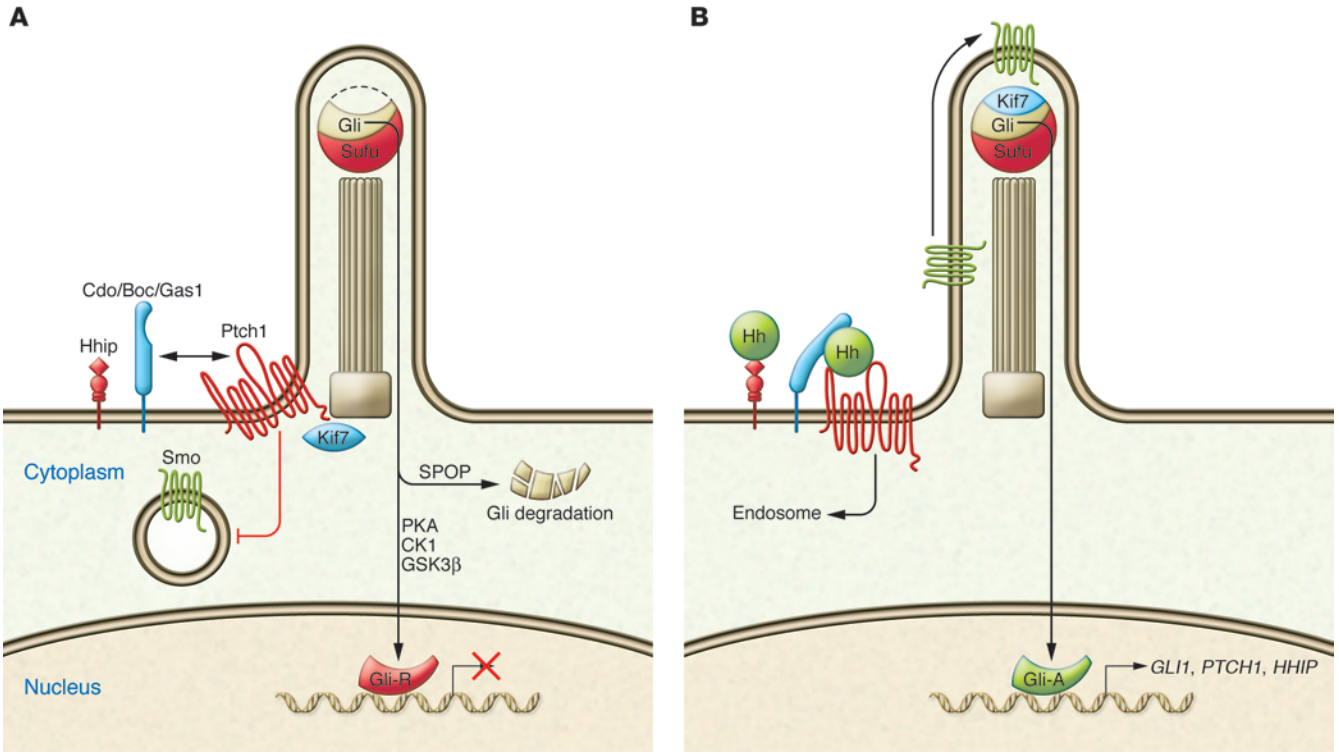

Figure 2
The Hh signaling pathway — a simplified model. (**A**) In its "off" state, Ptch1 represses Smo activity. Gli2 and Gli3, effectors of the Hh pathway, are phosphorylated by a kinase cascade, which includes PKA, CK1, and GSK3β, and are directed to the proteasomal degradation pathway via the SPOP complex. A fraction of the Gli2/3 protein is processed into a repressor form, Gli-R, which inhibits Hh target gene transcription. (**B**) Hh ligand binding to Ptch1 abrogates its inhibitory effect on Smo, allowing Smo to translocate into the primary cilium and induce accumulation of the Gli-Sufu complex at the tip of the primary cilium. Activation of the Hh pathway results in accumulation of Gli-A and initiation of the transcription of Hh target genes such as *PTCH1*, *GLI1*, and *HHIP*.

including HF SCs (Table 2), only cells originating in the IFE and the upper infundibulum produced full-blown BCCs. Importantly, the IFE-derived tumors also exhibited an HF-like protein expression pattern, demonstrating that the biochemical and morphological characterization of tumor cells may be misleading when used to identify the cellular origin of cancers (29, 43).

These same mouse models (employing conditional expression of SmoM2 in K14- or K15-positive cells; Table 2) were used to study the effect of wound healing on BCC development (45). It was previously reported that active wound repair recruits HF cells for re-epithelialization (46–48). In K15-SmoM2 skin, which targets SmoM2 expression mostly to the HFs, the HFs exhibited only occasional basaloid lesions in the bulge and the hair germ (HG) (43, 49). Intriguingly, during wound healing, the HF SmoM2-expressing cells are mobilized to the IFE, where they drive BCC formation and form tumors (45). Why does SmoM2 induce tumors in the IFE upon wounding but not in the intact bulge or HG? One possible explanation is that the bulge microenvironment suppresses SmoM2-mediated oncogenesis, as *Gli1* and *Gli2* mRNAs, which encode Hh pathway effector proteins downstream of Smo, are upregulated in IFE-associated tumors but not in HFs (45).

Our group has also addressed the question of the BCC cell of origin and the effect of wounding on tumor growth (ref. 50 and Table 2). Overexpression of human GLI1 under the control of the K5 promoter resulted in BCC formation, preferentially in the IFE, but also in the HF. Lineage-tracing of Lgr5$^+$ SCs, which give rise to the bulge and HG, showed that in this BCC model, HF- and IFE-associated lesions had distinct cells of origin, as no Lgr5$^+$-traced lesions were found in the IFE. However, Lgr5-labeled HF cells were able to give rise to BCCs in the IFE upon wounding, in line with the study by Wong et al. (45). In the *Lgr5Cre-Ptch1$^{fl/fl}$* mouse model, *Ptch1* deletion in Lgr5-expressing HF SCs resulted in the formation of locally restricted basaloid proliferations in the lower part of the HF. In wounded skin, Ptch1-deficient Lgr5$^+$ cells were again recruited to the wound sites in the IFE, where they induced de novo basaloid lesions (50). Thus, the concept that wounding is an important factor in tumor development, postulated 150 years ago (52), also appears applicable to BCC development.

Despite similar molecular pathogenesis, a considerable morphological heterogeneity exists among BCCs (53). How do these morphological differences occur? By comparing two different BCC models, Grachtchouk et al. found that *BK5-GLI2* mice (54) with strong Hh signaling developed full-featured BCCs, while the weaker Hh signal in *ΔK5-SMO-M2* mice resulted in follicular hamartomas (35). Furthermore, a recent, more detailed study from the same group revealed that activated SmoA1 expression in the HF does not give rise to BCC-like lesions in the HF and that low levels of GLI2ΔN expression throughout the basal compartment do not lead to nodular BCCs in the HF (in contrast to the model with high GLI2ΔN expression), but to slow-growing basaloid follicular ham-

Table 1
General mouse models used to study BCC formation

Cell targeting alleles	Effector alleles	Start of Hh pathway modulation	Additional treatment	Pathology	Reference
K14	Shh Tg	E0[A]	No	BCC-like lesions	89
hK5	hSMO-M2 Tg	E0[A]	No	BCC-like lesions	90
All	*Ptch1*$^{+/-}$	E0	No	Trichoblastomas	91
All	*Ptch1*$^{+/-}$	E0	UV, γ-IR, X-ray	BCC, trichoblastomas	91
BK5	hGLI1 Tg	E0[A]	No	BCC, trichoepitheliomas, cylindromas, trichoblastomas	36
BK5	mGli2 Tg	E0[A]	No	BCC	54
BK5	mGli2ΔN2 Tg	E0[A]	No	BCC, trichoblastomas, cylindromas, basaloid follicular hamartomas	92
ΔBK5	hSMO-M2 Tg	E0[A]	No	Basaloid follicular hamartomas	35
All	*Ptch*$^{+/-}$	E0	No	Basaloid hyperproliferations	93
All	*Ptch*$^{+/-}$	E0	X-ray	BCC (nodular and infiltrative)	93
K5tTA (bovine)	TREmGli2 Tg	E0[A]	DOX off	BCC	75
All	*Sufu*$^{+/-}$	E0	No	BCC, basaloid follicular hamartomas	94
K6a-Cre	*Ptch1*$^{fl/fl}$	E0[A]	No	ORS hyperplasia	27
K6a-Cre	*Ptch1*$^{fl/fl}$	E0	Retinoic acid[B]	BCC-like lesions	27
K14-Cre	*Ptch1*$^{fl/fl}$	E0[A]		BCC-like lesions	95
Mx1-Cre	*Ptch1*$^{fl/fl}$	Various	poly(I:C)[C]	BCC-like lesions	95
CAGGS-CreER	fl-STOP-fl-SmoM2-YFP[D]	P10	No	BCC-like lesions	96
K14-CreERT	fl-STOP-fl-SmoM2-YFP[D]	P30–P35	No	BCC-like tumors	97
K14-CreERT	fl-STOP-fl-SmoM2-YFP[D,E]	P30–P35	No	Inhibition of BCC-like tumors	97
K14-CreERT	CLEG2-cond[F]	P30–P35	No	BCC-like tumors	97
K14-CreERT	CLEG2-cond[E,F]	P30–P35	No	Enhanced BCC-like tumors	97

[A]Effective promoter activation is dependent on temporal regulation during embryogenesis (e.g., K14 starts at E9.5). [B]To activate K6 expression in the IFE and ORS at P32. [C]To activate Mx1 promoter via interferon response. [D]Fusion protein of mSmoM2 with YFP inserted into the *Rosa26* locus. [E]Additional allele is *Kif3a*$^{fl/-}$. [F]CLEG2 denotes Myc-tagged, constitutively active human GLI2.

artomas resembling the tumors found in Δ*K5-SMO-M2* mice (ref. 37 and Table 2). These observations support the suggestion that, downstream of Ptch1, the level of Hh pathway activation, rather than the exact molecular target, is crucial in determining the BCC subtype. It is worth noting that high levels of GLI2ΔN expression in the bulge and HG rapidly lead to nodular tumors, most likely initiated in the lower bulge and the HG (37), and the HG may also be the source for tumor-initiating cells in *Lgr5Cre-Ptch1*$^{fl/fl}$ mice (50). The lower bulge and HG harbor cells with active Hh signaling in telogen (55, 56), which may represent a cell population that is preferentially susceptible to Hh pathway–driven tumorigenesis.

The influence of the hair cycle phase on BCC growth is also important, and the authors of three studies have presented direct evidence that BCC development occurs preferentially, but not exclusively, during anagen phase (37, 57, 58). One reason may be that cells located in the outer root sheath (ORS) of anagen HFs can give rise directly to nodular BCCs, supporting the idea that this compartment contains cells capable of transformation by oncogenic Hh signaling and, therefore, provides an expanded pool of potential tumor progenitors.

Together, the results obtained using mouse models to study BCC development have so far revealed that oncogenic Hh signaling can drive BCC-like tumor formation in several different epithelial progenitor populations in skin, although the morphology and the final outcome of BCC development are influenced by the cell of origin, the mutated Hh pathway member, and the strength of oncogenic Hh signaling.

New therapies

Highly efficient treatment modalities such as surgery that aims at complete extirpation, radiotherapy, curettage, cryotherapy, photodynamic therapy, and topical applications of imiquimod or 5-fluorouracil are available and effective for the great majority of BCC patients (9, 59). However, the occurrence of locally aggressive and invasive tumors, a bleak prognosis upon metastatic spread, a significant rate of recurrence often associated with increased aggressiveness, as well as the multitude of tumors appearing in high-risk populations such as BCNS patients, provide compelling reasons to search for new preventive and therapeutic avenues (60).

Hh signaling as a target for new BCC therapies. The first evidence that the Hh signaling pathway is sensitive to inhibition by small molecules stemmed from the observation of cyclopia in lambs, induced by the maternal ingestion of corn lilies (*Veratrum californicum*) (61), followed by the demonstration that the active compound, cyclopamine, inhibits Hh signaling (62) and binds to SMO (63). Initial studies showed that, in addition to Hh inhibition in various in vitro systems, the oral administration of cyclopamine reduced the growth and development of BCCs in *Ptch1*$^{+/-}$ mice exposed to UV irradiation (64), and its topical application to human BCCs can induce regression (65).

New derivatives of cyclopamine with improved pharmaceutical properties are now in clinical trials (Table 3 and ref. 66). Excellent results were obtained with the orally administered SMO inhibitor GDC-0449 (vismodegib; Table 3) in a phase I trial of patients with locally advanced or metastatic BCC (67, 68). Phase II results

Table 2
Mouse models used to identify the putative BCC cell of origin

Cell targeting alleles	Effector alleles	Additional alleles	Start of Hh pathway modulation	Additional treatment	Pathology	BCC localization	Ref.
K14-CreER	fl-STOP-fl-SmoM2-YFP[A]	–	P23–P28	No	BCC (nodular)	**IFE** and infundibulum	43
Shh-CreER	fl-STOP-fl-SmoM2-YFP[A]	–	P25–P30	No	No morphological changes	No	43
K15-CreER	fl-STOP-fl-SmoM2-YFP[A]	–	P21–P23	No	Rare hyper/dysplastic changes	HF	43
K15-CreER	fl-STOP-fl-SmoM2-YFP[A]	–	P21–P23	No	Rare hyper/dysplastic changes	HF	43
K19-CreER	fl-STOP-fl-SmoM2-YFP[A]	–	P28–P32	No	Rare hyper/dysplastic changes	HF	43
K15-CrePR1[B]	*Ptch1*$^{+/-}$	R26-YFP	7.5 weeks	X-ray at 8 weeks	BCC	HF	32
K15-CrePR1[B]	*Ptch1*$^{+/-}$	*P53*$^{fl/fl}$	7.5 weeks	X-ray at 8 weeks	BCC	HF (enhanced)	32
K14-CreER2[B]	*Ptch1*$^{+/-}$	R26-YFP	7.5 weeks	X-ray at 8 weeks	BCC	(IFE, 90% HF-connected) and **HF**	32
K14-CreER2[B]	*Ptch1*$^{+/-}$	*P53*$^{fl/fl}$	7.5 weeks	X-ray at 8 weeks	BCC	IFE and HF	32
K5tTA (bovine)	TREGLI1 Tg	Lgr5-EGFP-IRES-CreERT2/ R26-LacZ[C]	P16[D]	DOX off	BCC-like lesions	**IFE** and HF	50
K5tTA (bovine)	TREGLI1 Tg	Lgr5-EGFP-IRES-CreERT2/ R26-LacZ[C]	P16[D]	DOX off and full-thickness wound	Enhanced size of BCC-like lesions	**IFE** and HF	50
BK5-Cre*PR	*Ptch*$^{fl/fl}$	–	P18–P24	No	BCC-like lesions	IFE and **HF**	50
BK5-Cre*PR	*Ptch*$^{fl/fl}$	–	P18–P24	Full-thickness wound	Enhanced size and number of BCC-like lesions	IFE and HF	50
Lgr5-EGFP-CreERT2	*Ptch1*$^{fl/fl}$	R26-LacZ	P14–P20	No	Locally restricted BCC-like lesions	HF	50
Lgr5-EGFP-CreERT2	*Ptch1*$^{fl/fl}$	R26-LacZ	P14–P20	Full-thickness wound	BCC-like lesions	IFE and HF	50
K15-CrePR	fl-STOP-fl-SmoM2-YFP[A]		7.5 weeks	No	Rare small basaloid proliferations	HF	45
K15-CrePR	fl-STOP-fl-SmoM2-YFP[A]	R26-LacZ	7.5 weeks	Full-thickness wound, 3 days[E]	BCC (IFE), rare small basaloid proliferations (HF)	**IFE** and HF	45
K15-CrePR	fl-STOP-fl-SmoM2-YFP[A]	R26-LacZ	7.5 weeks	Full-thickness wound, 5 weeks[E]	BCC (IFE), rare small basaloid proliferations (HF)	**IFE** and HF	45
K15-CrePR	fl-STOP-fl-SmoM2-YFP[A]		7.5 weeks	Anagen induction via HF depilation, 3 days[E]	Not tumor promoting	HF	45
K14-CreER	fl-STOP-fl-SmoM2-YFP[A]	R26-LacZ	7.5 weeks	No	BCC	IFE	45
K14-CreER	fl-STOP-fl-SmoM2-YFP[A]	R26-LacZ	7.5 weeks	Full-thickness wound, 3 days[E]	Not tumor initiation promoting	IFE	45
K15-CrePR1	tetO-GLI2ΔN Tg	R26-LSL-rtTA	7 weeks	High DOX dose	BCC (nodular)	HF	37
K15-CrePR1	tetO-GLI2ΔN Tg	R26-LSL-rtTA	8 weeks	High DOX dose and anagen	Acceleration of BCC	HF and ORS	37
Lgr5-EGFP-CreERT2	tetO-GLI2ΔN Tg	R26-LSL-rtTA	7 weeks	High DOX dose	BCC (nodular)	HF	37
Lgr5-EGFP-CreERT2	tetO-GLI2ΔN Tg	R26-LSL-rtTA	8 weeks	High DOX dose and anagen	Acceleration of BCC	HF and ORS	37
K14rtTA	tetO-GLI2ΔN Tg	–	7 weeks	High DOX dose	BCC (nodular, HF; superficial, IFE)	IFE, HF, SG	37
K5-CreER	tetO-GLI2ΔN Tg	R26-LSL-rtTA	7 weeks	High DOX dose	BCC (nodular, HF; superficial, IFE)	IFE, HF, SG	37
K5-Cre	tetO-GLI2ΔN Tg	R26-LSL-rtTA	7 weeks	Low DOX dose	Basaloid follicular hamartomas	**IFE** and possibly HF involvement	37
K15-CrePR1	SmoA1 (mouse)	R26-LSL-rtTA	7 weeks	High DOX dose	Modest hyperplasia	HF	37

SG, sebaceous gland. Preferential localization, when present, is indicated by bold font. [A]Fusion protein of mSmoM2 with YFP inserted into the *Rosa26* locus. [B]Only used for lineage tracing and p53 alleles; *Ptch1* heterozygous in all cells and all tissues. [C]Cre activated at P14. [D]Effective GLI1 protein expression: P28. [E]Time after SmoM2 induction.

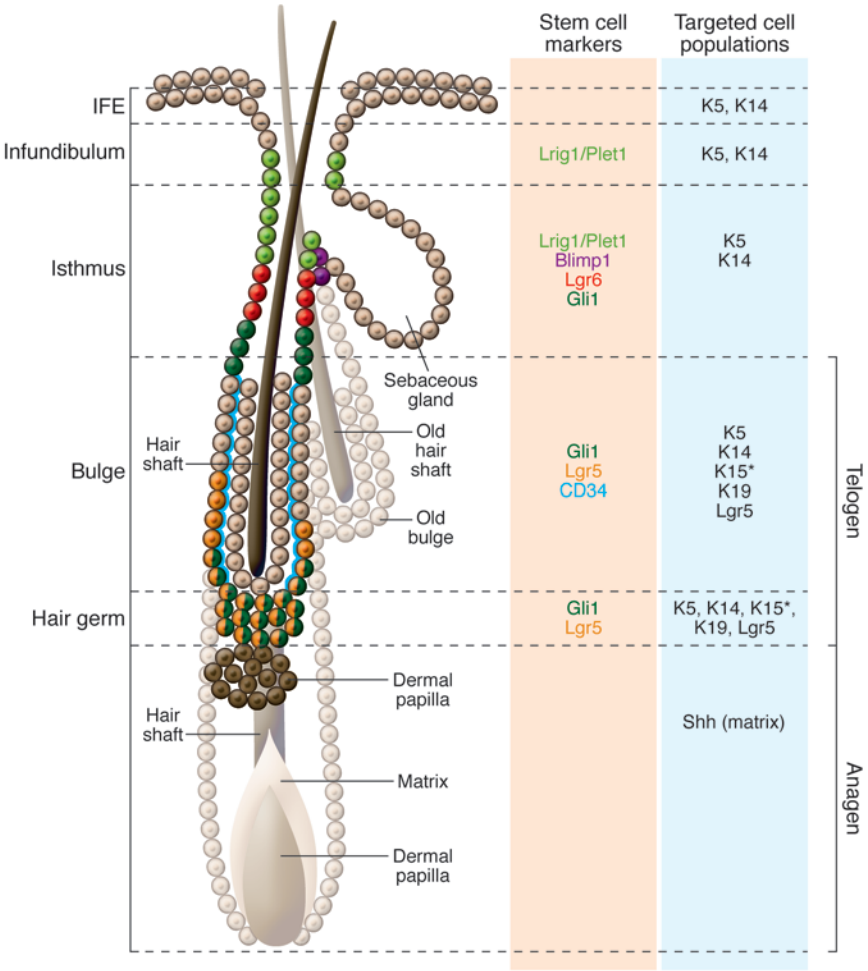

Figure 3
The HF and its morphological units, stem cell compartments, and targeted cell populations. The targeted cell populations are defined by endogenous or transgene promoter activities. K15* denotes a truncated version of the K15 promoter, restricted in its activity to the bulge area.

using GDC-0449 in patients with locally advanced or metastatic BCC (69) and in BCNS patients with multiple BCCs (70) were so promising that the Data Safety Monitoring Board recommended ending the placebo arm of the BCNS study in light of the differences between the study arms. Importantly, no resistance has so far been reported. In addition, a phase I study of IPI-926 (Table 3) given orally to patients with advanced types of BCC also resulted in a positive response rate (71).

In the studies investigating systemic treatment with SMO inhibitors, a common set of adverse effects has been observed, including muscle spasms, loss of taste (dysgeusia), hair loss, fatigue, nausea, and hyponatremia. It is likely that hair loss and altered taste, at least, are related directly to SMO inhibition, since Hh signaling is known to be active in HFs and taste buds (72, 73). One way to avoid or reduce such effects might be to use these inhibitors topically, thus limiting systemic exposure. A small, short-term study in BCNS patients with nodular and superficial BCC, employing twice-daily topical treatments of the SMO inhibitor LDE225 for four weeks, resulted in a positive response, and BCC regression correlated with a decrease in Hh target gene expression in most treated tumors (74). No treatment-related side effects were noted, consistent with low levels of systemic exposure to the inhibitor.

A potential caveat associated with the use of Hh pathway antagonists for the treatment of BCC is the possibility that, while treatment may result in a dramatic reduction in tumor mass, a small number of residual cells that are relatively insensitive to Hh signal inhibition may persist so that treatment may not be curative. The existence of such a cell population has been shown both in a mouse BCC model (75) and in a clinical trial of the SMO inhibitor LDE225 (ref. 74 and Table 3).

Another concern is the development over time of resistance to SMO inhibitors used in treatment, which may or may not involve mechanisms similar to those that render certain BCCs refractory to treatment from the start. Medulloblastoma is another tumor type in which Hh signaling is frequently activated by mutations in *PTCH1* or *SMO*, and the treatment of one patient with a medulloblastoma carrying *PTCH1* mutations with GDC-0449 led to rapid but transient tumor regression (76). Subsequently, it was found that an amino acid substitution in SMO that had no effect on Hh signaling but disrupted the ability of GDC-0449 to bind SMO and suppress this pathway was the underlying cause of the relapse (77). Studies in animal models confirmed that the development of resistance can be caused by mutations in *Smo* as well as by the amplification of downstream genes such as *Gli2* and cyclin D1 (78, 79). Potential methods of overcoming such resistance involve the use of alternative SMO inhibitors (79) such as the FDA-approved antifungal drug itraconazole, which was recently found to inhibit Hh signaling by binding to SMO at a site different from cyclopamine and to delay BCC development in $Ptch1^{+/-}$ mice (80). At present, itraconazole is being evaluated as a possible treatment for BCC

Table 3
Emerging BCC therapies

Agent/compound	Target	Condition/patient group studied	Study phase	ClinicalTrials.gov ID	Reference
SMO inhibitors in clinical trials					
GDC-0449[A]	SMO	BCNS patients	II	NCT00957229	70
GDC-0449	SMO	Locally advanced or metastatic BCC	II	NCT01367665	69
GDC-0449	SMO	Operable BCC	II	NCT01201915	–
GDC-0449	SMO	BCC	II	NCT00959647	–
GDC-0449	SMO	Advanced BCC	II	NCT00833417	–
GDC-0449	SMO	Locally advanced or metastatic BCC	II	NCT01160250	–
LDE-225	SMO	BCNS patients	II	NCT00961896	–
LDE-225	SMO	Advanced solid tumors (BCC, medulloblastoma)	I	NCT01208831	–
LDE-225	SMO	Advanced solid tumors (BCC, medulloblastoma)	I	NCT00880308	–
LDE-225	SMO	Sporadic superficial and nodular BCC	II	NCT01033019	–
LDE-225	SMO	Locally advanced or metastatic BCC	II	NCT01327053	–
LDE-225	SMO	BCNS patients	II	NCT01350115	–
LDE-225	SMO	BCNS patients	Preclinical	–	74
Cur61414	SMO	UV-treated *Ptch1*$^{+/-}$ mice; *Ptch1*$^{+/-}$/*K14-CreERT2-p53*$^{fl/fl}$ mice exposed to IR	Preclinical	–	98, 99
Cur61414	SMO	Superficial and nodular BCC[B]	I	–	99
IPI-926	SMO	BCC (including locally advanced or metastatic)	I	NCT00761696	71
BMS-833923	SMO	Advanced or metastatic BCC	I	NCT00670189	–
TAK-441	SMO	Advanced BCC	I	NCT01204073	–
Itraconazole	SMO	BCC	II	NCT01108094	–
Downstream Hh pathway inhibitors					
GANT 58, GANT 61	GLI	NA	Preclinical	–	81
Arsenic trioxide	GLI	NA	Preclinical	–	83, 84
HPI1/2/3	GLI	NA	Preclinical	–	82
HPI4	Ciliogenesis	NA	Preclinical	–	82
Other agents					
Vitamin D3	SMO/cell proliferation	BCC	III	NCT01358045	–
Tazarotene	RAR-β/RAR-γ	BCC on chest and back of BCNS patients	II	NCT00783965	–
Tazarotene	RAR-β/RAR-γ	BCC on face of BCNS patients	II	NCT00489086	–

[A]Drug name vismodegib. [B]BCCs failed to show signs of clinical response or significant GLI1 target gene inhibition, likely due to inadequate drug absorption.

in a phase II trial (Table 3). Alternatively, blocking other signaling pathways in resistant tumors may be effective, and in preclinical studies of medulloblastomas, PI3K inhibition has emerged as a promising possibility (78, 79).

In situations in which Hh pathway activation occurs downstream of SMO, targeting the final effectors in the pathway, such as the GLI transcription factors, would be preferable. The potential viability of this strategy has been demonstrated by the identification of small molecule inhibitors acting at the level of GLI, or at alternative steps downstream, and independent of SMO (refs. 81, 82, and Table 3). Interestingly, in two studies it has been found that arsenic trioxide, in clinical use for the treatment of acute promyelocytic leukemia, can inhibit Hh signaling at the GLI protein level, although the exact mechanism remains controversial (83, 84). However, given the diverse set of targets for arsenic trioxide, it will be challenging to delineate critical targets in an in vivo setting, and existing side effects may limit its attractiveness as a treatment for BCC; curiously, arsenic exposure is also a known risk factor in BCC development (85).

Given the key role of primary cilia in the transduction of the Hh signal (Figure 2), inhibitors of ciliogenesis or ciliary function represent a further means of intervention in BCC tumorigenesis, and a small molecule blocking ciliogenesis has been identified as an Hh inhibitor (ref. 82 and Table 3). Again, the multiple cellular effects expected as a result of cilia disruption will place obstacles in the way of obtaining specificity.

Other potential new BCC therapies. Vitamin D3 has been shown to block Hh signaling in vitro and in murine BCCs in vivo, presumably at the level of SMO, in a manner independent of vitamin D receptor (VDR) activation (86, 87). The enhanced differentiation of keratinocytes induced via VDR activation is an additional and well-established effect of vitamin D3. A phase III trial combining topical vitamin D3 therapy and treatment with an anti-inflammatory agent in patients with nodular BCC has been initiated (Table 3).

Finally, topical treatment with the RARB/RARG-selective retinoid tazarotene has been shown to reduce the number and size of early BCC lesions in irradiated *Ptch1*$^{+/-}$ mice (87, 88), and its efficacy in the control of BCC development in BCNS patients is under study in two phase II clinical trials (Table 3).

To summarize, early clinical results targeting the Hh signaling pathway are very promising, especially in regard to BCC treatment and chemoprevention in BCNS patients and for the treatment of locally aggressive or metastatic BCC. However, we still do not have the answer to several important questions: (a) Will treatment truly result in the eradication of BCCs, or will dormant tumor cells remain? (b) What are the major resistance mechanisms in BCCs? (c) Why are a substantial fraction of BCCs refractory to treatment

from the start? Moreover, the adverse effects that develop with systemic treatment are a significant concern, arguing for the development of local treatment options.

Perspectives

The great advances in our understanding of BCC biology, derived from deciphering its molecular genetics and from incisive studies using genetic mouse models that closely mimic the human disease, have been translated rapidly into new and promising targeted therapies. At the same time, it is important to realize that we are only just beginning to resolve the long-standing question about the BCC cell of origin. We know from studies in the mouse that SCs and progenitor cells present in the HF can serve as cells of origin, but that additional cells of origin must exist. The marked plasticity of skin epithelial stem cell populations, as revealed upon tissue injury, provides an additional layer of complexity.

Another challenging question begging an answer is the nature of the genetic events that cooperate with an activated Hh signaling pathway to determine BCC subtypes, ranging from the benign nodular and superficial forms to aggressive and, in rare cases, metastatic forms. Future studies will certainly provide answers to many of these questions, and it is to be hoped, moreover, that the lessons learned from treating BCC with Hh pathway inhibitors will pave the way for progress in the treatment of other tumors that depend on the presence of active Hh signaling.

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