Hedgehog signaling in basal cell carcinoma

Otsuka, Atsushi; Levesque, Mitchell P; Dummer, Reinhard; Kabashima, Kenji

Abstract: Basal cell carcinoma (BCC), the most common type of skin cancer, is occasionally aggressive with deep invasion, destruction of adjacent structures, recurrence and, on very rare occasions, regional and distant metastases. Mutations that occur in BCC in hedgehog (Hh) pathway genes primarily involve the genes encoding patched homolog (PTCH) and smoothened homolog (SMO). Several animal models have demonstrated the functional relevance of genetic alterations in the Hh pathway during tumorigenesis. Recently, targeted therapy has become available both commercially and in the context of human clinical trials. Interestingly, Hh pathway inhibitors not only suppress BCC progression but also promote acquired immune responses. Since immune responses are crucial for long-term tumor control, new clinical trials, such as those involving a combination of Hh inhibitors with immune modifiers, are needed to supplement standard methods of tumor control.

DOI: https://doi.org/10.1016/j.jdermsci.2015.02.007

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-116903
Accepted Version

Originally published at:
DOI: https://doi.org/10.1016/j.jdermsci.2015.02.007
Hedgehog signaling in basal cell carcinoma

Atsushi Otsuka, MD, PhD1; Mitchell P. Levesque PhD2; Reinhard Dummer, MD2; and
Kenji Kabashima, MD, PhD1

1Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto
606-8507, Japan

2Department of Dermatology, University Hospital Zurich, Gloriosastrasse 31, 8091 Zurich,
Switzerland

Correspondence to Atsushi Otsuka, MD, PhD
Department of Dermatology, Kyoto University Graduate School of Medicine
54 Shogoin Kawara, Sakyoku, Kyoto 606-8507, Japan
Tel: +81-75-751-3310, Fax: +81-75-761-3002, Email: otsukamn@kuhp.kyoto-u.ac.jp
ABSTRACT

Basal cell carcinoma (BCC), the most common type of skin cancer, is occasionally aggressive with deep invasion, destruction of adjacent structures, recurrence and, on very rare occasions, regional and distant metastases. Mutations that occur in BCC in hedgehog (Hh) pathway genes primarily involve the genes encoding patched homolog (PTCH) and smoothened homolog (SMO). Several animal models have demonstrated the functional relevance of genetic alterations in the Hh pathway during tumorigenesis. Recently, targeted therapy has become available both commercially and in the context of human clinical trials. Interestingly, Hh pathway inhibitors not only suppress BCC progression but also promote acquired immune responses. Since immune responses are crucial for long-term tumor control, new clinical trials, such as those involving a combination of Hh inhibitors with immune modifiers, are needed to supplement standard methods of tumor control.
Introduction

Basal cell carcinoma (BCC) is the most common cancer type and its incidence rate is increasing [1]. BCC characteristically arises in body areas that are exposed to the sun and is most common on the head and neck (80%), followed by the trunk (15%) and arms and legs [2]. BCC has also been reported in unusual sites, including the axillae, breasts, perianal area, genitalia, palms, and soles [3].

BCC is generally characterized by slow growth and minimal soft tissue invasiveness [4]. Since BCC has low metastatic potential, treatment focuses on local control. Treatment of BCC can be surgical or nonsurgical, such as conventional surgical excision or micrographic surgery, radiotherapy, photodynamic therapy, cryosurgery, or topical treatment, including 5-fluorouracil or toll-like receptor agonist imiquimod [5].

BCC is occasionally aggressive with deep invasion, destruction of adjacent structures, recurrence and, on very rare occasions, regional and distant metastasis [6]. A previous report showed that BCCs comprised 6.6% moderate (640 of 9652) and 0.6% (58 of 9652) of severe cases [7]. In 2012, the United States Food and Drug Administration (FDA) approved vismodegib as a first-generation hedgehog (Hh) pathway antagonist for the treatment of advanced or metastatic BCC. Vismodegib was also approved in the European Union, Switzerland, Canada, Australia, Mexico, Israel, South Korea and other countries in 2013. Vismodegib is an effective therapy that shrinks tumors to a manageable size. In this review, we will discuss the Hh pathway in BCC and new insights into Hh pathway inhibitors in adaptive immunity for BCC treatment.
**Hh signaling**

The Hh pathway plays a crucial role in patterning and organogenesis during early development, and is largely inactive in adults, except for its function in tissue repair and maintenance [8]. The central components of the Hh pathway consist of three secreted ligands (Sonic Hh, Indian Hh, and Desert Hh), a negative regulatory receptor (Patched [PTCH]), a positive regulatory receptor (smoothened [SMO]), and glioma-associated oncogene (GLI) transcription factors (GLI1, GLI2, and GLI3) [8, 9]. The primary cilium is a microtubule-based organelle that protrudes from the plasma membrane and acts as a sensor for extracellular signals, including the Hh pathway [10].

The precise mechanism of Hh signaling through SMO has been well studied. In the absence of Hh ligand, PTCH localizes in the cilia and represses SMO activity by preventing its trafficking and localization to the cilia (Figure 1A). GLI transcription factors are sequestered in the cytoplasm by several protein mediators, including protein kinase A (PKA) and suppressor of fused (SUFU) [11]. GLI undergoes proteasomal cleavage and the resulting repressor from GLI translocates to the nucleus and inhibits the translation of Hh target genes. On ligand binding, PTCH is displaced from the cilia, thereby allowing ciliary accumulation and activation of SMO. Activated GLIs, the final effectors of the pathway, translocate into the nucleus to induce the expression of various context-specific genes, which regulate cellular differentiation, proliferation, and survival (Figure 1B) [11].

**Hh signaling in BCC**
The relationship of Hh pathway activation and cancer has been examined since the report of germline loss-of-function mutations in PTCH in patients with nevoid basal cell carcinoma syndrome (NBCCS, Gorlin syndrome) [12]. NBCCS is an autosomal-dominant disease that is characterized by multiple developmental abnormalities and a predisposition to tumors, specifically BCC, medulloblastoma (MB), embryonal rhabdomyosarcoma, and meningioma [13]. Somatic mutations in PTCH have been identified in 90% of sporadic BCC [14], and gain-of-function mutations in SMO have been detected in BCC [15]. In particular, recurrent mutations in SMO and functional studies have demonstrated that these mutations, leading to aberrant activation of Hh signaling, promote tumor development (Figure 2A) [15]. Recently, Hh pathway mutations have been identified in large-scale whole-genome and whole-exome deep-sequencing studies across a wide range of cancers. Interestingly, somatic mutations in PTCH have been detected in other cancer types, such as ovarian and endometrial cancers [16]. In contrast with BCC and MB, these mutations are mainly missense. Their relevance in tumor development remains to be determined.

In addition to the SMO-dependent pathway, phosphatidylinositol 3-kinase (PI3K) also promotes Hh signaling in oncogenesis. S6 kinase 1 (S6K1) and atypical protein kinase C (aPKC), components that are downstream from PI3K, are reported to promote GLI-dependent transcription. S6K1 is also downstream of the mammalian target of rapamycin (mTOR) pathway and was found to be elevated in esophageal cancers resistant to SMO antagonists [17]. In addition, PI3K can promote 3-phosphoinositide-dependent kinase 1 (PDK1) activation and PDK1 can promote
mTOR and S6K1 activation. S6K1 promotes GLI-dependent transcription by phosphorylating GLI1, which prevents an inhibitory interaction with SUFU that allows GLI to enter into the nucleus and turn on target genes. aPKC is an Hh target gene that phosphorylates GLI1 at distinct sites from S6K1, activating GLI1 DNA binding and transcriptional activity to generate a positive-feedback loop that amplifies GLI-dependent transcription in BCC (Figure 2B) [17].

Mouse model of BCC

Several animal models have demonstrated the functional relevance of genetic alterations in the Hh pathway during tumorigenesis. Two spontaneous PTCH mutant animals have been reported, but these mice, such as PTCH<sup>mes/mes</sup> mice, do not develop BCC even after exposure to radiation in spite of these skin anomalies [18, 19]. On the other hand, two different conventional PTCH knockout mouse models, the PTCH<sup>neo12</sup> and PTCH<sup>neo67</sup> strains, in which exons 1 and 2 or exons 6 and 7, respectively, develop BCC [18, 20]. In addition to the several PTCH knockout mice that are known as BCC models, another BCC model exists that includes mice overexpressing Hh, oncogenic SMO, GLI1, or GLI2 specifically in the skin using the keratin (K) 5, 6, or 14 promoters [18]. The skin tumor subtypes range from follicular hamartoma and trichoepithelioma to nodular or invasive BCC depending on the gene and the targeted cell type [18].

Hh antagonist
The first well-studied therapy targeting the Hh pathway was cyclopamine, an endogenous steroidal plant alkaloid derived from corn lilies [21]. Since cyclopia is one of the defects in mice lacking sonic Hh, this phenotype provided an important connection between cyclopamine and Hh pathway activation [22]. A number of SMO antagonists, including vismodegib, were identified using in vitro screens to sift through thousands of compounds (Table 1, Figure 3).

Vismodegib is effective at suppressing BCC tumor growth and appears both tumoricidal and tumorstatic. Most BCC relapses after cessation of vismodegib, suggesting that the most efficient use of vismodegib as a therapeutic agent is to shrink tumors to a manageable level and then surgically excise any remaining tumor clones. Currently, a global single-arm open-label safety study on vismodegib in patients with advanced BCC (STEVIE study) is ongoing. SMO inhibitors that are in phase I or II clinical trials to treat advanced or metastatic BCC include sonidegib (LDE225), erismodegib, XL-139, LEQ506, itraconazole, and saridegib [16, 23]. Furthermore, there are several candidate components for BCC treatment, such as BEZ235, an inhibitor of mTOR signaling [24].

A previous study demonstrated that vismodegib showed a 30 and 60% response rate for metastatic and locally advanced BCC, respectively [25]. However, most responses were only partial. The most sensitive patient population were NBCCS patients who carry a PTCH mutation that predisposes them to developing hundreds of BCCs. NBCCS patients treated with vismodegib showed a 100% response rate. Slower-evolving tumors with low mutation rates, such as sporadic BCC or NBCCS,
will respond well to SMO inhibition, whereas metastatic BCCs with higher mutational rates have a higher likelihood of acquired resistance before or during drug treatment.

The most common toxicities reported with Hh pathway inhibitors include taste alteration (dysgeusia), alopecia, muscle spasms, anorexia, and fatigue [25]. However, the dose-limiting toxicities are highly variable across the class, probably because of differences in their structure-activity relationships, on-target potencies, or tissue distributions.

Promotion of acquired immune response by Hh signal inhibitors

Cancers have several mechanisms to escape immune surveillance [26]. Despite the presence of cancer-testis and other tumor antigens, BCC also escapes immune surveillance through the down-regulation of HLA class I expression [27]. Recently, we demonstrated that vismodegib promotes acquired immune response. In the context of HLA class I expression, cytotoxic CD8\(^+\) T cells play essential roles in anti-tumor effects for skin tumors [28, 29]. We have shown substantial alterations in the immune-microenvironment with an intra- and peritumoral increase of cytotoxic CD8\(^+\) T cells and an up-regulation of MHC class I during tumor regression under treatment with Hh pathway inhibitors [30]. Moreover, a reduction in primary cilia was observed after Hh pathway inhibitor treatment [30]. Before treatment, all BCC cells are ciliated, suggesting that they are responsive to Hh signaling. By inhibiting Hh signaling, the BCC cells lose their cilia and subsequently stop proliferating.

T cell activation requires both T cell receptor (TCR) and co-stimulatory molecule
ligation by professional antigen-presenting cells (APC), and the outcome of the stimulatory signal is influenced by the microenvironment of the T cell and the APC [31, 32]. The Hh signaling pathway reduced the strength of the TCR signal in mature peripheral T cells [33, 34]. Additionally, the repression of the Hh signaling pathway in T cells increased T cell activation [35]. This suggests that the Hh pathway inhibitor has direct effects on peripheral T cell and activates adaptive immune responses.

It is known that IFN-γ up-regulates MHC class I antigen presentation by inducing gene expression signatures that are related to MHC class I antigen processing and presentation, including activation of the JAK/STAT1 signal transduction pathway (Figure 4A) [36]. Potential cross-talk between IFN-γ and the Hh pathway was recently described by Laner-Plamberger et al. [37]. They demonstrated that the suppressor of cytokine signaling 1 (SOCS1) is a direct target of Hh/GLI signaling in human keratinocytes and medulloblastoma cells and a potent inhibitor of IFN-y-STAT1 signaling, which can induce cell cycle arrest, apoptosis, and anti-tumor immunity. It was shown that the transcription factors GLI1 and GLI2 activated the SOCS1 promoter and that STAT1 phosphorylation was reduced in cells with active Hh/GLI signaling (Figure 4B) [37]. During treatment with Hh pathway inhibitors, GLI was suppressed by proteosomal cleavage and did not activate the SOCS1 promoter. Up-regulation of MHC class I after treatment with Hh pathway inhibitors may be induced by this mechanism. To understand the precise mechanism, further studies are needed in the future.

Conclusion
BCCs are highly prevalent tumors that are treatable using traditional therapy, including both surgical and nonsurgical methods. Traditional therapies are not as effective, however, in treating multiple BCCs or those that become highly invasive or metastatic. The advancement of Hh pathway inhibitors, such as vismodegib, into clinical development has yielded good responses in mutation-driven tumors with activated Hh signaling. However, it appears that the clinical application of single-agent Hh pathway inhibitors might not be as broad as was initially expected.

In addition to the effect of Hh pathway inhibitors on BCC proliferation, we demonstrated that Hh pathway inhibitor treatment induced a recruitment of cytotoxic T cells into the tumor and up-regulation of MHC class I in BCCs. Reduction in the frequency of ciliated cells during the Hh pathway inhibitor treatment suggests that cilia are required for Hh inhibitor efficacy. For long-term tumor control, immune responses are crucial. It has been reported that immune modifiers, including imiquimod, are therapeutically beneficial [38]. New clinical trials, such as those involving a combination of Hh inhibitors with immune modifiers, are needed to supplement standard methods of tumor control.

ACKNOWLEDGEMENTS

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare of Japan. No additional external funding was received for
this study.

References


Table 1

Hh pathway antagonists that are currently in use or development for the treatment of BCCs

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visomodegib</td>
<td>SMO</td>
<td>[25]</td>
</tr>
<tr>
<td>Sonidegib</td>
<td>SMO</td>
<td>[39]</td>
</tr>
<tr>
<td>PF-04449913</td>
<td>SMO</td>
<td>[40]</td>
</tr>
<tr>
<td>Erismodegib</td>
<td>SMO</td>
<td>[41]</td>
</tr>
<tr>
<td>LEQ506</td>
<td>SMO</td>
<td>[42]</td>
</tr>
<tr>
<td>Saridegib</td>
<td>SMO</td>
<td>[43]</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>SMO</td>
<td>[44]</td>
</tr>
<tr>
<td>ATO</td>
<td>GLI</td>
<td>[44]</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>mTOR</td>
<td>[45]</td>
</tr>
</tbody>
</table>

SMO, Smoothened; ATO, arsenic trioxide; mTOR, mammalian target of rapamycin

Figure legends

Figure 1. Mechanism of the Hh pathway

(A) In the absence of Hh ligand, PTCH localizes in the cilia and represses SMO activity by preventing its trafficking and localization to the cilia. GLI transcription factors are sequestered in the cytoplasm by several protein mediators, including PKA and SUFU.
GLI undergoes proteasomal cleavage and the resulting repressor form GLI translocates to the nucleus and inhibits the translation of Hh target genes. (B) On ligand binding, PTCH is displaced from the cilia, thereby allowing ciliary accumulation and activation of SMO. Activated GLIs, the final effectors of the pathway, translocate into the nucleus to induce the expression of various context-specific genes that regulate cellular differentiation, proliferation, and survival.

Figure 2. Hh pathway in BCC

(A) Inactivating mutations in PTCH or the binding of Hh ligands to PTCH de-represses SMO, thereby allowing its translocation onto the tip of the primary cilium, leading to the transcriptional activation of Gli. Multiple ciliary proteins are involved in processing Hh signal transduction. The activation and nuclear translocation of Gli involve the dissociation of Gli from its endogenous inhibitor SUFU. (B) S6K1 and aPKC, which are downstream of PI3K, are reported to promote GLI-dependent transcription. S6K1 is also downstream of the mTOR pathway. PI3K can promote PDK1 activation and PDK1 can promote mTOR and S6K1 activation. S6K1 promotes GLI-dependent transcription by phosphorylating GLI1, which prevents an inhibitory interaction with SUFU that allows GLI to enter into the nucleus and turn on target genes. aPKC is an Hh target gene that phosphorylates GLI1 at distinct sites from S6K1, activating GLI1 DNA binding and transcriptional activity to generate a positive-feedback loop that amplifies GLI-dependent transcription in BCC.
Figure 3. Hh pathway antagonists for BCC treatment

Hh pathway activity can be inhibited through several mechanisms, including direct binding to SMO, modulation of primary cilia translocation of SMO, inhibition of the receptor–ligand interaction, and inhibition of GLI transcription factors.

Figure 4. Hypothesis of up-regulation of MHC class I during treatment with Hh pathway inhibitors

(A) IFN-γ up-regulates MHC class I antigen presentation by inducing gene expression signatures that are related to MHC class I antigen processing and presentation, including activation of the JAK/STAT1 signal transduction pathway. SOCS1 is a direct target of Hh/GLI signaling in human keratinocytes and medulloblastoma cells and a potent inhibitor of IFN-γ-STAT1 signaling, which can induce cell cycle arrest, apoptosis, and anti-tumor immunity. It was shown that the transcription factors GLI1 and GLI2 activated the SOCS1 promoter and that STAT1 phosphorylation was reduced in cells with active Hh/GLI signaling. (B) During treatment with Hh pathway inhibitors, GLI undergoes proteosomal cleavage and does not activate the SOCS1 promoter. Up-regulation of MHC class I after treatment with Hh pathway inhibitors may be induced by this mechanism.