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The hedgehog's tale: developing strategies for targeting cancer

Jessica M. Y. Ng and **Tom Curran**
Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, 4060 Colket Translational Research Bldg., 3501 Civic Center Blvd., Philadelphia, PA 19104

Jessica M. Y. Ng: ngj@email.chop.edu; Tom Curran: currant@email.chop.edu

Preface

Research into basic developmental biology has frequently yielded insights into cancer biology. This is particularly true for the hedgehog (HH) pathway. Activating mutations in the HH pathway cause a subset of sporadic and familial, skin (basal cell carcinoma) and brain (medulloblastoma) tumors. Furthermore, the growth of many human tumors is supported by HH pathway activity in stromal cells. Naturally occurring and synthetic inhibitors of HH signaling show great promise in animal models and in early clinical studies. However, it remains unclear how many cancers will ultimately benefit from these new, molecularly targeted therapies.

Cancer cells have long been known to express embryonic antigens and, in several instances, they have been shown to recapitulate developmental signaling pathways. Studies of cancer stem cells support the idea that tumors harbor hallmarks of early development in their gene expression repertoire[1]. However, prior attempts to translate these insights into improved cancer treatment, by targeting developmental signaling pathways with small molecule inhibitors, have met with mixed success. Recently, exciting findings from early-stage clinical trials (Table 1) of inhibitors of the well-known Hedgehog (HH) pathway (Box 1) have renewed hope that disruption of developmental signaling in tumors can be of therapeutic benefit[2–7]. HH inhibitors block both intrinsic signaling in cancer cells as well as extrinsic signaling to stromal cells to reduce tumor growth[8]. These two strategies exploit distinct oncogenic functions of the pathway[9]. The challenge currently facing the field is to distinguish which tumors will benefit from such interventions. Here, we present a critical review of the mechanisms and roles of the hedgehog pathway in tumorigenesis in light of recent results from the laboratory and clinic.

Box 1

Hedgehog Pathway in Development

The HH pathway was identified in *Drosophila melanogaster*[123], where it was ultimately shown to control segmental pattern formation. In mammals, three genes, Desert Hedgehog (DHH), Indian Hedgehog (IHH) and Sonic Hedgehog (SHH), function as ligands for Patched (PTCH1) in a wide range of developmental signaling roles[124]. In some cases, HH ligands clearly function as mitogens, whereas in others they promote differentiation[13]. The importance of HH signaling in mammalian development is underscored by observations that mutations in SHH cause holoprosencephaly (HPE)[125–127]. HPE is a developmental disorder affecting the midline of the face and nervous system. It is characterized by cleft lip and palate, single central incisor, impaired CNS septation, and in severe cases complete cyclopia. A similar constellation of phenotypes has long been associated with exposure of developing embryos to naturally occurring teratogenic alkaloids such as cyclopamine[128]. Indeed, the presence of these teratogens in extracts from *Veratrum Album,* commonly known as White Hellebore, part of Liliaceae (the lily family), used in ancient medicine, may have provided a source for

the legendary Cyclops[129]. Initially, the mechanism responsible for these effects was thought to relate to the ability of these compounds to inhibit cholesterol biosynthesis, however, subsequently it was demonstrated that they function as specific inhibitors of HH signaling[130, 131] by binding to SMO[132]. Thus, both the importance of the HH pathway in regulating growth, and the identification of a potential inhibitor, had their origins in developmental biology research.

The HH signaling pathway

A common feature of HH pathway signaling in development is that it acts in close association with several other biological signals, for example, bone morphogenetic proteins (BMPs)[10], parathyroid hormone[11], and retinoids[12]. Although the pathway has many components and multiple levels of regulation, in most circumstances, it functions in mammals as outlined in Figure 1. For the most updated, detailed review of HH signaling in metazoans see[13]. In brief, HH ligands bind to Patched (PTCH1), causing internalization and degradation, thereby releasing Smoothened (SMO) to enter the primary cilia where it promotes dissociation of a Suppressor-of-fused (SUFU)–glioma-associated oncogene homolog (GLI) complex. This results in nuclear translocation and activation of the GLI1 and GLI2 transcription factors, and degradation of the repressor forms of GLI (primarily GLI3). It is important to keep in mind that the details of these processes have yet to be worked out[14]. Activated GLI proteins stimulate transcription of HH pathway target genes, including GLI1, GLI2 and PTCH1. As all HH signaling through the canonical pathway requires SMO, small molecules such as cyclopamine, which inhibit SMO function, completely block all HH pathway signaling regardless of the ligand. These agents not only provide valuable tools for dissecting the biochemistry and biology of HH signaling, but they also enabled the development of unique molecular targeted therapies for cancer.

Hedgehog pathway mutations in cancer

The detection of loss-of-function mutations in the HH pathway in familial (Gorlin's syndrome) and sporadic basal cell carcinoma (BCC) and medulloblastoma (MB)[15–25] established a clear genetic contribution of HH pathway activity to oncogenesis. Since these initial findings, additional germline and sporadic mutations have been reported in SUFU[26, 27]. Recent findings have also used mutations in the HH pathway to identify the potential cell of origin of BCC and medulloblastoma. The relationship between cancer stem cells, normal stem cells and the cell of origin of specific tumors are often unclear[28], but given the prominent role HH signaling has in normal development, identifying these cell might prove to be especially informative in tumors that depend on HH pathway activity.

BCC

In the case of BCC, it has been estimated that essentially all tumors show evidence of constitutive HH pathway activity with 90% exhibiting loss of PTCH1 and 10% activating mutations in SMO[29]. Although the cell of origin of most cancers remains elusive, good candidates have been identified for BCC. Previously, it was suspected that BCC arose from bulge stem cells in the hair follicle[30]. However, targeted expression of an activated *Smo* gene in mice suggested that long-term resident progenitor cells of the interfollicular epidermis and upper infundibulum gave rise to tumors resembling BCC[31]. In contrast, in irradiated $Ptch1^{+/-}$ mice, BCC were found to arise from keratin-15 expressing stem cells of the follicular bulge[32]. One explanation for the discrepancies in these results is that PTCH1 and SMO have additional distinct functions beyond the HH pathway that also influence tumorigenesis. For example, PTCH1 can sequester cyclinB in the cytoplasm[33] and this was observed in BCC from $Ptch1^{+/-}$ mice, but not in those expressing activated SMO[32].

Furthermore, it appears that loss of p53 promotes BCC formation, in part, by increasing SMO expression[32]. The effect of p53 loss on SMO expression might also explain why homozygous loss of *Trp53* dramatically accelerates medulloblastoma formation in *Ptch1*$^{+/-}$ mice, whereas loss of *Arf* or heterozygous loss of *Trp53*, neither of which affect the transcriptional function of p53, do not increase tumorigenesis in *Ptch1*$^{+/-}$ mice [34]. These findings show that activation of the HH pathway through loss of PTCH1 or by expression of a mutated form of SMO are mechanistically distinct. As pointed out by Wang *et al*[32], although the HH pathway can be activated in cells that do not normally express SMO by expression of activated SMO or over expression of GLI2, loss of PTCH1 in cells that do not express SMO does not achieve the same effect.

The targeted expression of an activated form of GLI2 in stem cell populations in the skin generated a range of tumor phenotypes, depending on the cell of origin and level of GLI2 expression [35]. This study also showed that it is important to examine the histology of mouse skin tumors carefully as they may represent only a subset of the range of BCC types found in humans. Therefore, we should be cautious when comparing and interpreting data obtained from models based on different approaches.

Medulloblastoma

The situation in MB is less uniform, with up to 30% of tumors showing a gene expression signature indicative of HH pathway activation, but only half of these associated with loss of PTCH1, loss of SUFU or gain-of-function SMO mutations[36–38]. Thus, a subset of MB exhibits HH pathway activation without evidence of mutations in PTCH1, SMO or SUFU.

Historically, medulloblastoma research was confined to a handful of cell lines and xenografts, although the limitations of such models, including their low predictive value, were well recognized[39]. However, over the past several years, there has been explosive growth in the availability genetically engineered mouse (GEM) models for brain tumors. In MB, the majority of these models develop as a consequence of HH pathway activation[40]. Mice heterozygous for *Ptch1* provide a good model of Gorlin's syndrome, exhibiting several of the phenotypes, including an increased incidence of MB (up to 25%, depending on the genetic background of the mice)[40–43]. Tumor incidence increases to 100%, and the age of onset is decreased, in the absence of p53[34]. Significant acceleration is also observed following exposure to ionizing radiation[44]. Alterations in genes that function in DNA double-strand break repair and cell cycle control also collaborate with p53 loss to cause medulloblastoma in mice[45–47]. Mutations in DNA repair genes seem to function by increasing the likelihood of chromosomal alterations in *Ptch1* in mouse medulloblastoma[47], but mutations in DNA repair genes have not been described in human medulloblastoma. Additional mouse models of medulloblastoma have also been created by expressing transgenes with activating point mutations in SMO, that were first identified in BCC[48, 49], under the control of the *Neurod2* promoter[50, 51]. Tumors arising in one other mouse model, with heterozygous or homozygous loss of chemokine receptor type 6 (*Cxcr6*), exhibit an activated HH pathway and although *Ptch1* is not mutated its expression is suppressed[52]. In addition, targeted expression of GLI1, GLI2 or HH ligands can induce medulloblastoma and tumor formation in the skin[53–55]. Targeting expression of activated β-catenin to neural precursor cells in mice also results in the development of medulloblastoma, but in this case the WNT pathway is activated in tumors rather than the HH pathway[56]. At present, the available genetically modified mouse models do not recapitulate the full genetic diversity of human medulloblastoma, but they do reflect many of the properties of human medulloblastoma[40]. These models have made significant contributions to our understanding of the etiology of medulloblastoma and they have provided invaluable tools for proof-of-concept analysis of drugs that target the HH pathway.

Recent studies on the cell of origin of medulloblastoma have identified two potentially different tumor subtypes: that driven by HH pathway mutations and that driven by mutations in the WNT pathway. The origin of HH-medulloblastoma was suggested by the close resemblance of gene expression patterns in medulloblastoma cells with those of granule neuron progenitor cells[57]. Genetic studies showed that, while activation of the HH pathway in neural stem cells, as well as granule neuron progenitor cells, results in medulloblastoma formation, stem cells must first make the transition to committed granule neuron progenitors[58, 59]. In this case, it appears that oncogenesis results from constitutive activation of a signaling pathway, normally active in the cell of origin, which would otherwise be down regulated during the course of development. The WNT molecular subtype of medulloblastoma exhibits a distinct pattern of gene expression and appears to be derived from progenitor cells in the dorsal brainstem in which the HH pathway is inactive[56]. However, the situation might not be so clear-cut, as analysis of the medulloblastoma genome recently revealed that some tumors can harbor both HH and WNT pathway mutations simultaneously, indicating that there may be some overlap among the cells of origin[60].

Rhabdomyosarcoma

Soft tissue tumors very similar to human rhabdomyosarcoma (RMS) are also seen in *Ptch1*$^{+/-}$ mice, but the incidence is strongly influenced by genetic background[61]. Initial descriptions of Gorlin syndrome identified benign fetal rhabdomyoma and rare instances of several other tumors, including RMS[62]. Although it is often stated that RMS is associated with activating mutations in the hedgehog pathway, the role of the HH pathway in sporadic human RMS is not straightforward. RMS is a heterogeneous family of tumors, associated with the skeletal muscle lineage, encompassing several distinct molecular and histological subtypes, none of which have been specifically linked to HH pathway mutations[63]. DNA sequence analysis of the *PTCH1* coding region from 14 cases of RMS did not reveal any mutations[64]. Although another study reported mutations in *PTCH1* and *SUFU* in RMS, most of the analysis was restricted to loss of heterozygosity (LOH) analysis of *PTCH1* and *SMO* in rhabdomyoma and only one case of RMS[65]. Recently, low level amplification of GLI1 was reported in embryonal RMS and it was associated with increased expression of HH pathway target genes[66]. In addition, comparison of gene expression patterns in human embryonal RMS indicated that approximately 29% exhibited evidence of HH pathway activity but this was always in association with either p53 or RB pathway signatures[67]. However, the presence of the pathway signature does not necessarily mean that the tumor cells are dependent on SMO activity as analysis of the effects of cyclopamine on RMS in *Ptch1*$^{+/-}$ mice showed that, while loss of PTCH1 may contribute to tumor initiation, it is not required for tumor maintenance[68].

Other types of cancer with altered HH pathways

Several different molecular lesions in the HH pathway have been described in tumors and, in each case, they result in increased transcriptional activity of the GLI1 and GLI2 transcription factors. Indeed, the first indication that genes in the HH pathway were associated with human cancer was the observation that GLI1 was amplified in glioblastoma[69]. Although examples of GLI1 amplification have been described in childhood sarcoma[70], this does not appear to be a common primary mechanism underlying glioblastoma formation. The location of several other potential oncogenes (including MDM2 and CDK4) within the same amplicon has complicated interpretation of these data[71]. Translocations involving *GLI1* and *ACTB* (encoding β-actin) have defined a new class of pericytoma[72].

Many other tumors lacking HH pathway mutations have been reported to be sensitive to SMO inhibitors in vitro and in vivo[14]. This has led to estimates that up to 25% of human

tumors may depend on HH pathway activity for growth[73] and, as a consequence, a broad range of tumors was included in the early clinical trials of SMO inhibitors. Initially, these findings were interpreted to suggest that there was an autocrine feedback loop in some tumors expressing HH ligands[74–77] However, subsequently, it was shown in xenograft models that while some human tumors do make HH ligands, the target genes upregulated by these ligands are expressed in stromal cells of mouse origin[8]. In these cases, treatment with SMO inhibitors slowed tumor growth, but did not eliminate tumors under standard xenograft conditions[8]. In a mouse model of pancreatic cancer, inhibition of HH signaling enhanced the delivery and response to chemotherapy by depleting tumor-associated stromal tissue[78]. Thus, in addition to targeting tumor cells directly, SMO inhibitors may also show therapeutic benefit through effects on stromal cells.

Some leukemias were also reported to depend on SMO signaling for growth[79, 80], but this was disputed in subsequent studies on B-cell chronic lymphocytic leukemia indicating that although GLI1 had an autonomous role in promoting cell survival, this was not dependent on HH ligands or SMO[81]. The large body of work on the role of SMO signaling in cancer models supports a broader use of SMO inhibitors in tumors other than BCC and a subset of MB. The difficulty at present is to identify the tumors that would benefit from treatment, as the presence of HH ligands or a HH pathway signature alone, does not guarantee a response.

Molecular targeted therapies for HH pathway tumors

Currently, all of the HH pathway therapeutics in clinical development act by inhibiting SMO (Table I) and thus would be predicted to be ineffective against tumors harboring molecular lesions that lie downstream (including loss of SUFU or gain-of-function mutations in SMO that abrogate the inhibitor binding site). However, several groups are attempting to develop agents to target GLI that would have wider application[82]. In addition, arsenic tri-oxide (ATO) has recently been proposed to inhibit GLI proteins directly by two distinct mechanisms[83, 84]. ATO is proposed to block accumulation of GLI2 in cilia, ultimately resulting in reduced protein levels[83] and ATO is proposed to bind directly to GLI1, inhibiting its transcriptional function, even in the absence of cilia[84]. As ATO is an approved therapeutic, it may provide an alternative treatment for tumors that develop resistance to SMO inhibitors and could potentially be used in combination therapy with SMO inhibitors.

Preclinical development

Cyclopamine, and other naturally occurring inhibitors of SMO, are not suitable as therapeutic agents because of poor solubility, low potency, rapid clearance, non-specific toxicity and chemical instability[85]. This encouraged a search for novel inhibitors with preferential characteristics for drug development. HH-mediated GLI transcription activity provided a highly appropriate biomarker for identification of small molecule inhibitors as GLI1 and GLI2 contribute directly to oncogenic activity[55, 86–88]. Cell-based screening approaches, using GLI-dependent transcriptional reporters, proved remarkably adept at identifying small molecule inhibitors of SMO[89, 90]. Thus, it appears that SMO is a highly druggable target and a range of compounds, many with distinct structures from cyclopamine, have been uncovered that are capable of binding to the same site to block signaling (reviewed in[91]). Medicinal chemistry approaches optimized the properties of several lead compounds resulting in a 100-fold increase in potency compared to cyclopamine as measured in gene expression assays[8, 92, 93].

Pre-clinical proof-of-concept studies of SMO inhibitors, to establish efficacy in cancer models, have been both challenging and controversial. The advent of GEM models of cancer offered hope that new approaches would be more predictive than the venerable xenograft models. Indeed, a study of acute myeloid leukemia (AML) allograft transplant models,

expressing different translocation fusion genes, shows close concordance with responses seen in the respective pediatric populations[94]. Nevertheless, the issue remains hotly debated with strong proponents arguing in favor of xenograft models, others supporting GEM models and some adamant that mouse models of any kind cannot predict clinical outcome.

Most studies of HH pathway activity in cancer employed either human tumor cell lines or xenograft transplantation models treated with cyclopamine to demonstrate dependency on HH pathway activity. Reduced rates of cell proliferation or tumor growth were interpreted to mean that SMO activity was critical for growth of the respective tumors[74, 75, 95]. However, different groups have reported contradictory results, even when using the same cell lines or mouse strains carrying identical transplantable tumors[8, 96–100]. Taken together, a number of variables in the experimental approaches and application of the model systems used can account for these discrepancies.

There are no standard approaches to determining whether a particular cell line or tumor depends on HH pathway activity. Therefore, different marker genes have been used to assess pathway activity, and a range of quantitative and semi-quantitative approaches employed to measure gene expression levels, making it problematic to compare results from different studies. Cyclopamine is a toxic compound[85] and it can cause growth inhibition, independently of HH pathway activity, when used at high concentrations depending on the cell type[8, 101]. This means that it is critical to use cyclopamine at a concentration that inhibits the HH pathway but does not result in non-specific growth inhibition. This is very hard to do because there is no agreed up standard method to measure HH pathway activity. As a consequence, while some studies report concentrations of cyclopamine of 3 μM and above result in non-specific growth inhibition[8, 101], others claim that cyclopamine can function as a specific HH pathway inhibitor at levels up to 30 μM[100]. Although several studies employed complementary genetic approaches, such as short interfering RNAs to inhibit GLI1 or GLI2, as independent confirmation of the effects of cyclopamine, this approach does not demonstrate dependence on SMO. For example, GLI1 and GLI2 have been shown to function independently of HH pathway activity and SMO in B-cell chronic lymphocytic leukemia[81]. Thus, the fact that GLI1 and GLI2 are required for tumor cell viability does not necessarily mean that SMO is active in these cells and therefore does not confirm the effects of cyclopamine. In vivo, it is difficult to reach systemic levels of cyclopamine that completely inhibit HH pathway activity because of associated toxicities[85, 101]. Thus, it is likely that the role of HH pathway activity in human cancer has been overestimated as a consequence of the prevalent use of cyclopamine at toxic concentrations.

In many cases, alternate routes of administration were used to deliver cyclopamine. For example, in some instances cyclopamine was delivered by subcutaneous inoculation[95, 96]. This often caused ulceration at the injection site as the vehicle contained alcohol. This route is not directly comparable to systemic delivery and it is not clear if it resulted in the same amount of drug delivery to the tumor cells. When the route of administration was changed to oral delivery, it was not possible to achieve the same degree of tumor inhibition[102]. In most cases the level of cyclopamine achieved in tumor tissues was not determined, however, subcutaneous inoculation may have resulted in greater bioavailability, increased levels of exposure and higher specific, as well as non-specific, toxic effects.

Of much greater concern are studies in which cyclopamine was injected directly into the tumor mass[103]. In some studies the administration route was described as both proximal and directly into the tumor mass[104, 105]. In these cases, the actual concentration of cyclopamine to which tumor cells are exposed is extremely high and would likely result in non-specific toxicity. Finally, although the standard xenograft approach recommends treating tumors

only after they are fully established as transplants (approximately 200 – 400 mm^3) some studies used much smaller tumors, even as little as 10 mm^3, before they are fully established[105]. Thus, a series of methodological differences in preclinical experimental design, combined with the high degree of non-specific toxicity associated with cyclopamine, may account for some of the conflicting findings reported in the literature. These reports may have significantly overstated the potential of SMO inhibitors for treating human cancer.

Compounding these problems is the fact that pharmaceutical companies generally decline to supply compounds currently in development for such studies, because of fears that negative results would compromise the approval process. Therefore, most investigators relied on the use of cyclopamine, despite reservations about its properties, to test the contribution of SMO to tumor growth in vitro and in vivo. The notable exception was a set of compounds identified by Curis Inc., and subsequently developed in collaboration with Genentech Inc., that were used to show efficacy of SMO inhibitors in BCC explant cultures[29, 106] and in mouse models of medulloblastoma[52, 101]. One particular compound, a benzimidazole termed HhAntag, provided the most compelling preclinical data on the efficacy of SMO inhibitors. Oral delivery of HhAntag eradicated large medulloblastomas arising spontaneously in the cerebellum of *Ptch1*$^{+/-}$;*Trp53*$^{-/-}$mice[101]. However, tumor cell lines derived from these medulloblastomas, as well as allograft tumors made using these cell lines, were completely resistant to the inhibitory effects of HhAntag because the HH pathway was dramatically downregulated as soon as these cells were propagated in culture. By contrast, allografts derived directly from the medulloblastomas that were never grown in culture exhibited dramatic sensitivity, with large tumor masses (200 mm^3) regressing after only 4 days of treatment[52]. These model studies predicted that BCC and the HH pathway subtype of medulloblastoma would exhibit dramatic responses to SMO inhibitors in the clinic and led to the inclusion of patients with medulloblastoma in the initial clinical trials.

Clinical trials

The results of the first phase I trial of the SMO inhibitor GDC-0449 reported that 19 out of 33 patients with BCC, and one patient with medulloblastoma, exhibited either a partial or complete response to this novel therapy[6]. An unprecedented 50% response rate was observed in patients with metastatic BCC[3, 6]. A dramatic, albeit transient, responses was also reported in an adult with metastatic medulloblastoma[4], and encouraging results in a Phase I pediatric medulloblastoma clinical trial were reported at the 2010 American Society of Clinical Oncology meeting[107], indicating that the preclinical data on BCC and medulloblastoma were indicative of a response. In the one case of metastatic adult medulloblastoma, the patient subsequently relapsed because of mutation of the drug-binding site in SMO[2]. This unfortunate circumstance provided strong affirmation of SMO as a drug target and echoed early experiences with Imatinib (Gleevec), the prototypic molecular targeted therapy[108]. Resistance to inhibitors also arises readily in animal models as a consequence of mutations in SMO, amplification of GLI2 or amplification of *CCND1* (encoding cyclin D1)[2, 109, 110]. It is likely that drug resistance will be an important aspect of clinical treatment with HH pathway inhibitors and compounds have already been identified that can overcome resistance resulting from SMO mutations[110]. However, this approach will not be successful in resistant tumors that acquire mutations downstream of SMO. Some hope has been offered by the recent finding that blocking phosphatidylinositol-3 kinase (PI3K) activity inhibits the growth of certain resistant tumors[109].

In contrast to the dramatic clinical results reported in early BCC and medulloblastoma patients, no major responses to SMO inhibitors have yet been reported in other cancers[6]. Although Phase I trials are designed to test drug safety, not efficacy, the positive effects on BCC and medulloblastoma were very obvious. In addition, two trials of GDC-0449 have been closed to patient accrual (NCT00739661 and NTC00636610, listed on http://

clinicaltrials.gov). In the case of advanced ovarian cancer, GDC-0449 was being used in a maintenance setting as a single agent in a phase II clinical trial, but it did not sufficiently extend the median time to recurrence. In the case of colon carcinoma, a phase II combination therapy clinical trial of GDC-0449 plus bevacizumab (Avastin) failed because treatment did not extend the time from randomization to disease progression or death. A major caveat in the interpretation of the results of the initial clinical trials is that patients were not selected based on the presence of HH pathway activity in tumor tissue (Box 2). Previous experience with protein kinase inhibitors showed that therapeutic effects are often not detected without molecular stratification of patients. It is important to learn from these experiences by including tests that determine the suitability of patient populations for treatment with HH pathway inhibitors, as well as assays for monitoring the effect of treatment on the target at all stages of the drug development process[111].

Box 2

Personalizing HH-based therapies

One of the major challenges in the use of SMO inhibitors for treating cancer is how to identify the tumors that are capable of responding. Although genetic testing can reveal the presence of *PTCH1* mutations, this would only identify a subset of susceptible cases. Biomarkers for HH pathway activity can be employed, but this also identifies tumors with activating mutations in SMO, as well as those with lesions downstream of SMO, that would be resistant to treatment. Currently, RNA expression signatures are used as the primary biomarker, as there are no antibodies specific for HH pathway target genes that work in reliably immunohistochemistry assays[30]. However, additional biomarkers were recently proposed that could discriminate between medulloblastoma subsets[133, 134]. In the case of advanced BCC, molecular diagnostics is not a major issue as most tumors exhibit an activated HH pathway. However, it is a concern for medulloblastoma, in which only 30% of tumors have an activated HH pathway and only half of these have *PTCH1* mutations. In addition, different biomarkers will be needed to identify tumors in which HH signaling functions through stromal cells as the presence of HH ligands in these tumors is also not sufficient to predict responses[8]. The lack of appropriate biomarkers makes it challenging to develop robust criteria for stratification of patients with tumors other than BCC or medulloblastoma for treatment with SMO inhibitors. Therefore, it is important to revisit preclinical studies of SMO inhibitors in both genetic and xenograft models of these other tumors, to understand the mechanism of action and develop diagnostic markers.

Currently, seven compounds that bind and inhibit SMO are listed for use in a range of advanced cancers in more than 40 different clinical trials (Table 1). The most widely used compound is the first-in-class Vismodegib (GDC-0449), developed by Curis and Genentech[6, 92, 112].

In mammals, HH pathway activity depends on the primary cilia[113]. HH binding to PTCH1 allows SMO to translocate to the ciliary membrane where it relieves repression of GLI1 and GLI2 by SUFU. Although cyclopamine binding to SMO promotes translocation to the primary cilia through the intraflagellar transport pathway, it does not activate GLI1 or GLI2 [114, 115]. In contrast, the antagonists SANT-1 and SANT-2, which bind to the same site on SMO, do not promote cilial translocation of SMO[114, 115]. These findings imply that SMO has multiple conformations that could be exploited for drug development. Future research on the mechanisms responsible for SMO function and the downstream events leading to GLI activation will be particularly important if gain-of-function mutations in SMO occur frequently in drug-resistant tumors. At present, it is not clear which of the many SMO

inhibitors currently in drug development would be least affected by acquired mutations in SMO.

Side effects

SMO inhibitors have exhibited remarkably few and only relatively modest side effects in adult patients (Table 2)[6]. However, when given to young mice, for as little as 2–4 days, HhAntag caused dramatic and permanent defects in bone growth[116]. Short-term treatment of 10-day old mice resulted in malformation of the epiphysis and growth plate. The columnar organization of chondrocytes in the growth plate was disrupted, and the cartilage structure appeared dysplastic. These observations are consistent with the critical role the hedgehog pathway is known to have in bone development[117]. Deletion of Indian Hedgehog (*Ihh*) causes embryonic lethality in mice[118], conditional ablation results in a phenotype similar to that seen in mice treated with HhAntag[119], and hypomorphic mutations of *IHH* in humans cause acrocapitofemoral dysplasia [120]. The several roles that HH signaling has in development, including the postnatal formation of the cerebellum[121, 122], raise many concerns about potential side effects that may be seen in the youngest patients (Table 2).

Conclusions and perspectives

Given the dramatic responses reported in BCC and medulloblastoma in early trials[6], it is highly likely that SMO inhibitors will ultimately be approved as new therapeutic agents for treating cancer. This should be viewed as a success for basic, broad-based research in developmental biology, as well as cancer research, which laid a strong foundation for this translational opportunity. Credit should also be given to the many fruitful partnerships among investigators working in academia, biotechnology companies and in the pharmaceutical industry that brought these projects to fruition. However, there are many challenges ahead that illustrate common hurdles likely to be faced during development of other molecular targeted therapies for the treatment of cancer.

One major issue is that if SMO inhibitors were only effective in advanced BCC and a subset of medulloblastoma, the number of patients that could benefit from treatment would not provide an adequate return on investment from an economic perspective. Therefore, it is important to understand how these inhibitors could be used to treat other cancers, perhaps in combination with other therapies, which do not carry genetic lesions in the HH pathway. Similar problems may be encountered with other targeted therapies as genomic research continues to subdivide cancers into increasingly smaller molecular subtypes. The hope is that by understanding the mechanisms of action, and by proving efficacy of novel specific agents in rare genetically defined cancers, we will also learn how to treat the more prevalent forms. Clearly, improved understanding of cancer biology, particularly the interplay among cancer cells and stromal tissues, will help broaden the usefulness of such agents. The identification of reliable biomarkers, including non-invasive imaging approaches, will also be very important for selecting the patients who would benefit from treatment and to monitor responses to therapy.

Understanding resistance mechanisms and developing methods to overcome resistance to SMO inhibitors will also be important in the future. Currently, the patients being treated with SMO inhibitors in clinical trials have advanced disease, so they have already been treated with chemotherapy and in many cases radiation therapy. These mutagenic treatments increase the likelihood of developing resistance. In the case of BCC, early treatment, by surgical removal of lesions, is curative in the vast majority of cases. Therefore, it will be important to determine the circumstances in which treatment with a SMO inhibitor would be preferred over the surgical approach. In medulloblastoma, surgery is usually scheduled as soon as possible after diagnosis and is often followed with radiation and chemotherapy

treatment. However, in the future it may possible to identify and treat the HH pathway subtype of medulloblastoma with SMO inhibitors prior to these other interventions that, although often successful, can result in significant morbidity.

Glossary terms

Cancer stem cell hypothesis	This states that tumors harbor a subset of cells, sharing characteristics of normal stem cells that have a high capacity for self-renewal and an ability to differentiate into the many cell types that make up the bulk of the tumor mass
Acrocapitofemoral dysplasia	An autosomal recessive disorder associated with cone-shaped epiphyses in hands and hips caused by hypomorphic mutations in Indian Hedgehog (IHH)

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At a glance summary

The hedgehog (HH) pathway is an important regulator of embryogenesis and has also been implicated in tumor development. As all HH signaling through the canonical pathway requires smoothened (SMO), small molecules such as GDC-0449, which inhibit SMO function, completely block all HH pathway signaling regardless of the ligand.

Drugs based on cyclopamine and other compounds that target SMO have been developed and are currently in phase I and phase II clinical trials. Drugs that target other aspects of the HH signaling pathway are also in development.

Initial results suggest that SMO inhibitors will prove useful in the treatment of basal cell carcinoma and in the subtype of medulloblastoma that is dependent on HH signaling.

It is important to understand how HH inhibitors could be used to treat other cancers, perhaps in combination with other therapies, which do not carry genetic lesions in the HH pathway, but that rely on HH signaling for disease progression. Improved understanding of cancer biology, particularly the interplay among cancer cells and stromal tissues, will help broaden the usefulness of such agents.

The identification of reliable biomarkers that indicate patients most likely to benefit from HH inhibitors, including non-invasive imaging approaches, are essential.

Understanding resistance mechanisms and developing methods to overcome resistance to SMO inhibitors will also be important in the future.

The importance of HH pathways during development and studies in mice indicate that use of SMO inhibitors in children with medulloblastoma will need to be used with care, such that potential effects of skeletal and brain development are avoided.

Given the dramatic responses reported in BCC and medulloblastoma in early trials, it is highly likely that SMO inhibitors will ultimately be approved as new therapeutic agents for treating cancer. This should be viewed as a success for basic, broad-based research in developmental biology, as well as cancer research, which laid a strong foundation for this translational opportunity.

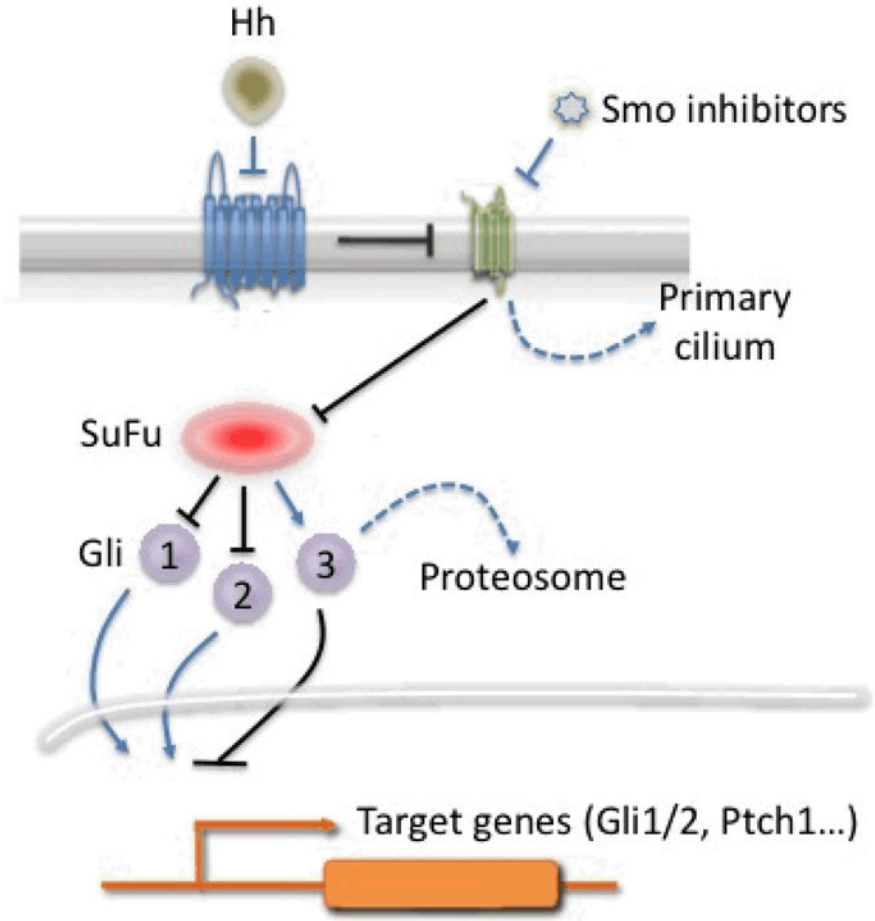

Figure 1. Summary of HH Signaling Pathway

Hedgehog (HH) ligands bind to Patched (PTCH1), causing internalization and degradation, and release the suppression of Smoothened (SMO) by PTCH1. SMO enters the primary cilia where it is activated. SMO then interacts with Suppressor-of-fused (SUFU), which, in turn, promotes activation and nuclear translocation of glioma associated oncogene homolog1 (GLI1) and GLI2 and degradation of GLI3.

Table 1

HH Pathway Inhibitors in Clinical Development[a]

Compound	Source	# Trials	Type
GDC-0449	Genentech	28	Phase I/II
LDE225	Novartis	5	Phase I/II
BMS-833923 (XL139)	BMS/Exelixis	5	Phase I/II
IPI-926	Infinity Pharm	2	Phase I/II
PF-04449913	Pfizer	2	Phase I
LEQ506	Novartis	1	Phase I
TAK-441	Millennium	1	Phase I

[a]Information obtained from http://clinicaltrials.gov

Table 2

Most Frequent Toxicities Associated with SMO Inhibitors[b]

Species and tissue	Effect
Mouse teeth	Reduced ameloblast progenitor population
	Loss of enamel from incisors
Mouse developing bone	Loss of proliferating chondrocytes
	Premature growth plate fusion
	Reduced thickness cortical bone
	Structural abnormalities in joints
Adult Humans	Muscle Spasms
	Dysgeusia (altered taste perception)
	Fatigue
	Alopecia (modest)
	Nausea

[b] Mouse data on HhAntag obtained from[116, 135] and human data on GDC-0449 obtained from[6]. No dose limiting toxicies were identified in the first reported clinical trial of GDC-0449[6].