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Smoothened Inhibitors in Cancer

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Abstract Smoothened (Smo) inhibitors are under intense development for the treatment of cancers linked to abnormal Hedgehog (Hh) signaling. The first inhibitor (vismodegib) was introduced in clinics for basal cell carcinoma and medulloblastomas associated with activating mutations of Hh signaling. In contrast, disappointing data are reported for cancers related to ligand overexpression. Here, we review recent preclinical and clinical data on the potential therapeutic importance of Smo and highlight the complexity of Smo pharmacology and its clinical implications.

Keywords Cancer stem cell, Hedgehog, Medulloblastoma, Resistance

Contents

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Abbreviations

7TM	7-Transmembrane domain
BCC	Basal cell carcinoma
Gli1–3	Glioma-associated oncogenes 1–3
GPCR	G-protein-coupled receptor
Hh	Hedgehog
PKA	Protein kinase A
Ptc	Patched
Smo	Smoothened
Sufu	Suppressor of fused

1 Introduction

Smoothened (Smo), a member of the G-protein-coupled receptor (GPCR) superfamily, is the main transducer of the Hedgehog (Hh) signaling pathway. This pathway is implicated in the maintenance of stem cells and tissue repair in the adult. However, aberrant control of this pathway is associated with tumorigenesis. Thus, intense academic and clinical research has focused on designing potent Smo inhibitors and determining their functionality for manipulating Smo activity in various cancers. A major breakthrough in the Hh field is the recent approval of Erivedge/vismodegib (GDC-0449, Genentech, Figure 1) by the FDA for treating metastatic basal cell carcinoma (BCC) and locally advanced BCC untreatable by surgery or radiation. Several clinical trials are underway in which Smo inhibitors are combined with other therapeutics for the treatment of a wide variety of solid tumors and blood malignancies [1–6]. Here, we discuss recent findings on Hh pathway activation and data from various clinical trials with Smo inhibitors targeted to blocking Hh signaling in cancer.

2 Smoothened, a Therapeutic Target for Cancer Therapy

2.1 Transduction of the Hedgehog Signal

In the absence of Hh ligands, the 12-pass transmembrane protein Patched (Ptc) negatively regulates Smo presumably via transporter-like activity. The binding of Hh to Ptc activates the canonical Hh signaling pathway by translocating Smo to the primary cilium. This initiates a complex signaling cascade mediated by the activation of the zinc finger transcription factors, glioma-associated oncogenes 1–3 (Gli1, Gli2, and Gli3), and translocation of their active forms to the nucleus leading to gene transcription [2, 7]. Interestingly, the primary cilium recently emerged as an important center for Hh pathway transduction in vertebrates (Figure 2). Trafficking

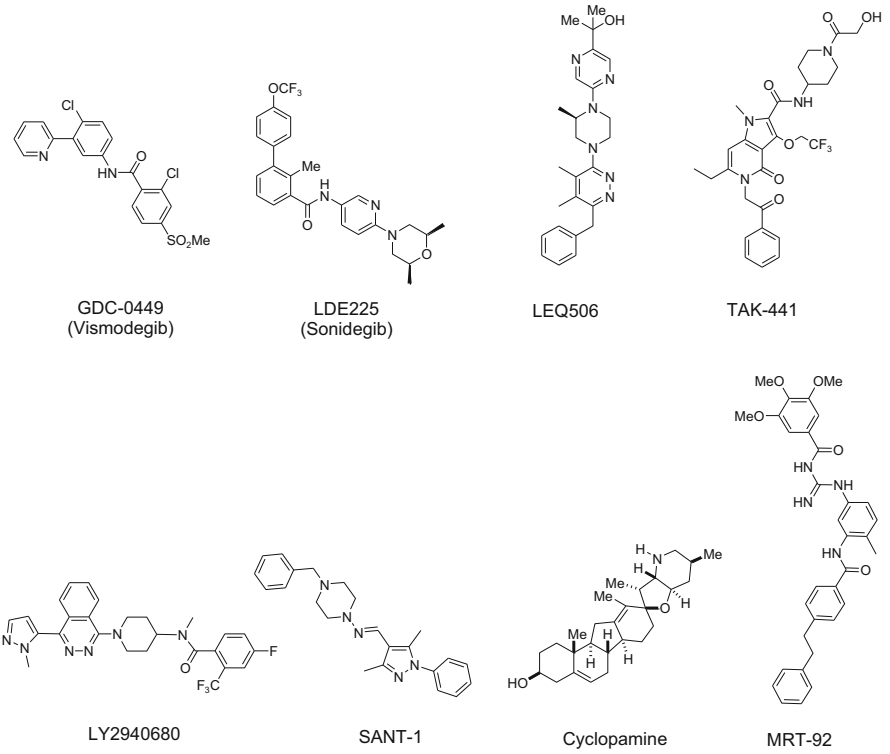

Fig. 1 Chemical structures of Smo antagonists

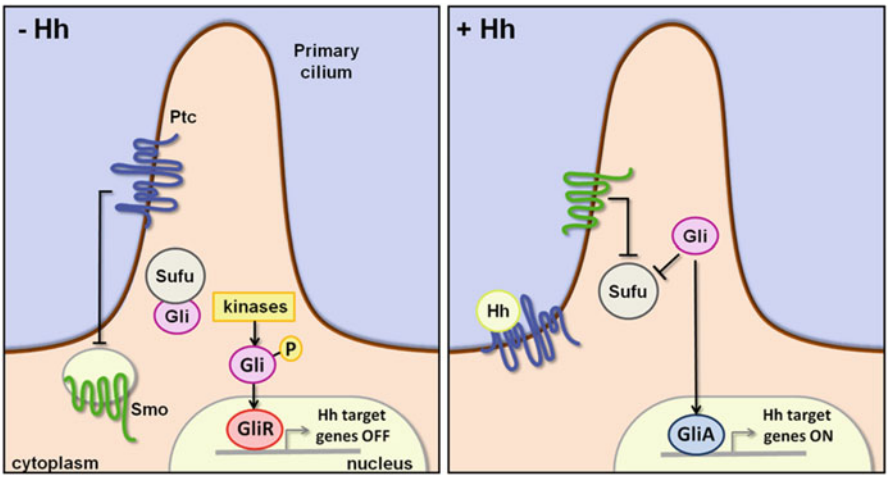

Fig. 2 Hedgehog signaling pathway at the primary cilium. In the absence of Hedgehog ligand (-Hh), the receptor Patched (Ptc), located in the cilium, inhibits Smoothened (Smo), a 7-transmembrane receptor mostly found outside the cilium, by a yet unknown mechanism. Repressor factors such as suppressor of fused (Sufu) and kinases, including protein kinase A

of Hh signaling proteins, along the cilia of stem and precursor cells, is the key step in the neural development of several genetic diseases and cancer (reviewed in [4, 8–10]).

2.2 Ligand-Independent Hh-Associated Cancers

The Hh signaling pathway is associated to cancer development due to the identification of germline loss-of-function *Ptc* mutations in patients with Gorlin syndrome (or nevoid basal cell carcinoma syndrome), an autosomal dominant disease [11]. These patients are predisposed to developing medulloblastoma, BCC, rhabdomyosarcoma, meningioma, as well as tumors localized to the jaw [1, 12, 13]. Somatic mutations of *Ptc* and *Smo* were identified in sporadic BCC and medulloblastomas [14–19]. Somatic gain-of-function mutations of *Smo* are also reported in meningiomas [20, 21] and are believed to increase tumorigenesis through the aberrant activation of Hh signaling [18, 22]. Similarly, somatic and germline mutations in *Suppressor of fused* (*Sufu*) are associated with medulloblastoma [23, 24]. The alteration of Hh signaling due to *Ptc* mutations was recently identified using an integrative deep-sequencing analysis of children with medulloblastoma [25–27]. Somatic *Ptc* mutations were also identified in ovarian and endometrial cancers, but their association with neoplasia requires further investigation [1].

Interestingly, *Ptc* heterozygous mice develop cerebellar tumors resembling human medulloblastoma. These tumor cells were used to develop a mouse model for investigating the potency of Smo inhibitors in blocking tumor progression [28]. The presence of primary cilia in specific variants of human medulloblastoma is also important from a therapeutic viewpoint. Ciliated medulloblastoma with high Hh signaling might be responsive to treatments that target the primary cilium [29].

Fig. 2 (continued) (PKA), promote Gli truncation and phosphorylation, respectively. These events lead to the generation of Gli repressor forms (GliR) and inhibition of Hh target genes. In the presence of Hh ligand (+Hh), Smo inhibition is relieved allowing its translocation and accumulation in the cilium. This leads to Sufu inhibition and Gli conversion into their activated forms (GliA). GliA enters the nucleus and activates transcription of Hh target genes including Ptc and Gli1

2.3 Ligand-Dependent Hh-Associated Cancers

Hh signaling is proposed to be responsible for the development of a variety of tumors through autocrine or paracrine ligand-dependent mechanisms. Secretion of one of the Hh peptides (Sonic, Indian, or Desert Hedgehog) from either the tumor or the stromal environment is implicated in the transformed phenotype. These tumors are called ligand dependent, and several Hh pathway activity models were discovered with therapeutic implications [3, 4, 30]. The pathway was also associated to blood malignancies. Several recent reviews describe the process of autocrine or paracrine Hh pathway activation in different cancers, in detail [1, 4, 28, 31]. Pharmacological treatment using Smo inhibitors (Figure 1) in mouse models of these cancers is also reported. However, conflicting views on the molecular mechanisms of action in tumor regression exist, including the potential off-target effects of some of these drugs [30].

2.4 Development of Smoothened Antagonists for Cancer Therapy

Cyclopamine, a natural and teratogenic alkaloid molecule, which can be purified from corn lilies, slows down tumor growth in animal models [32–35]. This molecule, which is well known for inducing cyclopia in newborn sheep, blocks canonical Hh signaling presumably by binding to the 7-transmembrane (7TM) domain of Smo [36, 37]. Cyclopamine was not developed for therapeutic use, but a more soluble and potent derivative (IPI-926, saridegib) has entered clinical trials for treating BCC and metastatic pancreatic cancer [38]. Several potent Smo inhibitors of different chemical classes were developed in recent years, both by academia and the pharmaceutical industry. Several of these molecules demonstrated efficacy in mouse xenografts, leading to clinical trials on a large range of metastatic and advanced cancers [1, 30, 32–35, 39, 40]. Data from five clinical trials suggest that Smo inhibitor side effects include hair loss, muscle spasms, taste disturbance, fatigue, nausea, and decrease in weight and appetite [1]. Although these side effects are often moderate, they may result in treatment interruption in patients.

This extensive research led to the recent approval of GDC-0449 for treating BCC and locally advanced BCC, untreatable by surgery and radiation [41, 42]. Vismodegib is now authorized in several countries including the Europe Union, Australia, and South Korea for treating metastatic and locally advanced BCC [5].

Smo inhibition by vismodegib blocks the transcription of tumor mediating genes associated to the Hh pathway [43, 44]. However, a patient with a metastatic form of medulloblastoma had a relapse after initially responding to the drug. This was due to an Smo mutation ($D473H^{6.55}$) in the sixth transmembrane domain that disrupted vismodegib binding [45]. Likewise, a mutation occurring at a homologous position

in mouse Smo was also observed in a vismodegib-resistant mouse model for medulloblastoma [46]. Furthermore, acquired resistance was also reported in BCC patients under vismodegib treatment [47–49]. Several Smo inhibitors such as sonidegib (LDE225, Novartis, Figure 1), BMS-833923, and saridegib are effective in BCC treatment and might be useful for treating Smo resistance. BCC patients, who had improved following vismodegib treatment, demonstrated limited benefit from saridegib. These results suggest an overlapping resistance mechanism, which is not yet investigated [38]. Analysis of resistance mechanisms in a medulloblastoma mouse model treated with LDE225 demonstrated activating Smo mutations, phosphatidylinositol 3-kinase upregulation, and *Gli2* amplification [50]. This suggests that besides resistance at the level of Smo itself, downstream Hh target gene amplification might also have clinical relevance. Thus, monitoring and establishing the resistance mechanisms associated with Smo inhibitor treatments in the ongoing trials will be important.

Sonidegib is currently in Phase III trials on medulloblastoma patients, selected for Smo inhibitor therapy based on a five-gene Hh signature [1]. Such an approach is expected to increase the number of patients positively responding to the treatment.

Smo inhibitors are being investigated in clinical trials on a variety of ligand-dependent tumors, but results are less successful (see also trial numbers NCT00822458, NCT01601184, NCT01239316, NCT01125800, NCT01208831, and NCT00880308, at *ClinicalTrials.gov*). Most of these trials are evaluating the effects of an Smo inhibitor with other therapeutic modalities. For example, treatment of metastatic pancreatic cancer patients with saridegib and gemcitabine revealed shorter median survival compared to those treated with gemcitabine alone [51]. Saridegib and TAK-441 have been discontinued, and negative results were obtained with vismodegib in patients with metastatic colorectal carcinoma and ovarian cancers [51–53]. It is important to understand the reasons for these failures, which might be linked to the mode of action of these compounds on Smo, Hh activation mechanisms in the tumor and trial design.

The antifungal compound itraconazole, an FDA-approved drug, inhibits Hh signaling and delays tumor growth, presumably by binding to hSmo at a site different from that of cyclopamine [54, 55]. This molecule is under investigation for BCC treatment [56].

3 Future Directions

X-ray structures of hSmo bound to several ligands have revealed two types of 7TM-directed antagonists: those binding mostly to extracellular loops (site 1, e.g., LY2940680) and those deeply penetrating the 7TM cavity (site 2, e.g., SANT-1). However, the existence of a third type of Smo antagonist was recently demonstrated. This class entirely fills the Smo binding cavity from the upper extracellular part to the lower cytoplasmic-proximal subpocket. One of these Smo inhibitors is

the acylguanidine, MRT-92, which was shown to inhibit the Hh canonical signaling pathway and rodent cerebellar granule cell proliferation induced by Hh pathway activation [55]. MRT-92 is one of the most potent Smo inhibitors known to date and displays low sensitivity to block the effects of Smo conformational states associated to noncanonical pathways [55, 57]. To better understand the failures of Smo inhibitors observed in the clinic, it is of utmost importance to relate the clinical efficacy of Smo antagonists to their binding mode and to check whether a highly potent type 3 antagonist like MRT-92 may confer some advantages over the existing type 1 or type 2 Smo antagonists.

The identification of canonical and noncanonical pathways mediated through Smo culminates with the hypothesis that Smo antagonists of one pathway can act as agonists in another pathway [2, 58, 59]. This is reminiscent of the signaling bias reported for an increasing number of molecules acting on GPCRs [60]. The recognition that several agonists do not stabilize the same active site, but rather unique active states of a given receptor, fits with most Smo modulator pharmacological data. Smo interacts with Gi family members, presumably to decrease cAMP levels [61, 62], and might be associated with multiple cellular signaling proteins [2]. Thus, it will be important to better understand Smo regulation by small molecules, biased signaling, and associated pathways, which should help identify potential therapeutic effects of Smo modulators.

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