Cellular and Molecular Life Sciences

REVIEW

CrossMark

Development of anticancer agents targeting the Hedgehog signaling

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Abstract Hedgehog signaling is an evolutionarily conserved pathway which is essential in embryonic and postnatal development as well as adult organ homeostasis. Abnormal regulation of Hedgehog signaling is implicated in many diseases including cancer. Consequently, substantial efforts have made in the past to develop potential therapeutic agents that specifically target the Hedgehog signaling for cancer treatment. Here, we review the therapeutic agents for inhibition of the Hedgehog signaling and their clinical advances in cancer treatment.

Introduction

Hedgehog (HH) signaling is an evolutionarily conserved pathway that is indispensable for developmental patterning and adult tissue homeostasis. HH signaling was first identified in *Drosophila Melanogaster* for its essential role in early embryo patterning, and subsequently has been reported in variety of vertebrates [1, 2]. In mammalians, three HH paralogues, Sonic Hedgehog (sHH), Indian Hedgehog (iHH), and Desert Hedgehog (dHH), have been reported, and each of them displays unique expression patterns and functions [3–5]. For instance, sHH is essential for correct formation of the limbs, phallus, somites, and neural tube in early embryogenesis [6–8], whereas iHH is restricted to chondrocyte development and dHH is limited to spermatogenesis and nerve sheath formation in Schwann cells [9–12]. All the three HH orthologues mediate the HH signaling in primary cilia of mammalian cells in a similar way. In the absence of the HH ligands, a 12-span transmembrane protein Patched (PTCH) is positioned in cilia and catalytically inhibits Smoothened (SMO), preventing SMO accumulation to cilia (Fig. 1). Full-length GLI (GLI2/3) proteins are then phosphorylated by protein kinase A (PKA), glycogen synthase kinase 3β (GSK3β), and casein kinase 1 (CK1), leading to proteolytic process to generate repressor GLI (GLI^R) for suppression of the HH target gene transcription [13–15] (Fig. 1). However, when extracellular HH ligands bind to PTCH, PTCH is displaced from cilia and inhibitory influence of PTCH on SMO is then removed. Activated SMO subsequently relocates to cilia to transduce the downstream signaling (Fig. 1). The complex of Suppressor of Fused (SUFU) and GLI is then disassociated within cilia, and the activated GLI proteins bypass proteolytic processing and translocate into nucleus to induce transcription of the HH target genes, including *GLI1, PTCH1, SNAIL*, and HH-interacting protein (*HHIP*), cyclin D1 (*CCND1*), c-Myc, BMI1 polycomb ring finger (*BMI1*) and B-cell CLL/lymphoma 2 (*BCL2*), etc [16–18]. SUFU is a negative regulator of the HH signaling by sequester GLI proteins in the cytoplasm to suppress GLI transcriptional activation [19, 20].

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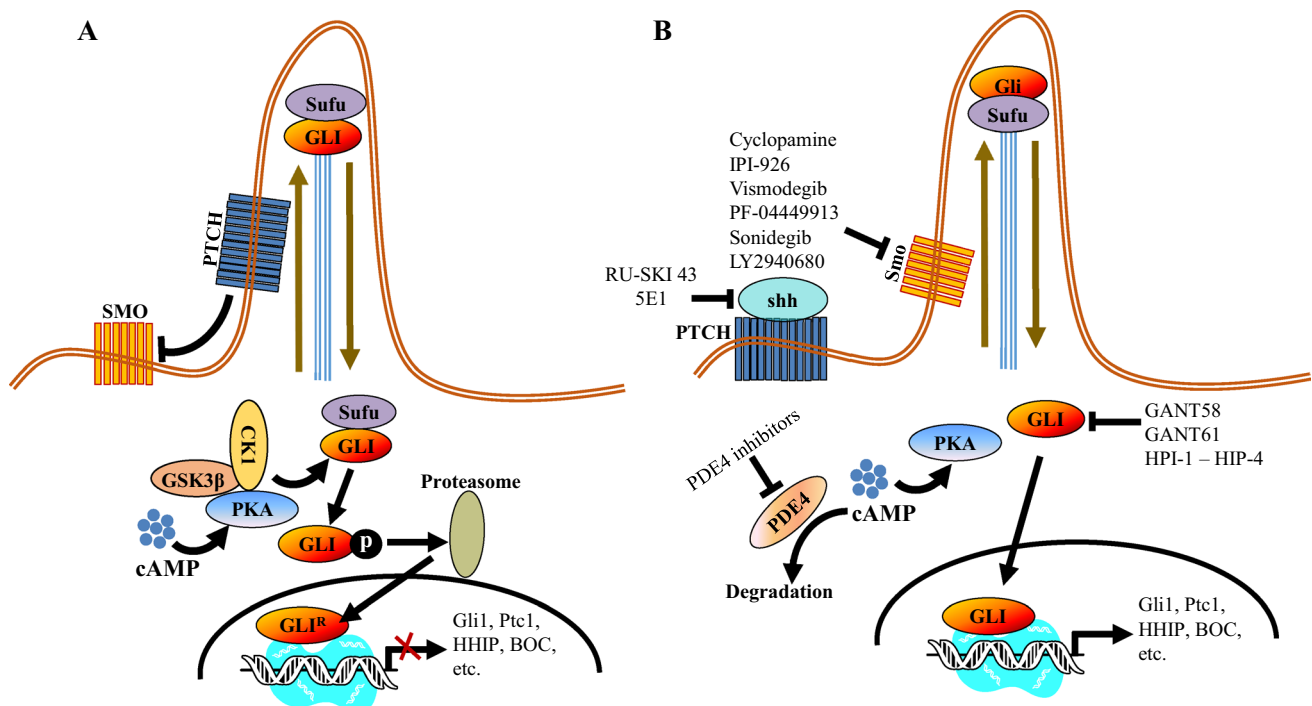
Fig. 1 Schematic representation of the HH signaling pathway and the oncology-indication drug candidates discussed in the paper. **a** In the absence of HH ligands, PTCH positioned in cilia prevents SMO accumulation in cilia and inhibits SMO activity. PKA, CK1 and GSK3β phosphorylate GLI2/3, which subsequently is proteolytically processed to an NH2-terminal truncated GLI2/3 repressor form for suppression of the HH signaling target gene expression. **b** In the presence of HH ligands, HH ligands bind and inactivate PTCH, and activated SMO then translocates to cilia, leading to disassociation of SUFU-GLI complex. The activated GLI 2/3 then translocates into the nucleus to induce the HH signaling target gene transcription

Abnormal activation of HH signaling is implicated in many types of cancer [21]. In addition, increasing evidence supports that the HH signaling also plays a critical role in maintaining "stemness" of cancer stem cells (CSCs), a subpopulation of tumor cells that are believed to account for tumor initiation, growth, and recurrence as well as drug resistance [22–27]. Thus, eradication of CSCs by targeting the HH signaling represents a potential effective therapeutic strategy for cancer, and significant efforts have been made in the past decades to develop HH signaling inhibitors. In this article, we review development of therapeutic agents targeting the HH pathway and their clinical advances in cancer treatment.

Hedgehog signaling pathway in cancer and cancer stem cells

Implication of HH signaling in cancer was first suggested in malignant glioma by identification of overexpression of HH target gene *GLI1* [28]. Further studies have led to proposal of three primary HH signaling models in cancer: ligand-independent mutation-driven signaling, ligand-dependent autocrine/juxtacrine signaling, and ligand-dependent

paracrine signaling [18, 21]. In the ligand-independent mutation-driven signaling model, PTCH inactivating mutations were identified in patients with basal cell nevus syndrome, a rare autosomal dominant disorder with a high risk of basal cell carcinoma, medulloblastoma and rhabdomyosarcoma [29–33]. Moreover, activating mutations in SMO or inactivating mutations in SuFu that can constitutively activate the HH signaling in the absence of HH ligands were also reported in sporadic basal cell carcinoma, chondrosarcoma, and medulloblastoma [34–38]. However, in the ligand-dependent autocrine/juxtacrine signaling model, elevated HH ligand expression in the tumor cells constitutively activates the HH pathway in themselves or the adjacent tumor cells to support tumor growth and survival [39–41]. This signaling model has been observed in many cancers, including lung cancer, esophagus cancer, digestive tract cancer, pancreas cancer, prostate cancer, breast cancer, and liver and brain cancer [23, 39–44]. In contrast, in the ligand-dependent paracrine signaling, tumor-produced HH ligands activate the HH signaling in the stromal microenvironment which then feeds back and contributes to tumor progression [45–47].

Recently, increasing evidence supports that CSCs, a small subset of cancer cells with capability of self-renewal

and differentiation into heterogeneous tumor cells, are responsible for tumor initiation, growth, and recurrence as well as drug resistance [48–50]. Further studies have supported that abnormal activation of HH signaling plays an essential role in CSC regulation and maintenance in various cancers including glioblastoma, lung squamous cell carcinoma, breast cancer, pancreatic adenocarcinoma, myeloma, and chronic myeloid leukemia (CML) [22–27]. For instance, activation of HH signaling enhanced multiple myeloma CSC expansion, whereas inhibition of the HH pathway effectively attenuated multiple myeloma CSC clonal expansion [51].

In summary, abnormal activation of HH signaling plays a critical role in tumorigenesis and CSC maintenance, and targeting the HH signaling pathway represents an important therapeutic strategy to treat cancer, and potentially disrupt CSCs' stemness and functions to improve overall cancer treatment outcomes.

Therapeutic agents targeting Hedgehog signaling

Given the critical roles of abnormal activation of the HH signaling in various types of cancer and CSCs, substantial efforts have been made to develop therapeutic agents to inhibit HH signaling by targeting various key components in the pathway cascade.

Target HH ligands

As the ligand-depend HH signaling activation is associated with various cancers, therapeutic reagents targeting HH ligands to inhibit dysregulated HH signaling activation have been highly sought after for cancer treatments. Monoclonal antibody 5E1, which blocks binding of all three mammalian HH orthologues to PTCH for HH signaling inhibition, was generated with mouse hybridoma using the rat SHH N-terminal domain as the antigen [52, 53]. In the preclinical studies, 5E1 has been shown to suppress growth of medulloblastoma and pancreatic tumors in mouse models, respectively [54, 55]. In addition to the biological antibody, a small molecule RUSKI-43 has also been developed to specifically target the hedgehog acyltransferase [56] (Table 1). Hedgehog acyltransferase is an essential enzyme for the SHH palmitoylation, a critical step to significantly enhance sHH ligand potency during sHH processing before it binds to the PCTH receptor [56–59]. RUSKI-43 was shown to reduce proliferation and anchorage-independent growth of breast cancer cells as well as inhibit pancreatic tumor growth in animal models [60, 61]. Nevertheless, a recent study has indicated that RUSKI-43 possesses cytotoxic activity unrelated to canonical sHH signaling and the authors also reported a preferred small molecule

RUSKI-201 which selectively inhibits catalytic function of Hedgehog acyltransferase [62]. To date, no clinical trials of 5E1, RUSKI-43, or RUSKI-201 have taken place yet.

Target SMO

SMO has been a primary target in development of the HH signaling inhibitors for decades. To date, numerous SMO inhibitors have been developed and two of them (Vismodegib and Sonidegib) have been approved by FDA for basal cell carcinoma (BCC) treatment (Table 1).

Cyclopamine and its derivatives

Cyclopamine isolated from lily *Veratrum Californicum* is an alkaloid targeting SMO for HH signaling inhibition [63, 64] (Table 1). Intensive preclinical studies have demonstrated that cyclopamine effectively inhibits growth of tumors, including human glioma, melanoma, colon, pancreatic, prostate cancers, small cell lung cancer, and medulloblastoma [40, 43, 65–67]. In addition, a topical cream containing cyclopamine was shown to regress human basal cell carcinomas [68]. Nevertheless, therapeutic potential of cyclopamine as an HH signaling inhibitor for human cancers was limited by its side effects, low solubility in normal saline, and other physiological solutions as well as instability under acidic conditions (human stomach environment) [69, 70]. To overcome these issues, several cyclopamine derivatives that display more-drug like properties have been developed, including KAAD-cyclopamine and IPI-926 (Saridegib) [64, 71, 72] (Table 1). Particularly, IPI-926 displays improved metabolic stability, pharmacokinetics, and potency over cyclopamine [73]. In vivo studies in animal models further demonstrated that IPI-926 effectively attenuated tumor growth in medulloblastoma, chondrosarcoma, and pancreatic cancer [71, 72, 74]. Subsequently, IPI-926 entered into clinical trials for various cancers, and it was shown well tolerated in clinical trials [75]. However, further Phase 2 trials of IPI-926 were terminated for patients with pancreatic cancer or myelofibrosis for their safety and poor clinical benefit.

Vismodegib (GDC-0449) and Sonidegib (LDE-225)

The SMO inhibitor, Vismodegib, is the first HH pathway inhibitor approved by FDA for treatment of metastatic BCC, or patients with recurrent, locally advanced BCC who are not candidates for surgery or radiation therapy [76, 77] (Table 1). Currently, clinical trials of vismodegib as a monotherapy or in combination with other therapeutic drugs are ongoing for various cancers, including medulloblastoma, metastatic pancreatic cancer, metastatic prostate cancer, intracranial meningioma, advanced head/neck

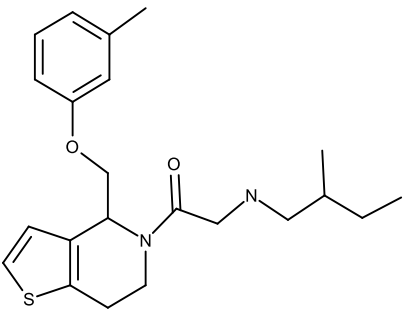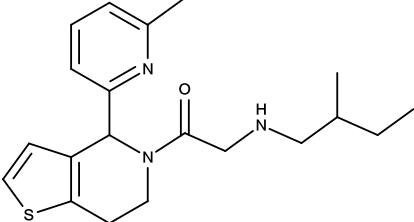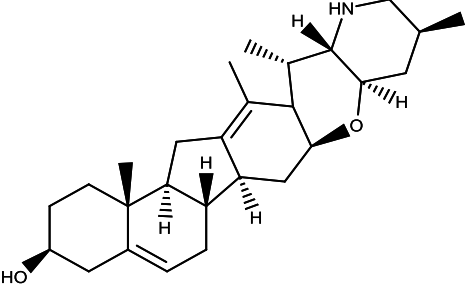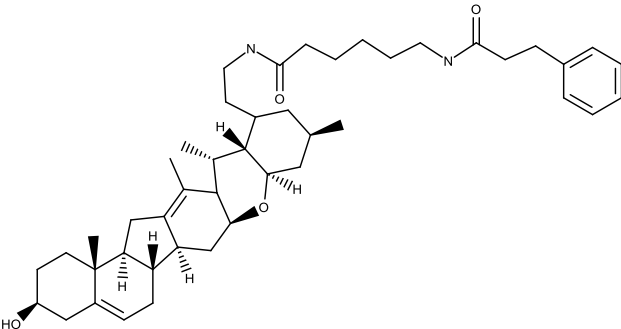**Table 1** Selected small molecules that inhibit the HH signaling
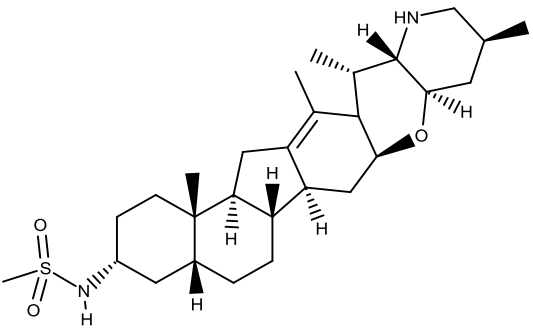
Structure	Name	Target	References
	RUSKI-43	SHH ligand	[56]
	RUSKI-201	SHH ligand	[62]
	Cyclopamine	SMO	[63]
	KAAD-cyclopamine	SMO	[64]
	IPI-926 (Saridegib)	SMO	[71]

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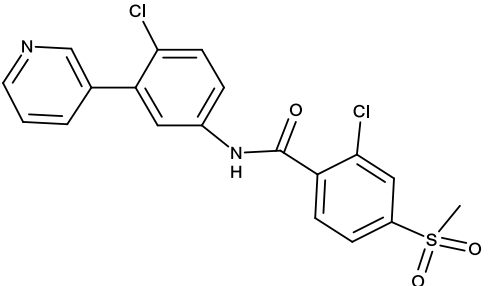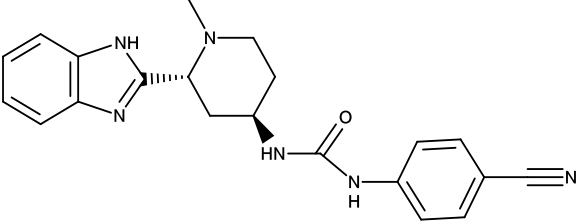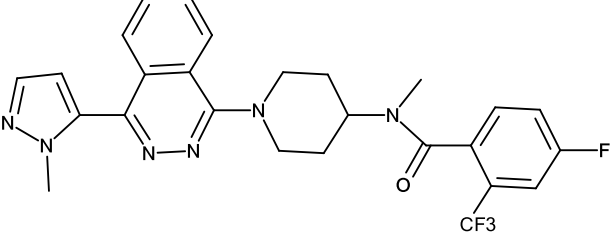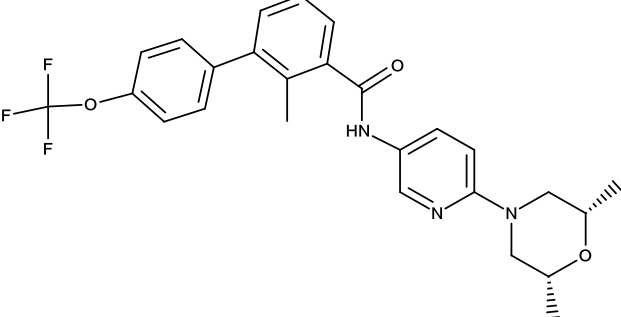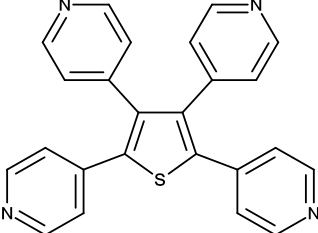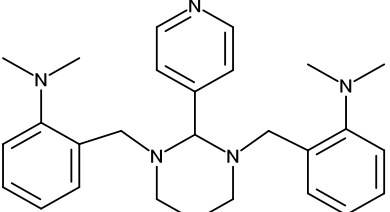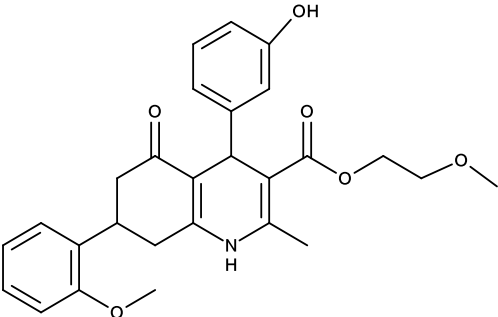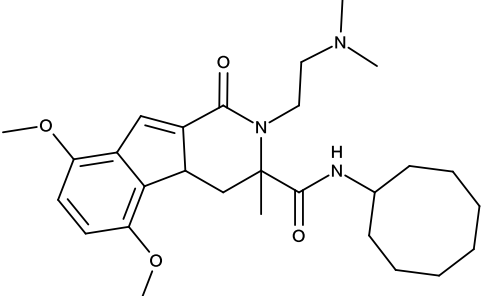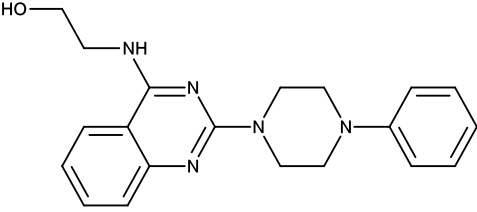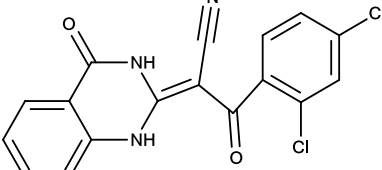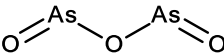
Structure	Name	Target	References
	Vismodegib (GDC-0449)	SMO	[76] [77]
	PF-04449913 (Glasdegib)	SMO	[81]
	LY2940680 (Taladegib)	SMO	[86] [87]
	Sonidegib (LDE-225)	SMO	[76] [77]
	GANT58	GLI	[92]
	GANT61	GLI	[92]

Table 1 (continued)

Structure	Name	Target	References
	HPI-1	GLI	[97]
	HPI-2	GLI	[97]
	HPI-3	GLI	[97]
	HPI-4	Ciliogenesis	[97]
	Arsenic trioxide	GLI	[98] [99]

basal cell carcinoma, recurrent glioblastoma, and acute myeloid leukemia [78]. In 2015, Sonidegib became the second SMO inhibitor approved by FDA to treat patients with locally recurrent advanced BCC following surgery or radiation therapy and those who are not candidates for surgery or radiation therapy (Table 1). Both Vismodegib and Sonidegib have displayed positive initial response in patients with BCC. In addition, clinical Phase I/II trials of Vismodegib and Sonidegib to treat solid tumors and hematological malignancies have been conducted or are underway [78, 79]. For instance, clinical Phase I study of Sonidegib demonstrated its acceptable safety profile and antitumor activity in patients with various malignancies, including medulloblastoma, lung cancer BCC and advanced solid tumor [80].

Other SMO inhibitors and clinical challenges of the SMO inhibitors

In addition, a number of other SMO inhibitors have been developed and entered to clinical trials including PF-04449913 (Glasdegib) and LY2940680 (Taladegib) (Table 1). For instance, Munchhof et al. reported an SMO inhibitor PF-04449913 which displays excellent potency and drug properties [81], and PF-04449913 was shown to attenuate the leukemia-initiation potential of acute myeloid leukemia cells in a serial transplantation mouse model [82]. The initial Phase I study of PF-04449913 supported its safety, tolerance, and potential efficacy in acute myeloid leukemia, myelodysplastic syndrome, myelofibrosis,

chronic myelomonocytic leukemia, and advanced solid tumors [83–85]. Subsequent phase II trials of PF-04449913 are underway for acute myeloid leukemia, high-risk myelodysplastic syndrome (NCT01546038), myelofibrosis previously treated with ruxolitinib (NCT02226172), and refractory/relapsed myelodysplastic syndrome or chronic myelomonocytic leukemia (NCT01842646). Moreover, LY2940680, another SMO inhibitor which binds to the extracellular end of the transmembrane-helix bundle of SMO for HH signaling inhibition, has been developed [86, 87]. Currently, LY2940680 is being tested in Phase I and Phase II trials for advanced solid tumors and esophageal cancers (NCT02530437).

Although development of SMO inhibitor-based drugs represents a great breakthrough in cancer treatments, they are also facing some formidable challenges. Other than their common clinical side effects, including fatigue, nausea, muscle cramps, and dysgeusia, therapeutic SMO inhibitors are often associated with the critical drug resistant problem [88–90]. For instance, Yauch et al. reported that medulloblastomas had a dramatic initial response to vismodegib, but subsequently acquired drug resistance through a new SMO mutation which can bypass the drug inhibition [88]. Similarly, two additional new SMO mutations that mediate resistance to vismodegib in BCC's patients have also been detected [89]. The fact that SMO quickly acquires oncogenic mutations for the drug resistance argues long-term benefits of SMO inhibitor-based drugs, supporting the idea that targeting downstream components of the HH signaling cascade may overcome drug resistance associated with the SMO inhibitors in cancer treatment [91].

Target GLI

GLI is a critical transcription factor positioned in very downstream of the HH signaling, and targeting GLI therefore represents an effective therapeutics to overcome the acquired drug resistance for the SMO inhibitors (Fig. 1). Consequently, efforts have been made to develop reagents targeting GLI for the HH pathway inhibition. For instance, Lauth and colleagues have identified two small molecule GLI inhibitors, GANT58 and GANT61 in a cell-based screen [92] (Table 1). Both GANT58 and GANT61 block GLI1 expressions, and particularly GANT61 appears to prevent DNA binding to GLI1 or destabilize the GLI1–DNA complex. In an in vivo xenograft studies, GANT61 showed strong attenuation for the growth of prostate cancer and rhabdomyosarcoma [92, 93]. Further studies also demonstrated that GANT61 robustly suppressed proliferation of colon cancer cells, ovarian cancer cells, and canine osteosarcoma cells [94–96]. Moreover, Hyman and colleagues identified a new class of HH pathway inhibitors termed HPI-1, HPI-2, HPI-3, and HPI-4 in a high-throughput screening [97] (Table 1). It has been shown that HPI-1 inhibits HH signaling through a mechanism that is potentiated by GLI phosphorylation, whereas both HPI-2 and HPI-3 block the conversion of full-length GLI 2 proteins into its active form for the HH signaling. Different from the rest of HIPs (HIP-1, HIP-2, and HIP-3), HPI-4 was shown to inhibit the HH signaling by disrupting ciliogenesis, an essential ciliary processes for GLI function in mammalians [97]. Finally, arsenic trioxide (ATO) is another GLI inhibitor which directly binds to GLI1 and GLI2 to inhibit GLI transcriptional activity, thus decreasing expression of endogenous GLI target genes [98, 99] (Table 1). ATO showed inhibitory activity in models of medulloblastoma and Ewing's sarcoma, osteosarcoma, acute promyelocytic leukemia, rhabdosarcoma, malignant pleural mesothelioma, prostate, and colon cancer cells [98–103]. In addition, ATO has been approved for treatment of acute promyelocytic leukemia [104], and currently, a few of clinical trials of ATO are underway for solid tumors and hematological malignancies.

Target phosphodiesterase-4 (PDE4)

PDE4 is an enzyme that specifically hydrolyzes cyclic AMP (cAMP), and PDE4 inhibitor-based dugs have previously been used to treat non-malignant diseases, such as depression, asthma, and pulmonary hypertension [105–107]. In addition, increasing evidence supports that PDE4 activation is also implicated in breast tumor, brain tumor, lung cancer and colorectal cancer, etc [108–111]. Nevertheless, the important role of PDE4 in the HH signaling was not recognized until very recently. In 2015, two groups demonstrated that PDE4 inhibition down-regulated the HH pathway to suppress the tumor growth [112, 113], suggesting that PDE4 may play a key role in the HH signaling. Subsequently, we elucidated mechanism of PDE4 inhibition for the HH signaling suppression by identification of Eggmanone (EGM), an extraordinarily selective PDE4 inhibitor in fibroblast cells and zebrafish models [114]. We showed that EGM specifically increases cAMP levels, resulting in activation of protein kinase K (PKA) which disrupts a process downstream of GLI ciliary trafficking for HH inhibition (Fig. 1) [114]. To date, despite some PDE4 inhibitor drugs have been approved by FDA, they are typically used for non-cancer treatments. For instance, Roflumilast was approved for treatment of severe chronic obstructive pulmonary disease and Otezla (apremilast) is used for treatments of active psoriatic arthritis and moderate-to-severe plaque psoriasis [115, 116]. Whether those PDE4 inhibitor drugs can benefit cancer patients remain unknown, and further clinical investigations are warranted. However, caution needs to be taken to target PDE4 for cancer treatment as PDE4 has four subtypes (PDE4A, PDE4B,

PDE4C, and PDE4D), and understanding the critical roles of PDE4 subtypes in cancer progression is essential to develop selective subtype-specific therapies in cancer treatments.

Conclusion

Abnormal activation of the HH signaling is involved in various types of cancer and CSCs. Therefore, targeting the key components of the HH pathway has become an important therapeutic strategy for cancer treatment. In the past few years, numerous HH signaling inhibitors, particularly by targeting SMO, have been developed, and two SMO inhibitors (Vismodegib and Sonidegib) have been approved by FDA for treatment of advanced or metastatic BCC. Despite their initial efficacies in cancer treatment, those SMO inhibitors are often associated with the drug resistant problem as cancer exposed to those SMO inhibitors can quickly acquire new SMO mutations to circumvent the drug inhibition for the HH signaling. Therefore, it has been proposed that targeting the downstream SMO in the HH signaling cascade may represent a valid anticancer therapeutic strategy. Consequently, a number of small molecules targeting GLI have been developed, and some of them have showed promising outcomes in both preclinical and clinical studies. In addition, the important role of PDE4 in the HH signaling pathway has recently been recognized, and the PDE4 may represent a promising new target to inhibit the HH signaling for cancer treatment.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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