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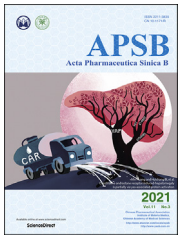

REVIEW

Hedgehog signaling in gastrointestinal carcinogenesis and the gastrointestinal tumor microenvironment

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Abstract The Hedgehog (HH) signaling pathway plays important roles in gastrointestinal carcinogenesis and the gastrointestinal tumor microenvironment (TME). Aberrant HH signaling activation may accelerate the growth of gastrointestinal tumors and lead to tumor immune tolerance and drug resistance. The interaction between HH signaling and the TME is intimately involved in these processes, for example, tumor growth, tumor immune tolerance, inflammation, and drug resistance. Evidence indicates that inflammatory factors in the TME, such as interleukin 6 (IL-6) and interferon-γ (IFN-γ), macrophages, and T cell-dependent immune responses, play a vital role in tumor growth by affecting the HH signaling pathway. Moreover, inhibition of proliferating cancer-associated fibroblasts (CAFs) and inflammatory factors can normalize the TME by suppressing HH signaling. Furthermore, aberrant HH

Abbreviations: 5-Fu, 5-fluorouracil; ALK5, TGF-β receptor I kinase; ATO, arsenic trioxide; BCC, basal cell carcinoma; BCL-2, B cell lymphoma 2; BMI-1, B cell-specific moloney murine leukemia virus insertion region-1; CAFs, cancer-associated fibroblasts; ch5E1, chimeric monoclonal antibody 5E1; CSCs, cancer stem cells; DHH, Desert Hedgehog; EGF, epidermal growth factor; FOLFOX, oxaliplatin; and leucovorin, GLI; glioma-associated oncogene homologue, GRK2; G protein coupled receptor kinase 2, HH; Hedgehog, HIF-1α; hypoxia-inducible factor 1α, IFN-γ: interferon-γ; IHH, Indian Hedgehog; IL-10/6, interleukin 10/6; ITCH, itchy E3 ubiquitin ligase; MDSCs, myeloid-derived suppressor cells; NK, natural killer; NOX4, NADPH Oxidase 4; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; PKA, protein kinase A; PTCH, Patched; ROS, reactive oxygen species; SHH, Sonic Hedgehog; SMAD3, mothers against decapentaplegic homolog 3; SMO, Smoothened; SNF5, sucrose non-fermenting 5; STAT3, signal transducer and activator of transcription 3; SUFU, Suppressor of Fused; TAMs, tumor-related macrophages; TGF-β, transforming growth factor β; TME, tumor microenvironment; VEGF, vascular endothelial growth factor; WNT, Wingless/Integrated; βArr2, β-arrestin2.

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signaling activation is favorable to both the proliferation of cancer stem cells (CSCs) and the drug resistance of gastrointestinal tumors. This review discusses the current understanding of the role and mechanism of aberrant HH signaling activation in gastrointestinal carcinogenesis, the gastrointestinal TME, tumor immune tolerance and drug resistance and highlights the underlying therapeutic opportunities.

1. Introduction

Hedgehog (HH) molecules are the key modulators that regulate diverse processes ranging from tissue patterning and cell differentiation to cancer initiation, progression, and metastasis[1]. In the gastrointestinal tract, HH signaling is extensively involved in gastrointestinal organogenesis and cancer development, especially in regulating the tumor microenvironment (TME)[2–4]. The gastrointestinal tract develops from the embryonic gut, which consists of an endoderm-derived epithelium surrounded by cells of mesodermal origin[5]. Normal activation of HH signaling during embryonic development has been documented to be related to the development of the gastrointestinal tract[6]. In the early stage, the widespread activation of Indian Hedgehog (IHH) and Sonic Hedgehog (SHH) in the endoderm is essential for the development of the gastrointestinal tract. In the late stage, changes in the expression of IHH and SHH promote the differentiation of gastrointestinal tract cells towards different lineages. In addition to its critical roles in normal gastrointestinal development, HH signaling, when aberrantly activated, is involved in the development, progression, metastasis, and drug resistance of gastrointestinal cancers[7].

The canonical HH signaling pathway is tightly regulated by a complex signaling network (Fig. 1). Following the binding of HH ligands [including IHH, SHH, and Desert Hedgehog (DHH) molecules] to their receptor Patched (PTCH), Smoothened (SMO) is derepressed to activate glioma-associated oncogene homologue (GLI) signal transduction and promote the transcription of downstream HH-related genes[8–14]. Among the GLI family of transcription factors (GLI1, GLI2 and GLI3), GLI1 is well known as an exclusively full-length transcriptional activator. Its activity is regulated by the Suppressor of Fused (SUFU) protein, vital suppressor of the HH signaling pathway[15,16]. In the absence of ligand binding, SUFU directly prevents the translocation of GLI proteins from the cytoplasm to the nucleus and thus inhibits HH signal transduction[2,17,18]. In addition, kinesin-like protein KIF7 is involved in the processing of GLI molecules[19]. The function of SMO requires the presence of β-arrestin2 (βArr2) and G protein-coupled receptor kinase 2 (GRK2)[20,21]. Other negative regulators of GLI molecules include RAB23, protein kinase A (PKA), SUFU, sucrose non-fermenting 5 (SNF5), β-TRCP, the itchy E3 ubiquitin ligase (ITCH), and so on[16,22–25]. Moreover, the HH pathway interacts with other signaling pathways, such as the transforming growth factor β (TGF-β) pathway, epidermal growth factor (EGF) pathway and Wingless/Integrated (WNT) pathway (Fig. 1)[26,27].

Recently, aberrant activation of the HH signaling pathway has been reported to play vital roles in both the carcinogenesis and pathogenesis of gastrointestinal tumors[2,6,28]. In addition, the role of HH activation in cancer stem cells (CSCs) is one of the mechanisms that lead to drug resistance in gastrointestinal tumors[29]. In this review, we aim to review the current understanding of the roles and mechanisms of aberrant HH signaling activation in gastrointestinal carcinogenesis and the gastrointestinal TME and to highlight the underlying therapeutic insights.

2. HH signaling in gastrointestinal tract carcinogenesis

In tumor tissues, aberrant activation of HH signaling can be observed in both cancer cells and the surrounding stroma. In cancer cells, HH ligand overexpression or mutations in HH signaling-related genes (SMO, PTCH1, GLI1 and GLI2) are involved in the pathogenesis of many epithelial cancers, including stomach, liver, esophageal, breast, and skin cancers[68,69]. In stromal cells, HH signaling activation favors the formation of an immunosuppressive environment to support tumor development and progression[70,71]. HH inhibitors can exert cytotoxic effects by directly targeting HH signaling activity in cancer cells and inhibit tumor growth[72]. The following sections discuss the role and mechanism of HH signaling activation in gastrointestinal cancers.

2.1. *The role of HH signaling in gastrointestinal carcinogenesis*

Aberrant HH signaling activation is associated with a variety of gastrointestinal diseases, including Pallister−Hall syndrome, gut malrotation, and gastric cancer[6]. Aberrant activation of HH signaling can promote the proliferation, metastasis, and drug resistance of gastric cancer cells and inhibit their apoptosis[7]. In gastritis, activation of HH signaling is required for the transformation of infiltrating myeloid cells to myeloid-derived suppressor cells (MDSCs), which help transformed cells evade immune surveillance, indicating the essential role of HH signaling activation in cancer development[73,74]. During *Helicobacter pylori* infection, the SHH ligand originating from gastric parietal cells is ectopically expressed to induce metaplasia, which can eventually lead to tumorigenesis[75,76]. In gastrointestinal cancer, the expression of SHH and IHH signaling pathway components and their target genes is upregulated[77,78]. Specifically, the expression of GLI1 is usually correlated with the degree of malignancy of gastric cancer, especially with lymph node metastasis[79].

In addition, aberrantly activated HH signaling interacts with signaling in immune checkpoint pathways to favor gastric cancer development. A recent study indicated that during *H. pylori* infection, the bacteria induce programmed cell death ligand-1 (PD-L1) expression on gastric epithelial cells and that this process is dependent on the HH signaling pathway, suggesting an interaction between HH signaling and this immune checkpoint pathway during tumor development[80]. This interaction is also observed in cancer cells. HH signaling activation has been

reported to induce the expression of PD-L1 on gastric cancer cells and thus help cancer cells evade immune surveillance, suggesting the potential immunosuppressive and the carcinogenic effects of aberrant HH signaling activation on gastrointestinal cancer[63].

2.2. *HH signaling and gastrointestinal CSCs*

Aberrant activation of the HH signaling pathway is involved in the growth and regulation of CSCs in gastrointestinal cancer. Upregulation of SHH and GLI1 is crucial for $CD44^+$ gastrointestinal CSCs to maintain their stem-like phenotype and malignant transformation ability, and the role of HH signaling activation in gastrointestinal CSCs has been observed in many studies[29,81–86]. For example, genistein can inhibit the activity of highly migratory $CD44^+$ cells by downregulating GLI1 expression. This study showed that GLI1 inhibition can attenuate the cancer stem-like properties of gastric cancer cells and thus reduce their invasive ability[84]. Upregulation of GLI2A, an activator of GLI2, rapidly accelerates gastric cancer development from $Lgr5^+$ CSCs[87]. Activation of GLI1 through the integrin $\alpha v\beta 3$/ERK1/2 pathway is reported to be crucial for maintaining the stem-like phenotype

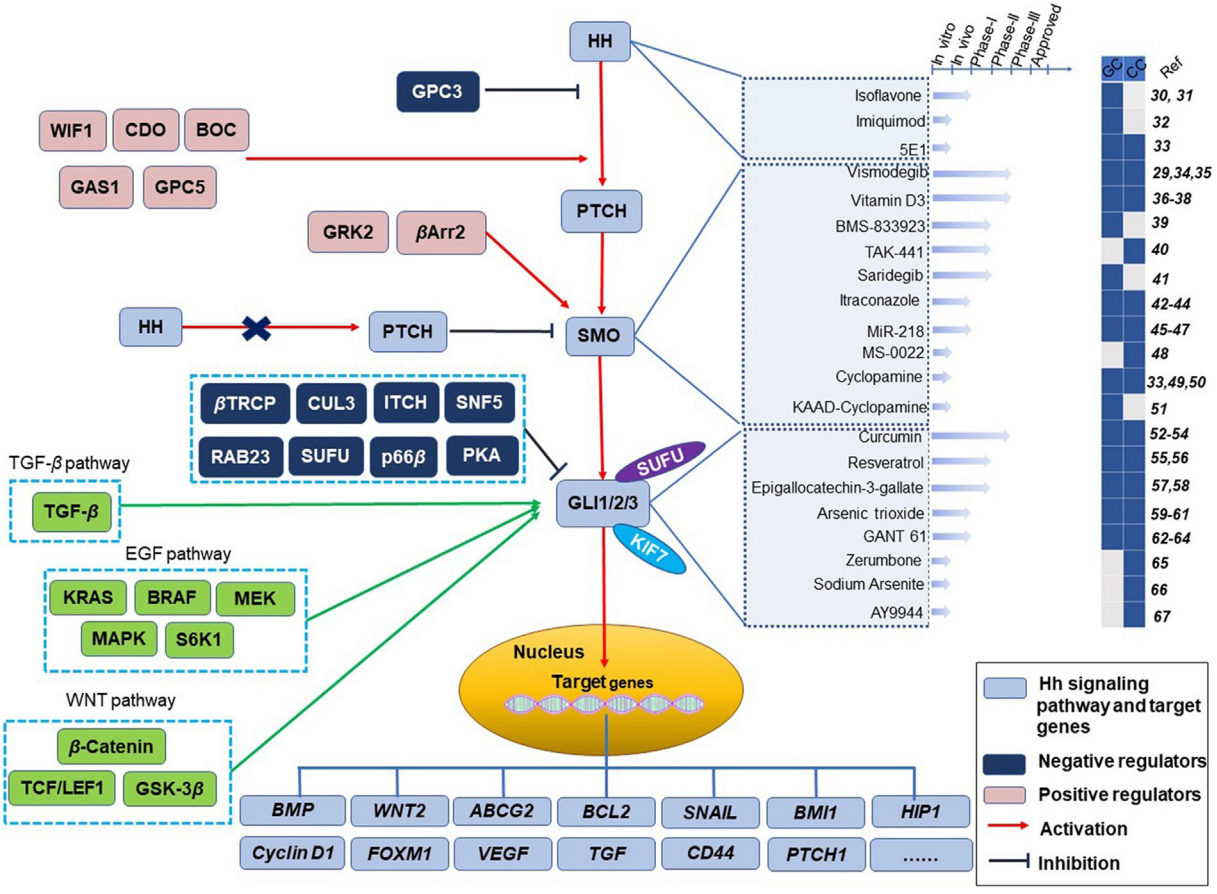

Figure 1 The Hedgehog (HH) signaling pathway and its related regulators, target genes and current therapeutic landscape. The coreceptors for HH include cell adhesion molecule-related/downregulated by oncogenes (CDO), brother of CDO (BOC), growth arrest-specific 1 (GAS1), glypican 3 (GPC3) and glypican 5 (GPC5). Wingless/Integrated (WNT) inhibitory factor-1 (WIF1) affects HH signaling *via* CDO, BOC or GPC5. The function of Smoothened (SMO) needs β-arrestin 2 (βArr2) and G protein-coupled receptor kinase 2 (GRK2). Suppressor of Fused (SUFU)/KIF7 is involved in the processing of glioma-associated oncogene homologue (GLI) molecules. Other negative regulators of GLI molecules include RAB23, protein kinase A (PKA), SUFU, sucrose non-fermenting 5 (SNF5), cullin-3 (CUL3), p66β, β-TRCP and Itch. Patched (PTCH) is shuttled out of the cilium and cannot inhibit SMO in the presence of HH, and HH binding promotes a conformational change in SMO. However, PTCH inhibits SMO signaling independent of HH ligand binding. The pathways interacting with the HH pathway, including the transforming growth factor β (TGF-β) pathway, epidermal growth factor (EGF) pathway and WNT pathway, are shown in green. HH inhibitors that have been effective in treating gastrointestinal tumors include isoflavone[30,31], imiquimod[32] and 5E1[33]. SMO inhibitors that have been effective in treating gastrointestinal tumors include vismodegib[29,34,35], vitamin D3[36–38], BMS-833923[39], TAK-441[40], saridegib[41], itraconazole[42–44], miR-218[45–47], MS-0022[48], cyclopamine[33,49,50] and KAAD-cyclopamine[51]. GLI inhibitors that have been effective in treating gastrointestinal tumors include curcumin[52–54], resveratrol[55,56], epigallocatechin-3-gallatel[57,58], arsenic trioxide[59–61], GANT 61[62–64], zerumbone[65], sodium arsenite[66] and AY9944[67]. Dark blue represents the type of tumor that the inhibitor is effective in. The arrows indicate progress. CC, colorectal cancer; GC, gastric cancer; *BMP*, bone morphogenetic protein; *FOXM1*, forkhead box protein M1; *VEGF*, vascular endothelial growth factor; MAPK, mitogen-activated protein kinase; TCF/LEF1, T-cell factor/lymphoid enhancer factor1; GSK-3β, glycogen synthase kinase-3β; *HIP1*, Huntingtin-interacting protein 1; *BMI-1*, B cell-specific Moloney murine leukemia virus insertion region-1; Ref, reference.

during gastric cancer metastasis[79]. In addition, GLI1 is co-expressed with CSC markers such as SOX9 and CD133 in tissues of colorectal adenocarcinoma patients, and inhibiting GLI1 expression can reduce the expression of these markers in gastric cancer cells, suggesting an important role of GLI1 in colorectal adenocarcinoma stemness[88,89]. Whole-transcriptome analysis revealed that the expression levels of both noncanonical PTCH1-dependent and SHH-dependent HH signaling components are upregulated in colorectal CSCs, and the expression levels of both PTCH1 and GLI1 are positively correlated with colorectal cancer stemness[90]. Moreover, other studies have shown that HH signaling activation affects the expression of CSC-related markers, such as B cell-specific Moloney murine leukemia virus insertion region-1 (BMI1), SNAIL, B cell lymphoma 2 (BCL-2), WNT2, and CD44 (Fig. 1)[29,81,91,92].

In summary, it is reasonable to believe that upregulation of SHH, GLI1 and PTCH1 can maintain the stem-like phenotype and malignant transformation ability of gastrointestinal CSCs.

2.3. *HH signaling and drug resistance in gastrointestinal cancer*

Activation of HH signaling is intimately involved in drug resistance in gastrointestinal cancer. A series of studies demonstrated that inhibition of GLI using pharmacological inhibitors (GANT61 and GDC-0449) or knockdown of GLI1/SHH can induce apoptosis and enhance the antitumor effect of chemotherapy in xenograft models[85]. GLI can modulate ABCG2 expression through transcriptional regulation to maintain 5-fluorouracil (5-Fu) resistance[92–95]. In addition, another recent study proposed that GLI2 is activated to promote chemoresistance *via* hypoxia-inducible factor (HIF-1α) and TGF-β2 in the hypoxic TME of colorectal cancer and that high expression levels of GLI2 are significantly related to post chemotherapy recurrence in colorectal cancer patients. These results suggest that GLI2 could be a biomarker for drug resistance in colorectal cancer[96]. Furthermore, evidence from a phase II clinical study indicates that vismodegib, an HH inhibitor, may reduce resistance to 5-Fu, oxaliplatin, and leucovorin (FOLFOX) therapy in advanced gastric cancers with high CD44 expression[29]. AY9944 and GANT61, two other HH inhibitors, can downregulate the expression of CSC markers (c-MYC, CD44, and Nanog) and significantly reduce resistance to 5-Fu and irinotecan in colorectal cancer[67,97]. Finally, the cooperative action of cyclopamine and paclitaxel has shown significant therapeutic effects on the enhancement of PD-1 checkpoint blockade treatment in solid tumors by augmenting $CD8^+$ T cell infiltration, suggesting that HH inhibitors can overcome resistance to immunotherapeutic drugs[98].

In conclusion, drug resistance resulting from HH signaling activation may be mediated by CSCs, and inhibiting HH signaling in CSCs can contribute to reducing drug resistance and enhancing the antitumor effect of chemotherapy or immunotherapy in gastrointestinal tumors.

3. HH signaling in the gastrointestinal TME

3.1. *The gastrointestinal TME*

The TME comprises various types of nontransformed cells (*e.g.*, endothelial cells, fibroblasts, and immune cells) and extracellular components (*e.g.*, inflammatory cytokines, various growth factors, and the extracellular matrix) that surround tumor cells and include a complex vascular network[99–101]. Accumulating evidence indicates that aberrant activation of the HH signaling pathway contributes to immune evasion and cancer development by modulating the TME. HIF-1α and TGF-β2 secreted by cancer-associated fibroblasts (CAFs), a main component of the TME, have been reported to upregulate the expression of GLI2 in CSCs, resulting in increased stemness/dedifferentiation and drug resistance[96]. Moreover, aberrant activation of HH signaling can affect many genes or cytokines, such as vascular endothelial growth factor (VEGF), angiogen-1, and angiogen-2 in endothelial cells; interleukin 6 (IL-6) in myofibroblasts; and BMI-1 and NANOG in CSCs. In fact, these genes or cytokines can promote tumor growth by maintaining an immunosuppressive environment[102–104]. Inhibition of HH pathway signaling reduces the solid tumor mass by eliminating tumor cells and stromal cells, indicating the important role of HH signaling in the TME[105].

3.2. *The interaction between HH signaling and CAFs promotes tumor proliferation*

CAFs belong to the intermediate mesenchymal cell population in solid tumors, with morphological and some functional characteristics like those of fibroblasts in normal tissues[106]. However, CAFs in the TME are also significantly involved in tumor development and progression by promoting the proliferation of tumor cells and assisting theirs to escape from immune killing[107]. Aberrant activated HH signaling and CAFs interact with each other and cooperatively promote tumor development[108]. On the one hand, tumor cell-derived SHH has been confirmed to modulate CAFs *via* paracrine activation of HH signaling in solid cancers, and inhibition of HH signaling by vismodegib remodels the TME by reducing the proliferation of CAFs[71,109]. Moreover, studies have shown that overexpression of SMO in CAFs contributes to HH signal transduction and GLI1 activation, a possible mechanism underlying paracrine activation of HH signaling in solid cancers[110]. In addition, other studies have shown that the HH inhibitors cyclopamine and vismodegib can decrease the population of stroma-producing CAFs in tumors[71,111]. Therefore, inhibition of CAF proliferation in tumors by HH signaling antagonists contributes to normalizing the TME, which in turn helps to overcome gastric cancer.

On the other hand, CAFs, which are the major source of proinflammatory cytokines in the TME, can produce tumor-associated cytokines such as IL-6, HIF-1α and TGF-β2 in gastrointestinal cancer. These cytokines can regulate SHH expression during tumor transformation and ultimately accelerate tumor development[96,112–116]. IL-6 has been found to promote carcinogenic HH/GLI signaling through signal transducer and activator of transcription 3 (STAT3) activation[117,118]. Other studies have shown that exosomal SHH derived from CAFs facilitates the proliferation and malignant progression of tumor cells[119]. Thus, aberrant activation of HH signaling can further promote the inflammatory response and change the microenvironment by increasing the levels of tumor-related inflammatory factors. Furthermore, CAFs from colorectal tumors can inhibit the activity of natural killer (NK) cells and the secretion interferon-γ (IFN-γ), which is related to their proinflammatory response to immune tolerance in the TME[120].

Therefore, it is reasonable to conclude that the crosstalk between CAFs and gastrointestinal tumors *via* aberrant activation of the HH signaling pathway is one of the factors that promote tumor

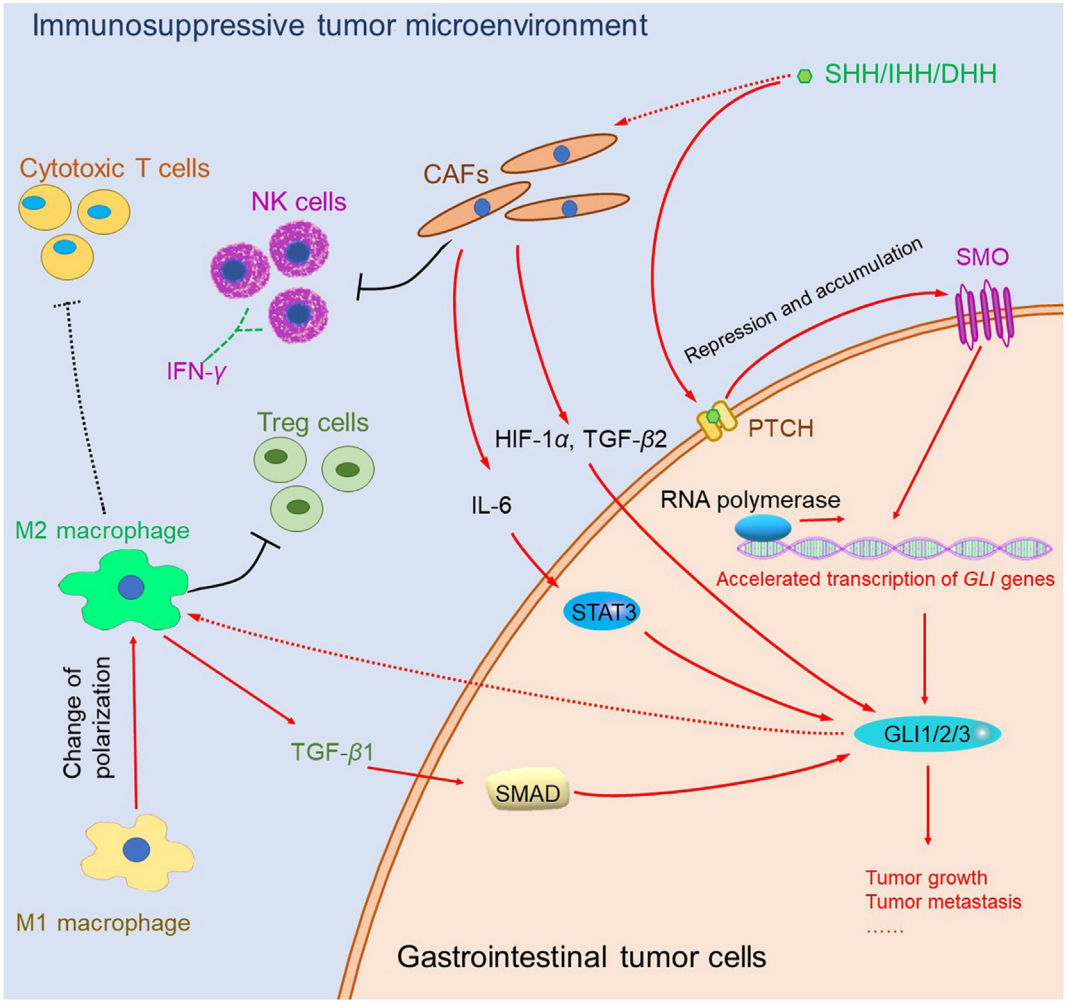

Figure 2 Interaction between Hedgehog (HH) signaling and the tumor microenvironment in gastrointestinal tumor cells. Red arrow: upregulation; black arrow: downregulation; green dashed arrow: secretion; solid line: direct interaction; dashed line: indirect interaction. CAFs, cancer-associated fibroblasts; DHH, Desert Hedgehog; IHH, Indian Hedgehog; SHH, Sonic Hedgehog; NK cells, natural killer cells; PTCH, Patched; SMO, Smoothened; TGF-β, Transforming growth factor β; IL-6, interleukin 6; IFN-γ, interferon γ; HIF-1α, hypoxia-inducible factor 1α; RNA, ribonucleic acid; STAT3, signal transducer and activator of transcription 3; SMAD, Drosophila mothers against decapentaplegic protein; GLI, glioma-associated oncogene homologue.

development, and strategies targeting CAF-tumor crosstalk *via* the HH signaling pathway may provide alternative approaches for overcoming gastric cancer.

3.3. The interaction among HH signaling, tumor-associated macrophages (TAMs), and immune cells promotes tumor immune tolerance

TAMs are divided mainly into the proinflammatory M1 subtype and the anti-inflammatory M2 subtype. Studies show that tumors with high expression of HH exhibit upregulation of inflammation-related genes and increased infiltration of TAMs, which may promote tumor growth by affecting the immune response[121]. On the one hand, activation of HH signaling can mediate the interaction between cancer cells and macrophages and stimulate selective polarization of macrophages towards M2 subtype[122]. Among the TAM subtypes, M2 TAMs can activate HH signaling by secreting interleukin 10 (IL-10) and TGF-β1 to regulate the immune response and facilitate the malignant progression of gastric cancer cells, and TGF-β-mediated HH signaling is activated *via* the TGF-β receptor I kinase (ALK5)/mothers against decapentaplegic homolog 3 (SMAD3) pathway[51,123]. In addition, the activation of HH signaling can induce the recruitment of M2 subtype to upregulate FOXP3 expression and increase Treg cells[124–127].

On the other hand, HH-induced M2 polarization can inhibit the recruitment of $CD8^+$ T cells and enhance the infiltration of suppressive regulatory T cells by inhibiting the production of CXCL9 and CXCL10 by TAMs, leading to TAM-mediated immunosuppression[98,122]. In addition, MDSCs, among other factors, contribute to tumor immunosuppression[128]. Activation of HH signaling can enhance the infiltration of MDSCs into the

TME, which further leads to tumor immunosuppression[129]. Studies have shown that overexpression of SHH in *H. pylori*-infected mice accelerates the emergence of MDSCs in the gastric corpus[73]. This immunosuppressive effect was confirmed by upregulation of PD-L1, which inactivates effector T cell function and allows the proliferation of gastric cancer cells[63,130]. Furthermore, inhibition of HH signaling with vismodegib led to the accumulation of cytotoxic T cells and decreases in the population of immunosuppressive M2 macrophages and MDSCs in the TME[70,131–134].

In summary, the above evidence indicates that TAMs affect the HH signaling pathway by secreting cytokines and that HH-induced M2 polarization can inhibit the accumulation of cytotoxic T cells and increase Treg cells. The overall effect of these events is the promotion of tumor growth *via* suppression of the immune response in gastrointestinal tumors. Inhibition of HH signaling can promote the infiltration of cytotoxic T cells, inhibit the accumulation of M2 macrophages and MDSCs, and then remodel the immunosuppressive state of the TME (Fig. 2).

4. HH signaling as a potential clinical prognostic and therapeutic target in gastrointestinal cancer

4.1. The expression level of HH signaling components has important prognostic value in gastrointestinal tumors

Clinically, the level of HH signaling pathway components is associated with the prognosis of gastrointestinal cancer patients[135]. Clinicopathological analysis of gastrointestinal cancers revealed that the HH pathway is abnormally modulated. A series of molecules in the HH signaling pathway, such as SHH, GLI1, GLI2, SMO and PTCH, have been reported to be related to the prognosis of gastric cancer patients. First, overexpression of SHH is a biomarker for poor prognosis in gastrointestinal cancer. In a study including 117 patients who underwent radical gastrectomy, the survival time of gastric cancer patients with high expression of SHH was significantly shortened[136]. In 228 human colon cancer biopsies, overexpression of SHH, PTCH or GLI1 was found to be an indicator of poor prognosis, and analysis of a database containing information for 735 colon and rectal cancers showed that SMO overexpression might be associated with poor prognosis in colorectal cancer[135,137]. Second, positive GLI1 expression is a reliable indicator of poor prognosis in patients with highly aggressive gastric cancer. In addition, a meta-analysis of 886 patients with gastric cancer showed that positive GLI1 expression is correlated with poor prognosis in gastric cancer patients[138]. The poor prognosis of patients with high expression levels of GLI1 may be related to reactive oxygen species (ROS) generated by NADPH oxidase 4 (NOX4); increased production of ROS in hypoxia causes GLI1 upregulation[139]. Finally, some studies have shown that SUFU as a negative regulator of HH signaling pathway is downregulated and negatively associated with the tumor stage in gastric cancer, supporting the potential of SUFU expression as another diagnostic or prognostic marker[140,141].

In addition to upregulation of the HH pathway, mutations in the key proteins in the HH pathway may also be important in gastrointestinal cancer development. An early study reported that 85% of basal cell carcinomas (BCCs) have HH pathway gene mutations, of which approximately 73%, 20% and 8% have *PTCH1*, *SMO* and *SUFU* driver mutations, respectively. This observation indicates that HH pathway gene mutations are the main driver mutations in BCC[142]. In a group of 39 gastrointestinal cancers, only three *SMO* mutations and one *PTCH1* mutation were found. However, the frequencies of these mutations were low in these patients, and, due to the small sample size, the role of these mutations could not be inferred[143]. Therefore, large-scale profiling studies are needed to help us understand the role of those mutations in gastrointestinal cancer.

In conclusion, the levels of HH signaling pathway components have substantial prognostic value in gastrointestinal tumors. The role of *SMO* and *PTCH1* mutations in gastrointestinal tumors and the clinical value of these mutations need to be analyzed in larger samples.

4.2. HH signaling pathway inhibitors

The HH signaling pathway is a promising therapeutic target in gastrointestinal tumors. Various molecules that inhibit this pathway have been evaluated in the treatment of gastrointestinal tumors. Most of these agents are designed to target the SHH, SMO and GLI proteins in the HH signaling pathway.

More inhibitors have been developed to target SMO than SHH or GLI. Cyclopamine, the first reported SMO inhibitor, exhibits inhibitory activity *in vitro* gastrointestinal tumors models[33,49,50]. Saridegib (IPI-926), a semisynthetic analog of cyclopamine, with improved chemical stability and biological activity, has entered a phase I clinical trial for the treatment of gastric cancer[144]. Vismodegib (GDC-0449), a potent inhibitor of SMO, exhibited antitumor efficacy in both preclinical studies and clinical trials[145]. Vismodegib and saridegib are currently approved in the United States and Europe for the treatment of adult patients with metastatic or locally advanced BCC[146,147]. Clinical studies showed high tumor control rates and low drug resistance in the designated BCC patient population[148]. Therefore, vismodegib and saridegib are effective and well-tolerated systemic treatments for specific BCC patients[149]. Other SMO inhibitors that have been effective in treating gastrointestinal tumors include miR-218, itraconazole, vitamin D3, BMS-833923 (XL139), TAK-441 and MS-0022 (Fig. 1).

Given the high tumor recurrence rate in clinical trials of SMO inhibitors, exploration of other components of the HH signaling pathway as therapeutic targets is urgently needed[150]. GLI proteins show advantages as new targets because they can be activated by both SHH ligand-dependent and SHH ligand-independent mechanisms[151]. GANT61, a GLI1 inhibitor, was designed to block the binding between GLI1 and DNA or to alter the conformation of the GLI1–DNA complex[152]. GANT61 has been observed to induce DNA damage and extensive cell death in human colon cancer cells *in vivo*[64]. Other inhibitors of GLI that have been studied in gastrointestinal tumors include curcumin, resveratrol, epigallocatechin-3-gallate, arsenic trioxide (ATO), zerumbone, AY9944 and sodium arsenite (Fig. 1).

The chimeric monoclonal antibody 5E1 (ch5E1) has been shown to inhibit the SHH signaling pathway by binding to SHH, resulting in inhibitory effects on gastrointestinal tumor cells *in vitro*[34,153,154]. Other inhibitors of SHH include imiquimod and isoflavones (genistein), which have been used *in vitro* and *in vivo*, respectively, in gastrointestinal tumor studies (Fig. 1).

In summary, only a few inhibitors have entered clinical trials (the most advanced are in phase II) for the treatment of

Table 1 Clinical trials of Hedgehog pathway modulators discussed in gastrointestinal disease (data from ClinicalTrials. gov[39]).

Drug	Target	Disease indication	Maximum developmental stage	ClinicalTrials.gov identifier
Vismodegib (GDC-0449)	SMO (antagonist)	Metastatic colorectal cancer	Phase II	NCT00636610
Sonidegib (LDE225)	SMO (antagonist)	Certain cancers (including metastatic colorectal cancer, gastric cancer, gastroesophageal junction cancer)	Phase I	NCT01576666
Vismodegib (GDC-0449)	SMO (antagonist)	Advanced stomach cancer or gastroesophageal junction cancer	Phase II	NCT00982592
Vitamin D3	SMO (antagonist)	Stage IV colorectal cancer	Phase II	NCT01074216
TAK-441	SMO (antagonist)	Advanced nonhematologic malignancies	Phase I	NCT01204073
BMS-833923	SMO (antagonist)	In inoperable, metastatic gastric, gastroesophageal, or esophageal adenocarcinomas	Phase I	NCT00909402
Curcumin	GLI (antagonist)	Unresectable colorectal cancer	Phase II	NCT02439385
Resveratrol	GLI (antagonist)	Colorectal cancer	Phase I	NCT00433576
Epigallocatechin-3-gallate	GLI (antagonist)	Colorectal cancer	Phase I	NCT02891538

SMO, Smoothened; GLI, glioma-associated oncogene homologue.

gastrointestinal tumors. Research and development progress on HH-targeting drugs in gastrointestinal cancer lags behind that in other types of cancer. A deeper understanding of the role and mechanism of HH signaling and a comprehensive characterization of current HH inhibitors may provide novel insights for accelerated investigations targeting HH signaling as a therapeutic strategy in gastrointestinal cancer.

4.3. HH signaling and TME as potential therapeutic targets in gastrointestinal cancer

Due to its extensive involvement in carcinogenesis, tumor development, and progression, the HH signaling pathway has recently been intensively investigated in anticancer drug discovery pipelines, leading to two U.S. Food and Drug Administration (FDA)-approved small molecule drugs and dozens of clinical trial-stage inhibitors[155–157]. These drugs and inhibitors cover a wide range of indications, including BCC, medulloblastoma, pancreatic tumors, and non-small cell lung cancer[157–159]. The critical role of HH provides a promising therapeutic opportunity in gastrointestinal cancer. Unlike for BCC, no specific HH signaling-based clinical therapies for gastrointestinal cancer are currently available. These findings highlight the urgency and importance of discovering of HH signaling-based therapies for gastrointestinal cancer. Currently, 8 HH inhibitors have been evaluated in phase I or phase II clinical trials for gastrointestinal cancer (Table 1), although none have entered phase III clinical trials or have been approved. Unfortunately, the results from a phase II clinical trial (NCT00982592) show that the addition of vismodegib to FOLFOX chemotherapy failed to achieve significant improvement in progression-free survival in gastrointestinal cancer[160]. Therefore, other mechanisms may influence the therapeutic effect of HH pathway inhibitors in gastrointestinal tumors.

Considering the role of HH signaling activation in the TME, adopting a more comprehensive consideration of the TME may provide a new opportunity for the design of effective HH-based therapies for gastrointestinal cancer[161]. *In vivo*, pharmacological inhibition of HH signaling can suppress tumor growth by modulating the TME, an approach that could be exploited in rational therapeutic design. For example, the SMO inhibitor vismodegib normalizes the TME in breast cancer by reducing the proliferation of CAFs and thus significantly improves the efficacy of Abraxane and Doxil in xenograft tumors[71]. In addition, vismodegib can ameliorate the immunosuppressive TME, which stems from M2 subtype TAMs and regulatory T cells[134]. Based on the modulatory effect of HH signaling activation on the TME, HH inhibitors can be combined with immunotherapies to improve therapeutic efficacy by increasing the infiltration of cytotoxic T cells into tumors. Recently, a nanodrug that carried the HH inhibitor cyclopamine and paclitaxel to alter the TME of pancreatic cancer was designed to enhance the efficacy of immune checkpoint blockade[98]. Moreover, inhibiting HH activity in the stroma has been reported to increase the density of the intratumoral vasculature and thus allow enhanced infiltration of cytotoxic $CD8^+$ T cells, which finally increases the antitumor activity of anti-programmed cell death-1 (PD-1) antibodies. This evidence indicates that inhibiting HH signaling may be a strategy to balance or reboot the tumor immune status and therefore allow the effective use of immunotherapies such as PD-1 and PD-L1 inhibitors in gastrointestinal cancer.

In summary, owing to the important roles of HH signaling activation in the gastrointestinal TME, a combined approach of targeting the HH signaling pathway and modifying TME may present a promising therapeutic opportunity for gastrointestinal cancer. Additional clinical studies are anticipated.

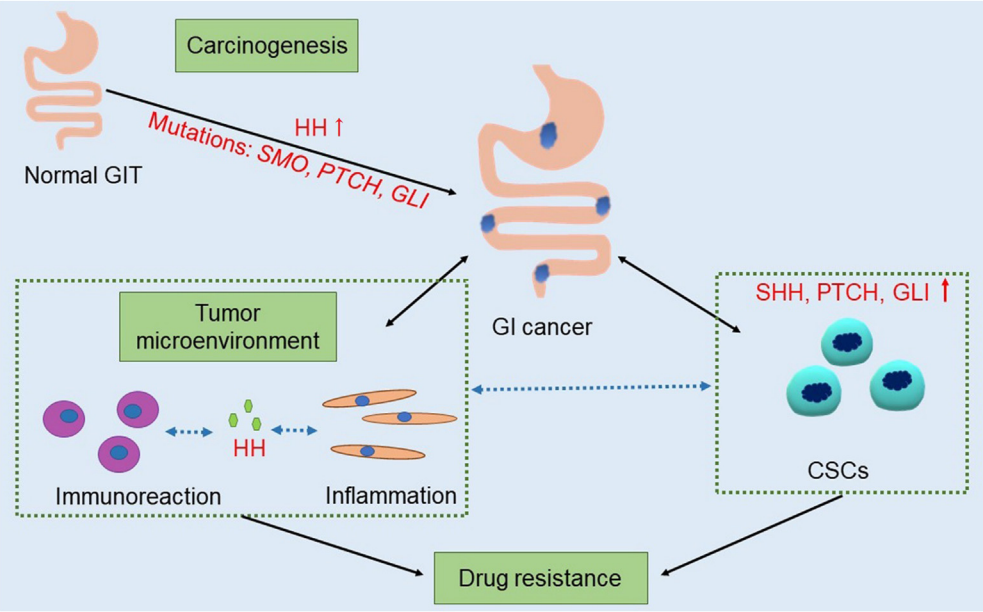

Figure 3 The Hedgehog signaling pathway plays an important role in gastrointestinal carcinogenesis, the tumor microenvironment and cancer stem cells. GIT, gastrointestinal tract; GI, gastrointestinal; CSCs, cancer stem cells; HH, Hedgehog; SHH, Sonic Hedgehog; SMO, Smoothened; PTCH, Patched; GLI, glioma-associated oncogene homologue.

5. Conclusions

In conclusion, aberrant activation of the HH signaling pathway plays a central role in both gastrointestinal carcinogenesis and gastrointestinal TME (Fig. 3). In fact, abnormal HH signaling activation not only causes uncontrolled proliferation of tumor cells but also contributes to the establishment of an immunosuppressive TME by regulating macrophage and T cell-dependent immune responses as well as the expression of tumor-related inflammatory factors. Moreover, HH contributes to the proliferation of gastrointestinal CSCs, a mechanism underlying drug resistance. Therefore, the results of current studies demonstrate that HH is not only a promoter of CSC proliferation but also a critical immunosuppressive and inflammatory factor in the TME, an observation that highlights a potential therapeutic approach for gastrointestinal tumors through inhibition of HH signaling.

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Author contributions

Jinghui Zhang, Jiajun Fan, Xian Zeng, Kai Yin, and Dianwen Ju wrote the paper; Mingming Nie, Jingyun Luan, and Yichen Wang collected data and contributed to the literature search. Jinghui Zhang, Jiajun Fan, and Xian Zeng draw the illustrations. Kai Yin, and Dianwen Ju edited the paper.

Conflicts of interest

The authors declare no conflicts of interest.

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