Review Article

Targeting glioma stem cells via the Hedgehog signaling pathway

Yang Liu, Xing Liu, Ling-Chao Chen, Wen-Zhong Du, Yu-Qiong Cui, Xing-Yin Piao, Yong-Li Li, Chuan-Lu Jiang

Department of Neurosurgery, The Second Affiliated Hospital of Harbin Medical University, Harbin 150086, Heilongjiang, China.

ABSTRACT

Cancer is one of the leading causes of death worldwide. Gliomas are among the most devastating tumor types, and current clinical therapies are unsatisfactory. Recent reports revealed the importance of glioma-propagating cells in the malignancy of gliomas. These cells, also referred to as glioma stem cells (GSCs), share similarities with neural stem cells (NSCs). The Hedgehog (Hh) signaling pathway controls tissue polarity, patterning maintenance, and maintenance of NSCs during embryonic development. Aberrant activation of the Hh pathway resulting from mutation and deregulation has recently been recognized to cause tumorigenesis in a wide variety of tissues, including gliomas and GSCs. In this review, we explore the role of the Hh signaling pathway in GSCs and its potential as a therapeutic strategy.

Key words: Cancer stem cells, glioma, Hedgehog, microRNA

INTRODUCTION

Cancer is estimated to be the leading cause of death worldwide by the World Health Organization (WHO).[1] Gliomas are one of the most lethal adult brain tumors. Although substantial progress has been made, there is still a lack of effective therapy. It is reported that patients with low-grade gliomas can survive for years, while patients with glioblastoma (WHO grade IV) survive for only 1-2 years after diagnosis.[2]

Tumors are composed of a heterogeneous group of cells. Some tumor cell fractions have the ability to initiate tumors in xenograft models, whereas other fractions do not.[3] These cells, capable of sphere-like growth *in vitro* and tumor formation *in vivo*, are defined as cancer stem cells (CSCs) and share similarities with normal neural stem cells (NSCs). It has been hypothesized that these cells are involved in radio- and chemo-resistance, as well as tumor recurrence.

The Hedgehog (Hh) signaling pathway plays a pivotal role in embryonic development, including the formation and maintenance of glioma stem cells (GSCs).[4] Since GSCs are important biological factors responsible for cancer invasion, metastasis, drug resistance, and relapse, Hh signaling is believed to be an important target for cancer therapy. Recently, both natural and synthesized small-molecule inhibitors of Hh signaling have been investigated as potential cancer treatments. However, targeting only one molecule may be insufficient. Therefore, strategies using a combination of natural products and chemotherapeutics with different targets may improve the overall survival of patients with gliomas.

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CANCER STEM CELLS IN GLIOMA

Cancer stem cells and the niche

The concept of CSCs has been in use for over 50 years. In the 1990s, Lapidot *et al*.[5] identified leukemia stem cells capable of generating human acute myeloid leukemia after transplantation. Within gliomas, stem-like cells with the ability to self-renew, differentiate into multiple lineages, and initiate tumors are known as GSCs. Subsequently, GSCs capable of self-renewal and producing glioma-initiating cells were identified using a limiting dilution analysis.[6] The presence of GSCs and the increasing evidence of radio-resistance and chemo-resistance indicated that GSCs may contribute to tumor maintenance and recurrence, and that targeting GSCs may be a promising therapeutic intervention.[7,8] Stem cells reside in specialized niches, which could regulate their proliferation and differentiation.[9] More than one

Corresponding Author: Dr. Chuan-Lu Jiang, Department of Neurosurgery, The Second Affiliated Hospital of Harbin Medical University, No. 246 Xuefu Road, Nangang, Harbin 150086, Heilongjiang, China. E-mail: jcl6688@163.com

study has demonstrated the presence of GSCs near blood vessels, consistent with the perivascular niche for NSCs.[10,11] Alternatively, Li *et al*.[12] demonstrated that GSCs could also be found in regions of necrosis, which are hypoxic, suggesting that there may be more than one niche. Hypoxia promotes the self-renewal capability of both the stem cell and nonstem cell populations, and also promotes the conversion of nonstem cells into stem cells by up-regulating the expression of important stem cell factors,[13] indicating that this niche may be important for GSCs. Therapies that target cytokines, such as hypoxia-inducible factor-1α (HIF-1α) and HIF-2α, or cells in the niche, such as glia cells and endothelial cells, may, therefore, show promise.

Glioma stem cells and resistance to therapy

Surgical resection, radiation, and chemotherapy are still the mainstay treatments for gliomas and are associated with a clear improvement in overall survival in patients with high-grade gliomas. However, recurrence of the tumor is common following conventional therapy. Antiangiogenic therapy against vascular endothelial growth factor is another frequently used therapeutic treatment, but drug resistance is common.[14] The mechanisms of resistance are not understood in complete detail and may be multi-factorial. The proposal that GSCs are a prerequisite for tumor formation suggests that chemo- and radio-resistant GSCs are the main cause of recurrence.[15,16] Liu *et al*.[17] found that $CD133^+$ cells were resistant to chemotherapeutic agents, whereas $CD133^-$ cells sorted from the same primary glioma cultures were not. Furthermore, Bao *et al*.[7] determined that ionizing radiation treatment enriched the $CD133^+$ population in human gliomas. Although CD133 does not identify all GSCs, these data suggest that GSCs play a key role in resistance to traditional therapies. The mechanism for resistance is complicated. Bao *et al*.[7] demonstrated that $CD133^+$ cells contribute to glioma radio-resistance via preferential activation of DNA damage checkpoints, and that their resistance could be partially reversed by inhibitors of Chk1 and Chk2. Other researchers demonstrated that the bone morphogenetic proteins and cannabinoids inhibit the tumorigenic potential of GSCs and promote their differentiation.[18] Radiation treatment may expand the GSC population, enhance the aggressiveness of tumors, and induce expression of GSC marker proteins, such as CD133 and nestin, as well as proteins involved in self-renewal, such as Notch2 and Sox2.[19] The GSC niche may enhance the radio-resistance of GSCs as well.[20] Besides their relative radio-resistance, GSCs can express high levels of multiple genes associated with drug resistance[21] and show significant resistance to chemotherapeutic agents.[17] Temozolomide (TMZ) treatment, which could eliminate O6-methylguanine-DNA methyltransferase-negative cells, increased the stem population.[22] Increased expression of drug transporters, such as ATP-binding cassette (ABC) transporters, could lead to chemotherapeutic agents being pumped out of tumor cells and may be another mechanism of chemo-resistance. Recent studies have found that the expression of ABC transporters is increased in stem cells.[23] Therefore, focusing on the connection between GSCs and their niche may help to elucidate the mechanisms behind the treatment-resistant phenotype of GSCs, which may lead to solutions that reduce the resistance of gliomas to therapy and improve clinical outcome. This information may also be useful in the pursuit of effective therapeutic strategies for the treatment of radiation-associated injuries.

Cell sorting and culture

Several methods may be used in order to obtain purified GSCs for study. In some studies, fluorescence-activated cell sorting and magnetic activated cell sorting have been used to separate GSCs from other cell types.[12,24] Glioma cell lines and clinical samples have also been used to isolate and culture GSCs.[25,26] However, there is currently no standard definition of what constitutes a GSC. Specific markers for identifying GSCs are, therefore, required. The current definition of GSCs is based on their capacity for self-renewal, long-term proliferation, and tumor formation *in vivo*. The ideal marker, therefore, is a molecule that is specifically expressed on GSCs and functionally associated with GSC maintenance.

CD133 has been widely used as a marker for GSC sorting. However, NSCs also express CD133, which limits its utility and reliability as a target. Some studies have also suggested that $CD133^-$ cells have the capability to act as GSCs.[13] Moreover, $CD133^-$ glioma cells can give rise to $CD133^+$ GSCs.[13] Other surface molecules such as CD15 (SSEA-1),[27] A2B5,[28,29] and L1CAM[30] have been used as markers but are not widely accepted. Interestingly, both $CD133^+$ $A2B5^-$ and $CD133^-$ $A2B5^+$ cells have been shown to exhibit characteristics of GSCs.[31] Therefore, these markers may only label specific sub-populations of GSCs. In recent times, one group exploited the intrinsic auto-fluorescence properties and distinctive morphology of a subpopulation of cells ($FL1^+$) in order to isolate them from human gliomas. $FL1^+$ cells are capable of self-renewal *in vitro*, tumorigenesis *in vivo*, and preferentially express stem cell genes, but expression of FL1 did not correlate with the expression of other proposed GSC markers.[32] This finding deserves special attention as it may provide a new way to identify GSCs.

HEDGEHOG IN GLIOMA

Hedgehog signaling pathway

Hedgehog is a highly conserved signaling pathway and a key regulator of embryonic development, including the processes of cell differentiation, proliferation, and tissue patterning.[33,34] In adults, Hh plays an important role in the maintenance of stem cells, tissue repair, and regeneration. The Hh family consists of three secreted proteins, sonic Hedgehog (Shh), Indian Hedgehog (Ihh) and desert Hedgehog. Two molecules that are important for Hh signaling are patched (Ptch) and smoothened (Smo). In the absence of Hh, Ptch inhibits the activity of Smo, a receptor-like protein with seven transmembrane domains. In the presence of activated Smo, a complex consisting of the glioma-associated oncogene family zinc finger (Gli) and the suppressor of fused (Sufu), an important negative regulator of Hh signaling, enters the nucleus, leading to nuclear translocation and activation of the Gli1 and Gli2 transcription factors, as well as degradation of Gli3. Activated Gli subsequently promotes the transcription of Hh target genes [Figure 1]. Three types of Gli transcription factors, Gli1, Gli2, and Gli3, have been identified in mammals. Gli1 and Gli2 are activators of Hh target genes, while Gli3 mainly appears to be a repressor. The function of Hh signaling is very complicated and critical. Thus, it is important to elucidate the function and molecular regulation of Hh signaling, especially in GSCs.

Functional studies of Hedgehog in glioma stem cells

Aberrant activation of Hh signaling has been shown to be associated with the formation of gliomas. Several studies have investigated the role of Hh-Gli signaling in GSCs and found that it regulates self-renewal and tumorigenic potential in GSCs.[22,35-37] Importantly, inhibition of Hh-Gli signaling enhances the ability of TMZ to inhibit GSC proliferation and induce cell death.[38] Several studies have demonstrated that inhibition of Hh signaling blocks tumor growth and influences both proliferation and malignancy.[39] The

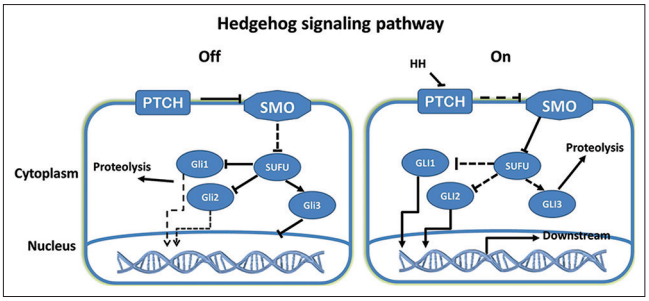

Figure 1: The off and on states of the Hedgehog (Hh) signaling pathway. Patched (Ptch) inhibits the activity of smoothened, which prevents the suppressor of fused-Gli1/2 complex from entering the nucleus and promotes Gli3 nuclear accumulation, leading to low expression of Hh target genes. Hh binding to Ptch activates the Hh signaling pathway by promoting Gli1/2 expression

Shh pathway plays an important role in the migratory ability of cells derived from $CD133^-$ glioblastoma cells.[35] Furthermore, the Hh inhibitor cyclopamine has been shown to improve the effect of radiation on GSCs. All of the studies mentioned above suggest that Hh signaling is one of the critical pathways for the maintenance, proliferation, migration, and tumorigenic potential of GSCs. Thus, targeting this pathway with pharmacologic inhibitors may inhibit GSC growth and improve the efficacy of conventional therapies.

The regulatory role of microRNA

MicroRNAs (miRNAs) are a class of small noncoding cellular RNAs that bind to cis-regulatory elements located primarily in the 3' untranslated regions of target mRNAs, resulting in their translational inhibition or degradation. The function of some miRNAs has been determined to be important for neural development.[40] Other studies have indicated that miRNAs play a potentially important role in glioma biology. The relationship between the Hh pathway and miRNA is currently being investigated.

It has been shown that stable miR-302-367 cluster expression is sufficient to suppress the stem cell-like signature, self-renewal, and infiltration of cells by inhibition of the CXCR4 pathway. Furthermore, inhibition of CXCR4 leads to disruption of the Shh-Gli-Nanog network, which is involved in self-renewal and expression of the embryonic stem cell-like signature.[41,42] Wu *et al*.[43] suggested that miR-5 can specifically suppress Hh signaling by directly targeting Smo in Drosophila. In addition, miR-125b and miR-326 have been identified as suppressors of Smo, and miR-324-5p targets the downstream transcription factor Gli1. Down-regulation of these miRNAs allows high levels of Hh-dependent gene expression leading to tumor cell proliferation.[44] Furthermore, functional analyses have shown that miR-326 may be regulated by Shh activation and act as a negative modulator of Shh signaling by directly targeting Smo and Gli2.[45] Other studies have demonstrated that miR-214 can inhibit Sufu, allowing maximal activation of Gli-mediated transcription,[46] and miR-941 targets key components of the Hh-signaling pathway, including Smo, Sufu, and Gli1.[47] Moreover, miR-212 was found to be involved in tumorigenesis, and the oncogenic activity of miR-212 was partly due to suppression of Ptch1.[48] Although many miRNAs have been shown to regulate the Hh pathway as upstream factors, the Hh pathway in turn has been shown to regulate the mir-29b-1/mir-29a promoter [Table 1 and Figure 2].[49]

The studies mentioned above show that miRNAs affect the expression of numerous genes involved in glioma

Table 1: MicroRNAs and their targets in Hh signaling pathway	
MicroRNA	**Target**
miR-302~367	CXCR4 Shh-Gli-Nanog
miR-125b	Smo
miR-326	Smo, Gli2
miR-324-5p	Smo, Gli1
miR-214	Sufu
miR-941	Smo, Sufu, Gli1
miR-212	Ptch1

Hh: Hedgehog; Shh: sonic Hedgehog; Ptch: patched; Smo: smoothened; Sufu: suppressor of fused

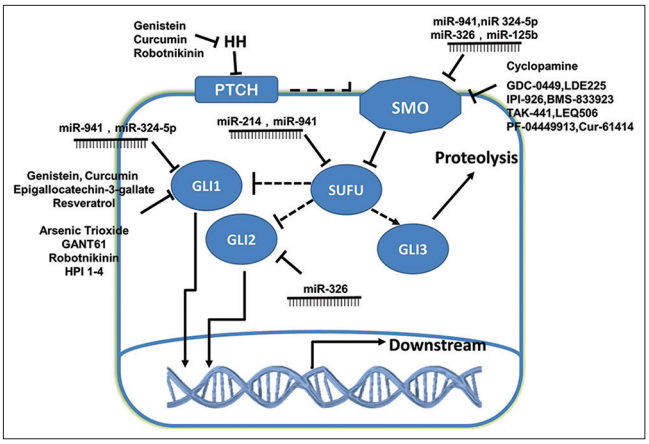

Figure 2: Inhibition of the Hedgehog signaling pathway. A selection of currently known inhibitors, including microRNAs and small molecular inhibitors (both natural and synthetic) are shown, along with their corresponding targets

biology. Identifying the roles that different miRNAs play may help to understand the mechanisms leading to glioma-propagation and provide new therapeutic strategies. In particular, miRNAs that affect the Hh pathway should be investigated in more detail.

Cross-talk with other pathways

Cross-talk between the Hh signaling pathway and other embryonic signaling pathways, such as the Notch and Wnt pathways, has been reported not only in glioma cell lines, but in other cancers as well. Cross-talk between signaling pathways has the potential profoundly to add to the complexity of cellular responses to external stimuli. Wnt signaling directs the development of a variety of organ systems during embryogenesis. In adults, Wnt signaling has a key role in the regulation of tissue self-renewal. Over the past several years, various discoveries have suggested that there are fundamental similarities between the Wnt and Hh signaling pathways.[50] Both pathways are activated by a membrane protein (Frizzled or Smo) and prevent phosphorylation-dependent proteolysis of key effector (β-catenin or cubitus interruptus), which converts a DNA-binding protein from a repressor into an activator of transcription. In addition, silencing of both pathways in the absence of ligand requires Slimb-β-TRCP-FWD-1, which is a component of the SCF ubiquitin ligase complex, and the protein kinases glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1). It has been observed that molecules involved in Wnt signaling, such as GSK-3 β, regulate the Hh signaling pathway.[51] In turn, activation of Gli may stimulate the transcription of Wnt ligands. It has been found that GSK-3 β phosphorylates and stabilizes Sufu, leading to inhibition of Hh activation.[52] Moreover, the Hh pathway was found to inhibit Wnt signaling, as a result, of Gli1 induction through up-regulation of secreted frizzled-related protein 1.[53]

Notch signaling is another conserved developmental signaling pathway that is important for embryogenesis, cellular homeostasis and stem cell renewal.[54] Notch receptor activation induces the expression of hairy and enhancer of split 3 (Hes3) and Shh through rapid activation of cytoplasmic signals, including the serine/threonine kinase Akt, the transcription factor STAT3, and the mammalian target of rapamycin (mTOR), leading to the promotion of NSC survival.[55] Simultaneously, Shh induces the expression of another specific target gene, Hes1, and Smo function has been found to be necessary for Shh-induced up-regulation of Hes1.[56] Moreover, inhibition of Shh and Notch may enhance the sensitivity of $CD133^+$ GSCs to TMZ;[38] and the Shh, Notch, and Wnt pathways combined may regulate self-renewal and differentiation of breast CSCs and progenitor cells.[57]

Aberrant activation of epidermal growth factor receptor (EGFR) signaling has been implicated in a number of human malignancies, which has made EGFR a prime molecular target in chemotherapy.[58] Several studies suggest that the combination of specific EGFR and Hh inhibitors may provide a therapeutic benefit. For instance, the combination of the selective EGFR inhibitor gefitinib and the Smo antagonist cyclopamine, with or without the chemotherapeutic drug docetaxel, inhibits cell growth and induces apoptosis.[59] EGFR synergizes with Gli1 and Gli2 to selectively activate transcription of Gli target genes via stimulation of RAS/RAF/MEK/ERK signaling.[60] Moreover, EGF has already been shown to have the capability to stimulate the proliferative activity of Shh on NSCs and to enhance the invasive properties of epidermal cells expressing Shh.[61,62] Further investigation is required, however, in order to understand these interactions in more detail.

Hedgehog signaling may also be involved in cross-talk with other pathways, such as transforming growth factor-β (TGF-β) and AKT signaling. TGF-β has been shown to promote Gli2-mediated expression of parathyroid hormone-related protein.[63] Other studies have indicated that co-activation of the Hh and AKT pathways promote tumorigenesis.[64] The results of these

studies, in addition to those mentioned above, indicate the existence of cross-talk between Hh signaling and several other pathways. It is, therefore, possible that combination therapies targeting both Hh and other pathways may provide additional benefits to patients in comparison with individual treatments.

Hedgehog as a therapeutic target in glioma

In recent times, several inhibitors of Hh signaling have been synthesized or discovered for use in studies of cancer treatment *in vitro* and *in vivo*. By targeting important molecules in the Hh signaling pathway, Hh inhibitors down-regulate the activity of Hh signaling in cancer cells, resulting in the inhibition of cancer cell growth and tumor progression. GDC-0449 (vismodegib) is a small-molecule inhibitor specifically designed to target Smo. In preclinical experiments, GDC-0449 has been shown to inhibit the activation of the Hh pathway, leading to the inhibition of tumor growth initiated by mutations of Ptch or by increased levels of Hh ligands.[65] A search of the clinicaltrials.gov database identified 38 clinical trials of GDC-0449 focused on the treatment of different malignancies. In one trial, for example, GDC-0449 was tested in combination with Avastin (bevacizumab) and traditional chemotherapy in metastatic colorectal cancer. Treatment with GDC-0449 resulted in a reduction of symptoms, and the data suggests that GDC-0449 may be safely used in combination with conventional agents. Unfortunately, mutations in Smo and its downstream targets are common, and may lead to GDC-0449 resistance. However, it has been shown that resistant medulloblastomas are sensitive to PI3K inhibition, which may indicate that combined therapy is necessary.[66]

LDE225, another Hh inhibitor specifically targeting Smo, has also been shown to reduce Hh-dependent proliferation. The main side effects include nausea, vomiting, anorexia, fatigue, muscle cramps, and dysgeusia. During the course of LDE225 treatments, resistance to the drug was observed. Possible mechanisms for this resistance include Gli2 amplification and Smo mutations, leading to reactivation of Hh signaling. Similar to the GDC-0449 study, a combination treatment of LDE225 with PI3K inhibitor delayed the development of resistance.[67] Thus, combined therapy targeting multiple pathways needs more investigation.

IPI-926 (saridegib) is a unique, selective, and potent molecule that inhibits Smo. IPI-926 is orally bioavailable and has demonstrated biological activity in multiple preclinical animal models of cancer. IPI-926 appears to down-regulate Hh signaling, leading to inhibition of the potential for self-renewal. Drug resistance was observed after extended treatment periods, but was primarily caused by increased expression and activity of P-glycoprotein drug transporters rather than the emergence of genetic mutations that prevent drug-target interactions.[68] BMS-833923 is another Hh inhibitor that acts by binding to Smo. Clinical trials have been conducted to evaluate the effects, safety, tolerability, and pharmacokinetics of BMS-833923 alone or in combination with other drugs. Resistance to this drug and the mechanisms behind it still need to be studied, however. In addition, other synthetic Smo antagonists including TAK-441, LEQ506, PF-04449913, and Cur-61414 have already been tested in clinical trials in order to determine dosage levels and evaluate safety [Table 2 and Figure 2].

Currently, most drugs targeted against the Hh pathway function by inhibiting Smo and thus lead to the suppression of tumor proliferation. However, Hh signaling could also be altered by targeting components located downstream of Smo. Accordingly, several groups are attempting to develop agents that target Gli or other molecules in the Hh pathway. For example, GANT61 is an Hh inhibitor targeting Gli1 and Gli2. GANT61 has been shown to effectively down-regulate Gli expression, inhibit cell proliferation and migration, and induce G1 arrest and apoptosis.[69] GANT61 may also decrease cell invasiveness by inhibiting Gli2 in human bladder transitional cell carcinoma.[70] Another potential therapeutic agent is arsenic trioxide (ATO). ATO has been proposed to block the accumulation of Gli2, resulting in reduced protein levels,[71] and to bind directly to Gli1, inhibiting its transcriptional function.[72] Furthermore, four Hh pathway inhibitors (HPIs) have been identified that act downstream of Sufu to modulate Gli processing, activation, and/or trafficking, including small molecule antagonist of ciliogenesis. HPI-1 has been shown to inhibit activation of the Hh pathway induced by overexpression of Gli1. HPI-2, on the other hand, inhibits Hh target gene expression in cells lacking Sufu function or overexpressing Gli2, but is less effective against exogenous Gli1. HPI-3 likely blocks activation

Table 2: Synthetic inhibitors of Hedgehog signaling pathway

Synthetic inhibitors	Target
GDC-0449 (Erivedge, vismodegib)	Smo
LDE225	Smo
IPI-926 (Saridegib)	Smo
BMS-833923	Smo
TAK-441	Smo
LEQ506	Smo
PF-04449913	Smo
Cur-61414	Smo
Arsenic trioxide	Gli
GANT61	Gli
Robotnikinin	Shh
HPI 1-4	Gli

HPI: Hedgehog pathway inhibitor; Shh: sonic Hedgehog; Smo: smoothened

by Gli2 as well, albeit through a different mechanism. HPI-4 appears to act by perturbing ciliogenesis.[73] Another synthetic molecule, named Robotnikinin, specifically binds to extracellular Shh and blocks Shh-signaling in cell lines, human primary keratinocytes and a synthetic model of human skin,[74] which may represent an alternative treatment for tumors resistant to Smo inhibitors.

In addition to synthetic drugs, several natural molecules have been found to target the Hh pathway. Genistein is an isoflavone found in soybeans and most soy-protein products. Both *in vitro* and *vivo* studies have demonstrated that genistein may inhibit Gli1 mRNA expression and down-regulate Gli reporter activity, leading to significant inhibition of prostate cancer cell proliferation.[75] Cyclopamine, derived from Veratrum californicum, has been showed directly to bind to Smo, blocking the Hh pathway and preventing transcription.[76] In addition, cyclopamine reduces neurosphere formation in glioblastoma cell lines.[77] Epigallocatechin-3-gallate (EGCG) is one of the most potent anticarcinogenic compounds known as catechins found in green tea. Studies indicate that EGCG could decrease the expression of Gli1 mRNA and inhibit Gli reporter activity.[75] Another study found that EGCG down-regulates Ihh, Gli1, and Bcl-2 expression, which may inhibit cell proliferation and induce apoptosis.[78] Another natural inhibitor of the Hh pathway is resveratrol, a stilbenoid found in the skin of red grapes and peanuts. Experimental studies have shown that resveratrol suppresses cancer cell proliferation and induces apoptosis partially through the down-regulation of Gli1 mRNA expression and the inhibition of Gli reporter activity.[75] Curcumin (diferuloylmethane), derived from *Curcuma longa*, inhibits the nuclear factor-κB, PI3K/Akt, and activator protein-1 signaling pathways, resulting in antiinflammatory, antioxidant and anticancer effects. Curcumin also regulates Hh signaling by down-regulating Shh and Gli1.[79] In addition, curcumin reduces the protein levels of β-catenin and its downstream targets, c-Myc and cyclin D1, suggesting that curcumin could interrupt the cross-talk between Hh and Wnt signaling.[79] In an advanced model of pancreatic cancer, cyclopamine alone was not sufficient to deplete the number of CSCs. Following treatment with a combination of the conventional chemotherapy agent gemcitabine and the mTOR inhibitor rapamycin, however, CSCs were virtually undetectable both *in vitro* and *in vivo*.[80] Natural molecules targeting a variety of different pathways involved in cancer proliferation have been identified, and may lead to effective therapies for glioma or other malignancies. It is possible, however, that combination therapy may be needed for treatment of gliomas. Therefore, the exact mechanism of each inhibitor should be investigated, and the effects and defects of combined therapy should be evaluated in clinical trials [Table 3 and Figure 2].

Table 3: Hh related natural products

Natural compounds	Target
Cyclopamine	Smo
Genistein	Gli1
Curcumin (diferuloylmethane)	Shh, Gli1
Epigallocatechin-3-gallate	Ihh, Gli1
Resveratrol	Gli1

Hh: Hedgehog; Ihh: indian Hedgehog; Shh: sonic Hedgehog; Smo: smoothened

CONCLUSION

the Hh signaling pathway plays a pivotal role in the process of embryonic development. Aberrant activation of Hh signaling contributes to cancer development, progression, and the processes of cancer invasion and metastasis, leading to the formation of gliomas. GSCs are at the core of glioma biology and play an important role in cancer invasion, metastasis, drug resistance and tumor recurrence. Thus, strategies specifically targeting Hh signaling in GSCs could lead to promising therapies that inhibit tumor initiation and progression. A variety of synthetic molecules and natural products are currently under investigation in both fundamental research studies and clinical trials. Although some benefits have been observed, there are still problems that remain to be solved. In particular, further studies are needed to (1) identify more effective methods of differentiating GSCs from NSCs; (2) evaluate the benefits of combination therapy with HPIs and conventional chemotherapeutic agents; and (3) determine the mechanisms behind nutraceuticals that inhibit the Hh pathway for the prevention of human malignancies *in vitro*, *in vivo*, and in clinical trials.

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