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Regulation of Hedgehog Signaling in Cancer by Natural and Dietary Compounds

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Abstract

The aberrant Hedgehog (Hh) signaling induced by mutations or overexpression of the signaling mediators has been implicated in cancer, associated with processes including inflammation, tumor cell growth, invasion and metastasis, as well as cancer stemness. Small molecules targeting the regulatory components of the Hh signaling pathway, especially Smoothened (Smo), have been developed for the treatment of cancer. However, acquired resistance to a Smo inhibitor vismodegib observed in clinical trials suggests that other Hh signaling components need to be explored as potential anticancer targets. Natural and dietary compounds provide a resource for the development of potent agents affecting intracellular signaling cascades, and numerous studies have been conducted to evaluate the efficacy of natural products in targeting the Hh signaling pathway. In this review, we summarize the role of Hh signaling in tumorigenesis, discuss results from recent studies investigating the effect of natural products and dietary components on Hh signaling in cancer, and provide insight on novel small molecules as potential Hh signaling inhibitors.

Keywords

Cancer; Hedgehog; Gli; Natural inhibitors; Smoothened

1. Introduction

The Hedgehog (Hh) signaling pathway was first identified in *Drosophila* [1]. It is known to be involved in various developmental processes such as tissue patterning and organogenesis during embryogenesis [2–4] as well as in tissue regeneration and repair after injury [5, 6]. Although Hh signaling is important during development, its dysregulation has been

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Conflict of interest

The authors have no conflict of interest.

implicated in hyperproliferative disorders including cancer [7–9]. Genetic mutations of Hh signaling mediators and their hyperactivation have been associated with development of basal cell carcinoma (BCC), medulloblastoma, breast, pancreatic, prostate and lung cancers [10]. Consequently, Hh signaling has been explored for cancer prevention and treatment [11, 12]. Clinical development of agents targeting a Hh signaling component Smoothened (Smo) resulted in approval of vismodegib and sonidegib for the treatment of BCC by the United States Food and Drug Administration (FDA) in 2012 and 2015, respectively. However, development of resistance to vismodegib reported in patients with advanced BCC and medulloblastoma [13, 14] underscores the need for alternative approaches targeting different mediators of the Hh signaling pathway.

Because of the role of the Hh signaling in cancer, naturally occurring compounds and dietary components inhibiting aberrant Hh signaling have been investigated for cancer prevention and therapy during the past decade [15, 16]. We retrieved articles from the PubMed database using the keywords “hedgehog and cancer”, “Smo and cancer”, and “Gli and cancer” and searched for natural products and dietary components reported to regulate Hh signaling in cancer. In this article, we review the role of Hh signaling in processes associated with carcinogenesis, such as inflammation, tumor growth, invasion, metastasis and stemness. In addition, we summarize results from *in vitro* and *in vivo* studies investigating natural products and dietary components as inhibitors of Hh signaling.

2. Hh Signaling

Hh signaling is activated upon the interaction between Hh ligands, such as Sonic Hedgehog (Shh), Desert Hedgehog (Dhh), and Indian Hedgehog (Ihh), and the membrane-bound cell surface receptor, Patched (Ptch) [17, 18]. In the absence of Hh ligands, Ptch keeps G protein-coupled receptor Smo from entering the primary cilium, where the suppressor of fused (SuFu) forms complex with glioma associated oncogene (Gli) 2 and 3 [19]. Gli can be phosphorylated by protein kinase A (PKA), casein kinase (CK)-1, and glycogen synthase kinase (GSK)-3β and partially degraded by proteasome in the base of the primary cilium [20–23]. Recently, the ciliary G-protein coupled receptor Gpr161 was found to increase the level of cAMP, resulting in PKA activation [24]. This observation suggests that cilia has a possible role in repressing Gli in the absence of Hh ligand, and in converting inactive Gli to an active form in the presence of the ligand. After partial removal of the C-terminal domain, the repressor form of Gli translocates to the nucleus to act as a transcriptional repressor to turn off Hh signaling.

During activation of the Hh signaling pathway, Hh ligands bind to the Ptch receptor to form a complex which is then degraded in lysosomes, and released Smo is relocalized at the tip of the cilium to activate downstream signaling [25]. Although the precise mechanism of Smo activation is not clearly understood, recent studies suggest that covalent modification of Smo on the Asp95 residue by cholesterol induce conformational changes in response to Hh ligands [26, 27]. After Smo activation, Gli2/3 escapes from SuFu complex and Gli2 as an activated form of Gli (Gli-A) induces transcription of the target genes. One of the target genes, Gli1, further amplifies the Hh signaling; Gli1 expression level has been suggested as an indicator of Hh signaling activity [28]. Other Gli targets include genes involved in cell

proliferation (MYC, CCND1, CCND2, FOXM1)[29–32], stem cell regeneration (JAG2, FST) [30, 33], and cell survival (BCL2, c-FLIP) [34, 35].

In addition to the ligand and receptor-dependent mechanisms, Hh signaling mediators, especially Gli, are known to be regulated by different cellular networks including mitogen-activated protein kinases (MAPK), phosphatidylinositol-3-kinase (PI3K)/AKT, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β [36–41]. The activation of PI3K/AKT also leads to Gli1/2 up-regulation, where a Gli inhibitor and an AKT inhibitor synergistically suppress tumor growth *in vitro* and *in vivo* [40]. Recently, TNF-α was found to induce Gli1 phosphorylation through mammalian target of rapamycin (mTOR)/S6 kinase (S6K) 1 in esophageal adenocarcinoma [39]. Additionally, interaction of β-catenin with Gli1 and induction of Gli1/2 by TGF-β through Smad3 were implicated in regulation of Hh signaling [36, 41]. After it was reported that activated MEK1 induces the expression of the Gli protein, and the N-terminus of Gli1 is an important region for extracellular signal-regulated kinase (ERK) 1/2 [37], interactions between Hh signaling and ERK1/2, ERK5, c-Jun N-terminal kinase (JNK), and p38 have been demonstrated in different cancers (reviewed in [42]).

3. The role of Hh signaling in carcinogenesis

The underlying mechanisms of Hh signaling in cancer development have been extensively reviewed [43] and include i) mutation-driven ligand-independent Hh activation in BCC and medulloblastoma; ii) ligand-dependent autocrine Hh activation in lung, breast, stomach, and prostate cancer, iii) ligand-dependent paracrine Hh activation in pancreatic cancer, iv) ligand-dependent inverse paracrine Hh activation in B-cell lymphoma, multiple myeloma, and leukemia [10]. Here, we discuss how Hh signaling is involved in the process of tumor development and metastasis.

Hh signaling in inflammation

Inflammation is known to be associated with cancer development by driving several processes including proliferation, angiogenesis, and metastasis [44]. Recent studies have shown that Hh signaling is activated during inflammation. In *Helicobacter pylori*-induced gastric inflammation, nuclear factor-κB (NF-κB) is activated to induce gene expression of Shh, Ptch, and Gli [45, 46]. Further, upregulated cytokines, such as IL-6, IL-1β and TNF-α, have been associated with uncontrolled activation of Hh signaling [47]. The inhibition of Hh signaling by a Smo inhibitor reduces activated macrophages and decreases the expression of pro-inflammatory molecules such as TNF-α, IL-1β and IL-6 in hepatic inflammation [48]. These results indicate that Hh signaling is associated with inflammatory responses that contribute to carcinogenesis.

Hh signaling in cancer cell growth

Hh signaling regulates cell proliferation through modulating the cell cycle- and apoptosis-related genes. In particular, cyclin D and cyclin E involved in the G1/S transition are known to be transcription targets of Hh/Gli signaling in mammalian cells [49–51]. Ptch has been shown to regulate cyclin B/mitosis-promoting-factor (MPF) complex where MPF is required

for the G2/M transition in most cell types [52]. Shh, an Hh ligand, blocks cyclin-dependent kinase inhibitor p21-induced cell cycle arrest [53]. In addition, Hh signaling enhances cell survival by inhibiting caspase 8 signaling through regulating cFLIP and FAS, as well as activating BCL2 promoter [34, 35, 54]. Recently, it was reported that Shh promotes tumor cell survival by inhibiting a Shh receptor, cell adhesion molecule-related/down-regulated by oncogenes (CDON) [55]. Hh signaling inhibitors, such as cyclopamine and vismodegib targeting Smo, and GANT (GLI ANTagonist) 61 targeting Gli, were reported to inhibit cell proliferation through cell cycle arrest and apoptosis in different cancer models [56–58].

Hh signaling in angiogenesis

Tumor progression requires the formation of new blood vessels to supply oxygen and nutrients mediated by vascular endothelial growth factor (VEGF) signaling [59]. Activation of the Hh pathway was found to enhance vascularization by regulating VEGF and VEGF receptor in triple negative breast cancer [60, 61] and hepatocellular carcinoma [62]. Ectopic overexpression of Shh in colon xenografts increased tumor blood vessel density and angiogenesis via Hh-induced VEGF [63]. Harris *et al.* reported that constitutive expression of Shh enhanced vascularization in breast cancer by upregulating a Hh signaling target gene, cysteine-rich angiogenic inducer 61 (cyr61), although in a VEGF-independent mechanism [64]. Overall, these results suggest an important role of Hh signaling in regulating angiogenesis.

Hh signaling in invasion and metastasis

Tumors metastasize by invading the basement membrane, extravasating into circulatory system including lymph and blood vessels, and intravasating to distant locations [65]. Gli1 was found to directly bind the promoter region of human CXCR4 gene and stimulate ERK phosphorylation in breast cancer which results in cellular invasiveness and metastasis [66]. Further, TNF-α induced Gli1 expression increased the migration and invasion of breast cancer cells by activating MMP-9 [67]. In gastric cancer, Shh activated PI3K/AKT signaling and enhanced cellular motility and invasiveness [68]. Chong *et al* reported that galectin-1, which stimulates invasiveness of gastric cancer, increased the expression of Gli1 independently of Smo and further promoted metastasis [69]. In glioblastoma, Shh dose-dependently upregulated the expression of MMP-2 and MMP-9, leading to enhanced cell migration and invasion [70].

Hh signaling in cancer stem cells

Cancer stem cells (CSC) have been functionally defined by their capacity to undergo self-renewal and differentiation that may participate in tumor relapse and drug resistance [71]. The involvement of Hh signaling in CSC has been suggested in studies of multiple human cancers (reviewed in [72]). Activated Hh signaling in CSC was found in glioblastoma [73], breast cancer [74], colon cancer [75], and pancreatic cancer [76], where the suppression of Hh mediators by inhibitors, a ligand-neutralizing antibody, and/or siRNA treatment resulted in inhibition of stem-like properties. Hh signaling is activated in Bcr-Abl positive leukemia stem cells (LSC), and pharmacological inhibition of Smo reduced LSC *in vivo* [77], suggesting that Smo inhibition could be an effective treatment strategy in reducing tumor relapse and drug resistance in chronic myeloid leukemia.

4. Regulation of Hh signaling by natural and dietary compounds

Natural and dietary compounds generally target multiple signaling pathways and are not known to be specific or direct modulators of individual signaling pathways. Only a limited number of studies have attempted to identify direct molecular targets of natural and dietary compounds in Hh signaling. However, it may be worth the effort because natural compounds often uncover novel mechanisms or chemical structures that are useful as platforms for drug development. Here, we provide an overview of studies with natural and dietary compounds active in modulating the Hh signaling pathway. The studies use both non-specific tumor models as well as models specific to molecules involved in Hh signaling pathways. The effects of natural products and dietary components reported to inhibit Hh signaling from *in vitro* and *in vivo* studies are summarized in Tables 1 and 2, respectively.

4.1. Direct inhibitors of Hh signaling from natural and dietary sources

Berberine—This isoquinoline alkaloid from the *Berberis* species was reported to suppress Gli1 transcriptional activity induced by a Shh ligand or a Smo agonist (SAG) [78]. Berberine inhibited Hh signaling activity by targeting Smo, most likely by directly binding to Smo on the same site as cyclopamine, and suppressesed Hh-dependent medulloblastoma growth *in vitro* and *in vivo* [78].

Cyclopamine and jervine—Cyclopamine and jervine, natural steroidal alkaloids isolated from *Veratrum californicum*, are the first small molecule Hh inhibitors identified to bind to the transmembrane domain of Smo [79]. Jervine, a metabolically more stable analog of cyclopamine, is 5- to 10-fold less potent in inhibiting Smo than cyclopamine [80]. As a lead natural Smo inhibitor, cyclopamine suppressed tumor growth in animal models [75, 81–84], and topical application of cyclopamine regressed BCC development in patients [85]. However, its insolubility in water, poor stability, and relatively high toxicity led to the development of pharmacologically more useful inhibitors [86–88]. Based on the mechanisms targeting the transmembrane domain of Smo, two novel synthetic Smo inhibitors, vismodegib and sonidegib, were developed and recently approved by the FDA for the treatment of locally advanced or metastatic BCC [89, 90]. Since cyclopamine and related compounds have been extensively described as inhibitors of Hh signaling in recent literature, we limit discussion on these compounds in this review.

Glabrescione B—Glabrescione B, identified from the seeds of *Derris glabrescens,* was recently shown by NMR spectroscopy to directly interact with K340 and K350 in zinc finger (ZD) domain 4 of Gli1. Because ZD4 and ZD5 domains of Gli1 can bind to a specific sequence of DNA, glabrescione B interferes with Gli1/DNA binding resulting in impairment of Gli1-dependent transcriptional activity. In biological assays, glabrescione B suppressed Gli1 target genes in Gli1-overexpressed HEK293T cells, $Smo^{-/-}$ mouse embryonic fibroblasts (MEF), $Ptch^{-/-}$ MEF and $SuFu^{-/-}$ MEF cells [91]. In allograft animal models where primary medulloblastoma cells from $Ptch^{+/-}$ mice and Gli1-dependent BCC cells (ASZ001) were grafted, glabrescione B inhibited tumor growth and decreased the expression of Gli1 and its target genes [91].

Vitamin D3—Bijlsma *et al.* first reported that vitamin D3 directly binds Smo at the same site as cyclopamine in yeast model transformed with Smo using Scatchard analysis, and its treatment of zebrafish embryos mimicked the *smo*$^{-/-}$ phenotype such as U-shaped somites and aberrant extension of the yolk tube [92]. From a structure-activity relationship study, A-ring of vitamin D3 was important in direct binding to Smo and inhibiting Hh signaling [93, 94]. Vitamin D3 further showed inhibition of Hh signaling in BCC cells (ASZ) in a vitamin D receptor (VDR) independent way, and its topical application reduced Gli1 mRNA expression and proliferation of BCC cells in Ptch$^{+/-}$K14-CreER p53 fl/fl mice [95]. In addition, in renal cell carcinoma, vitamin D3 inhibited cellular growth and suppressed the expression of Gli2, an effect that was diminished when Smo was not expressed [96]. Oral administration or intraperitoneal injection of vitamin D3 also suppressed tumor growth in the xenograft model and decreased the expression of Gli2 in tumor tissue lacking VDR [96]. Active form of vitamin D3, calcitriol, inhibited cell proliferation *in vitro* and growth of BCC in Ptch mutant mice by targeting Smo [97]. Although vitamin D3 and its metabolites were reported to inhibit Hh signaling in a VDR-independent manner [95–97], it was recently demonstrated that VDR enhances the expression of SuFu through regulating miR-214 in breast cancer cells [98]. Overall, these findings suggest the interplay between vitamin D/VDR axis and Hh signaling in cancer.

4.2. Potential inhibitors of Hh signaling from natural and dietary sources

Curcumin and bisdemethoxycurcumin—Curcumin, a main active ingredient of *Curcuma Longa* (turmeric), induced cell cycle arrest and apoptosis via down-regulating the Hh signaling mediators including Gli1 in medulloblastoma and glioma cells [99, 100]. It suppressed the transcriptional activity of Gli1 and inhibited growth of mouse prostate cancer cells [101]. Recently, several studies demonstrated that curcumin, via inhibiting the Hh signaling pathway, reversed epithelial-mesenchymal transition (EMT) induced by TGF-β1 or hypoxia in pancreatic cancer cells [102, 103] and by γ-irradiation in glioma cells [104]. In a tumorsphere culture of lung CSC, curcumin suppressed formation of the tumorsphere and increased expression of stem cell markers, CD133, CD44, aldehyde dehydrogenase (ALDH) 1, Nanog, and Oct4, as well as expression of Gli and Smo, all of which were induced by Smo activator purmorphamine [105].

Epigallocatechin gallate (EGCG)—EGCG, a well-known catechin in green tea, was found to down-regulate the expression of Gli1 and inhibit the proliferation of mouse prostate cancer cells [101] and human chondrosarcoma cells [106]. In pancreatic CSC, EGCG inhibited cellular self-renewal capacity through regulating stem cell markers, Nanog, c-Myc and Oct4, as well as Hh signaling mediators, Smo, Ptch and Gli1/2 [107]. In an animal model of carcinogen-induced liver cancer, oral administration of EGCG reduced the population of α-fetoprotein- and CD44-positive cells and inhibited the expression of Gli1, Smo, cyclin D1, cMyc, and EGFR [108, 109].

Genistein and daidzein—Genistein, one of major isoflavones in soy products, inhibited transcriptional activity and expression of Gli1 in prostate cancer cells [101]. An additional study reported that genistein suppressed tumorsphere formation and decreased Gli1 and CD44 expression [110]. In a xenograft model of docetaxel-resistant prostate cancer cells,

genistein inhibited tumor growth and down-regulated the expression of Gli1 and CD44 in tumor tissues whereas docetaxel showed no effect [110]. In MCF-7 breast cancer cells, genistein reduced the size and number of tumorspheres, decreased the percentage of the $CD44^+/CD24^-$ subpopulation, and inhibited the expression of Smo and Gli1 [111]. This finding was further confirmed in MCF-7 xenograft tumors by demonstrating that genistein decreased tumor weight, and reduced the expression of Smo, Gli1, and a key stem cell marker ALDH1 [111]. Similar results showing regulation of Gli1 and CD44 expression and CSC properties by genistein were reported from a study of gastric cancer [112]. Another isoflavone, daidzein, was found to reverse cellular migration and invasion stimulated by TNF-α via inhibiting Gli1 expression and its transcriptional activity as well as MMP-9 activity in estrogen receptor (ER)-negative breast cancer cells [67].

Resveratrol—The compound, a stilbenoid found in grapes, blueberries and peanuts, inhibits Gli1 transcriptional activity [101]. Recent studies demonstrated resveratrol-mediated suppression of proliferation and induction of apoptosis in pancreatic cancer by modulating the expression of Gli1, Ptch and Smo [113]. Resveratrol inhibited the invasion capacity of gastric cancer cells by blocking the expression of Gli1, Snail, and N-cadherin and by increasing levels of E-cadherin [114]. In addition, hypoxia-stimulated Hh activation and invasiveness was suppressed by resveratrol in pancreatic cancer cells [115]. It is noteworthy that all studies of resveratrol targeting the Hh signaling have been conducted in cultured cells but not *in vivo*.

Silibinin—The compound present in seeds of milk thistles inhibited cell proliferation, induced apoptosis, and reduced Gli1 expression in renal cell carcinoma cells [116]. Silibinin decreased expression of phosphorylated AKT, mTOR, Gli1 and BCL2 in a renal cell carcinoma xenograft model [116]. Importantly, it is recently reported that silibinin inhibited the growth of Smo inhibitor-resistant basal cell carcinoma cells via targeting EGFR-MAPK-AKT, suggesting the possible combination of Smo inhibitors and other Hh targeting natural molecules [117].

Sulforaphane and sulforaphene—Sulforaphane, commonly found in cruciferous vegetables, suppressed the expression of Smo and Gli as well as Nanog and Oct4 in pancreatic cancer cells, which may indicate depletion of CSC [118]. A subsequent study using a xenograft model implanted with $CD133^+/CD44^+/CD24^+/ESA^+$ pancreatic CSC showed that oral administration of sulforaphane inhibited tumor growth and expression of Smo, Gli, Nanog, and Oct4 [119]. A sulforaphane analog, sulforaphene, was also found to inhibit Hh signaling mainly through reducing Gli1 expression and altering its localization which resulted in decreased migration and invasion of breast cancer cells [120].

Zerumbone and gedunin—Zerumbone, a sesquiterpene identified from the subtropical ginger *Zingiber zerumbet*, was reported to suppress the expression of chemokine receptor 4 (CXCR4) [121], a direct target of Gli1 involved in migration and metastasis of breast cancer cells [66]. These results suggest that zerumbone may regulate metastasis through Gli1/CXCR4 in breast cancer. Gedunin, a tetranotriterpenoid identified from *Azadirachta indica* known as Neem, inhibited proliferation, migration and metastasis of pancreatic cancer cells

and reduced both endogenous and Shh-stimulated levels of Ptch, Smo, Gli1, Shh, and SuFu [122]. Gedunin also reduced tumor growth in a xenograft model and decreased the levels of Hh mediators and EMT markers such as Notch-2, Snail, N-cadherin and Vimentin [122].

Others—Ishibashi *et al* employed a tetracyclin-regulated Gli1 expression/Gli1-luciferase assay system in HaCaT cells to screen the effects of natural components on Hh signaling and further to test their growth inhibitory effects in pancreatic (PANC1) and prostate (DU145) cancer cells [123–129]. The compounds identified as suppressors of Gli1 expression and transcriptional activity and inhibitors of cell proliferation included acoschimperoside P, 2'-acetate from *Vallaris glabra*, betulinic acid and colubrinic acid from *Zizyphus cambodiana*, gitoxigenin analogues from *Adenium obesum*, taepeenin D, (+)-drim-8-ene and quercetin 3-O-beta-D-glucopyranosyl-4-O-beta-D-glucopyranoside from *Acacia pennata*, staurosporinone and physalin F & B from *Crinum asiaticum,* physalin H from *Solanum nigrum,* and vitetrifolin from *Vitex negundo* [123–129]. Arcyriaflavin C from *Tubifera casparyi* also suppressed the transcriptional activity of Gli1 without affecting cell viability [124]. Importantly, physalin H and vitetrifolin blocked the direct interaction between Gli1 and DNA containing Gli1 binding site, suggesting Gli as a molecular target [127, 128]. Deguelin, a natural rotenoid derived from plants including *Derris trifoliate,* was reported to up-regulate SuFu and Ptch1/2, down-regulate Gli1, and inhibit proliferation, migration, and invasion in pancreatic cancer cells [130]. Ellagic acid, produced by hydrolysis of tannins from different fruits and vegetables, inhibited pancreatic tumor growth when orally administered and suppressed the expression of Gli1 and Gli2 in tumor tissues [131]. It was recently reported that crocetinic acid purified from crocetin inhibited the sphere formation of pancreatic cancer cells and decreased the expression of Shh, Smo, Gli1 and SuFu [132]. Germacranolide, a sesquiterpene lactone from *Siegesbeckia glabrescens*, suppressed the expression of Gli1 and Gli1-luciferase activity in pancreatic cancer cells [133]. Apigenin, baicalein, and quercetin inhibited cell growth and Gli1 expression in TRAMP-C2 cells although they did not affect Shh-induced Gli transcriptional activity in Shh Light II cells [101]. Recently, Infante *et al*. employed *in silico* screening of an *in house* compound library against the crystallographic structure of Smo bound to cyclopamine [134]. Based on the virtual hits fitting the Smo binding site and interaction with Smo residues, N219, Y394, K395, R400 and E518, the Smo antagonists were selected by using the FRED docking program and by ranking the Chemgauss4 score [134]. The biological function of selected molecules were then confirmed in Gli-responsive luciferase assay system, and isosophoranone, sorocein A, kuwanol E, and derrustone were found to exert an inhibitory activity [134]. Overall, modulation of the specific molecules in the Hh signaling pathway by natural and dietary inhibitors does not necessarily indicate that these compounds are specific or direct Hh inhibitors. The tumor inhibitory effects of these natural products and dietary components in the Hh-specific model systems need to be further examined to confirm whether molecular mechanisms involved are dependent on Hh signaling.

Conclusion and Future Directions

The role of Hh signaling in carcinogenesis has been demonstrated in experimental models and confirmed by clinical efficacy of two FDA-approved selective Smo inhibitors,

vismodegib and sonidegib. However, the acquired resistance to vismodegib in cancer patients demonstrates clinical limits of targeting Smo and sheds light on the roles of different mediators of the Hh signaling pathway. As reviewed in this article, numerous studies have evaluated the effects of natural products and dietary components on Hh signaling through Smo, Gli, SuFu and other factors. Results from these studies can provide new insights into the development of promising agents for cancer prevention and treatment. However, there are several important issues to highlight before considering inhibition of Hh signaling by natural products and dietary components as a viable cancer preventive strategy.

First, although results from numerous studies of natural products have demonstrated their inhibitory role in Hh signaling, many have not been proven to be direct inhibitors of the Hh signaling molecules. Because Hh signaling can be modulated by both canonical regulation and interaction with different cellular pathways, it is critical to conduct detailed investigations using appropriate *in vitro* and *in vivo* models to identify the natural components' direct cellular targets. Second, many natural products are poorly bioavailable and metabolized by the intestinal microflora and/or hepatic metabolizing enzymes. In addition, the concentrations used in some *in vitro* studies may not be achievable in physiological conditions. Therefore, the natural products' blood levels necessary for activity need to be determined. Third, experience from clinical trials with Smo-targeted drugs showed the importance of selecting cancer patients with aberrantly activated Hh pathway to achieve tumor response. Thus, it is important to systematically characterize the Hh signaling profiles in cancer cells and in animal tumor models. Fourth, combining natural products to maximize inhibition of Hh signaling may be necessary to provide optimal efficacy while overcoming resistance.

Overall, despite the outlined challenges, exploring natural products and dietary components that target the complex network of signaling molecules in the Hh pathway is a promising direction in the effort of searching for novel agents to prevent and treat cancer.

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Abbreviations:

ALDH	aldehyde dehydrogenase
BCC	basal cell carcinoma
CDON	cell adhesion molecule-related/down-regulated by oncogenes
CK	casein kinase
CSC	cancer stem cells

CXCR4	chemokine receptor 4
Dhh	Desert Hedgehog
EMT	epithelial-mesenchymal transition
ERK	extracellular signal-regulated kinase
GANT	Gli antagonist
Gli	glioma associated oncogene
GSK	glycogen synthase kinase
Hh	Hedgehog
Ihh	Indian Hedgehog
JNK	c-Jun N-terminal kinase
LSC	leukemia stem cells
MAPK	mitogen-activated protein kinase
MEF	mouse embryonic fibroblasts
MMP	matrix metalloproteinase
MPF	mitosis promoting factor
NFκB	nuclear factor-κB
PI3K	phosphatidylinositol-3-kinase
PKA	protein kinase A
S6K	S6 kinase
SAG	Smo agonist
Shh	Sonic Hedgehog
Smo	Smoothened
SuFu	suppressor of fused
TGF	transforming growth factor
TNF	tumor necrosis factor
mTOR	mammalian target of rapamycin
VEGF	vascular endothelial growth factor
VDR	vitamin D receptor

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Table 1.

Natural and dietary compounds regulating Hedgehog signaling *in vitro*

Compound	Treatment (μM)	Experimental model	Proposed targets/Mechanism of action	Effect	Ref
Direct inhibitors of Hh signaling					
Berberine	1–20	Medulloblastoma cells from allografts in Ptch +/− p53−/−mice NIH-3T3	Competitive binding with cyclopamine to Smo	↓Proliferation	[78]
Glabrescione B	1–10	Medulloblastoma cells from Ptch+/− mice Basal cell carcinoma (ASZ001)	↓Gli1/DNA interaction	↓Tumorsphere formation ↓Proliferation	[91]
Vitamin D3	5, 10	Murine basal cell carcinoma (ASZ, BSZ, and CSZ)	↓Gli1	↓Proliferation	[95]
	20, 30	Renal cell carcinoma (786-O, A498, ACHN, Caki-1)	↓Gli2	↓Proliferation	[96]
	50	Breast cancer (WT-145, MCF7, T47D)	↓miR-199a/miR-214 ↑SuFu	↓Proliferation	[98]
Potential inhibitors of Hh signaling					
Acoschimperoside P, 2'-acetate	0.75–6	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Gli1, ↓Ptch1	↓Proliferation	[126]
Apigenin	1–100	Prostate cancer (TRAMP-C2)	↓Gli1	↓Proliferation	[101]
Arcryaflavin C	1.5–100	HaCaT-GLI1-Luc cells	↓Gli1-transcriptional activity		[124]
Baicalein	1–100	Prostate cancer (TRAMP-C2)	↓Gli1	↓Proliferation	[101]
Betulinic acid	16– 66	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Gli1, ↓Ptch1	↓Proliferation ↑Apoptosis	[125]
Colubrinic acid	16– 66	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Gli1, ↓ Ptch1	↓Proliferation ↑Apoptosis	[125]
Crocetinic acid	1, 10	Pancreatic ductal adenocarcinoma (MiaPaCa-2, BxPC-3, Capan-1, ASPC-1)	↓Shh, ↓Gli1, ↓Smo	↑Apoptosis ↓Pancosphere ↓Stemness	[132]
Curcumin	5–40	Lung cancer (A549, H1299)	↓ CD133, CD44, ALDH1, Nanog, Oct4, β-catenin ↓ Shh, Smo, Gli1, Gli2	↓CSC formation ↓CD133+ cells ↑Apoptosis ↓Proliferation	[105]
	20	Pancreatic cancer (Panc-1)	↓Shh, Smo, Gli	↓Hypoxia induced EMT, proliferation, Invasion, migration	[102]
	10–30	Pancreatic cancer (Panc-1)	↓Shh, Gli1, Vimentin ↑E-cad	↓TGF-β induced EMT, Invasion, migration	[103]
	20	Glioma (U251, LN229)	↓Gli1, ↑SuFu, ↑E-Cad	γ-irradiation induced EMT, Invasion, migration	[104]
	5–40	Glioma (U87, T98G)	↓Shh, Gli1	↓Cell viability ↑Apoptosis	[99]
	10–40	Medulloblastoma (DAOY and primary cells)	↓Shh, Gli1	↓Cell viability ↑Apoptosis	[100]
	1–100	Prostate cancer (TRAMP-C2) NIH3T3 Shh-Light II cells	↓Gli1 ↓Gli1-transcriptional activity	↓Cell viability	[101]

Compound	Treatment (μM)	Experimental model	Proposed targets/Mechanism of action	Effect	Ref
Daidzein	30	Breast cancer (MCF10DCIS.com)	↓Gli1, Smo	↓TNF-α induced migration, invasion	[67]
Deguelin	5–20	Pancreatic cancer (Bxpc-3, Panc-1)	↑SuFu, ↑Ptch1, Ptch2 ↓Gli1	↑Apoptosis ↓Invasion, migration	[130]
Derrustone	30	NIH3T3 Shh-Light II cells	↓Gli1 transcriptional activity	-	[134]
(+)-Drim-8-ene	4.0–60	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Gli1, ↓Ptch, ↓Bcl2 ↓Gli-transcriptional activity	↓Proliferation	[123]
EGCG	20–60	Pancreatic cancer stem cells (CD133+/CD44+/CD24+/ESA+)	↓Nanog, Oct4, Smo, Ptch, Gli, Snail, ZEB1, Slug	↓Size and Colony formation of Spheroids ↑Apoptosis	[107]
	1–4	Chondrosarcoma (SW1353, CRL-7891)	↓ Ptch, ↓Gli	↓Cell viability ↑Apoptosis	[106]
	1–100	Prostate cancer (TRAMP-C2) NIH3T3 Shh-Light II cells	↓ Gli ↓Gli1-transcriptional activity	↓Proliferation	[101]
Gedunin	15, 25	Pancreatic cancer (HPAC, PANC-1, MIAPaCa-2) Normal pancreatic epithelial cells (hTERT HPNE)	↓Gli1, Ptch1, Ptch2, Shh, ↑SuFu ↓Notch-2, Snail, N-cadherin	↓Proliferation ↑Apoptosis ↓Migration ↓Metastasis	[122]
Genistein	5–30	Breast cancer (MCF7)	↓ Smo, Gli1	↓Proliferation ↑Apoptosis ↓CSC formation	[111]
	15, 30	Prostate cancer (22RV1, DU145)	↓CD44, Gli1	↓Tumorsphere formation	[110]
	10	Gastric cancer (MKN45)	↓Gli1, ↓CD44, ↓OCT4	↓Tumorsphere formation ↓CSC properties	[112]
	1–100	Prostate cancer (TRAMP-C2, PC3) NIH3T3 Shh-Light II cells	↓Gli1 ↓Gli1-transcriptional activity	↓Proliferation	[101]
Germacranolide	1–20	Pancreatic cancer (PANC1, AsPC-1)	↓Gli1	↓Proliferation	[133]
Gitoxigenin analogues	0.031–0.5	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1)	↓Ptch, Bcl2 ↓Gli-transcriptional activity	↓Proliferation	[129]
Isosophoranone	30	NIH3T3 Shh-Light II cells	↓Gli1 transcriptional activity	–	[134]
Kuwanol E	30	NIH3T3 Shh-Light II cells	↓Gli1 transcriptional activity	–	[134]
Physalin F & B	2–8	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1)	↓Gli1, Gli2, Ptch1 ↓Gli-transcriptional activity	↓Proliferation	[124]
Physalin H	0.75–3	HaCaT-GLI1-Luc cells	↓Ptch1 ↓Gli1/DNA binding	–	[128]
Quercetin	1–100	Prostate cancer (TRAMP-C2)	↓Gli1	↓Proliferation	[101]
Quercetin 3-o-beta-d-glucopyranosyl-4-o-beta-d-glucopyranoside	10–40	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Gli1, ↓Ptch, ↓Bcl2 ↓Gli-transcriptional activity	↓Proliferation	[123]
Resveratrol	12.5–50	Pancreatic cancer (BxPC-3, Panc-1)	↓Smo and Gli	↓Hypoxia-induced Invasion, migration	[115]
	55	Gastric cancer (SGC-7901)	↓ Gli1, Snail, N-cad ↑E-cad	↓Invasion, metastasis ↓EMT	[114]
	50–200	Pancreatic cancer (MIA PaCa-2)	↓ Ihh, Ptch, Smo	↓Cell viability ↑Apoptosis	[113]
	1–100	Prostate cancer (TRAMP-C2) NIH3T3 Shh-Light II cells	↓Gli1 ↓Gli1 transcriptional activity	↓Proliferation	[101]
Silibinin	50–200	Renal cell carcinoma (769-P, 786-O, ACHN, OS-RC-2)	↓Gli1	↓Proliferation ↑Apoptosis	[116]

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Compound	Treatment (μM)	Experimental model	Proposed targets/Mechanism of action	Effect	Ref
Sorocein A	30	NIH3T3 Shh-Light II cells	↓Gli1 transcriptional activity	-	[134]
Sulforaphane	5–20	Pancreatic cancer stem cells	↓ Smo, Gli1, Gli2 ↓ Nanog, Oct4	↓Cell viability ↑Apoptosis	[118]
Sulforaphene	1–10	Breast cancer (SUM159)	↓Gli1 expression, nuclear translocation	↓Invasion, metastasis	[120]
Sutherlandioside D	0.01–10	Prostate cancer (PC3, LNCaP) Mouse prostate cancer (RAMP-C2)	↓Gli1, ↓Ptch1	↓Proliferation	[135]
Staurosporinone	2–8	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1)	↓Gli1, ↓Gli2, ↓Ptch1 ↓Gli-transcriptional activity	↓Proliferation	[124]
Taepeenin D	0.5–8.0	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Gli1, ↓Ptch, ↓Bcl2 ↓Gli-transcriptional activity	↓Proliferation	[123]
Vitetrifolin D	12.3–49.3	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1) Prostate cancer (DU145)	↓Ptch1 ↓Gli1/DNA binding	↓Proliferation	[127]
Zerumbone	0.73–23	HaCaT-GLI1-Luc cells Pancreatic cancer (PANC-1)	↓Gli1, Gli2, Ptch1 ↓Gli-transcriptional activity	↓Proliferation	[124]

ALDH, Aldehyde dehydrogenase; CK2α, Casein kinase 2α; CSC, Cancer stem cells; E-cad, E-cadherin; EGCG,pigallocatechin gallate; EMT, Epithelial-mesenchymal transition; Gli, Glioma-associated oncogene; Ihh, Indian hedgehog; N-cad, N-cadherin; Oct4, octamer-binding transcription factor 4; Ptch, Patched; Shh, Sonic hedgehog; Smo, Smoothened; SuFu, Suppressor of fused; TGF, Transforming growth factor; TNF, Tumor necrosis factor; ZEB1, Zinc finger E-box-binding homeobox 1.

Table 2.

Natural and dietary compounds regulating Hedgehog signaling *in vivo*

Compound	In vivo model	Treatment	Effect and Target	Ref
Berberine	Primary intracranial medulloblastoma cells from Ptch +/− p53−/−mouse s.c. in athymic nude mice	100 mg/kg BW, p.o., daily for 3 weeks	↓Gli1, ↓Ptch ↓Tumor growth (37.5% reduction)	[78]
Curcumin	U87-Luc (3×10^5 cells) intracranial injection to female nude mice	60 mg/kg BW, i.p., daily for 40 days	↓Gli1 ↓Tumor growth (71.4% reduction)	[99]
EGCG	N-Nitrosodiethylamine (NDEA) into oral cavity of female Swiss albino mice	8 μg/kg BW, p.o., up to 30 weeks	↓Gli1, Smo, CD44, Cyclin D1, c-Myc, EGFR ↓BrdU incorporation ↓Dysplasia progression	[109]
	CCl_4/NDEA in female Swiss albino mice	8 μg/kg BW, p.o., up to 30 weeks	↑Ptch1 ↓Smo, Gli1, CD44 Cyclin D1, c-Myc, EGFR ↓BrdU incorporation ↓Dysplasia progression	[108]
Ellagic acid	Pancreatic cancer cells PANC-1 (2×10^6 cells) s.c. in BALB/c nude mice	40 mg/kg BW, p.o., 5 days a week for 6 weeks	↓Gli1, Gli2 ↓Tumor growth and metastasis (41.2% reduction)	[131]
Gedunin	Pancreatic cancer cells HPAC (1×10^6 cells) s.c. in female athymic nude mice	20 mg/kg BW, i.p., 5 days a week for 1 month	↓Gli1, Ptch1, Ptch2, Shh ↑SuFu ↓Tumor growth (82.2% reduction)	[122]
Genistein	Breast cancer cells MCF-7 (1×10^6 cells), mammary fat pad injection in female nude mice	20 and 50 mg/kg BW, i.p., daily for 2 weeks	↓Smo, Gli1, ALDH ↓Tumor growth (46% and 68% reduction, respectively)	[111]
	Tumorsphere (10^4 cells) from prostate cancer cells 22RV1 s.c. in male BALB/c nude mice	10 mg/kg BW, i.p., daily for 2 weeks	↓Gli1, CD44 ↓Tumor growth (58.3% reduction)	[110]
	Tumorsphere (10^5 cells) from prostate cancer cells DU145 s.c. in male BALB/c nude mice	10 mg/kg BW, i.p., daily for 2 weeks	↓Gli1, CD44 ↓Tumor growth (57.1% reduction)	[110]
Glabrescione B	Medulloblastoma (2×10^6 cells) from Ptch +/− mice s.c. in female BALB/c nude mice	75 μmol/kg BW, daily for 18 days	↓Gli1, Ptch1 ↓Tumor growth (63.6% reduction)	[91]
	Basal cell carcinoma ASZ001 (2×10^6 cells) s.c. in female NOD/SCID mice	100 μmol/kg BW, daily for 18 days	↓Gli1, PTCH1 ↓Tumor growth (71.4% reduction)	[91]
Silibinin	Renal cell carcinoma 786-O cells s.c. in male BALB/c nude mice	200 mg/kg, p.o., daily for 30 days	↓Gli1, ↓Gli2 ↓Tumor growth (64.9% reduction)	[116]
Sulforaphane	Orthotopic implantation of pancreatic cancer stem cells ($CD133^+/CD44^+/CD24^+/ESA^+$, 1×10^3 cells) in the pancreas of male NOD/SCID/IL2R gamma mice	20 mg/kg BW, p.o. 5 days a week for 6 weeks	↓Smo, Gli1, Gli2 ↓Nanog, Oct4, PDGFRα., VEGF, ZEB1 ↑E-cad ↓Tumor weight (45.0% reduction)	[119]
Vitamin D3	Renal cell carcinoma 786-O cells s.c. in male athymic nude mice	250 IU/mouse, every 2 weeks, i.p. Up to 12 weeks (Prophylactic, therapeutic treatment)	↓Gli2 ↓Tumor growth (92.0% and 81.4% reduction, respectively)	[96]
	Renal cell carcinoma 786-O cells s.c. in male athymic nude mice	10,000 IU/kg BW diet. Up to 12 weeks (Prophylactic, therapeutic treatment)	↓Gli2 ↓Tumor growth (45.0% and 25.0% reduction, respectively)	[96]
	Ionizing radiation treated Ptch1+/− K14-Cre-ER p53 fl/fl mice developing basal cell carcinoma	Topical application of vitamin D3 (1.3 and 2.6 mg/kg BW) up to 30 days	↓Gli1 ↓Ki67 expression	[95]

ALDH, Aldehyde dehydrogenase; BW, Body weight; E-cad, E-cadherin; EGCG, Epigallocatechin gallate; EGFR, Epidermal growth factor receptor; Gli, Glioma-associated oncogene; i.p., intraperitoneal injection; Oct4, octamer-binding transcription factor 4; PDGFR, Platelet-derived growth factor receptor; p.o., per os (oral administration); Ptch, Patched; s.c., subcutaneous injection; Shh, Sonic hedgehog; Smo, Smoothened; SuFu, Suppressor of fused; VEGF, Vascular endothelial growth factor; ZEB1, Zinc finger E-box-binding homeobox 1.