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Targeting Hedgehog signaling pathway: paving the road for cancer therapy

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Abstract

The Hedgehog pathway is essential for embryonic development but also for tissue and organ homeostasis in adult organisms. Activation of this pathway leads to the expression of target genes involved in proliferation, angiogenesis and stem cell self-renewal. Moreover, abnormal persistence of Hedgehog signaling is directly involved in a wide range of human cancers. Development of novel strategies targeting the Hedgehog pathway has become a subject of increased interest in anticancer therapy. These data are sustained by pre-clinical studies demonstrating that Hedgehog pathway inhibitors could represent an effective strategy against a heterogeneous panel of malignancies. Limited activity in other tumor types could be explained by the existence of crosstalk between the Hedgehog pathway and other signaling pathways that can compensate for its function. This review describes the Hedgehog pathway in detail, with its physiological roles during embryogenesis and adult tissues, and summarizing the preclinical evidence on its inhibition, the crosstalk between Hedgehog and other cancer-related pathways and finally the potential therapeutic effects of emerging compounds.

Keywords: Anticancer Therapy, Embryonic development, Hedgehog signaling, Self-renewal

Introduction

Despite advancements in preclinical and clinical research, cancer incidence remains in full view due to a range of heterogeneous risk factors including location, ethnicity, gender, dietary habits, genetic predisposition, environmental exposure and socio-economic factors. Therefore, cancer research is still one of the top research subjects, with current interest focusing on personalized and targeted therapeutic approaches, novel diagnosis and prognosis methods and improved clinical management (1).

The installation and development of malignant entities is governed by a high number of interconnected pathological signaling pathways such as the extracellular signaling Wingless-type (Wnt) and Hedgehog (Hh) pathways, linked to direct embryonic growth and modeling and abnormally synchronized in cancer (2). In fact, the Hh signaling pathway is fundamental in regulating cell growth and differentiation, as well as maintaining homeostasis in several tissues and organs, by influencing the activity of stem cells in vertebrates and invertebrate organisms (2-4). It is well-known that stem cells possess the capacity for continuous division, being able to shift into different cell types through differentiation mediated by a number of cell signaling pathways including Wnt, Hh, and Notch (5). Sonic hedgehog (SHh), Indian hedgehog (IHh), and Desert hedgehog (DHh) comprise the three mammalian Hh genes playing major roles in designing and modeling many tissues and organs (6). Of these tissues, the Hh gene was characterized as an obligatory signaling protein for the specification of positional distinctiveness in the *Drosophila* embryo (7). Porter et al. (8) reported that the peptide formed through intramolecular cleavage and lipid modification reactions in the secretory pathway is responsible for the total signaling actions of the Hh gene. Zhu et al. (2) recently showed that the expression of Hh canonical pathway genes, namely Smoothened (Smo) and Gli1, are mediated by fibroblast growth factor (bFGF) which in turn accelerates fibroblast migration.

On the other hand, when the Hh pathway is activated in an unusual manner, it may

facilitate a number of tumor types, by favoring the process of tumorigenesis, and metastasis (5, 9). Actually, cancer stem cells are quite similar to regular stem cells, in terms of their self-renewal capacity. Several studies have demonstrated that the Hh pathway is actively involved in and can control self-renewal pathways within cancer stem cells, particularly in leukemia, other blood cancers, and breast cancer (10-12). Since a significant number of molecules targeting said pathway are already at the clinical testing stage, especially for the niche of hematological malignancies, we aimed to review the Hh signaling pathway in detail as one of the key targets for cancer therapy and development of more effective and promising cancer inhibitory strategies. Therefore, this review covers all mechanistic and functional details related to Hh pathway along with its inhibitors and clinical potential.

Hedgehog pathway

The Hedgehog pathway (Figure 1) derives its name from Hh gene mutant Drosophila, which presents a spiked phenotype resembling the animal "hedgehog" (13). The Hh proteins control embryonic development in vertebrates, where the signaling mechanisms mediated by the proteins are multiple and increasingly variable. For instance, they can act as morphogens (by mediating the morphogenesis process in a concentration dependent manner) or mitogens (by controlling cell proliferation through the mitosis process) (14). The Hh gene has three homologues in mammals: IHh (Indian hedgehog), SHh (Sonic hedgehog) and DHh (Desert hedgehog), with Sonic hedgehog being the most studied.

The IHh homologue is produced by the chondrocytes (cells of the cartilage) and is involved in controlling their differentiation with a role in regulating osteoarthritis. The transmembrane proteins PTCH1 (Patched 1) and Smo (Smoothened) respond to IHh and mediate the IHh signaling mechanism. The PTCH1 protein generally inhibits Smo in the absence of IHh by suppressing the downstream transcription factors Gli 1, 2 and 3 (Gli Zinc

finger). However, in the presence of IHh the signaling mechanism is activated and the inhibition of Smo by PTCH1 is relieved. Next, the expression of Gli transcription factors is enhanced, being further translocated within the nucleus where they enhance the transcription of downstream target genes (Figure 1) (15). Regulatory mechanisms can also be found at the Gli transcription factor level, where Gli2 and Gli3 can be found in active or inactive forms. Where Hh is absent, Gli3 is cleaved through proteolytic mechanisms into the $Gli3^{R}$ (Gli3 repressor form), where Gli2 is further degraded. When Hh is expressed, the cleavage of Gli2 is blocked and can be N-terminally truncated to generate an inactive form ($Gli2^{A}$) (16).

The SHh pathway (SHh is named after Sega's jump 'n' run character) mainly controls the growth and development of the embryo and is also involved in mechanisms related to the central nervous system. This pathway is mediated by the signaling molecule SHh, which mediates the expression of transcription factors depending on its deposition in the neural tube. Similar to the IHh pathway, the expression of SHh, removes the inhibition of PTCH1 on Smo and enhances the expression of Gli transcription factors (Figure 1) (17). The activity of SHh as a morphogen is concentration dependent, where the signaling is specifically directed dependent on the concentration of the SHh protein: for instance ventral neuron, motor neuron and floor plate cell development takes place at low, high and very high concentrations respectively (18). On the other hand, the DHh pathway mediated by the DHh protein (which is expressed in the testis) controls the spermatogenesis process by maintaining the male germ line cell. The DHh pathway also operates through PTCH1 and Smo protein regulation (Figure 1) (19).

The PTCH1 mediator has two additional homologues PTCH2 and HHIP1 (HH-interacting protein-1), with all three proteins playing central roles in mediating the Hh ligand signaling which occurs during the development of the embryo (20). The Smo protein is a nodal point within the Hh signaling pathway, since it mediates the response associated with the Hh ligand. While this integral membrane protein has structural similarities with the GPCR (G-

protein coupled receptors), there is little evidence to indicate a direct coupling with GPCR. In Drosophila the Smo protein accumulates in the cell membrane at the moment of Hh activation, while in vertebrates the protein gets internalized after activation of the pathway (21). There are many essential proteins which directly phosphorylate Smo, activating the protein and allowing for initiation of the Hh pathway, such as CK1 (Casein kinase 1) and Grk2 (G-protein-coupled receptor kinase 2) (22).

Since the Hh signaling pathway is vital for a heterogeneous range of developmental processes, different transcription factors act in coordination to regulate the expression of said Hh protein. If any mutation occurs in these transcription factors, this will affect the expression of Hh proteins and result in developmental related conditions such as epilepsy, tibial hypoplasia, polydactyly and X-linked lissencephaly, neurological disorders and cancer (23).

Hedgehog pathway in embryonic development

It is now well established that the Hedgehog pathway solves a role as one of the essential signaling mechanisms for the modulation of cellular growth and differentiation during embryogenesis. Operating through time- and position-dependent mechanisms, this pathway mostly guarantees the correct size, cellular content and position achievement of organs during embryonic development (24).

As a mitogen, the Sonic Hedgehog signaling pathway promotes proliferative and differentiation processes in specific groups of cells from ectoderm, mesoderm and endoderm tissues (25-28). In addition, this pathway is involved in the formation of teeth and lungs and midline facial development, and most importantly the induction of neural tissue by mesodermal notochord (29). Beside its regulatory role on cell proliferation, Sonic Hedgehog can also promote proliferation and survival of neural progenitor cells in the ventral spinal cord (30). This pathway controls different sets of homeodomain proteins in distinct progenitor cells

through utilization of Gli^A and Gli^R (31). Gli^A and Gli^R similarly affect a wide range of targets during Sonic Hedgehog induced development of sclerotome. Nevertheless, the way through which the expression of these sets of target genes is regulated is yet to be fully understood.

Through regulating the proliferation and differentiation of chondrocytes, the Indian Hedgehog signaling pathway mostly controls skeletal development during embryogenesis. Additionally, the pathway is involved in visceral endoderm differentiation, hematopoiesis and vasculogenesis (32). Finally, the Schwann cell derived Desert Hedgehog signaling pathway results in the creation of perineurium through induction of mesenchymal cell transitions (33).

Key components of the Hedgehog pathway in vertebrates

The vital physiological roles of the Hh signaling pathway has inspired many scientists to investigate its key components and the interplay between them. This complicated signaling mechanism regulates proliferation, differentiation, and tissue patterning during embryogenesis and can be reactivated in adults as part of processes of repair and regeneration (34). So far, canonical (mediated by Gli family of transcription factors) together with non-canonical pathways (mediated by Gli independent mechanisms) have been proposed for Hh protein signal transduction which is mostly enriched in the cilia.

Prior to secretion, all Hh proteins undergo covalent attachment of a cholesterol molecule to the C-terminal residue, after which Hh acyltransferase (Hhat) transfers a palmitoyl group to the amino termini. These lapidated morphogens are secreted to the cell surface, where they undergo a multimerization process; in this form they interact with Hh receptors, serving as long range signaling molecules (35-37). Sheddases such as the glycoprotein Scube2 (signal peptide, cubulin domain, epidermal growth factor-like protein 2) and glycosylphosphatidylinositol (GPI)-linked glypican (Gpc) heparan sulfate proteoglycans (HSPGs) have been proven to be regulators of Hh activity. Grobe and colleagues proposed that Gpc HSPG control the release

of SHh from secreting cells in preclinical models, where purified heparane sulfate has been found to directly trigger SHh processing (37). Other approaches have shown that Hhat, the enzyme responsible for the transfer of palmitate upon SHh, could function as a potent therapeutic target in pancreatic cancer (38). Magee and colleagues offer significant insights regarding Hhat activity and structure, with potential benefits for a more informed development of Hhat targeting agents. They reveled that the enzyme is composed of ten transmemebrane domains and is palmitoylated on numerous cysteines with cytosolic localization, which aids in structural stability. Furthermore, mutation within the catalytic domain can result in complete depletion of Hhat palmitoylation (39).

Three main mechanisms have been proposed for the secretion of active forms of hedgehog ligands: first, construction of a multimeric molecule with lipid moieties placed on the inside, making a soluble Hh protein which can diffuse from the membrane; second, function of dispatched proteins through packaging multimeric Hh or proton promoting transportation and third, movement of multimeric Hh by Tout-velo dependent mechanisms (40). Subsequently, Hh ligands bind to their transmembrane receptors PTCH 1 and 2 or a G-protein-coupled-receptor resembling protein (GPCR class F) named Smo (41). Specific co-receptors such as cell adhesion molecules may also be down-regulated by oncogenes such as (Cdo), brother of Cdo (Boc), and growth arrest–specific gene 1 (GAS-1), co-receptors which enhance the Hh ligand binding to PTCH. Contrarily, Hhip protein competes with Hh ligands for PTCH binding (34, 42, 43).

Canonical Hedgehog signaling pathway

The canonical signaling pathway is mainly activated by binding modified Hh ligands to their PTCH receptor, hindering the inhibitory effect of PTCH on Smo, and allowing Smo to enter the primary cilium to regulate the downstream cascade. Smo generates intracellular signals

which activate glioma-associated (Gli) transcription factors by modulating membrane associated protein complexes containing the protein kinase Fused (Fu), suppressor of Fused (SuFu) and the kinesin related protein costal 2 (Cos2). While the function of Fu in mammals is unclear, SuFu plays a significant role in regulating the stability of Gli factors in vertebrates, as a deficiency in these results in several development defects. SuFu forms complexes with Gli proteins which further accumulate in primary cilium following Hh stimulation, detaching after phosphorylation. These modified Gli proteins translocate from primary cilium to cytoplasm and from here to the nucleus, promoting expression of Hh target genes including Hh feedback pathway (e.g., GLI1, PTCH1), proliferation (e.g., MYC, Cyclin-D1), angiogenesis (e.g., ANG1/2), apoptosis (e.g., Bcl-2), epithelial-to-mesenchymal transition (EMT) (e.g., SNAIL), or stem cell self-renewal (e.g., NANOG, SOX2) (18, 42, 44-46).

The Gli family consists of three members: Gli 1, Gli 2, and Gli 3 through which the Hh target genes are regulated. A proteolytic process manipulates activity of Gli 3 and to some extent Gli 2 to display a dual function, both as repressors and activators of Hh target genes. When Hh stimulation is absent, proteolytic events remove the transactivation domain of Gli 3 in the primary cilium. The Gli 3 repressor protein then translocates to the nucleus, inhibiting the transcription of Hh target genes. Although the mechanism of Gli activator formation is yet to be entirely elucidated, some studies have proposed that this may occur through deactivation of G-protein-coupled receptor 161 (GPCR161) in the cilium, which further inhibits Hh signaling through PKA and Gli 3 repressor formation (47). In contrast with Gli 2 and 3, Gli 1 is limited to activator functions.

Non-canonical Hh signaling pathways

Non-canonical Hh signaling pathways are generally categorized into two types: Type I or PTCH-dependent pathway and Type II or Smo-dependent pathway. These two types are further

presented in more detail:

Type-I

Type-I non-canonical signaling is dependent on PTCH1 and Hh ligands, but independent from Smo, and is mostly involved in processes of apoptosis and proliferation. *In vitro* studies have showed that PTCH1 has apoptosis inducing properties and functions as a dependent receptor. If Hh ligands are not present, PTCH1 binds to the pro-apoptotic complex of Caspase-9, a regulating protein for apoptosis related cell death, DRAL, and TUCAN-1 to maintain Cyclin B outside the nucleus. In the presence of Hh ligands, the linkage between PTCH1 and Cyclin B1 is disrupted and a new complex is formed between PTCH1 and G-protein receptor kinase-2 (GRK2). This process results in nuclear translocation of Cyclin B1 and increased cell proliferation and survival (42) through induction genes encoding Cyclin D1 and N-Myc (42, 48)

Type-II

Type-II non-canonical Hh signaling has been found to regulate actin cytoskeleton and calcium release in fibroblasts and neurons. The Smo and Gi protein mediated activation of the Rho small GTPase subfamilies including RhoA and Rac1, have been shown to be involved in cytoskeletal rearrangement processes. T lymphoma invasion and metastasis protein (Tiam-1), is a guanine exchange factor for Rac1. In response to Hh stimulation, the complex Rac1-Tiam-1 is removed and Rac1 is further activated. Smo and Gi mediated phospholipase C-γ (PLC-γ) activation has been shown to result in formation of inositol 3-phosphate (IP3) which in turn increases intracellular calcium ions with effects upon different activities in neurons, such as proliferation, apoptosis, differentiation and migration (42, 47, 49-53).

Other studies have also proposed regulatory roles for Hh proteins in axon guidance through activation of Src family kinases (SFK) in a Smo dependent manner. Due to the unsuccessful

identification of Src as a component of the canonical pathway through Hh receptors, and lack of phenotypes indicative of dysregulated canonical signaling in mutant mice, this regulation has been attributed to the non-canonical signaling pathway (54, 55).

Furthermore, non-canonical Smo and Gi mechanisms have also been shown to be involved in metabolic reprogramming of mouse embryonic fibroblasts and adipocytes. The activation of AMP-activated protein kinase (AMPK) following activation of Smo in these cells results in a rapid increase in glucose uptake (56, 57).

Hedgehog signaling in cancer

Deregulation in Hh signaling has been highlighted in cancer, and one third of all malignancies are currently believed to be dependent on the aberrant functioning of the pathway. Three types of activated signaling mechanisms are currently associated with cancer development: Type I – ligand independent and autonomous Hh pathway, Type II – ligand dependent oncogenic Hh pathway (autocrine/juxtacrine mode), Type IIIa/b – ligand dependent oncogenic Hh pathway (paracrine or reverse paracrine mode) (58).

The activation of Hh signaling in a ligand independent manner, defined as Type I, is mainly determined by the acquisition of activator mutations within Smo, or inactivation mutations in the negative modulators - Sufu or Ptch1. These genomic modifications allow Hh cascade signaling without the presence of a specific ligand (59). Patients with autosomal dominant disorders - BCNS (Gorlin syndrome) – frequently display a mutation of Ptch1; these patients are also associated with a high risk of acquiring sporadic basal cell carcinoma (BCC) and other malignancies such as medulloblastomas (MBs) and meningiomas (60). Similarly, patients diagnosed with BCC or presence of MB had associated mutations in activators of inhibitors of Hh signaling (58).

Type II – the ligand dependent oncogenic Hh pathway (autocrine/juxtacrine mode)is ,

as the name suggests, activated upon ligand binding in a cell-autonomous manner, where production ligands can stimulate the activity of the cell of origin or of surrounding cells. Over-activation of such stimulation has been reported in numerous malignancies such as pancreatic, esophageal and stomach cancers (61), lung (62), prostate (63), breast (64) and colorectal cancers (65), melanoma (66) and glioma (67). Colorectal cancer has been associated with contradictory results in terms of SHh expression; some studies report increased expression, suggesting that secretion of SHh is an essential feature of cancer development (68, 69), while others concluded that the Hh pathway is inactive in this malignancy (61, 70). These contradictory data reports could highlight a tumor dependent context of Hh activation, where patient stratification could become an essential element in selection of potential experimental treatments.

Type IIIa/b – the ligand dependent oncogenic Hh pathway (paracrine or reverse paracrine mode) is mediated by paracrine activation and is usually encountered in embryonic development, but also in installation and progression of cancer (58). Specifically, Hh ligands secreted by malignant cells activate the Ptch1 receptor found on tumor associated stromal cells; in turn, the stromal cells emit growth factors such as VEGF, PDGF, BMP and IGF stimulating the proliferation and differentiation of cancer cells (71). In special contexts, reverse paracrine signaling can be found in hematological malignancies, where the tumor cells are stimulated by Hh ligands secreted in lymph node or bone marrow stromal cells. In this case, the stromal cells can become a therapeutic target due to their significant involvement in the establishment of pro-malignant environments (72).

Clinical impacts of Hedgehog inhibition

In the past decade, strategies for inhibiting Hh signaling pathway have been studied intensively (Table 1) but often with contradictory results. One of the first and most studied inhibitors of

the tumor-promoting Hh signaling pathway has been Vismodegib (also called HhAntag691, and GDC-0449), a molecule that serves as a cyclopamine-competitive antagonist of Smo. This low molecular weight compound entered clinical trials about ten years ago and is used to treat some types of solid tumors (73). In 2009, Von Hoff et al. published the results of an open-label, multicenter (three centers), two stage phase 1 clinical trial aimed at studying the adverse effects of the use of Vismodegib, at three increasing doses/day (150, 270 and 540 mg) orally administered to 33 patients (8 women and 25 men) with metastatic (18 patients) or locally advanced (15 patients) basal-cell carcinoma. This kind of tumor, which is associated with mutations in Hh pathway signaling, is successfully treated with surgery, radio- and chemo-therapy in the early stages. However, advanced and metastatic basal-cell carcinoma are usually unresponsive to standard therapies, with a median time of survival of about 8 months. The study showed that of 33 patients, 18 had a response, 11 presented stable conditions for about 11 months, and four did not show any effects and progression of the disease was registered. An interesting point to consider is that one of these last patients did not show significant Hh signaling pathway activation. These results justify the failure of the therapeutic strategy in this case. On the contrary, two of these four patients showed an increase in the HH signaling pathway, suggesting that other mechanisms lay behind Vismodegib ineffectiveness. Based on the results of this clinical trial, the authors concluded that the use of a Hh signaling pathway inhibitor could be a good strategy against advanced and/or metastatic basal-cell carcinoma unresponsive to standard therapies (74).

Three years later, Kaye et al. investigated the effects of Vismodegib administered to patients affected by epithelial ovarian carcinoma, fallopian tube carcinoma, or primary peritoneal carcinoma (75). These types of cancer are considered to be part of the same category of malignancies and are treated with the same standard therapy including surgery and chemotherapy (platinum agents in combination with taxane). The aim of this phase II,

randomized, double-blind, placebo controlled clinical trial was to investigate progression-free survival (PFS) following Vismodegib maintenance therapy (150 mg/day for 14 weeks after chemotherapy) in 104 patients (18 years or older), in which a second or third complete remission (that means no symptoms suggestive of persistent cancer) was achieved and measured by radiographic assessment (computed tomography scan of the chest/abdomen/pelvis). Unfortunately, the results showed that in the selected population no statistical differences were registered in PFS, though PFS was greater for the treated patient group (7.5 months) in comparison with the placebo one (5.8 months). Moreover, Hh ligand expression was found in only 13.5% of archival tumor tissue, suggesting that no correlation can be established between Hh ligand overexpression and clinical benefits. This result is in agreement with that reached by another placebo controlled trial conducted on patients affected by metastatic colorectal cancer who received Vismodegib or placebo in combination with bevacizumab and chemotherapy (76).

More recently, Vismodegib was administered in combination with Gemcitabine in a pilot clinical trial that enrolled 23 patients affected by metastatic pancreatic adenocarcinoma. This type of malignancy is the most frequent form of pancreas cancer and is ranked in fourth place for cancer mortality in Europe and United States, with a survival rate lower than any other cancer. Unfortunately, surgery, radio- and chemo-therapy are frequently ineffective in metastatic pancreatic adenocarcinoma(77). In this single arm pilot clinical study, patients were given 150 mg/day of Vismodegib orally for four weeks (Cycle 1). Then, the same patients were treated weekly intravenously (1000 mg/m^2) with a combination of Vismodegib and Gencitabine, for four further weeks (Cycle 2). The results showed that this combination therapy induced a decrease in target genes within the Hh signaling pathway (i.e. Gli1 and PTCH1). Nevertheless, no significant differences were found for the other parameters studied, including SHh, Gli1, and PTCH1 levels before and after the treatment, and no dissimilarities were found

in PFS and overall survival in comparison with a sole Gemcitabine treatment in the same population. These results countered a negative attitude towards this type of therapy in patients suffering from metastatic pancreatic cancer (78). More recently, the same conclusions were achieved by Catenacci et al. whom reported that the combination of Vismodegib and Gencitabine did not lead to additional benefits (in terms of PFS and overall survival) in comparison with sole Gemcitabine in a randomized phase II clinical trial comprising of 106 patients with metastatic pancreatic cancer (79).

This same research group continued the investigation of this therapeutic strategy with the study of IPI-926, another Hh inhibitor, in combination with a mixture of chemotherapy drugs including 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) in patients with advanced pancreatic cancer. In this case too, the authors concluded that while antitumor activity and safety were registered with this combination, they were rather skeptical about the development of Hh inhibitors for the treatment of pancreatic cancer (80).

Natural and synthetic inhibitors of Hedgehog pathway

Natural compounds have emerged as potent therapeutic alternatives or adjutants for different types of cancer (81-84). Several natural and synthetic compounds have been proposed as being effective as Hh pathway inhibitors, many of them targeting the Smo receptor. In this context, several synthetic drugs that target Smo have been developed, including the aformentioned Vismodegib (GDC-0449), which is still in the clinical trial phase for advanced and metastatic basal cell carcinoma (85). Another Hh pathway inhibitor, Erismodegib (Sonidegib, NVP-LDE225), inhibits the proliferation, migration and/or invasion of renal cell carcinoma cells (RCC) by reducing the activation of proteins and acting as a Smo antagonist (86). An orally bioavailable Smo antagonist, BMS-833923 (XL139), yielded a significant decrease in cellular proliferation and tumor growth in preclinical testing (*in vitro* and *in vivo*)

(87, 88).

LEQ506, TAK-441 and NVP-LEQ506 possess varying levels of *in vitro* and *in vivo* effects (89-91) through the same principle - smoothened antagonists. On the other hand, a benzimidazole derivative Hh antagonist, showed the strongest efficacy in animal models (92, 93). IPI-926 (a Smo antagonist) in conjunction with gemcitabine, demonstrated a decrease in fibrotic reaction (34, 94). In cell viability assays, another compound, robotnikinin, was found to act as a Hh pathway inhibitor by binding the amino terminal region of SHh (SHhN) (95, 96). Taladegib (LY2940680) was reported to restrain Smo-resistant mutant cells (97) and Itraconazole showed the capability to bind the Smo receptor and inhibit its accumulation in the cilium (98). A member of the aminoproline group of compounds, CUR61414, reduced tumor growth through binding of Smo and inhibition of the Hh signaling pathway (92). PF-04449913 was identified as a selective Hh signaling antagonist that binds to Smo and blocks signal transduction, reducing tumor growth in *in vivo* colorectal and pancreatic cancer models when used in combination with other anticancer agents (88, 99).

Cyclopamine, a steroidal alkaloid type secondary metabolite from *Veratrum californicum* Durand (Melanthiaceae), serves as an anti-Hh constituent blocking the activation of Smo (96, 100). According to previous studies, cyclopamine was shown to inhibit the development of human hepatocellular carcinomas *in vitro* and *in vivo* via inhibition of SHh signaling (98, 101-103). Moreover, cyclopamine effectively targeted cancer stem cells (CSCs) from pancreatic and breast cancers as well as glioblastoma and multiple myeloma (104-106). Due to the poor oral bioavailability and specificity of the compound, other cyclopamine-derivatives have been investigated to target Smo and inhibit Hh signaling (107). Thus, an orally bioavailable Smo antagonist drug, Saridegib (IPI-926), a semi-synthetic cyclopamine derivative, was developed to treat metastatic solid tumors and BCC (108).

More recently, inhibitors of Gli transcription factors have gained momentum over the

Smo antagonists. Lack of specificity, drug resistance and possible side effects of Smo antagonists were the main reasons for this shift. Direct and indirect Gli inhibitors, epigenetic drugs and Gli-regulated signaling inhibitors are strategies for Gli-dependent output**.** Common traits of direct Gli inhibitors is their inhibition capacity on Hh target gene expression $Sufu^{-/-}$ cells (109).

Gli-antagonists, namely GANT-58 and GANT-61, were identified from cellular screening assays (109). The Gli inhibitory action of arsenic trioxide (As_2O_3) was also demonstrated through regulation of transcriptional activity without changing the DNA binding capacity (98, 110, 111), a fact that highlighted its therapeutic use against malignant diseases associated with SHh pathway activation (86, 97). Imiquimod constitutes an additional example of a Gli modulator by interfering with the activity of toll like receptor (TLR) 7 and TLR8 as an agonist, further stimulating PKA via adenosine receptors (ADORAs). Finally, PKA activation facilitates Gli2 phosphorylation and further degradation (112). Nanoquinacrine (NQC) acts by stimulating the expression of Gli inhibitors and through destabilization of Gli1-DNA binding, contributing to impaired Gl1 dependent tumor development and proliferation (113). RU-SKI 43 was associated with inhibiting pancreatic cancer cell proliferation in preclinical studies, also reducing Gli1 activation due to direct inhibition of Hedgehog acyltransferase (Hhat) – an enzyme with processing roles in SHh. Indirect effects were also shown on Akt and mTOR pathways that accentuated the antiproliferative function of the small molecule (38).

Targeting the Hh pathway seems to be a promising therapeutic option for multiple types of cancer; however, as in the case of numerous other drugs, there is the possibility of acquisition of drug resistance or mutation within the therapeutic target. A potent perspective in inhibiting Hh signaling could be represented by concomitant targeting of different spots within the pathway (eg. simultaneous inhibition of Smo and Gli) by using combination therapeutics, decreasing the chances of alternative pathway activation and installation of drug resistance.

Moreover, a combination strategy could significantly decrease the necessary doses for both agents; since Hh signaling is an essential process for turnover of various stem cells – e.g. bone marrow and skin – we are still unaware of its long term effect upon healthy entities and overall function of the organism. Moreover, due to the incipient character of the clinical testing, there are no data on the long terms effect of Hh inhibitors and eventual toxicity (114, 115)). In these terms, a combination strategy of different Hh inhibitors for differential targets within the pathway could significantly decrease the downsides of current monotherapeutic perspectives.

The inhibition of Gli transcription factors can occur via various mechanisms and can also be modulated by natural compounds (116). These compounds have the advantage of reduced toxicity and long term side effects, and minimal impact upon healthy cells. However, their mechanisms of action are not as strict as in the case of small molecule inhibitors designed for specific targeting of an aberrant molecule. There are currently a limited number of studies that focus on the association of natural derivatives with specific targets within Hedgehog signaling, although their identification could have a significant impact in possible adjuvant therapeutic options (117).

An isoflavone type secondary metabolite, Glabrescione B (GlaB), from *Derris glabrescens* (Benth.) J.F. Macbr. from the family Fabaceae, was found to possess the capacity to bind the Gli1 zinc finger domain and to further block DNA binding capacity (118). Pyrvinium, an anthelmintic drug approved by the FDA, was shown to possess strong inhibitory action on the Hedgehog pathway (119). FN1-8 was demonstrated to be active against diverse cancer cells characterized by Gli activation, including colon, pancreatic and prostate malignancies (120). Natural compounds, namely zerumbone, physalin, staurosporinone and arcyriaflavin C, were characterized as molecules with Gli transcription inhibition activity (121). Zerumbone, the main compound of *Zingiber zerumbet* (L.) Sm. (Zingiberaceae) extract, enhanced apoptosis and inhibited invasion of cancer cells, revealing antitumor effects when

administrated in leukemia and breast, liver, lung and pancreatic cancers via inhibition of Gli1 and Gli2, as well as SHh signaling gene-mediated transcription (121-125). Among the other natural Hh inhibitors there is curcumin, a very well-known agent with anti-cancer effects from the same plant family, Zingiberaceae. It is derived from the plant *Curcuma longa* L. and has been investigated as a cancer preventive agent in recent years (126, 127). This compound was reported to inhibit prostate cancer cell growth by inhibiting SHh signaling via downregulation of SHh pathway proteins, as well as enhancing the antitumor activity of cisplatin and □-rays (128, 129). Moreover, genistein, one of the soy isoflavones from *Glycine max* (L.) Merr. (Fabaceae), was shown to display high antiproliferative action in breast, prostate, gastric, bladder colon and skin cancer cells (130). Genistein inhibited prostate cancer cell growth and exerted anti-CSC effect via inhibition of SHh signaling (128, 131).

Resveratrol, a polyphenolic compound obtained from *Vitis vinifera* L. (Vitaceae) and *Polygonum cuspidatum* Siebold & Zucc. (Polygonaceae), was associated with the ability to inhibit human cancer cell proliferation including chronic myeloid leukemia (CML) cells and reduce carcinogenesis *in vivo* through inhibition of the SHh pathway and mediation of Bcr-Abl expression (128, 132-134).

Norcantharidin is a demethylated analog derived from cantharidin which was isolated from *Mylabris phalerata* Pall. and was reported to induce cell anoikis and apoptotic processes, also inhibiting the invasion, angiogenesis and metastasis processes (135, 136) and combating the installation of mutidrug resistance through inhibition of SHh signaling and expression of downstream multidrug resistance genes (128).

Epigallocatechin-3-gallate (EGCG) from *Camellia sinensis* (L.) Kuntze (Theaceae), was reported to possess inhibitory abilities upon the SHh pathway, inducing apoptosis and suppressing proliferation in human chondrosarcoma cells (137). In addition, EGCG was demonstrated to reduce prostate cancer cell growth via suppression of Gli1 transcript (128) and

self-renewal abilities of pancreatic CSCs by inhibiting the SHh pathway components and Gli transcriptional activity (138).

Withaferin A and its derivatives from the leaves of *Withania somnifera* were identified as Gli1-mediated transcriptional inhibitors. The compounds displayed cytotoxic effects on Hh signaling-positive cancer cell lines including DU145, MCF7 and PANC-1 by suppressing target proteins within the Hh signaling pathway, Hh ligand receptor PTCH and anti-apoptosis protein BCL-2. In addition, Withaferin A showed inhibitory action on the formation of the Gli1-DNA complex (139).

Berberine, an isoquinoline alkaloid isolated from *Berberis* species, inhibits Hh signaling through modulation of Smo, most probably through direct targeting. A similar mechanism of action was found for Vitamin D3, which was found to bind Smo at the same site with cyclopamine, further inhibiting Hh signaling (117).

Even if the classical concept associates Gli inhibition with beneficial effects in cancer treatment, more insights are required on the mechanisms involved before progressing into clinical trials. Specifically, impairment of the Hh/Gli pathway could sustain the progression of disease, as this pathway is involved in repair and regeneration processes. For example, inhibition of Hh signaling in bladder cancer stroma resulted in accelerated progression of the malignancy (140). Also, there are several other natural agents considered to have activity upon Hh signaling; these potential compounds were extensively reviewed by Bao et al. (117).

Epigenetic drugs, such as HDAC inhibitors, regulate gene expression by affecting the activity of histone or DNA modifying enzymes and their associated transcriptional response (141). BET bromodomain protein inhibition is another epigenetic approach for blocking the Hedgehog pathway at the downstream level. For instance, JQ1 has been developed as a selective inhibitor of BET bromodomains, showing inhibitory ability upon cell viability and proliferation *in vitro* and *in vivo* in models of Smo-antagonist resistant medulloblastoma (142,

143).

SHh crosstalk with TGF/SMAD signaling

Based on the insights gleaned from research over the course of decades, it has become evident that cancer cells and bone microenvironments interact with each other through various signaling molecules (144). Cancer cells produce angiogenic factors and bone resorbing factors which significantly enhance the proliferation of cancer cells within the bone microenvironment. More excitingly, the bone tissue acts as a repository for growth factors, such as bone morphogenetic proteins (BMPs) and transforming growth factor (TGF)-β. BMP-4 was noted to upregulate the SHh mRNA in prostate cancer CWR22 cells, an event that induces osteoblastic lesions when injected into tibia of immunodeficient animal models. BMP-4, BMP-6 and BMP-9 upregulated SHh mRNA expression in LNCaP cells in a dose dependent manner, though the effects of BMP-4 and BMP-6 were more pronounced that the ones associated with BMP-9. Mechanistically it was shown that BMP-4 increases SHh production through SMAD4 modulation in prostate cancer cells (144). As expected, BPM-4 mediated increase in SHh was notably reduced in SMAD4 silenced LNCaP cells. BMPs were reported as being involved in osteoblastic differentiation of stromal cells, and treatment of stromal MC3T3-E1 cells with SHh considerably increased the BMP-responsive reporter activity induced by BMP-4. SHh markedly increased BMP signaling in MC3T3-E1 cells by enhancing the expression levels of BMP signaling modulators. SHh significantly upregulated activin receptor and SMAD1 expression in MC3T3-E1 cells. Furthermore, BMP-4 mediated phosphorylation of the C-terminal region of SMAD1 increased notably in MC3T3-E1 cells treated with SHh (144). Yet another important observation was that LNCaP cells produced SHh in response to BMPs. Moreover, BMPs and BMP-induced SHh worked synergistically and facilitated the osteoblastic differentiation of MC3T3-E1 cells. BMP-4 also inhibited the

growth of LNCaP in monoculture conditions. However, when MC3T3-E1 cells were co-cultured with LNCaP, BMP-4 mediated growth inhibition was abolished. Surprisingly, BMP-4 promoted LNCaP growth, suggesting that MC3T3-E1 cells supported growth and/or survival of LNCaP cells (144).

SHh crosstalk with PDGFR

Treatment of BRAF (V600E) metastatic melanoma with BRAF inhibitors is challenging due to acquired and intrinsic drug resistance. It has previously been convincingly determined that the use of BRAF inhibitors triggers SHh pathway activation with consequences on the upregulation of PDGFRα (Platelet-derived growth factor receptor α) (145). Due to activation of the SHh pathway in BRAF inhibitor treated melanoma cells, SHh inhibitors were tested. Data clearly suggested that SHh and BRAF inhibitors synergistically inhibit SHh and PDGFRα in melanoma cells. SHh inhibitor (40 mg/kg/day) enhanced the efficacy of vemurafenib to inhibit tumor growth of M21 cells in SCID mice (145).

PDGRFα was noted to be significantly downregulated in sulforaphane administered xenografted mice. Gli1 and Gli2 were also observed to be repressed in mice xenografted with pancreatic cancer stem cells (146).

Notch signaling

The notch signaling pathway participates in multiple cell processes including differentiation, proliferation, and survival (147). *Notch* genes encode transmembrane receptors which have been shown to be upregulated together with their ligands in many types of cancer (148, 149). A well-established crosstalk between Notch signaling and the Hedgehog pathway has been reported by various authors in breast, multipotent mesodermal, glioblastoma and in prostate cancer cells with acquired resistance to docetaxel (150-153). It has been suggested that Notch

can directly suppresses the Hedgehog pathway via the repressive transcription factor Hes1, by inhibiting Gli1 transcription (153). In an *in vivo* animal model of ovarian cancer, Jagged1, a Notch ligand, reduced tumor growth partially through a crosstalk mechanism with the Gli2 mediator (154).

It has been reported that $CD133^+$ glioma stem cells are resistant to temozolomide (TMZ) therapy (155). Dose dependent TMZ treatment of $CD133^+$ cells enhanced activities of the Notch and SHh pathways (156). Moreover, 500 μmol/L TMZ induced a significant upregulation of Gli1, HES1 and HES5 transcripts. A considerably higher apoptotic rate was noted in $CD133^+$ cells in response to parallel treatment with GSI-1 (Notch inhibitor), cyclopamine (SHh pathway inhibitor) and TMZ (156).

Crosstalk with different molecules

WW domain containing oxidoreductase gene (WOX1) is a tumor suppressor that is frequently downregulated in glioblastoma cells. There was a significantly enhanced response to radiation therapy in glioblastoma cells reconstituted with WOX1. Another interesting finding of this research was that use of signaling inhibitor sensitized WOX1 expressing glioblastoma cells to radiation therapy (157).

The Integrin Beta-4 (ITGB4)/ FAK signaling axis plays a contributory role in regulation of migration and invasion of ovarian cancer cells induced by SHh. There was a 3-fold increase in ITGB4 in SHh treated ovarian SKOV3 cancer cells (158). GANT61, an inhibitor of Gli1 and Gli2 was noted to be effective against SKOV3 cancer cells. ITGB4 was markedly reduced in cells treated with GANT61. Phosphorylation of FAK (Focal adhesion kinase) at 397^{th} tyrosine residue was notably enhanced in SKOV3 cells stimulated with SHh. However, despite stimulation with SHh, treatment of SKOV3 cells with an anti-ITGB4 blocking antibody dramatically reduced FAK (Tyr397) phosphorylation (158). GANT61 (25 mg/kg, three times

per week) significantly reduced tumor growth in mice subcutaneously implanted with SKOV3 cells (158).

Crosstalk between Hedgehog inhibition and other signaling pathways

The Hedgehog pathway is a highly evolutionarily conserved signaling pathway (159). In mammals, this pathway controls embryonic development but can also be over-activated in a wide diversity of human cancers, with great importance in the self renewal capacity of cancer stem-like cells (160). The downstream activation of Gli transcription factors leads to the transcription of gene products that promote cell proliferation, cell renewal and survival (Figure 2) (161). Given the important role of this pathway in some cancers, the inhibition of the Hedgehog pathway is a potential and promising target for anticancer therapy. While the Hedgehog pathway contributes to tumorigenesis, many other pathways are involved in the sustenance of the carcinogenesis related processes, suggesting that the combined inhibition of the Hedgehog pathway and connected signaling pathways would be a better and effective strategy against some types of cancer.

Crosstalk between Hedgehog signaling and a wide range of other signaling pathways has been evidenced. One of the most investigated connections consists of functional association of the Hh signaling with epidermal growth factor receptor (EGFR), as this latter is involved in the activation of many other signaling pathways such as phosphoinositide 3-kinase (PI3K)/AKT/mTOR, mitogen-activated protein kinases (MAPKs), signal transducer and activator of transcription (STAT), SRC/FAK pathway or phospholipase C (162). Through its downstream effects, EGFR signaling regulates a broad spectrum of biological processes such as cell proliferation and survival. Aberrant activation of EGFR signaling is directly linked with the development and growth of tumor cells (162, 163). A wide range of in *vitro* studies have shown that Hedgehog and EGFR pathways synergize in the stimulation of cell proliferation

and invasion. Indeed, alteration of at least one of the two signaling pathways is found in about one-third of all cancers; moreover, deregulation of both in the same tumor is common (46). For example, cooperation between both pathways has been evidenced in neural stem cells, in medulloblastoma cells, in HaCaT keratinocytes and in prostate cancer cells (164-167). Cooperation between EGFR signaling and Hedgehog has been highlighted via induction of the RAS/RAF/mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling (168, 169). In an *in vivo* animal human model of non small-cell lung cancer (NSCLC) with acquired resistance to EGFR-inhibitors, Hedgehog signaling demonstrated a significant role in mediating the resistance to the inhibitors, via favoring of the epithelial to mesenchymal transition (EMT) (170). This cooperation was also found in head and neck squamous cell cancer (HNSCC) where significant reduction in cell proliferation and colony formation was reported after dual targeting of EGFR and Hedgehog pathways (171).

The RAS/RAF/MEK/ERK pathway is a central controller of cell proliferation and survival and, consequently, alterations to some of its components lead to hyperactivation of the pathway, an event related to numerous types of cancer (172). High rates of alterations to this pathway are found in leukemia, colorectal, pancreatic and lung cancers (173). Moreover, synergistic activation of both RAS/RAF/MEK/ERK and Hedgehog pathways are reported in a diverse spectrum of malignancies (173). Experimental evidence shows that co-activation of both pathways resulted in the formation of pancreatic intraepithelial neoplasias in a transgenic mouse model (174). In another study, mice lacking Gli1 presented a reduced progression of KRAS-induced pancreatic preneoplastic lesions (175). In pancreatic cancer cells, KRAS was shown to be capable of Hedgehog pathway activation via MEK/ERK1/2, increasing the levels and the transcriptional activity of Gli1 levels. This activation was blocked by siRNA targeted towards KRAS inhibition, or by pharmacological inhibition of MEK (176). In addition, many studies have provided evidence of crosstalk between aberrant activation of the Hedgehog

pathway and ERK1/2 in different types of cancer (see (177) for review).

The PI3K/AKT/mTOR signaling pathway constitutes an important mechanisms that regulates numerous cellular processes such as growth, proliferation, differentiation and apoptosis (178). Consequently, deregulation of this pathway favors the survival of cancer cells and their proliferation and progression into cancer (179). Additionally, treatment of HeLa cells with rapamycin, an inhibitor of mTORC1, has been shown to regulate nuclear localization and transcriptional activity of Gli3, suggesting a crosstalk between PI3K/AKT/mTOR and Hedgehog signaling (180). A synergistic relationship was also reported between both pathways in embryonic development and in Hedgehog-dependent tumors (181). Cooperation between PI3K/AKT and Hedgehog pathways has been shown to promote cancer cell survival, proliferation and metastasis in esophageal (182, 183), pancreatic (3, 184), soft-tissue sarcoma (185), or biliary tract cancer cells (186). Another interesting study on immunohistochemical analysis of primary human gastric tumor biopsies found that activation of the Hedgehog pathway was directly correlated with lymph node metastasis through PI3K/Akt interaction (187).

The Wnt/β-catenin signaling pathway is involved in determination of cell fate and organ development during embryonic growth, but also participates in the regulation of tissue renewal in adults (188). Alterations of the Wnt/β-catenin pathway and its components have been related to carcinogenesis mechanisms (189-191). Moreover, signaling interference between Wnt and Hedgehog pathways has been found in gastric and prostatic cancer cells (192, 193). An over-expression of Gli1 in endometrial cancer cell lines leads to an increased expression of nuclear β-catenin, evidencing a direct interaction of Gli1 with β-catenin (194). However, a great heterogeneity in gene expression and interactions in the activation of Wnt and Hedgehog pathways was found in stage III serous ovarian cancer (195). These data suggest that such variability could be one of the main causes for drug resistance in this type of cancer.

In addition to these signaling pathways, other pathways could also synergistically interact with the Hedgehog signaling pathway in different types of cancer. Among these signaling pathways, the androgen receptor (196), hypoxia inducible factor (HIF)-1〈 (197), interleukin (IL)-6/IL-6r/gp130 (198) or transforming growth factor-beta TGFβ (199) pathways bear particular mention.

Trickier aspects of Hedgehog inhibition Results obtained in preclinical experiments represent evidence that stands at the base of the claims that the Hedgehog signaling forms a pivotal component in the onset, pathogenesis, self-renewal, and chemotherapy resistance in several typologies of human cancers. Therefore, the main associated proteins (SHh, Smo, and Gli1/2) have been characterized as key targets for the development of novel and targeted cancer therapeutics. Actually many SHh-SMO-Gli pathway inhibitors have been investigated in preclinical investigations and clinical trials, and this is of particular interest as both downstream effectors (Smo and Gli1/2) could be activated by SHh-independent stimuli. The safety of Hedgehog signaling inhibition has been reported in several studies (200-206), where nearly all patients treated showed at least one treatment-emergent adverse effect, most of them mild or moderate, with a high incidence rate (95–100%) across studies, due to the inhibition of the Hedgehog pathway in normal tissues. Commonly observed adverse effects in patients include muscle spasms, weight loss, ageusia/dysgeusia, asthenia, and alopecia. While these adverse effects are of mild or moderate intensity, their long-term nature can lead to a substantial decrease in quality of life, leading to cases of discontinuation of the treatment, and in some cases even interruption. For instance, muscle spasms (with mild or moderate intensity) can occur in any location, but these occur more frequently at the level of the lower leg and foot, with high incidence in elderly individuals and are also more frequent at night (207). These effects are due to the activation of non-canonical Smo/Ca^{2+}/AMPK signaling and inhibition of

the canonical Smo signaling pathway, with an increase of Ca^{2+} influx in the cells and subsequent contractions (57). The inhibition of the Hedgehog pathway also has a fundamental role in the differentiation and maintenance of taste buds (208, 209). In fact, Vismodegib-treated patients report taste disturbances in ~ 50-71% of the cases, due to a decrease in the number of SHh-expressing type IV taste cells (210) critical for the differentiation of taste bud in adults. Another side effect of Vismodegib treatment is alopecia, which occurs in ~46-66% of the patients and has a longer time to onset (at least 2 months after the start of treatment) and it is related to the follicles being prevented from transition to the anagen phase after shedding of hair in the telogen phase.

Despite significant progress within the development of synthetic Hh inhibitors, acquisition of drug resistance remains a stringent issue. Independent studies have observed these mechanisms in their attempt to inhibit Smo activity (211-213). Therefore, several preclinical and clinical tests have revealed the appearance of drug-refractory tumors that provide new perspectives on the development of Hh inhibition based strategies. Specifically, within the first clinical tests, there was a case of a significant initial response from a MB patient treated with Vismodegib that presented tumor relapse within a short time period (90). Subsequent molecular characterizations revealed the presence of a de novo Smo missense mutation (D473H) that impeded the binding of the drug to the actual target; this mutation has no further effect on the actual pathway (213). This initial observation stimulated additional investigations, where the acquisition of Vismodegib resistance was shown to mainly rely on Smo mutations that hamper drug binding, and other genomic modifications that results in Sufu loss of function of Gli2 activation (214, 215). Similar mechanisms were also shown for sonidegib resistant tumors (mutations within Smo and Gli2 gain of function) in preclinical models (216). Cross-resistance was encountered for different Smo antagonists *in vitro* and in clinical trials (215, 217). An important role in the efficiency of Hh inhibitors is also attributed

to intra-tumor heterogeneity, where cells can differ in their response in concordance with their genomic pattern and translated molecules for targeting (214).

Besides modifications of direct targets, drug resistance can occur due to feedback mechanisms represented by alterations of outside pathways. PI3K-mTOR signaling was found to be overexpressed in sonidegib resistant tumors compared to sensitive ones (216). BRD4 (218), aPKC-ι/λ (219), and PDE4 (220) were identified as potent supplementary targets in reducing acquired resistance to Smo inhibitors.

These mechanisms underline the importance of patient stratification, the inclusion of the tumor heterogeneity aspects into studies and the implementation of drug combinations that will limit the acquired resistance or activation of alternative compensatory mechanisms.

Conclusion and future prospects The Hh pathway is highly repressed in most tissues in adults, although it can be reactivated in situations of tissue repair and/or regeneration. Moreover, in case of aberrant expression, the proliferative activity of the Hh pathway can play a significant role in the development and progression of many types of cancer. Diverse studies have highlighted the utility of inhibition of the Hh pathway against various malignancies; although the results have not always been satisfactory or conclusive. The complexity and heterogeneity of the pathways implicated in the development and progression of cancer, and the existence of an interrelationship and cross-talk mechanism between them, significantly limits the utility of single specific Hh inhibitors. Further research for the development of new inhibitors of the Hh pathway and additional clinical studies combining Hh inhibitors with other molecules impairing activity upon additional interconnected pathways may extend the therapeutic range of Hh related therapy in a therapeutic cancer perspective.

Take-home message

Cancer is a growing issue in today's population, associated, among other factors, with the increase in life expectancy. Although cancer research has progressed immensely, a great deal of knowledge, especially that related to molecular mechanics, is still required in order to develop novel, targeted and superior effective therapies. The search for new therapeutic targets that allow the cure or the delay of malignant progression represents a key point that must be addressed by science. The Hh pathway may appear to be a promising candidate for some types of cancer, however, we are still far from finding effective therapies with minimal side effects but potent malignant impairing ability. The low specificity and the existence of significant side effects of many anticancer agents make it important to look for other options such as natural products that are better tolerated by the population segment at risk and are to be proven to be effective.

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Conflicts of interest statement

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References

1. I. Varlamis, I. Apostolakis, D. Sifaki-Pistolla, N. Dey, V. Georgoulias, C. Lionis, Application of data mining techniques and data analysis methods to measure cancer morbidity and mortality data in a regional cancer registry: The case of the island of Crete, Greece, Comput Methods Programs Biomed. 145(2017)73-83.

2. Z.X. Zhu, C.C. Sun, Y.T. Zhu, Y. Wang, T. Wang, L.S. Chi, et al., Hedgehog signaling contributes to basic fibroblast growth factor-regulated fibroblast migration, Exp. Cell Res. 355(2017)83-94.

3. K. Hao, X.D. Tian, C.F. Qin, X.H. Xie, Y.M. Yang, Hedgehog signaling pathway regulates human pancreatic cancer cell proliferation and metastasis, Oncol. Rep. 29(2013)1124-1132.

4. H. Chen, Q. Zuo, Y. Wang, M.F. Ahmed, K. Jin, J. Song, et al., Regulation of Hedgehog Signaling in Chicken Embryonic Stem Cells Differentiation Into Male Germ Cells (Gallus), J. Cell. Biochem. 118(2017)1379-1386.

5. S.F. Aval, H. Lotfi, R. Sheervalilou, N. Zarghami, Tuning of major signaling networks (TGF-β, Wnt, Notch and Hedgehog) by miRNAs in human stem cells commitment to different lineages: Possible clinical application, Biomed. Pharmacother. 91(2017)849-860.

6. J. Taipale, P.A. Beachy, The Hedgehog and Wnt signalling pathways in cancer, Nature 411(2001) 349-354.

7. L.V. Goodrich, M.P. Scott, Hedgehog and patched in neural development and disease Neuron 21(1998)1243-1257.

8. J.A. Porter, K.E. Young, P.A. Beachy, Cholesterol modification of hedgehog signaling proteins in animal development, Science 274(1996) 255.

9. J.M. Bailey, P.K. Singh, M.A. Hollingsworth, Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenic proteins, J. Cell. Biochem. 102(2007) 829-839.

10. A. Borah, S. Raveendran, A. Rochani, T. Maekawa, D. Kumar, Targeting self-renewal pathways in cancer stem cells: clinical implications for cancer therapy, Oncogenesis 4(2015)e177.

11. M. Singh, P. Chaudhry, A.A. Merchant, Primary cilia are present on human blood and bone marrow cells and mediate Hedgehog signaling. Exp. Hematol. 44(2016)1181-7. e2.

12. G.A. Horne, M. Copland, Approaches for targeting self-renewal pathways in cancer stem cells: implications for hematological treatments, Exp. Opin. Drug Discov. 12(2017) 465-474.

13. M. Varjosalo, J. Taipale, Hedgehog: functions and mechanisms, Genes Dev. 22(2008) 2454-2472.

14. Gupta S, Takebe N, LoRusso P. Review: Targeting the Hedgehog pathway in cancer, Ther. Adv. Med. Oncol. 2(2010)237-250.

15. F. Shuang, Y. Zhou, S.X. Hou, J.L. Zhu, Y. Liu, C.L. Zhang, et al., Indian Hedgehog signaling pathway members are associated with magnetic resonance imaging manifestations and pathological scores in lumbar facet joint osteoarthritis, Sci. Rep. 2015;5(2015)10290.

16. Y. Pan, Y. Gong, H. Ruan, L. Pan, X. Wu, C. Tang, et al., Sonic hedgehog through Gli2 and Gli3 is required for the proper development of placental labyrinth, Cell Death Dis. 6(2016)e1653.

17. J. Liu, X. Wang, J. Li, H. Wang, G. Wei, J. Yan, Reconstruction of the gene regulatory network involved in the sonic hedgehog pathway with a potential role in early development of the mouse brain, PLoS Comput. Biol. 10(2014):e1003884.

18. N.J. Mobassarah, Z. Choudhry, A.A. Rikani, A.M. Choudhry, S. Tariq, F. Zakaria, et al., Sonic hedgehog signalling pathway: a complex network, Ann. Neurosci. 21(2014).

19. W.A. O'Hara, W.J. Azar, R.R. Behringer, M.B. Renfree, A.J. Pask, Desert hedgehog is a mammal-specific gene expressed during testicular and ovarian development in a marsupial, BMC Dev. Biol.11(2011)72.

20. A.M. Holtz, S.C. Griffiths, S.J. Davis, B. Bishop, C. Siebold, B.L. Allen, Secreted HHIP1 interacts with heparan sulfate and regulates Hedgehog ligand localization and function, J. Cell. Biol. 209(2015) 739-758.

21. D. Huangfu, K.V. Anderson, Signaling from Smo to Ci/Gli: conservation and divergence of Hedgehog pathways from Drosophila to vertebrates, Development. 133(2006)3-14.

22. Z. Zhao, R.T.H. Lee, G.V. Pusapati, A. Iyu, R. Rohatgi, P.W. Ingham, An essential role for Grk2 in Hedgehog signalling downstream of Smoothened, EMBO Rep. 17(2016)739-752.

23. S.A. Ramsbottom, M.E. Pownall, Regulation of hedgehog signalling inside and outside the cell, J. Dev. Biol. 4(2016)23.

24. I. Caro, J.A. Low, The role of the hedgehog signaling pathway in the development of basal cell carcinoma and opportunities for treatment, Clin. Cancer Res. 16(2016)3335-3339.

25. D. Duprez, C. Fournier-Thibault, N. Le Douarin, Sonic Hedgehog induces proliferation of committed skeletal muscle cells in the chick limb. Development. 125(1998) 495-505.

26. E.M. Levine, H. Roelink, J. Turner, T.A. Reh, Sonic hedgehog promotes rod photoreceptor differentiation in mammalian retinal cells in vitro. J. Neurosci. 17(1997)6277-6288.

27. J. Ericson, J. Muhr, M. Placzek, T. Lints, T. Jessel, T. Edlund, Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube, Cell. 81(1995)747-756.

28. D.J. Roberts, R.L. Johnson, A.C. Burke, C.E. Nelson, B.A. Morgan, C. Tabin, Sonic hedgehog is an endodermal signal inducing Bmp-4 and Hox genes during induction and regionalization of the chick hindgut, Development 121(1995) 3163-3174.

29. L. Wilson, M. Maden, The mechanisms of dorsoventral patterning in the vertebrate neural tube, Dev. Biol. 282(2005) 1-13.

30. Y. Litingtung, C. Chiang, Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3, Nat. Neurosci. 3(2000)979-85.

31. M. Wijgerde, J.A. McMahon, M. Rule, A.P. McMahon, A direct requirement for Hedgehog signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord, Genes Dev. 16(2002) 2849-2864.

32. M.A. Dyer, S.M. Farrington, D. Mohn, J.R. Munday, M.H. Baron, Indian hedgehog activates hematopoiesis and vasculogenesis and can respecify prospective neurectodermal cell fate in the mouse embryo, Development128(2001) 1717-1730.

33. F.C. Kelleher, D. Fennelly, M. Rafferty, Common critical pathways in embryogenesis and cancer, Acta oncol. 45(2006) 375-388.

34. P. Heretsch, L. Tzagkaroulaki, A. Giannis, Modulators of the hedgehog signaling pathway, Bioorg. Med. Chem. 18(2010) 6613-6624.

35. L. Lum, P.A. Beachy, The Hedgehog response network: sensors, switches, and routers. Science 304(2004)1755-1759.

36. Jr M.M. Cohen, Hedgehog signaling update. Am. J. Med. Genet. Part A. 152(2010) 1875-1914.

37. C. Ortmann, U. Pickhinke, S. Exner, S. Ohlig, R. Lawrence, H. Jboor, et al., Sonic hedgehog processing and release are regulated by glypican heparan sulfate proteoglycans, J Cell. Sci. 128(2015) 2374-2385.

38. E. Petrova, A. Matevossian, M. Resh, Hedgehog acyltransferase as a target in pancreatic ductal adenocarcinoma, Oncogene 34(2015):263.

39. A.D. Konitsiotis, B. Jovanović, P. Ciepla, M. Spitaler, T. Lanyon-Hogg, E.W. Tate, et al., Topological analysis of Hedgehog acyltransferase, a multipalmitoylated transmembrane protein, J. Biol. Chem., 290(2015)3293-3307.

40. M.M. Cohen, The hedgehog signaling network. Am. J. Med. Genet. Part A. 2003;123(2003)5-28.

41. A.M. Arensdorf, S. Marada, S.K. Ogden, Smoothened Regulation: A Tale of Two Signals, Trends Pharmacol. Sci. 37(2016)62-72.

42. D.J. Robbins, D.L. Fei, N.A. Riobo, The Hedgehog signal transduction network, Sci. Signal. 5(2012)re6.

43. B.Z. Stanton, L.F. Peng, Small-molecule modulators of the Sonic Hedgehog signaling pathway, Mol. BioSyst. 6(2010)44-54.

44. C.C. Hui, S. Angers, Gli proteins in development and disease, Ann. Rev. cell. dev. biol. 27(2011)513-37.

45. S.J. Scales, F.J. de Sauvage, Mechanisms of Hedgehog pathway activation in cancer and implications for therapy, Trends Pharmacol. Sci. 30(2009) 303-312.

46. B. Stecca, A.R. i Altaba, Context-dependent regulation of the GLI code in cancer by HEDGEHOG and non-HEDGEHOG signals, J. Mol. Cell Biology. 2(2010)84-95.

47. R. Teperino, F. Aberger, H. Esterbauer, N. Riobo, J.A. Pospisilik, Canonical and non-canonical Hedgehog signalling and the control of metabolism, Semin. Cell. Dev. Biol. 33(2014)81-92.

48. N. Dahmane, A. Ruiz-i-Altaba, Sonic hedgehog regulates the growth and patterning of the cerebellum, Development 126(1999) 3089-3100.

49. D. Brennan, X. Chen, L. Cheng, N.A. Riobo, Noncanonical hedgehog signaling, Vitam. Horm. 88(2012)55.

50. P. Chinchilla, L. Xiao, M.G. Kazanietz, N.A. Riobo, Hedgehog proteins activate pro-angiogenic responses in endothelial cells through non-canonical signaling pathways, Cell Cycle. 9(2010)570-579.

51. M.A. Renault, J. Roncalli, J. Tongers, T. Thorne, E. Klyachko, S. Misener, et al., Sonic hedgehog induces angiogenesis via Rho kinase-dependent signaling in endothelial cells, J. Mol. Cell. Cardiol. 49(2010)490-498.

52. A.H. Polizio, P. Chinchilla, X. Chen, S. Kim, D.R. Manning, N.A. Riobo, Heterotrimeric Gi proteins link Hedgehog signaling to activation of Rho small GTPases to promote fibroblast migration, J. Biol. Chem. 286(2011)19589-11596.

53. N. Sasaki, J. Kurisu, M. Kengaku, Sonic hedgehog signaling regulates actin cytoskeleton via Tiam1–Rac1 cascade during spine formation, Mol. Cell. Neurosci.45(2010)335-44.

54. F. Charron, M. Tessier-Lavigne, Novel brain wiring functions for classical morphogens: a role as graded positional cues in axon guidance. Development 132(2005) 2251-2262.

55. G. Kuo, L. Arnaud, P. Kronstad-O'Brien, J.A. Cooper, Absence of Fyn and Src causes a reeler-like phenotype, J. Neurosci. 25(2005) 8578-8586.

56. C. Guido, D. Whitaker-Menezes, C. Capparelli, R. Balliet, Z. Lin, R.G. Pestell, et al., Metabolic reprogramming of cancer-associated fibroblasts by TGF-β drives tumor growth: connecting TGF-β signaling with “Warburg-like” cancer metabolism and L-lactate production, Cell Cycle. 11(2012) 3019-3035.

57. R. Teperino, S. Amann, M. Bayer, S.L. McGee, A. Loipetzberger, T. Connor, et al., Hedgehog partial agonism drives Warburg-like metabolism in muscle and brown fat, Cell 151(2012) 414-426.

58. A.M. Skoda, D. Simovic, V. Karin, V. Kardum, S. Vranic, L. Serman, The role of the Hedgehog signaling pathway in cancer: A comprehensive review, Bosnian J. Basic Med. Sci. 18(2018)8.

59. J. Reifenberger, M. Wolter, C. Knobbe, B. Köhler, A. Schönicke, C. Scharwächter, et al. Somatic mutations in the PTCH, SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas, Br. J. Dermatol. 152(2005)43-51.

60. H. Hahn, C. Wicking, P.G. Zaphiropoulos, M.R. Gailani, S. Shanley, A. Chidambaram, et al. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome, Cell. 85(1996)841-51.

61. D.M. Berman, S.S. Karhadkar, A. Maitra, R.M. De Oca, M.R. Gerstenblith, K. Briggs, et al., Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours, Nature 425(2003)846.

62. D.N. Watkins, D.M. Berman, S.G. Burkholder, B. Wang, P.A. Beachy, S.B. Baylin, Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer, Nature 422(2003)313-317.

63. T. Sheng, C. Li, X. Zhang, S. Chi, N. He, K. Chen, et al., Activation of the hedgehog pathway in advanced prostate cancer, Mol. Cancer 3(2004)29.

64. M. Kubo, M. Nakamura, A. Tasaki, N. Yamanaka, H. Nakashima, M. Nomura, et al. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer, Cancer Res. 64(2004) 6071-6074.

65. A. Gulino, E. Ferretti, E. De Smaele, Hedgehog signalling in colon cancer and stem cells, EMBO Mol. Med.1(2009) 300-302.

66. K.E. O'Reilly, E.V.S. de Miera, M.F. Segura, E. Friedman, L. Poliseno, S.W. Han, et al., Hedgehog pathway blockade inhibits melanoma cell growth in vitro and in vivo, Pharmaceuticals 6(2013) 1429-1450.

67. O.J. Becher, D. Hambardzumyan, E.I. Fomchenko, H. Momota, L. Mainwaring, A.M. Bleau, et al., Gli activity correlates with tumor grade in platelet-derived growth factor–induced gliomas, Cancer Res. 68(2008) 2241-2249.

68. M. Monzo, I. Moreno, R. Artells, R. Ibeas, A. Navarro, J. Moreno, et al., Sonic hedgehog mRNA expression by real-time quantitative PCR in normal and tumor tissues from colorectal cancer patients, Cancer Lett. 233(2006)117-123.

69. R. Douard, S. Moutereau, P. Pernet, M. Chimingqi, Y. Allory, P. Manivet, et al., Sonic Hedgehog–dependent proliferation in a series of patients with colorectal cancer, Surgery 139(2006) 665-670.

70. G.R. Van Den Brink, S.A. Bleuming, J.C. Hardwick, B.L. Schepman, G.J. Offerhaus, J.J. Keller, et al. Indian Hedgehog is an antagonist of Wnt signaling in colonic epithelial cell differentiation, Nat. Genet. 36(2004) 277-282.

71. J. Jiang, C.C. Hui, Hedgehog signaling in development and cancer, Dev. Cell. 15(2008) 801-812.

72. J.W. Theunissen, F.J. de Sauvage. Paracrine Hedgehog signaling in cancer, Cancer Res. 69(2009):6007-6010.

73. Y. Zhang, J. Laterra, M.G. Pomper, Hedgehog pathway inhibitor HhAntag691 is a potent inhibitor of ABCG2/BCRP and ABCB1/Pgp, Neoplasia 11(2009) 96-101.

74. D.D. Von Hoff, P.M. LoRusso, C.M. Rudin, J.C. Reddy, R.L. Yauch, R. Tibes, et al., Inhibition of the hedgehog pathway in advanced basal-cell carcinoma, N. Engl. J. Med. 361(2009)1164-1172.

75. S.B. Kaye, L. Fehrenbacher, R. Holloway, A. Amit, B. Karlan, B. Slomovitz, et al., 2012. A phase II, randomized, placebo-controlled study of vismodegib as maintenance therapy in patients with ovarian cancer in second or third complete remission, Clin. Cancer Res. 18(2012) 6509-6518.

76. J. Berlin, J. Bendell, L. Hart, I. Firdaus, I. Gore, R. Hermann, et al., editors. A phase 2, randomized, double-blind, placebo-controlled study of hedgehog pathway inhibitor (HPI) GDC-0449 in patients with previously untreated metastatic colorectal cancer (mCRC). ANNALS OF ONCOLOGY; 2010: OXFORD UNIV PRESS GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.

77. J. Ferlay, C. Partensky, F. Bray, More deaths from pancreatic cancer than breast cancer in the EU by 2017. Acta Oncol. 55(2016)1158-1160.

78. E.J. Kim, V. Sahai, E.V. Abel, K.A. Griffith, J.K. Greenson, N. Takebe, et al., Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma, Clin. Cancer Res. 20(2014)5937-5945.

79. D.V. Catenacci, M.R. Junttila, T. Karrison, N. Bahary, M.N. Horiba, S.R. Nattam, et al., Randomized phase Ib/II study of gemcitabine plus placebo or vismodegib, a hedgehog pathway inhibitor, in patients with metastatic pancreatic cancer, J. Clin. Oncol. 33(2015) 4284-4292.

80. A.H. Ko, N. LoConte, M.A. Tempero, E.J. Walker, R.K. Kelley, S. Lewis, et al., A phase I study of FOLFIRINOX plus IPI-926, a hedgehog pathway inhibitor, for advanced pancreatic adenocarcinoma, Pancreas 45(2016) 370-375.

81. C. Braicu, V. Pilecki, O. Balacescu, A. Irimie, I.B. Neagoe, The relationships between biological activities and structure of flavan-3-ols, Int. J. Mol. Sci. 12(2011) 9342-9353.

82. D. Gulei, N. Mehterov, S.M. Nabavi, A.G. Atanasov, I. Berindan-Neagoe, Targeting ncRNAs by plant secondary metabolites: The ncRNAs game in the balance towards malignancy inhibition, Biotechnol. Adv. 36(2018)1779-1799.

83. L. Budisan, D. Gulei, O.M. Zanoaga, A.I. Irimie, C. Sergiu, C. Braicu, et al., Dietary Intervention by Phytochemicals and Their Role in Modulating Coding and Non-Coding Genes in Cancer, Int. J. Mol. Sci. 18(2017).

84. A.I. Irimie, C. Braicu, O. Zanoaga, V. Pileczki, C. Gherman, I. Berindan-Neagoe, et al., Epigallocatechin-3-gallate suppresses cell proliferation and promotes apoptosis and autophagy in oral cancer SSC-4 cells. OncoTargets Ther. 8 (2015) 461-70.

85. E. De Smaele, E. Ferretti, A. Gulino, Vismodegib, a small-molecule inhibitor of the hedgehog pathway for the treatment of advanced cancers. Curr. Opin. Invest. Drugs 11(2010) 707-718.

86. C. D'Amato, R. Rosa, R. Marciano, V. D'Amato, L. Formisano, L. Nappi, et al., Inhibition of Hedgehog signalling by NVP-LDE225 (Erismodegib) interferes with growth and invasion of human renal cell carcinoma cells, Br. J. Cancer 111(2014) 1168-1179.

87. D. Riedlinger, M. Bahra, S. Boas-Knoop, S. Lippert, M. Bradtmöller, K. Guse, et al., Hedgehog pathway as a potential treatment target in human cholangiocarcinoma, J.Hepato-biliary-pancreatic Sci. 21(2014)607-615.

88. L. Di Magno, S. Coni, L. Di Marcotullio, G. Canettieri, Digging a hole under Hedgehog: downstream inhibition as an emerging anticancer strategy, Biochim. Biophysic. Acta (BBA)-Rev. Cancer 1856(2015) 62-72.

89. J.M. Ng, T. Curran, The Hedgehog's tale: developing strategies for targeting cancer, Nat. Rev. Cancer. 11(2011) 493-501.

90. C.M. Rudin, C.L. Hann, J. Laterra, R.L. Yauch, C.A. Callahan, L. Fu, et al., Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449, N. Engl. J. Med. 361(2009) 1173-1178.

91. S. Peukert, F. He, M. Dai, R. Zhang, Y. Sun, K. Miller-Moslin, et al., Discovery of NVP-LEQ506, a Second-Generation Inhibitor of Smoothened, ChemMedChem 8(2013) 1261-1265.

92. J.A. Williams, O.M. Guicherit, B.I. Zaharian, Y. Xu, L. Chai, H. Wichterle, et al., Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions, Proc. Natl. Acad. Sci. 100(2003) 4616-4621.

93. J.T. Romer, H. Kimura, S. Magdaleno, K. Sasai, C. Fuller, H. Baines, et al., Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1+/− p53−/− mice, Cancer Cell 6(2004) 229-240.

94. K.P. Olive, M.A. Jacobetz, C.J. Davidson, A. Gopinathan, D. McIntyre, D. Honess, et al., Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer, Science 324(2009) 1457-1461.

95. B.Z. Stanton, L.F. Peng, N. Maloof, K. Nakai, X. Wang, J.L. Duffner, et al., A small molecule that binds Hedgehog and blocks its signaling in human cells, Nat. Chem. Biol. 5(2009) 154-156.

96. G.P. Raju, D. Pham, Hedgehog inhibition as an anti-cancer strategy, Vitam. Horm. 88(2011)507-22.

97. V. Justilien, A.P. Fields, Molecular pathways: novel approaches for improved therapeutic targeting of Hedgehog signaling in cancer stem cells, Clin. Cancer Res. 21(2015) 505-513.

98. J. Kim, J.Y. Tang, R. Gong, J. Kim, J.J. Lee, K.V. Clemons, et al., Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth, Cancer Cell 17(2010) 388-399.

99. A.J. Wagner, W.A. Messersmith, M.N. Shaik, S. Li, X. Zheng, K.R. McLachlan, et al., A phase I study of PF-04449913, an oral hedgehog inhibitor, in patients with advanced solid tumors, Clin. Cancer Res. 21(2015)1044-51

100. J.K. Chen, J. Taipale, M.K. Cooper, P.A. Beachy, Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened, Genes Dev. 16(2002) 2743-2748.

101. W.T. Cheng, K. Xu, D.Y. Tian, Z.G. Zhang, L.J. Liu, Y. Chen, Role of Hedgehog signaling pathway in proliferation and invasiveness of hepatocellular carcinoma cells, Int. J. Oncol.34(2009)829.

102. X.L. Chen, Q.Y. Cheng, M.R. She, Q. Wang, X.H. Huang, L.Q. Cao, et al. Expression of sonic hedgehog signaling components in hepatocellular carcinoma and cyclopamine-induced apoptosis through Bcl-2 downregulation in vitro, Arch. Med. Res.;41(2010):315-23.

103. K.S. Jeng, I. Sheen, W.J. Jeng, M.C. Yu, H.H. Tsai, F.Y. Chang, et al., Blockade of the sonic hedgehog pathway effectively inhibits the growth of hepatoma in mice: An in vivo study, Oncol. Lett. 4(2012)1158-1162.

104. E.E. Bar, A. Chaudhry, A. Lin, X. Fan, K. Schreck, W. Matsui, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma, Stem Cells. 25(2007) 2524-2533.

105. D. Subramaniam, S. Ramalingam, C.W. Houchen, S. Anant, Cancer stem cells: a novel paradigm for cancer prevention and treatment, Mini Rev. Med. Chem. 10(2010) 359-371.

106. Y.C. Huang, K. Chao, H.F. Liao, Y.J. Chen, Targeting sonic hedgehog signaling by compounds and derivatives from natural products, Evid. Based Complement. Alternat. Med. 2013(2013) 748587.

107. T.L. Lin, W. Matsui, Hedgehog pathway as a drug target: Smoothened inhibitors in development. Onco Targets Ther. 5(2012) 47-58.

108. A. Jimeno, G.J. Weiss, W.H. Miller, S. Gettinger, B.J. Eigl, A.L.S. Chang, et al., Phase I study of the Hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors, Clin. Cancer Res. 19(2013)2766-2774.

109. M. Lauth, Å. Bergström, T. Shimokawa, R. Toftgård, Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc. Natl. Acad. Sci. 104(2007) 8455-8460.

110. E.M. Beauchamp, L. Ringer, G. Bulut, K.P. Sajwan, M.D. Hall, Y.C. Lee, et al., Arsenic trioxide inhibits human cancer cell growth and tumor development in mice by blocking Hedgehog/GLI pathway, J. Clin. Invest. 121(2011)148-160.

111. G.P. Raju, Arsenic: a potentially useful poison for Hedgehog-driven cancers, J. Clin. Invest. 121(2011) 14-16.

112. F. Wolff, A. Loipetzberger, W. Gruber, H. Esterbauer, F. Aberger, A. Frischauf, Imiquimod directly inhibits Hedgehog signalling by stimulating adenosine receptor/protein kinase A-mediated GLI phosphorylation, Oncogene. 32(2013)5574.

113. A. Nayak, S.R. Satapathy, D. Das, S. Siddharth, N. Tripathi, P.V. Bharatam, et al., Nanoquinacrine induced apoptosis in cervical cancer stem cells through the inhibition of hedgehog-GLI1 cascade: Role of GLI-1, Sci. Rep. 6(2016)20600.

114. G.M. Selvarajan Sandhiya, S.S. Kumar, S.A. Dkhar, The dawn of hedgehog inhibitors: Vismodegib, J. Pharmacol. Pharmacother. 4(2013)4.

115. K. Garber, Hedgehog drugs begin to show results, Oxford University Press; 2008.

116. J.M. Hyman, A.J. Firestone, V.M. Heine, Y. Zhao, C.A. Ocasio, K. Han, et al., Small-molecule inhibitors reveal multiple strategies for Hedgehog pathway blockade, Proc. Natl. Acad. Sci.106(2009)14132-14137.

117. C Bao, P Kramata, HJ Lee, N Suh. Regulation of Hedgehog signaling in cancer by natural and dietary compounds, Mol. Nutr. Food Res. 62(2018) 1700621.

118. P. Infante, M. Mori, R. Alfonsi, F. Ghirga, F. Aiello, S. Toscano, et al., Gli1/DNA interaction is a druggable target for Hedgehog-dependent tumors, EMBO J. 34(2015) 200-217.

119. B. Li, D.L. Fei, C.A. Flaveny, N. Dahmane, V. Baubet, Z. Wang, et al., Pyrvinium attenuates Hedgehog signaling downstream of smoothened, Cancer Res. 74(2014) 4811-4821.

120. G. Bosco-Clément, F. Zhang, Z. Chen, H.M. Zhou, H. Li, I. Mikami, et al., Targeting Gli transcription activation by small molecule suppresses tumor growth, Oncogene 33(2014) 2087-2097.

121. T. Hosoya, M.A. Arai, T. Koyano, T. Kowithayakorn, M. Ishibashi, Naturally Occurring Small-Molecule Inhibitors of Hedgehog/GLI-Mediated Transcription, ChemBioChem 9(2008) 1082-1092.

122. M. Xian, K. Ito, T. Nakazato, T. Shimizu, C.K. Chen, K. Yamato, et al., Zerumbone, a bioactive sesquiterpene, induces G2/M cell cycle arrest and apoptosis in leukemia cells via a Fas-and mitochondria-mediated pathway, Cancer Sci. 98(2007) 118-126.

123. M. Kim, S. Miyamoto, Y. Yasui, T. Oyama, A. Murakami, T. Tanaka, Zerumbone, a tropical ginger sesquiterpene, inhibits colon and lung carcinogenesis in mice, Int. J. Cancer 124(2009)264-271.

124. S.S. Sakinah, S.T. Handayani, L.A. Hawariah, Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio, Cancer Cell Int. 7(2007)4.

125. B. Sung, S. Jhurani, K.S. Ahn, Y. Mastuo, T. Yi, S. Guha, et al., Zerumbone down-regulates chemokine receptor CXCR4 expression leading to inhibition of CXCL12-induced invasion of breast and pancreatic tumor cells, Cancer Res. 68(2008) 8938-8944.

126. Y. Li, M.S. Wicha, S.J. Schwartz, D. Sun, Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds, J. Nutr. Biochem. 22(2011) 799-806.

127. Z.M. Shao, Z.Z. Shen, C.H. Liu, M.R. Sartippour, V.L. Go, D. Heber, et al., Curcumin exerts multiple suppressive effects on human breast carcinoma cells, Int. J. Cancer 98(2002) 234-240.

128. A. Ślusarz, N.S. Shenouda, M.S. Sakla, S.K. Drenkhahn, A.S. Narula, R.S. MacDonald, et al., Common botanical compounds inhibit the hedgehog signaling pathway in prostate cancer, Cancer Res. 70(2010) 3382-3390.

129. M.H. Elamin, Z. Shinwari, S.F. Hendrayani, H. Al-Hindi, E. Al-Shail, A. Al-kofide, et al., Curcumin inhibits the Sonic Hedgehog signaling pathway and triggers apoptosis in medulloblastoma cells, Mol. Carcinogen. 49(2010) 302-314.

130. S. Barnes, Effect of genistein on in vitro and in vivo models of cancer, J. Nutr. 125(1995)777S.

131. L. Zhang, L. Li, M. Jiao, D. Wu, K. Wu, X. Li, et al., Genistein inhibits the stemness properties of prostate cancer cells through targeting Hedgehog–Gli1 pathway, Cancer Lett. 323(2012) 48-57.

132. B.B. Aggarwal, A. Bhardwaj, R.S. Aggarwal, N.P. Seeram, S. Shishodia, Y. Takada, Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies, Anticancer Res. 24(2004) 2783-840.

133. K.B. Harikumar, B.B. Aggarwal, Resveratrol: a multitargeted agent for age-associated chronic diseases, Cell cycle 7(2008) 1020-1035.

134. A. Bishayee, Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials, Cancer Prev. Res. 2(2009) 409-418.

135. Y.J. Chen, C.D. Kuo, Y.M. Tsai, C.C. Yu, G.S. Wang, H.F. Liao, Norcantharidin induces anoikis through Jun-N-terminal kinase activation in CT26 colorectal cancer cells, Anti-cancer Drugs 19(2008) 55-64.

136. Y.J. Chen, Y.M. Tsai, C.D. Kuo, K.L. Ku, H.S. Shie, H.F. Liao, Norcantharidin is a small-molecule synthetic compound with anti-angiogenesis effect, Life Sci. 85(2009) 642-651.

137. G.Q. Tang, T.Q. Yan, W. Guo, T.T. Ren, C.L. Peng, H. Zhao, et al., (−)-Epigallocatechin-3-gallate induces apoptosis and suppresses proliferation by inhibiting the human Indian Hedgehog pathway in human chondrosarcoma cells, J. Cancer Res. Clin. Oncol. 136(2010) 1179-1185.

138. S.N. Tang, J. Fu, D. Nall, M. Rodova, S. Shankar, R.K. Srivastava, Inhibition of sonic hedgehog pathway and pluripotency maintaining factors regulate human pancreatic cancer stem cell characteristics, Int. J. Cancer. 131(2012) 30-40.

139. T. Yoneyama, M.A. Arai, S.K. Sadhu, F. Ahmed, M. Ishibashi, Hedgehog inhibitors from Withania somnifera. Bioorg. Med. Chem. Lett. 25(2015) 3541-3544.

140. M. Didiasova, L. Schaefer, M. Wygrecka, T. Sanda, Targeting GLI Transcription Factors in Cancer, Molecules 23(2018).

141. A. Nebbioso, V. Carafa, R. Benedetti, L. Altucci, Trials with ‘epigenetic’drugs: an update, Mol. Oncol. 6(2012) 657-682.

142. Y. Tang, S. Gholamin, S. Schubert, M.I. Willardson, A. Lee, P. Bandopadhayay, et al., Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition, Nat. Med. 2014 (2014) 732-740.

143. P. Filippakopoulos, J. Qi, S. Picaud, Y. Shen, W.B. Smith, O. Fedorov, et al., Selective inhibition of BET bromodomains, Nature 468(2010) 1067-1073.

144. H. Nishimori, S. Ehata, H.I. Suzuki, Y. Katsuno, K. Miyazono, Prostate cancer cells and bone stromal cells mutually interact with each other through bone morphogenetic protein-mediated signals, J. Biol. Chem. 287(2012) 20037-20046.

145. F. Sabbatino, Y. Wang, X. Wang, K.T. Flaherty, D. Pepin, G. Scognamiglio, et al., PDGFRα up-regulation mediated by sonic hedgehog pathway activation leads to BRAF inhibitor resistance in melanoma cells with BRAF mutation, Oncotarget 5(2014) 1926-1941.

146. S.H. Li, J. Fu, D.N. Watkins, R.K. Srivastava, S. Shankar, Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog–GLI pathway, Mol. Cell. Biochem. 373(2013) 217-227.

147. T. Borggrefe, F. Oswald, The Notch signaling pathway: transcriptional regulation at Notch target genes, Cell. Mol. Life Sci. 66(2009) 1631-46.

148. R. Kopan, M.X.G. Ilagan, The canonical Notch signaling pathway: unfolding the activation mechanism, Cell 137(2009) 216-233.

149. L. Miele, H. Miao, B. Nickoloff, NOTCH signaling as a novel cancer therapeutic target, Curr. Cancer Drug Targets 6(2006) 313-23.

150. J. Domingo-Domenech, S.J. Vidal, V. Rodriguez-Bravo, M. Castillo-Martin, S.A. Quinn, R. Rodriguez-Barrueco, et al., Suppression of acquired docetaxel resistance in prostate cancer through depletion of notch-and hedgehog-dependent tumor-initiating cells, Cancer Cell 22(2012) 373-388.

151. S. Guo, M. Liu, R.R. Gonzalez-Perez, Role of Notch and its oncogenic signaling crosstalk in breast cancer, Biochim. Biophysic. Acta (BBA) Rev. Cancer. 1815(2011) 197-213.

152. W. Ingram, K. McCue, T. Tran, A. Hallahan, B. Wainwright, Sonic Hedgehog regulates Hes1 through a novel mechanism that is independent of canonical Notch pathway signalling, Oncogene 27(2008)1489-1500.

153. K.C. Schreck, P. Taylor, L. Marchionni, V. Gopalakrishnan, E.E. Bar, N. Gaiano, et al., The Notch target Hes1 directly modulates Gli1 expression and Hedgehog signaling: a potential mechanism of therapeutic resistance, Clin. Cancer Res. 16(2010)6060-6070.

154. A.D. Steg, A.A. Katre, B. Goodman, H.D. Han, A.M. Nick, R.L. Stone, et al., Targeting the notch ligand JAGGED1 in both tumor cells and stroma in ovarian cancer, Clin. Cancer Res. 17(2011) 5674-5685.

155. M.D. Aldea, B. Petrushev, O. Soritau, C.I. Tomuleasa, I. Berindan-Neagoe, A.G. Filip, et al., Metformin plus sorafenib highly impacts temozolomide resistant glioblastoma stem-like cells. J. BUON 19(2014) 502-511.

156. I.V. Ulasov, S. Nandi, M. Dey, A.M. Sonabend, M.S. Lesniak, Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133+ glioma stem cells to temozolomide therapy, Mol. Med. 17(2011) 103.

157. M.F. Chiang, H.H. Chen, C.W. Chi, C.I. Sze, M.L. Hsu, H.R. Shieh, et al., Modulation of Sonic hedgehog signaling and WW domain containing oxidoreductase WOX1 expression enhances radiosensitivity of human glioblastoma cells, Exp. Biol. Med. 240(2015) 392-399.

158. Q. Chen, R. Xu, C. Zeng, Q. Lu, D. Huang, C. Shi, et al., Down-regulation of Gli transcription factor leads to the inhibition of migration and invasion of ovarian cancer cells via integrin β4-mediated FAK signaling, PloS one 9(2014)e88386.

159. P.W. Ingham, A.P. McMahon. Hedgehog signaling in animal development: paradigms and principles, Genes Dev. 15(2001) 3059-3087.

160. R. McMillan, W. Matsui, Molecular pathways: the hedgehog signaling pathway in cancer, Clin. Cancer Res. 18(2012) 4883-4888.

161. K.E. Ryan, C. Chiang, Hedgehog secretion and signal transduction in vertebrates, J. Biol. Chem. 287(2012) 17905-17913.

162. M. Sibilia, R. Kroismayr, B.M. Lichtenberger, A. Natarajan, M. Hecking, M. Holcmann, The epidermal growth factor receptor: from development to tumorigenesis. Differentiation 75(2007) 770-787.

163. N.E. Hynes, H.A. Lane, ERBB receptors and cancer: the complexity of targeted inhibitors, Nat. Rev. Cancer 5(2005) 341-354.

164. F. Götschel, D. Berg, W. Gruber, C. Bender, M. Eberl, M. Friedel, et al., Synergism between Hedgehog-GLI and EGFR signaling in Hedgehog-responsive human medulloblastoma cells induces downregulation of canonical Hedgehog-target genes and stabilized expression of GLI1, PloS one 8(2013) e65403.

165. M. Mimeault, S.L. Johansson, G. Vankatraman, E. Moore, J.P. Henichart, P. Depreux, et al., Combined targeting of epidermal growth factor receptor and hedgehog signaling by gefitinib and cyclopamine cooperatively improves the cytotoxic effects of docetaxel on metastatic prostate cancer cells, Mol. Cancer Ther. 6(2007) 967-978.

166. V. Palma, D.A. Lim, N. Dahmane, P. Sánchez, T.C. Brionne, C.D. Herzberg, et al., Sonic hedgehog controls stem cell behavior in the postnatal and adult brain, Development 132(2005) 335-344.

167. R.L. Bigelow, E.Y. Jen, M. Delehedde, N.S. Chari, T.J. McDonnell, Sonic hedgehog induces epidermal growth factor dependent matrix infiltration in HaCaT keratinocytes, J. Invest. Dermatol. 124(2005) 457-465.

168. H. Schnidar, M. Eberl, S. Klingler, D. Mangelberger, M. Kasper, C. Hauser-Kronberger, et al., Epidermal growth factor receptor signaling synergizes with

Hedgehog/GLI in oncogenic transformation via activation of the MEK/ERK/JUN pathway. Cancer Res. 69(2009) 1284-1292.

169. M. Kasper, H. Schnidar, G.W. Neill, M. Hanneder, S. Klingler, L. Blaas, et al., Selective modulation of Hedgehog/GLI target gene expression by epidermal growth factor signaling in human keratinocytes, Mol. Cell. Biol. 26(2006) 6283-6298.

170. C.M. Della Corte, U. Malapelle, E. Vigliar, F. Pepe, G. Troncone, V. Ciaramella, et al., Efficacy of continuous EGFR-inhibition and role of Hedgehog in EGFR acquired resistance in human lung cancer cells with activating mutation of EGFR, Oncotarget 8(2017) 23020.

171. H. Liebig, G. Günther, M. Kolb, C. Mozet, A. Boehm, A. Dietz, et al., Reduced proliferation and colony formation of head and neck squamous cell carcinoma (HNSCC) after dual targeting of EGFR and hedgehog pathways, Cancer Chemother. Pharmacol. 79(2017) 411-420.

172. C. Frémin, S. Meloche, From basic research to clinical development of MEK1/2 inhibitors for cancer therapy, J. Hematol. Oncol. 3(2010) 8.

173. M. Lauth, 1. Abstract 2. Introduction 3. RAS signaling 3.1. RAS and cancer 4. The Hedgehog signaling pathway 4.1. Hedgehog and cancer 5. The RAS-HH crosstalk 5.1. Molecular crosstalk between RAS and HH, Front. Biosci. 16(2011)2259-2270.

174. M.P. di Magliano, S. Sekine, A. Ermilov, J. Ferris, A.A. Dlugosz, M. Hebrok, Hedgehog/Ras interactions regulate early stages of pancreatic cancer, Genes Dev. 20(2006) 3161-3173.

175. L.D. Mills, Y. Zhang, R.J. Marler, M. Herreros-Villanueva, L. Zhang, L.L. Almada, et al., Loss of the transcription factor GLI1 identifies a signaling network in the tumor microenvironment mediating KRAS oncogene-induced transformation, J. Biol. Chem. 288(2013) 11786-11794.

176. Z. Ji, F.C. Mei, J. Xie, X. Cheng, Oncogenic KRAS activates hedgehog signaling pathway in pancreatic cancer cells, J. Biol. Chem. 282(2007) 14048-14055.

177. E. Rovida, B. Stecca, editors. Mitogen-activated protein kinases and Hedgehog-GLI signaling in cancer: A crosstalk providing therapeutic opportunities? Semin. Cancer Biol. 35 (2015)154-167.

178. J.A. Engelman, Targeting PI3K signalling in cancer: opportunities, challenges and limitations, Nat. Rev. Cancer 9(2009) 550-562.

179. M.Y. Follo, L. Manzoli, A. Poli, J.A. McCubrey, L. Cocco, PLC and PI3K/Akt/mTOR signalling in disease and cancer, Adv. Biol. Regul. 57(2015)10-16.

180. S. Krauß, J. Foerster, R. Schneider, S. Schweiger, Protein phosphatase 2A and rapamycin regulate the nuclear localization and activity of the transcription factor GLI3, Cancer Res. 68(2008) 4658-4665.

181. N.A. Riobó, K. Lu, X. Ai, G.M. Haines, C.P. Emerson, Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling, Proc. Natl. Acad. Sci. U.S.A. 103(2006) 4505-4510.

182. Y. Wang, Q. Ding, C.J. Yen, W. Xia, J.G. Izzo, J.Y. Lang, et al., The crosstalk of mTOR/S6K1 and Hedgehog pathways, Cancer Cell 21(2012) 374-387.

183. L. Wei, Z. Xu, Cross-signaling among phosphinositide-3 kinase, mitogen-activated protein kinase and sonic hedgehog pathways exists in esophageal cancer, Int. J. Cancer. 129(2011)275-284.

184. N. Sharma, R. Nanta, J. Sharma, S. Gunewardena, K.P. Singh, S. Shankar, et al., PI3K/AKT/mTOR and sonic hedgehog pathways cooperate together to inhibit human pancreatic cancer stem cell characteristics and tumor growth, Oncotarget 6(2015) 32039-32060.

185. S.Z. Kaylani, J. Xu, R.K. Srivastava, L. Kopelovich, J.G. Pressey, M. Athar, Rapamycin targeting mTOR and hedgehog signaling pathways blocks human rhabdomyosarcoma growth in xenograft murine model, Biochem. Biophysic. Res. Commun. 435(2013) 557-561.

186. M. Zuo, A. Rashid, C. Churi, J. Vauthey, P. Chang, Y. Li, et al., Novel therapeutic strategy targeting the Hedgehog signalling and mTOR pathways in biliary tract cancer, Br. J. Cancer 112(2015) 1042-1051.

187. Y.A. Yoo, M.H. Kang, H.J. Lee, B.H. Kim, J.K. Park, H.K. Kim, et al., Sonic hedgehog pathway promotes metastasis and lymphangiogenesis via activation of Akt, EMT, and MMP-9 pathway in gastric cancer, Cancer Res. 71(2011)7061-7070.

188. Y.M. Kim, M. Kahn, The role of the Wnt signaling pathway in cancer stem cells: prospects for drug development, Res. Rep. Biochem. 4(2014) 1.

189. J.A. McCubrey, D. Rakus, A. Gizak, L.S. Steelman, S.L. Abrams, K. Lertpiriyapong, et al., Effects of mutations in Wnt/β-catenin, hedgehog, Notch and PI3K pathways on GSK-3 activity—Diverse effects on cell growth, metabolism and cancer, Biochim. Biophysic. Acta (BBA) Mol. Cell Res. 1863(2016) 2942-2976.

190. L. Song, Z.Y. Li, W.P. Liu, M.R. Zhao, Crosstalk between Wnt/β-catenin and Hedgehog/Gli signaling pathways in colon cancer and implications for therapy, Cancer Biol. Ther. 16(2015) 1-7.

191. R.Z. Karim, M. Gary, T.C. Putti, R.A. Scolyer, C.S. Lee, The significance of the Wnt pathway in the pathology of human cancers, Pathology 36(2004) 120-128.

192. A. Farooqi, S. Mukhtar, A. Riaz, S. Waseem, S. Minhaj, B. Dilawar, et al., Wnt and SHH in prostate cancer: trouble mongers occupy the TRAIL towards apoptosis, Cell Prolifer. 44(2011) 508-515.

193. J.H. Kim, H.S. Shin, S.H. Lee, I. Lee, Y.S. Lee, J.C. Park, et al., Contrasting activity of Hedgehog and Wnt pathways according to gastric cancer cell differentiation: relevance of crosstalk mechanisms. Cancer Sci. 101(2010) 328-335.

194. X. Liao, M.K. Siu, C.W. Au, Q.K. Chan, H.Y. Chan, E.S. Wong, et al., Aberrant activation of hedgehog signaling pathway contributes to endometrial carcinogenesis through β-catenin, Modern Pathol. 22(2009)839-847.

195. S. Schmid, M. Bieber, F. Zhang, M. Zhang, B. He, D. Jablons, et al., Wnt and hedgehog gene pathway expression in serous ovarian cancer, Int. J. Gynecol. Cancer 21(2011) 975.

196. P.S. Gowda, J.D. Deng, S. Mishra, A. Bandyopadhyay, S. Liang, S. Lin, et al., Inhibition of hedgehog and androgen receptor signaling pathways produced synergistic suppression of castration-resistant prostate cancer progression, Mol. Cancer Res. 11(2013) 1448-1461.

197. H. Onishi, T. Morisaki, F. Nakao, S. Odate, T. Morisaki, M. Katano, Protein-bound polysaccharide decreases invasiveness and proliferation in pancreatic cancer by inhibition of hedgehog signaling and HIF-1α pathways under hypoxia, Cancer Lett. 335(2013) 289-298.

198. N. Kangwan, Y.J. Kim, Y.M. Han, M. Jeong, J.M. Park, E.J. Go, et al., Sonic hedgehog inhibitors prevent colitis-associated cancer via orchestrated mechanisms of IL-6/gp130 inhibition, 15-PGDH induction, Bcl-2 abrogation, and tumorsphere inhibition. Oncotarget 7(2016) 7667.

199. D. Javelaud, M.J. Pierrat, A. Mauviel, Crosstalk between TGF-β and hedgehog signaling in cancer, FEBS Lett. 586(2012) 2016-2025.

200. M.R. Migden, A. Guminski, R. Gutzmer, L. Dirix, K.D. Lewis, P. Combemale, et al., Treatment with two different doses of sonidegib in patients with locally advanced or

metastatic basal cell carcinoma (BOLT): a multicentre, randomised, double-blind phase 2 trial, Lancet Oncol. 16(2015) 716-728.

201. A. Sekulic, M.R. Migden, A.E. Oro, L. Dirix, K.D. Lewis, J.D. Hainsworth, et al., Efficacy and safety of vismodegib in advanced basal-cell carcinoma, N. Engl. J. Med. 366(2012) 2171-2179.

202. A. Sekulic, M.R. Migden, K. Lewis, J.D. Hainsworth, J.A. Solomon, S. Yoo, et al., Pivotal ERIVANCE basal cell carcinoma (BCC) study: 12-month update of efficacy and safety of vismodegib in advanced BCC, J. Am. Acad. Dermatol. 72(2015) 1021-6. e8.

203. A. Sekulic, M.R. Migden, N. Basset-Seguin, C. Garbe, A. Gesierich, C. Lao, et al., Long-term safety and efficacy of vismodegib in patients with advanced basal cell carcinoma: Final update (30-month) of the pivotal ERIVANCE BCC study, BMC Cancer 17(2017)332.

204. A.L.S. Chang, J.A. Solomon, J.D. Hainsworth, L. Goldberg, E. McKenna, B.M. Day, et al. Expanded access study of patients with advanced basal cell carcinoma treated with the Hedgehog pathway inhibitor, vismodegib, J. Am. Acad. Dermatol. 70(2014) 60-69.

205. H. Sofen, K.G. Gross, L.H. Goldberg, H. Sharata, T.K. Hamilton, B. Egbert, et al., A phase II, multicenter, open-label, 3-cohort trial evaluating the efficacy and safety of vismodegib in operable basal cell carcinoma, J. Am. Acad. Dermatol. 73(2015) 99-105. e1.

206. N. Basset-Seguin, A. Hauschild, J.J. Grob, R. Kunstfeld, B. Dréno, L. Mortier, et al., Vismodegib in patients with advanced basal cell carcinoma (STEVIE): a pre-planned interim analysis of an international, open-label trial, Lancet Oncol. 16(2015) 729-736.

207. A. Abdulla, P. Jones, V. Pearce, Leg cramps in the elderly: prevalence, drug and disease associations, Int. J. Clin. Practice. 53(1998) 494-496.

208. H.X. Liu, A. Ermilov, M. Grachtchouk, L. Li, D.L. Gumucio, A.A. Dlugosz, et al., Multiple Shh signaling centers participate in fungiform papilla and taste bud formation and maintenance, Dev. Biol. 382(2013) 82-97.

209. L.A. Barlow, Progress and renewal in gustation: new insights into taste bud development. Development 142(2015) 3620-3629.

210. H. Yang, W.N. Cong, J.S. Yoon, J.M. Egan, Vismodegib, an antagonist of hedgehog signaling, directly alters taste molecular signaling in taste buds, Cancer Med. 4(2015) 245-252.

211. A.L.S. Chang, A.E. Oro, Initial assessment of tumor regrowth after vismodegib in advanced basal cell carcinoma, Arch. Dermatol. 148(2012) 1324-1325.

212. A. Iarrobino, J.L. Messina, R. Kudchadkar, V.K. Sondak, Emergence of a squamous cell carcinoma phenotype following treatment of metastatic basal cell carcinoma with vismodegib, J. Am. Acad. Dermatol. 69(2013)e33-e4.

213. R.L. Yauch, G.J. Dijkgraaf, B. Alicke, T. Januario, C.P. Ahn, T. Holcomb, et al., Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma, Science 326(2009) 572-574.

214. S.X. Atwood, K.Y. Sarin, R.J. Whitson, J.R. Li, G. Kim, M. Rezaee, et al. Smoothened variants explain the majority of drug resistance in basal cell carcinoma, Cancer Cell 27(2015) 342-353.

215. H.J. Sharpe, G. Pau, G.J. Dijkgraaf, N. Basset-Seguin, Z. Modrusan, T. Januario, et al. Genomic analysis of smoothened inhibitor resistance in basal cell carcinoma, Cancer Cell 27(2015)327-341.

216. S. Buonamici, J. Williams, M. Morrissey, A. Wang, R. Guo, A. Vattay, et al., Interfering with resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma, Sci. Transl. Med. 2(2010) 51ra70-51ra70.

217. C. Danial, K.Y. Sarin, A.E. Oro, A.L.S. Chang, An investigator-initiated open-label trial of sonidegib in advanced basal cell carcinoma patients resistant to vismodegib, Clin. Cancer Res. 22(2016) 1325-1329.

218. Y. Tang, S. Gholamin, S. Schubert, M.I. Willardson, A. Lee, P. Bandopadhayay, et al., Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition, Nat. Med. 20(2014)732-740.

219. S.X. Atwood, M. Li, A. Lee, J.Y. Tang, A.E. Oro, GLI activation by atypical protein kinase C ι/λ regulates the growth of basal cell carcinomas, Nature 494(2013)484-488.

220. C.H. Williams, J.E. Hempel, J. Hao, A.Y. Frist, M.M. Williams, J.T. Fleming, et al., An in vivo chemical genetic screen identifies phosphodiesterase 4 as a pharmacological target for hedgehog signaling inhibition, Cell Rep. 11(2015) 43-50.

221. P.M. LoRusso, C.M. Rudin, J.C. Reddy, R. Tibes, G.J. Weiss, M.J. Borad, et al., Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors, Clin. Cancer Res. 17(2011) 2502-2511.

222. E.J. Kim, V. Sahai, E.V. Abel, K.A. Griffith, J.K. Greenson, N. Takebe, et al., Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. Clin. Cancer Res. 20(2014) 5937-5945.

223. C. D'Amato, R. Rosa, R. Marciano, V. D'Amato, L. Formisano, L. Nappi, et al., Inhibition of Hedgehog signalling by NVP-LDE225 (Erismodegib) interferes with growth and invasion of human renal cell carcinoma cells. Br. J. Cancer. 111(2014) 1168-1179.

224. D. Riedlinger, M. Bahra, S. Boas-Knoop, S. Lippert, M. Bradtmoller, K. Guse, et al., Hedgehog pathway as a potential treatment target in human cholangiocarcinoma, J. Hepatobiliary Pancreat. Sci. 21(2014) 607-615.

225. C.M. Rudin, C.L. Hann, J. Laterra, R.L. Yauch, C.A. Callahan, L. Fu, et al., Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449, N. Engl. J. Med.361(2009) 1173-1178.

226. S. Peukert, F. He, M. Dai, R. Zhang, Y. Sun, K. Miller-Moslin, et al., Discovery of NVP-LEQ506, a second-generation inhibitor of smoothened, ChemMedChem 8(2013)1261-1265.

227. J.T. Romer, H. Kimura, S. Magdaleno, K. Sasai, C. Fuller, H. Baines, et al., Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/-)p53(-/-) mice, Cancer Cell 6(2004)229-240.

228. K.P. Olive, M.A. Jacobetz, C.J. Davidson, A. Gopinathan, D. McIntyre, D. Honess, et al., Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer, Science 324(2009)1457-1461.

229. Stanton BZ, Peng LF, Maloof N, Nakai K, Wang X, Duffner JL, et al. A small molecule that binds Hedgehog and blocks its signaling in human cells, Nat. Chem. Biol. 5(2009)154-156.

230. J. Kim, J.Y. Tang, R. Gong, J. Kim, J.J. Lee, K.V. Clemons, et al., Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth. Cancer Cell 17(2010) 388-399.

231. J.A. Williams, O.M. Guicherit, B.I. Zaharian, Y. Xu, L. Chai, H. Wichterle, et al., Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions, Proc. Natl. Acad. Sci. U.S.A. 100(2003) 4616-4621.

232. A.J. Wagner, W.A. Messersmith, M.N. Shaik, S. Li, X. Zheng, K.R. McLachlan, et al., A phase I study of PF-04449913, an oral hedgehog inhibitor, in patients with advanced solid tumors, Clin. Cancer Res. 21(2015) 1044-1051.

233. A.J. Jackson-Fisher, M.J. McMahon, J. Lam, C. Li, L. Engstrom, K. Tsaparikos, et al. Abstract 4504: PF-04449913, a small molecule inhibitor of Hedgehog signaling, is effective in inhibiting tumor growth in preclinical models, 2011. 4504- p.

234. Y. Minami, H. Minami, T. Miyamoto, G. Yoshimoto, Y. Kobayashi, W. Munakata, et al., Phase I study of glasdegib (PF-04449913), an oral smoothened inhibitor, in Japanese patients with select hematologic malignancies, Cancer Sci. 108(2017) 1628-1633.

235. A. Jimeno, G.J. Weiss, W.H. Miller, Jr., S. Gettinger, B.J. Eigl, A.L. Chang, et al., Phase I study of the Hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors, Clin. Cancer Res. 19(2013) 2766-2774.

236. M. Lauth, A. Bergstrom, T. Shimokawa, R. Toftgard, Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists, Proc. Natl. Acad. Sci. U.S.A. 104(2007) 8455-8460.

237. P. Infante, M. Mori, R. Alfonsi, F. Ghirga, F. Aiello, S. Toscano, et al., Gli1/DNA interaction is a druggable target for Hedgehog-dependent tumors, EMBO J. 34(2015) 200-217.

238. B. Li, D.L. Fei, C.A. Flaveny, N. Dahmane, V. Baubet, Z. Wang, et al., Pyrvinium attenuates Hedgehog signaling downstream of smoothened, Cancer Res. 74(2014) 4811-4821.

239. G. Bosco-Clement, F. Zhang, Z. Chen, H.M. Zhou, H. Li, I. Mikami, et al., Targeting Gli transcription activation by small molecule suppresses tumor growth, Oncogene 33(2014) 2087-2097.

240. A. Slusarz, N.S. Shenouda, M.S. Sakla, S.K. Drenkhahn, A.S. Narula, R.S. MacDonald, et al., Common botanical compounds inhibit the hedgehog signaling pathway in prostate cancer, Cancer Res. 70(2010) 3382-3390.

241. L. Zhang, L. Li, M. Jiao, D. Wu, K. Wu, X. Li, et al., Genistein inhibits the stemness properties of prostate cancer cells through targeting Hedgehog-Gli1 pathway, Cancer Lett. 323(2012) 48-57.

242. M.H. Elamin, Z. Shinwari, S.F. Hendrayani, H. Al-Hindi, E. Al-Shail, Y. Khafaga, et al. Curcumin inhibits the Sonic Hedgehog signaling pathway and triggers apoptosis in medulloblastoma cells, Mol. Carcinogen. 49(2010) 302-314.

243. G.Q. Tang, T.Q. Yan, W. Guo, T.T. Ren, C.L. Peng, H. Zhao, et al., (-)-Epigallocatechin-3-gallate induces apoptosis and suppresses proliferation by inhibiting the human Indian Hedgehog pathway in human chondrosarcoma cells, J. Cancer Res. Clin. Oncol. 136(2010) 1179-1185.

244. S.N. Tang, J. Fu, D. Nall, M. Rodova, S. Shankar, R.K. Srivastava, Inhibition of sonic hedgehog pathway and pluripotency maintaining factors regulate human pancreatic cancer stem cell characteristics, Int. J. Cancer 131(2012) 30-40.

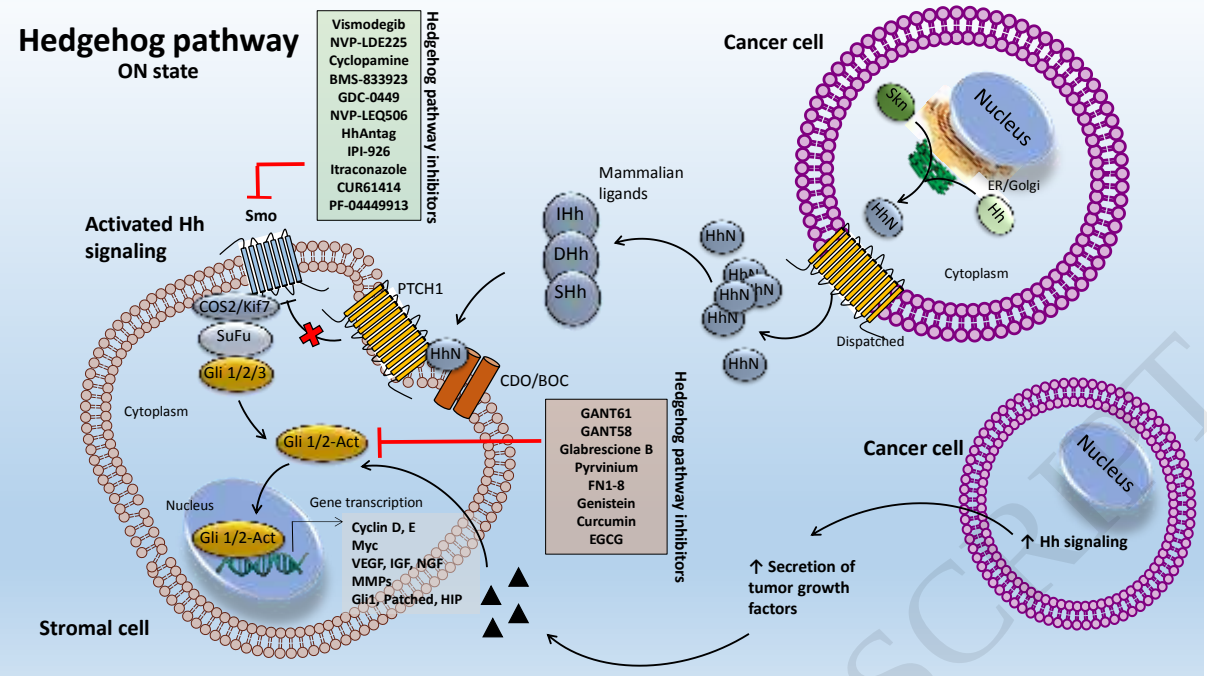

Figure 1. **Overview of the Hedgehog Pathway**. Under normal conditions, the PTCH 1 inhibits the Smo protein; In the presence of the Hh ligands (IHh, SHh and DHh), the inhibition of SMO is relieved and the translocation of Gli initiates the transcription of target genes. [PTCH1- patched 1; Smo- smoothened; Gli-glioma-associated transcription factors; IHh- Indian hedgehog; SHh- Sonic hedgehog; DHh- Desert hedgehog]

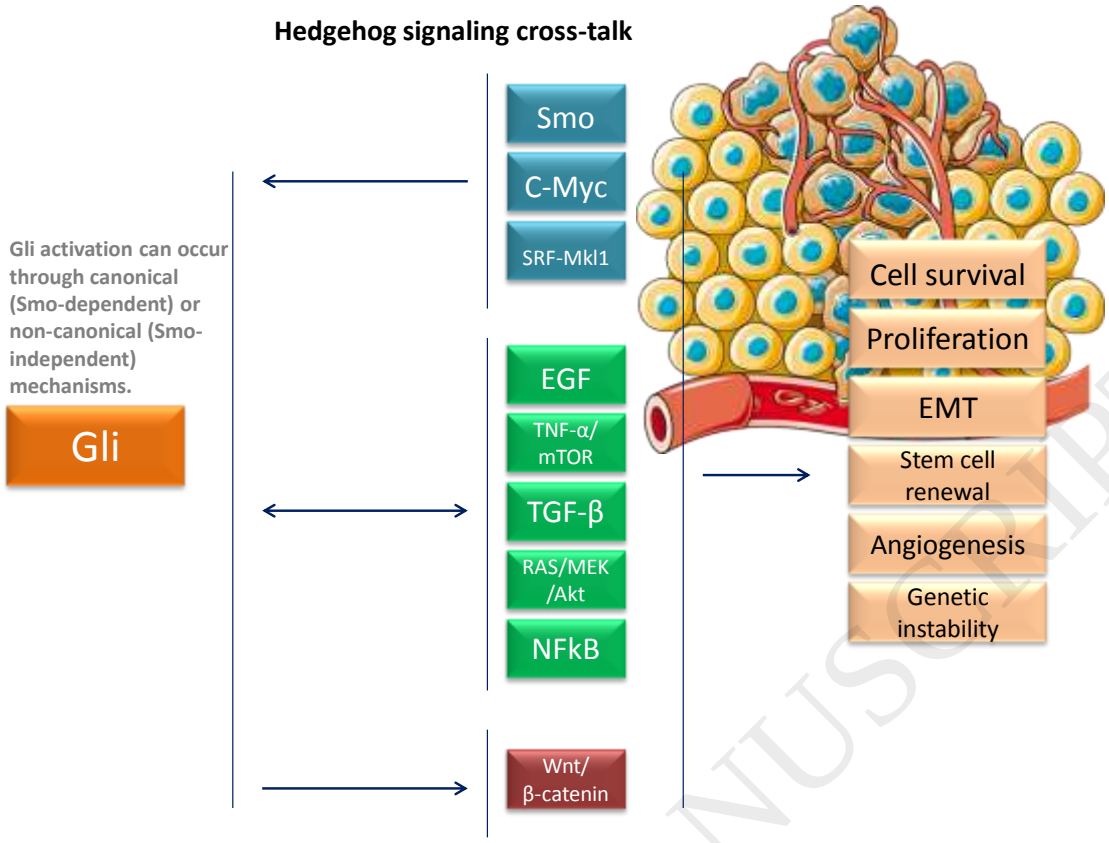

Figure 2. Hedgehog signaling cross-talk in cancer. The activation of Gli transcription factors can occur through canonical or non-canonical, respectively Smo-dependent and Smo-independent mechanisms, transforming these molecules in central points in Hedgehog signaling. In cancer, Gli become molecular hubs due to the crosstalk with different pathways that conclude in cell survival, proliferation, EMT, stem cell self renewal, angiogenesis and genetic instability.

Table 1. Active agents against the Hedgehog pathway

Therapeutic compound	Hedgehog pathway target	Pathology	Effects	Ref
Vismodegib (GDC-0449)	Smoothened (Smo)	Solid tumors (clinical trial)	Tolerance: good; Recommended dose: 150 mg/d; Tumor response: in 20 patients out of 68; Responsive pathology: advanced basal cell carcinoma (BCC)	(221)
Vismodegib (GDC-0449) combined with gemcitabine	Gli1, PTCH1	Metastatic pancreatic adenocarcinoma	Gli1 (95.6% patients) and PTCH1 (82.6% patients) downregulation; inhibited fibrosis (45.4%); no changes in cancer stem cells; GDC-0449 in combination with gemcitabine have not shown an superior action compared to gemcitabine	(222)
NVP-LDE225 (Erismodegib)	Smoothened (Smo)	Renal cell carcinoma (RCC)	Superior affects in combination with everolimus or sunitinib than in monotherapeutic approaches; Reduced migration and invasion *in vitro* and *in vivo;* Decreased proliferation *in vitro* and *in vivo;* Effects observed even in SuR cells (sunitinib-resistant); Impaired paxillin expression; *In vivo:* reduced lung metastatic potential and increased survival	(223)
Cyclopamine **BMS-833923** **BMS-833923** combined with gemcitabine	Smoothened (Smo)	Cholangiocarcinoma (CCC)	Increased PTCH1, Gli1, and SHh in patients tissue samples; Reduced EGI-1 cells proliferation after BMS-833923 treatment, not so significant with Cyclopamine; Inhibition of Gli1 and PTCH1 expression *in vitro* after BMS-833923 treatment; Decreased tumor growth *in vivo* after BMS-833923 and gemcitabine combination, but only with slight differences on expression of the target genes;	(224)

			Superior effects in the case of the combined treatment than in the monotherapeutic strategy; No side effects	
GDC-0449	Smoothened (Smo)	Medulloblastoma (case report)	Patient clinical characteristics: 26-year-old man, medulloblastoma confirmed at 22 years old, initial treatment: resection, irradiation and treatment with carboplatin, etoposide, cyclophosphamide, and vincristine, recurrence after 2 years; GDC-0449 treatment results: reduction of specific symptoms and also tumor, but with transitory characteristics; mutation of PTCH1 gene before treatment	(225)
NVP-LEQ506	Smoothened (Smo)	Medulloblastoma	Second generation inhibitor with activity on D473H cell line – mutant Smo (cell line isolated from the patient with recurrence after vincristine treatment – patient presented in the upper rows of the table); Active also in animal models of medulloblastoma - Ptch+/− Hic−/−	(226)
HhAntag	Smoothened (Smo)	Medulloblastoma	Experimental model: Ptc1+/−p53−/− medulloblastoma mice; Inhibition of SHh pathway; Suppression of Gli1, Sfrp1, Math1, and Ptc2 expression; no changes in wt *Ptc1 expression; significant reduction of the tumor mass and increased cancer free survival*	(227)
IPI-926 and gemcitabine	Smoothened (Smo)	Pancreatic ductal adenocarcinoma (PDA)	Enhanced delivery of gemcitabine concluded with transitory stabilization of the disease	(228)
Robotnikinin	Sonic Hedgehog (SHh)	Human skin cells and tissue	Inhibitory action on SHh signaling; No activity in *in vitro* models lacking Ptc1 receptor; Inhibition of	(229)

			Gli1/Gli2;	
Itraconazole	Smoothened (Smo)	Medulloblastoma	Hh impairing; No activity on the biosynthesis of cholesterol; Inhibitory action mediated by low density lipoprotein; Different inhibitory mechanism on Smo than one encountered in the case of cyclopamine administration; Tumor growth inhibition *in vivo*	(230)
CUR61414	Smoothened (Smo)	Basal cell carcinoma (BCC)	Inhibition of Hh signaling in cells characterized by inactive Ptch-1 (common model for BCC); Impairment of basaloid lesions, including the UV induced ones in mouse skin	(231)
PF-04449913	Smoothened (Smo)	Advanced tumors (clinical trial)	Good tolerance response from patients in the range of 80 to 320 mg once daily; No severe adverse effects; >80% Gli1 inhibition in skin tissue; 34.8% of patients were characterized by stable disease, but no tested subject was associated with partial or complete response	(232)
		Medulloblastoma, colon and pancreatic cancer	Significant tumor regression in Ptch1+/-p53 mouse model of medulloblastoma and also in PDX models for the same pathology (dose dependent action; Inhibition of Gli1 expression and additional related target genes; Tumor regression in combination with chemotherapy in PDX models of colon and pancreatic cancer (63% and 73% inhibition).	(233)
		Hematological malignancies (clinical trial)	Previous experimental model: PDX of CD34+ imatinib-resistant chronic myeloid leukemia (CML) cells - inhibition of leukemic stem cell maintenance; Significant effects upon all malignancies	(234)

			studied; G1/2 severity adverse side effects;	
IPI-926 (Saridegib)	Smoothened (Smo)	Solid tumors (clinical trial)	*IPI-926 – cyclopamine derivate with superior properties; Previous studies – efficient in B837Tx medulloblastoma allograft model, SCLC tumors and pancreatic cancer models; Range of tolerated doses: 20-160 mg; Most responsive patients: BCC ones; No hematological toxicities, no severe side effects;	(235)
GANT61 and **GANT58**	GLI	Hepatocarcinoma, leukemia; pancreatic adenocarcinoma, prostate carcinoma	Dose dependent interference with GLI1/2 transcription; GANT61 – impairment of GLI1/2 expression (HEK293 cells); Administration of GANT inhibitors in Sufu−/− cells resulted in reduction of *Gli1* and *Hip1 transcript levels* (no effects when cyclopamine was administrated) and also Hip1 protein level; Specificity for Hh signaling; Inhibited colony formation in NIH 3T3 cells; PANC1 and 22Rv1 cells treated with GANT resulted in GLI1 and PTCH expression reduction (not the case of cyclopamine); Significant inhibition of cell growth in PANC1 and 22Rv1 cells (GLI1-positive); Restrictive inhibition in GLI1 low cells: HepG2 and Jurkat; *In vivo* inhibition of tumor growth in xenograft model of prostate cancer	(236)
Glabrescione B (GlaB)	Gli1 zinc finger	Medulloblastoma	Inhibition of Gli1 in Smo−/− MEF cells with induced Gli1 expression; Direct structural inhibition of Gli1; Suppression of Hh signaling and implicit growth of cerebellum-derived normal	(237)

			progenitors in a Gli1 dependent manner (*in vitro* and *in vivo*); Inhibition of medulloblastoma cell growth *in vitro* and *in vivo*	
Pyrvinium	Casein Kinase-1α (CK1α)	Medulloblastoma	Inhibition of Hh signaling in stimulated cells (Light-II cells) concomitant with reduction of Gli1 and Ptch1; Reduced proliferation in primary cerebellar granular precursor cells; Inhibition of Hh in a Gli1 and Gli2 dependent manner (increased degradation of Gli TFs) mediated by CK1α; Growth inhibition of medulloblastoma model (Ptch+/− derived medulloblastoma allograft)	(238)
FN1-8	Gli/TAF9	Lung cancer	Inhibited Gli-mediated transcription activity (both Gli1/Gli2 and Gli/TAF9 dependent); *Increased Gli1/2 leveles in NSCLC tissue samples; Impairment of lung cancer cell proliferation; Inhibition of Gli function after TGFβ stimulation, not the case for cyclopamine; Reduced proliferation in a deficient Smo pancreatic cell line (BXPC3); not so accentuated effects in a mutant Smo pancreatic cell line with unaltered SHh pathway (CFPAC); Inhibition of tumor growth in an animal model of lung cancer (subcutaneous tumors - H460 and A549 cell lines)	(239)
Genistein	Gli1	Prostate cancer	Reduction of cell growth - most potent effects within a total of seven tested botanical agents; Inhibition of Hh signaling through reduction of Gli1 transcript levels; Reduction of SHh-stimulated Gli reporter activity	(240)

		Prostate cancer	Inhibition of cancer stem cells properties toghther with the formation of tumorsphere; Significant reduction of tumor parameters *in vivo* and survival in combination with docetaxel; Inhibition of SHh-stimulated Gli1 reporter activity; Impairment of Gli1 expression at both transcript and protein level; Decrease in CD44 levels (CSCs marker)	(241)
Curcumin	Gli1	Prostate cancer	Reduction of cell growth; Inhibition of Hh signaling through reduction of Gli1 transcript levels – most efficient within a total of seven tested botanical agents; Reduction of SHh-stimulated Gli reporter activity	(240)
		Medulloblastoma	Reduction of SHh–Gli1 network; Reduced protein level of SHh combined with reduced Gli1 and PTCH1; Inhibitory effect on β-catenin, phosphorylated Akt and NF-κB with consequences on C-myc, N-myc, and Cyclin D1 levels; Increased apoptosis via Bcl-2	(242)
Resveratrol	Gli1	Prostate cancer	Reduction of cell growth; Inhibition of Hh signaling through reduction of Gli1 transcript levels - most potent effects within a total of seven tested botanical agents	(240)
EGCG	Gli1	Prostate cancer	Reduction of cell growth; Reduction of SHh-stimulated Gli reporter activity	(240)
	Ptch and Gli-1	Chondrosarcoma	In vitro experimental model: SW1353 and CRL-7891 cells; Decreased growth and cell proliferation after EGCG administration; Increased apoptosis as shown by the morphological and structural parameters; Stimulation of	(243)

			Bax expression and inhibition of Bcl-2; No effects on Caspase-3; Downregulation of Ptch and Gli-1 transcript levels, effects present also at the protein level;	
	Smo, Gli1 and Gli2	Pancreatic cancer	Inhibition of Nanog, c-Myc and Oct-4 expression; reduction of CSCs self-renewal properties; Increased apoptosis correlated with the expression of Bcl-2 and XIAP and Caspase-3 markers; Downregulation of Smo, Gli1 and Gli2 and also of Gli transcriptional activity; Reduced migration through impairment of epithelial to mesenchymal markers: Snail, Slug and ZEB1	(244)