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Review

Hh-Gli Signaling Pathway Functions and Its Therapeutic Potential in Cancer

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Hh-Gli signaling pathway is one of the major and highly conserved pathways regulating embryogenesis and organ development. Its aberrant functioning is associated with malformations and cancer. Major proteins involved in the pathway are Hh, Ptch, Smo, and Gli1. Recent discoveries showed that the pathway is involved in many common and lethal tumors, such as breast, lung, prostate and digestive tract cancer. In these cases, tumor growth appears to require abnormal Hh stimulation from the surrounding cells, which causes a strong intracellular response of the pathway. Extensive involvement of the Hh-Gli pathway in tumor development has promoted it into an important target for cancer therapy, by discovery of a small molecule, cyclopamine, which was shown to block Smo. Successful tumor growth inhibition was accomplished both in cell cultures and on animal models. Current research includes analogous molecules, and alternative strategies of intervention on the other elements of the Hh-Gli pathway.

Keywords
Hh-Gli signaling pathway
cancer
development
therapy

INTRODUCTION

The Hh-Gli signaling pathway, also known as Hh-Ptc or Hh/Ptc/Smo, is highly conserved through evolution, and plays a critical role in early development of embryos and organs, both in insects and in mammals. Disturbances in the functioning of the Hh-Gli pathway cause various developmental deformities, and are involved in cancerogenesis. The distinct role of the Hh-Gli pathway in tumors linked to Gorlin syndrome (also known as NBCCS, Nevoid Basal Cell Carcinoma Syndrome) has been known for more than ten years, but its involvement in more common and deadly tumors such as breast, lung, prostate and digestive tract cancer became apparent only in last several years. Recently the Hh-Gli signaling pathway has been associated with two more human cancers, glioma,[1] and melanomas.[2]

The Hh-Gli pathway in adult organism is inactive in most cell types. It is normally activated only during development of particular organs. In such instances, a secreted protein Hh (Hedgehog) binds to a transmembrane protein Ptc (Patched), which releases its repression of Smo (Smoothened, also a transmembrane protein). This triggers a signaling cascade within the cell, causing expression of target genes. Among those genes is the PTC itself, which limits and finally blocks the pathway activity (Figure 1).

The Hh-Gli signaling is essential for proliferation of neural precursor populations in the developing central nervous system (CNS). Hh-Gli signaling modulates normal dorsal brain growth,[3] regulates number of embryonic and postnatal neocortical cells with stem cell properties,[4] and is required to maintain progenitor cells in the postnatal

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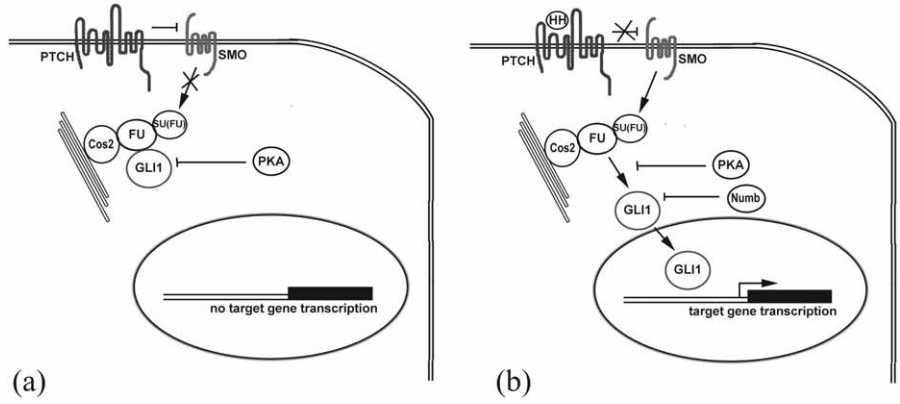

Figure 1. A simplified diagram of the Hh-Gli pathway: (a) in the absence of Hh, Ptch represses Smo and the pathway is inactive, Cos2 keeps the Hedgehog signaling complex tethered to the microtubules, Gli1 is bound in the cytoplasm and there is no transcription of target genes; (b) in the presence of Hh, Hh binds to Ptch, Smo is relieved and activates a cascade that leads to trascription of the pathway target genes.

telencephalon.[5] Hh-Gli signaling represents a large set of gene regulation networks that control stem cell fate throughout development and into adulthood.[6]

Many details of complex interactions within the Hh-Gli pathway are still poorly understood, in particular in case of its abnormal activation involved in malignant processes. Nonetheless, successful inhibitions of the Hh-Gli pathway have been accomplished in some tumors in laboratory conditions.

DETAILS OF THE PATHWAY MECHANISM

In humans, three different homologues of secreted ligand hedgehog (Hh) are known: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh) and Desert Hedgehog (Dhh), all of them are tissue specific. Their activity is dependent on two posttranslational modifications of the protein, one is the binding of cholesterol to the C-terminus, of the N-terminal fragment of the newly synthesized Hh protein (Hh-N), and the other is palmitoylation of one cystein residue in the protein.

Any of these isoforms binds to the receptor Patched (Ptch), which is a 12-pass transmembrane protein. The Patched gene (PTCH), which belongs to a segment polarity family, has two human homologues; after discovery of PTCH2, the PTCH gene is sometimes referred to as PTCH1.

Hh binding causes Ptch to relieve its repression of Smoothened (Smo). This is achieved catalytically via a small molecule, but the identity of this small regulating molecule is still unknown. Smo is a seven-pass transmembrane protein similar to G-protein coupled receptors, and appears to have no other homologues.[7]

Smo activates downstream pathway components by phosphorylation. These components include Fused (Fu), Suppressor of Fused (Su(Fu)), Costal2 (Cos2) and Gli proteins.[8] Cos2 is a kinesin-like protein which in its low phosphorylated state keeps this complex bound to microtubules. When Smo is phosphorylated by a G-protein coupled receptor kinase 2 (Grk2), it associates with Cos2; Fu is phosphorylated and this leads to release of Gli. Fu can also bind directly to Smo and to Su(Fu). Su(Fu) acts as a negative regulator of the pathway.[9,10]

The zinc finger transcription factors Gli1, Gli2 and Gli3 mediate Hh signaling during development, but their expression is also found in several human tumors, including basal cell carcinomas, medulloblastomas and sarcomas. Many of Gli1 targets are associated with cell proliferation, indicating that this oncogene induces cell transformation through multiple downstream pathways.[11] There are three GLI transcription factors in vertebrates: Gli1 appears to act as a transcriptional activator and is universally induced in Hh-responding cells, whereas Gli2 and Gli3 can act as activators or repressors of transcription depending on the cellular context. In particular, Gli1 induces the expression of individual PTCH transcripts in a cell type-specific manner, by direct interaction of Gli1 and the PTCH promoter region, where Gli1-binding sites have been found.[12] Therefore, sequence alterations or mere epigenetic events (such as methylation) in the promoter region can impair Ptch regulatory function.[13]

Other target genes important for the oncogenic function of the Hh-Gli pathway may be those involved in controlling cell proliferation (cyclin D, cyclin E, Myc, β-catenin and the components of the epidermal growth factor pathway) and in angiogenesis (components of the platelet-derived growth factor and of the vascular-epithelial growth factor pathway).[14,15]

Also, a number of additional genes have recently been reported to be associated with the pathway. Among them, Hippi is an adapter protein that mediates pro-apoptotic signaling to the extrinsic cell death pathway. In the absence of Hippi Hh-Gli pathway is downregulated and errors occur during embryonic development: defective left-right axis patterning and defects in neural tube develop-

ment.[16] Numb is a developmental protein that has critical roles in cell-fate determination and differentiation. It functions as an adapter protein during ubiquitination. Gli1 is targeted by Numb for Itch-dependent ubiquitination, which suppresses hedgehog signal, arrests growth and promotes cell differentitation.[17]

DEVELOPMENTAL ROLE CONSERVED THROUGH EVOLUTION

Hh-Gli signaling pathway was first discovered in fruit fly (*Drosophila melanogaster*), and the proteins were named after phenotypical characteristics their deletion induced in fruit flies. Hedgehog was named this way because the fruit fly larva with mutated HH gene showed unusual ridges on its back similar to hedgehog spines. Patched and Smoothened were named after the phenotypical characteristics of the fruit fly wings.[18] Only Gli was named after glioblastoma, because amplification of this gene was found in glioblastoma patients.

The entire pathway is highly conserved through evolution, from nematodes to vertebrates, and plays a major role in embryonic development. Nematodes seem to lack many components of the pathway, including Hh itself, though there are two Ptc related genes. There are also some differences between mammals, zebrafish and *Drosophila melanogaster.*[18]

Recently, it has been shown that Shh gene experienced intensified molecular evolution in primates, and according to the nature of the changes it is postulated that these changes enable more complex post-translational modifications in primates and lineage leading to humans.[19]

Hh-Gli signaling pathway is one of the major pathways regulating embryogenesis. It is involved in various tissues in various stages of development, and acts as a mitogenic, morphogenic or differentiation factor. Shh controls the development of the neural tube, limbs, digestive system, lungs, hair follicles, eyes and left/right side symmetry. Ihh is expressed in the digestive system and cartilage, and regulates bone development. Dhh is involved in Sertoli cells in the testes, where it is involved in differentiation of germline cells,[20] as well as secreted from Schwann cells where it regulates the formation of surrounding tissues.[21]

Malfunctioning or aberrant activation of the pathway in the wrong tissue or in the wrong stage of development leads to malformations and disease, and complete lack of Shh or Ptch genes terminates the embryo early in development.

ROLE IN HUMAN DISEASES

There are two hypothetical models of abnormal Hh-Gli pathway activation that can be distinguished in research papers published on this subject in the last several years. One model suggests constitutive activation of the pathway within the cell, and does not require outside stimulation by Hh signal. This model is associated with *e.g.* basocellular carcinoma and ovarian fibromas. The other model suggests abnormal Hh stimulation from the surrounding cells, which causes a strong intracellular response of the pathway. This model is recently associated with more deadly tumors such as lung and breast cancer.[22,23]

Hh-Gli pathway malfunctioning in humans was initially associated with a medical condition called Gorlin syndrome. This syndrome is characterized by multiple developmental anomalies, such as cleft lip or palate, macrocephaly, pits on palms and soles, spina bifida; but also with various tumors: basocellular carcinoma of the skin (BCC), medulloblastoma, rhabdomyosarcoma, ovarian fibroma and dermoids, jaw cysts and meningioma. Gorlin syndrome is caused by a germline mutation in PTCH gene, whose haploinsufficiency leads to malformations, and a somatic mutation of the second allele leads to tumor formation.

Many tumors associated with Gorlin syndrome can also appear sporadically. In those cases the involvement of the pathway has also been shown, proving the importance of this pathway not only in embryonic development but also in the homeostasis of the adult organism.

Although this pathway was mostly associated with benign tumors and tumors with low metastatic potential, in recent years its role in various fatal tumors is becoming more and more prominent. An increased activity of GLI1, PTCH and HH genes has been detected in a series of tumors not related to Gorlin syndrome (lung, breast, prostate, pancreas *etc.*).[22,23] It is suggested that, in these tumors, the mechanism of Hh-Gli pathway activation is dependent on the increased expression of HH gene and ligand conformation (vital for a successful binding to Ptc). According to some estimates, the pathway may be involved in one third of all lethal tumors.

THERAPEUTIC POTENTIAL

Initial knowledge about possible antagonists of this pathway came from studies of malformed sheep in California. It has been noticed that sheep grazing on the hillsides often gave birth to malformed lambs, mostly bearing cyclopia. The main cause of this condition was a mountain lily, *Veratrum californicum,* more specifically an alkaloid compound extracted from it named cyclopamine (Figure 2). Cyclopamine was shown to block Smo and successful tumor growth inhibition was accomplished both in cell cultures and on animal models.[24] Other similar natural or synthetic alkaloids were found to inhibit the pathway.

So far, the research in inhibition of the Hh-Gli pathway was mostly based on antagonizing Smo with cyclopamine and its analogues (Table I); however, the question

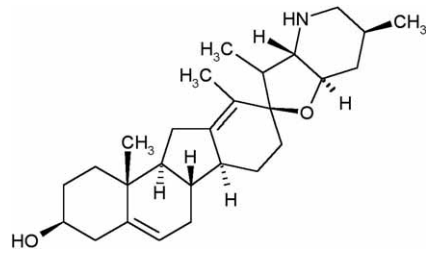

Figure 2. The cyclopamine molecule.

TABLE I. Recent research on Hh-Gli pathway inhibition in human tumors[a]

Tumor	Inhibitors	Reference
Medulloblastoma	Cyclopamine, Hh-Antag691	3, 25, 26, 27
Glioma	Cyclopamine	1, 25
Basal cell carcinoma	Cyclopamine, Cur61414	28, 29
Oral squamous cell carcinoma	Cyclopamine	30
Small cell lung cancer	Cyclopamine, Hh antibody	23
Pancreatic cancer	Cyclopamine, Hh antibody	31, 32, 33, 34
Digestive tract cancer	Cyclopamine, Hh antibody	33
Prostate cancer	Cyclopamine, Hh antibody, RNA interference	35, 36, 37
Breast cancer	Cyclopamine	22
Colorectal cancer	Cyclopamine	38
CNS tumors	Gli-antisense	25

[a]Adopted from Ref. 25.

remains whether this will be applicable *in vivo* on human tumors. Influencing Smo in the Hh-Gli signaling pathway has the obvious advantage of not being dependent on pathway activation with Hh. The drawback is the fact that it leads to a general violent Hh-Gli pathway shutdown and does not act on the cause of the abnormal activation of the pathway.

Alternative strategies of intervention on the other elements of the Hh-Gli pathway have only recently began to be researched (the best results were achieved by blocking Shh binding to Ptc with anti-Shh antibodies).[35] In a model of ligand-dependent activation it might be best act on the source – to suppress Hh gene expression or impede protein conformation – and in the case of a ligand-independent model to boost Ptc gene expression or continuously replace the protein.

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SAŽETAK

Signalni put Hh-Gli i mogućnosti primjene u terapiji raka

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Signalni put Hh-Gli jedan je od najvažnijih i visoko očuvanih puteva koji reguliraju embriogenezu i razvoj organa. Njegovo se poremećeno funkcioniranje povezuje s malformacijama i rakom. Glavni proteini uključeni u taj put jesu: Hh, Ptch, Smo i Gli1. Poremećeno fukcioniranje Hh-Gli puta kod ljudi u početku se povezivalo s Gorlinovim sindromom, koji se pripisuje germinativnoj mutaciji jednog alela PTCH gena. Znatno su zanimljivija nedavna otkrića da je signalni put uključen u mnoge druge uobičajene i letalne tumore, kao što su rak dojke, pluća, prostate i probavnog trakta. U tim se slučajevima čini da rast tumora zahtijeva abnormalnu Hh stimulaciju iz okolnih stanica, koja uzrokuje snažan međustanični odziv signalnog puta. Velika zastupljenost signalnog puta Hh-Gli u razvoju tumora promovirala ga je u važnu metu za terapiju raka, što je bilo potpomognuto otkrićem ciklopamina. Pokazalo se da ta mala molekula blokira Smo, čime je ostvarena uspješna inhibicija tumorskog rasta kako u staničnim kulturama tako i na životinjskim modelima. Današnja istraživanja uključuju ne samo analogne molekule nego i alternativne strategije djelovanja na druge elemente signalnog puta Hh-Gli.