Journal of Biology

BioMed Central

Minireview

The therapeutic potential of modulators of the Hedgehog-Gli signaling pathway

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Published: 6 November 2002

Journal of Biology 2002, **1**:9

The electronic version of this article is the complete one and can be found online at http://jbiol.com/content/1/2/9

 ISSN 1475–4924

Abstract

The discovery of small molecules that act as agonists and antagonists of the Hedgehog-Gli signaling pathway, which plays important roles in the embryo and adult, opens a new avenue for the treatment of diseases caused by aberrant suppression or activation of this complex pathway.

The Hedgehog-Gli signaling pathway regulates numerous events during the normal development of many cell types and organs, including the brain, bone, skin, gonads, lung, prostate, gastrointestinal tract and blood. The *hedgehog (hh)* gene - like many of the components of the signaling pathway triggered by Hedgehog (Hh) protein - was first identified in *Drosophila*, where it affects pattern formation very early in embryonic development. The binding of Hh to cell membranes triggers a signaling cascade that results in the regulation of transcription by zinc-finger transcription factors of the Gli family.

Of the three *hh*-family genes in mammals - *Sonic hedgehog* (*Shh*), *Indian hedgehog* (*Ihh*) and *Desert hedgehog* (*Dhh*) - *Shh* has been the most studied, mainly because it is expressed in various tissues but also because experiments with Shh protein are generally also applicable to other members of the family. The correct regulation of the Hh-Gli signaling pathway is essential not only for normal development but also to prevent a number of human diseases associated with abnormally increased or decreased signaling. Here, we discuss the potential use of small-molecule modulators of the Hh-signaling system, including those reported by Frank-Kamenetsky *et al.* in this issue [1], as therapeutic agents.

Hedgehogs are secreted glycoproteins that act through the transmembrane proteins Patched1 (Ptc1) and Smoothened (Smo) to activate an intricate intracellular signal-transduction pathway (Figure 1). Hh binds Ptc1, a protein with 12 transmembrane domains, and this releases the basal repression that Ptc1 exerts on Smo, a 7-transmembrane-domain protein that has homology to G-protein-coupled receptors. Inside the cell, a multimolecular complex, including Costal2 (Cos2), Fused (Fu) and suppressor of Fused (Su(Fu)), responds to the activation of Smo [2,3] in such a way as to modify the activity of the Gli proteins (reviewed in [4]). 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 particular cellular context. The fate of Gli proteins, which appear to reside in the cytoplasm in their inactive state, depends on the state of Hh signaling. In the absence

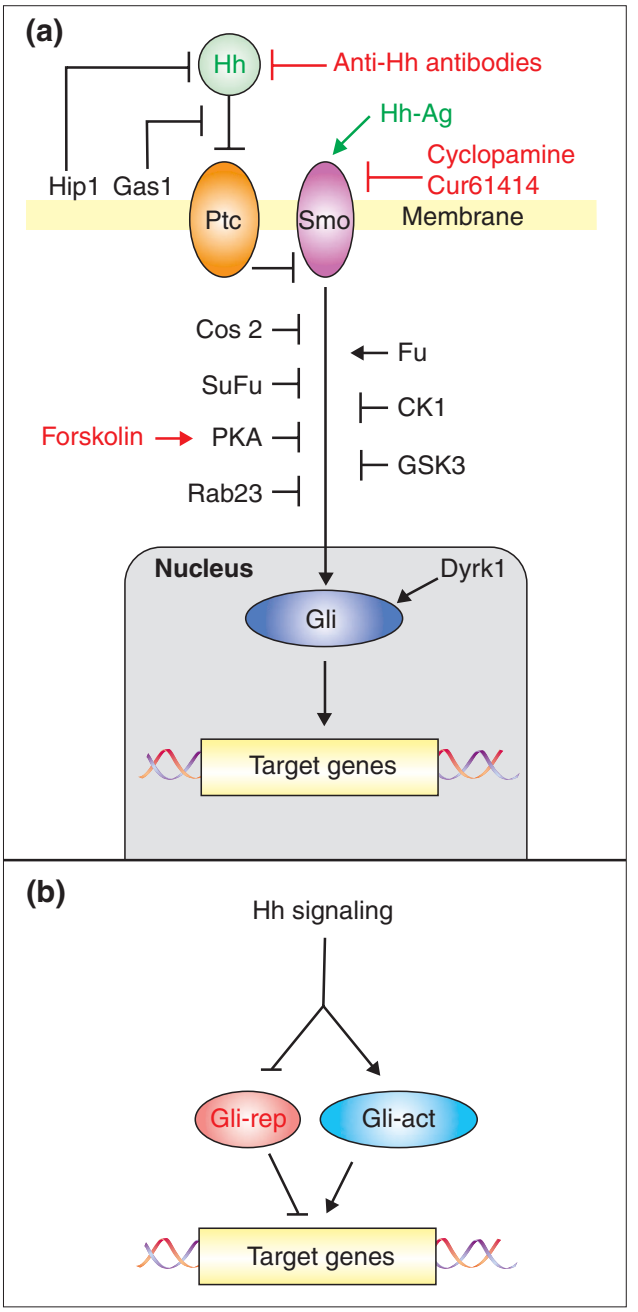

Figure 1
The Hh-signaling pathway. **(a)** A diagram of the Hh-signaling pathway, showing the site of action of the agonists (green) and antagonists (red) discussed in the text, as well as many additional factors that affect the pathway. Abbreviations: CK1, Casein kinase 1; Cos2, Costal2 ; Dyrk1, dual-specificity Yak1-related kinase 1; GSK3, Glycogen synthase kinase 3; Fu, Fused; Gas1, growth arrest specific 1; Hh, Hedgehog; Hip, Hedgehog-interacting protein 1; Rab23, a Rab-family Ras-like GTPase associated with vesicle traffic; Ptc, Patched1; PKA, Protein kinase A; Smo, Smoothened; SuFu, Suppressor of Fused. **(b)** A schematic generalized view of the regulation of Gli activator (Gli-act) and Gli repressor (Gli-rep) forms by Hh signaling. See [2-4] for further details.

of Hh, Gli3 is processed into a smaller, nuclear transcriptional repressor that lacks the carboxy-terminal domain of full-length Gli3 (Gli-rep in Figure 1). Upon activation of Smo (and Hh signaling), Gli3 protein cleavage is prevented and an apparent full-length form with transcription-activating function is generated (Gli-act in Figure 1). Gli2 also encodes a repressor function in its carboxy-terminally truncated form, but its formation does not appear to be regulated by Hh signaling.

Mutations in components of the HH-GLI pathway in humans (human gene and protein names are given in capitals) lead to several diseases that result from either loss of function or ectopic activation of the pathway (reviewed in [5]). For example, haploinsufficiency of *SHH* or mutation in the human *PTCH1* gene are associated with holoprosencephaly, a common syndrome affecting development of the forebrain and mid-face [6-8]. Moreover, ectopic expression of Shh, Gli1 or Gli2 in model systems leads to the formation of tumors that resemble basal cell carcinomas (BCCs) ([9-12]; reviewed in [13]), and sporadic human BCCs consistently express GLI, suggesting that all sporadic BCCs have this pathway active [10]. Similarly, human mutations in the *Suppressor of Fused - SU(FU)* - gene predispose the carrier to medulloblastoma [14]; sporadic medulloblastomas can carry *PTCH1* mutations and express *GLI1* - again suggesting that they harbor an active pathway - and *Ptc*$^{+/-}$ mice can develop medulloblastomas ([15-19]; reviewed in [13]).

From an examination of the different mutations that cause aberrant suppression or activation of the HH-GLI pathway in humans, it seems clear that the development of small molecules that could act as agonists or antagonists of the function of proteins such as PTCH1, SMO or GLI might provide an effective therapeutic approach. One such drug could be SHH protein itself, a natural agonist. For example, it has been reported that injection of Shh into the striatum reduces behavioral deficits in a rat model of Parkinson's disease [20], that Shh can induce dopaminergic neuronal differentiation [21,22] and that Shh is a neuroprotective agent [23]. But Shh has a relatively short half-life in serum [24] and its therapeutic effects have been difficult to evaluate *in vivo*. The use of synthetic Hh agonists could therefore provide a viable alternative to Shh protein. Frank-Kamenetsky *et al.* [1] have now identified a synthetic non-peptidyl small molecule that faithfully activates the Hh-Gli pathway, triggering the known biological effects of Hh signaling. They have shown that this agonist promotes proliferation and differentiation in a cell-type-specific manner *in vitro*, while *in vivo* it rescues developmental defects of *Shh*-null mouse embryos. But this agonist, unlike Shh protein, appears to bypass the Ptc1-regulatory step, by interacting directly with Smo (see

Figure 1). Similar results with a near-identical agonist have now been obtained by another group [25]. From a therapeutic point of view, the fact that the molecule retains its activity after oral administration is a great advantage and, if its ability to cross the blood-brain and placental barriers occurs in humans, it could be a very valuable therapeutic agent. Nevertheless, systemic side effects are to be expected, as there are many HH-responsive cell populations in the body.

Treatment of human diseases resulting from ectopic HH-GLI pathway activation, such as those caused by oncogenic mutations in *SMOH* and *PTCH1* or in any element of the pathway that results in activation of GLI function, requires the use of pathway antagonists. Up to now, inhibition of ectopic activity has been achieved by treatment with signaling antagonists that block the pathway at different levels (Table 1): first, blocking anti-Shh antibodies that act extracellularly [26]; second, cyclopamine, a plant alkaloid [27,28] that acts at the level of Smo in the cell membrane [29]; third, forskolin, an intracellular activator of protein kinase A (PKA) that is a cytoplasmic inhibitor of the pathway (see, for example, [30]); and fourth, Gli-repressor proteins that act within the nucleus to inhibit positive GLI function from mediating the HH signal [31] (Figure 1). Therapeutic use of anti-SHH antibodies is limited to diseases characterized by misexpression of the ligand and cannot generally be applied to tumors, because these do not consistently express *SHH* (see, for example, [10]). Use of forskolin is likely to lead to numerous side effects, given the widespread activity of PKA. In contrast, the use of the small molecule cyclopamine holds great promise.

Table 1

Examples of diseases caused by loss of or ectopic function of the HH-GLI signaling pathway, and the possible agents that could, in principle, be used as therapeutics

Disease type		Potential therapeutic	
Gain-of-function:	Basal cell carcinoma	**Antagonist:**	Anti-HH antibodies
	Medulloblastoma		Forskolin
	Rhabdomyosarcoma		Cyclopamine
			Cur61414
			GLI repressors
Loss-of-function:	Holoprosencephaly	**Agonist:**	SHH
			Hh-Ag*

*Hh-Ag is the Hh agonist described by Frank-Kamenetsky *et al.* [1].

A number of studies suggest that cyclopamine specifically inhibits Smo activity [27-29] and that it can affect disease states caused by activation of the HH-GLI pathway. For example, the proliferation of a number of human brain-tumor cell lines and primary tumor cultures, including those from medulloblastomas and some gliomas [18] as well as medulloblastoma allografts [32], are inhibited by treatment with cyclopamine. This suggests that pathway activation is required for tumor maintenance. Other experiments suggest that the activity of Gli proteins, the terminal elements of the pathway, is sufficient to induce tumor development ([10-12]; reviewed in [13]). Thus, HH-pathway activity may be involved in the initiation as well as the maintenance of different tumors. This provides an additional opportunity to inhibit the growth of a number of tumors in different organs and tissues, such as basal cell carcinoma in the skin and medulloblastoma in the brain, with the same agent. Cyclopamine could be such an agent if the diseases to be treated arise from activation of the HH-signaling pathway at the level of SMOH or above. In addition, Frank-Kamenetsky *et al.* [1] report the use of a new, synthetic, small-molecule inhibitor, Cur61414, which has inhibitory properties similar to those of cyclopamine and also acts at the level of Smo [33]. Whether Cur61414, or four additional small-molecule antagonists (SANT1-4) that also act on Smo and were recently identified [25], will prove to be better and easier to use than cyclopamine remains to be determined, but testing them against skin [33] and brain tumors is warranted from a biological point of view.

Finally, given that carboxy-terminally truncated repressor forms of GLI3 are potent inhibitors of the activating output of the HH-signaling pathway [31,34,35], these could be used as antagonists for the treatment of tumors. The difficulty of delivering them into cells might require the development of *in vivo* transducing strategies, taking advantage, for example, of the ability of the Penetratin peptide to cross cell membranes while loaded with cargo [36]. It also suggests that it would be useful to search for and design small molecules that inhibit GLI's transcription-activating function, perhaps by promoting endogenous GLI-repressor formation. This may be very difficult, but such drugs would be very specific and would be usable in cases where the cancer is due to mutation in the pathway at any level, from the extracellular ligand, the HH proteins, to the final mediators, the GLI proteins.

Agents that inhibit HH signaling may induce the regression of tumors that are dependent on a deregulated HH-GLI pathway, but these agents are likely also to affect the behavior of other normal pathway-dependent cells in the patient. This may, however, be a small price to pay in

order to combat cancer, and the agents may have fewer side effects than current non-specific cytotoxic anti-cancer chemotherapies.

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