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Non-cell-autonomous signaling by Shh in tumors: challenges and opportunities for therapeutic targets

Maarten F Bijlsma, **Henk Roelink**[†]

University of California Berkeley, Department of Molecular and Cell Biology, Berkeley, USA

Abstract

Importance of the field: The Hedgehog (Hh) pathway is required during many developmental events; in adults the Hedgehog pathway is involved in the maintenance of several stem cell niches. It is therefore not surprising that aberrantly regulated Hh pathway activity can cause birth defects in the developing organism, as well as neoplastic disease later in life.

Areas covered in this review: As a consequence of the involvement in pathogenesis, the Hh pathway components are subject to an intense scrutiny as potential targets for therapeutic agents. We aim to provide an overview of the biology of the Hh proteins and the cellular response, in conjunction with potential therapeutic interventions.

What the reader will gain: Specifically, we focus on the recently discovered non-cell-autonomous Shh signaling used by tumors and the implications of this for the design of treatment strategies. This should provide the reader with up-to-date knowledge on the role of the Hh pathway in tumor progression and the options to treat these malignancies.

Take home message: An important concept that we advocate in this review is the need to recognize the need to target both the stromal and the tumor compartment in malignancies that rely on paracrine Shh signaling.

Keywords

cancer; Disp1; Hedgehog; Shh; Smo; tumor biology

1. Background

As a developing organism grows more complex, gradients of signaling molecules shape many features by exposing cells in these gradients to distinct concentrations of these signals, termed *morphogens*. Members of four families of signaling molecules constitute the vast majority of morphogens; the fibroblast growth factor (FGF), TGF, (including the bone morphogenetic proteins (BMPs) and Activins), Wnt, and Hedgehog (Hh) families [1].

The Hh family is relatively small, with one ligand present in flies, and three in amniotes; Sonic-, Indian- and Desert Hedgehog. Unlike the many families of morphogens, all Hh ligands signal via the same receptor complex. Removal of the G-protein coupled receptor

[†]Author for correspondence: University of California Berkeley, Department of Molecular and Cell Biology, Berkeley, CA 94720-3204, USA, Tel: +1 510 642 5108; roelink@berkeley.edu.

(GPCR) Smoothened (Smo) prevents signaling by all Hh ligands, and *Smo*−/− embryos display a severe phenotype characterized by the absence of bilateral asymmetry, digit patterning and ventral neural tissue, consistent with a role for Hh signaling at these sites [2]. Several birth defects concerning digit number and identity, as well as some debilitating birth defects involving incorrect neural development can be ascribed to insufficient Sonic Hedgehog (Shh) signaling.

Hh signaling remains important into adulthood, where it is involved at many locations subject to continual tissue renewal, such as the skin and the lining of the digestive tract [3,4]. Not surprisingly, these sites are particularly sensitive to tumor formation due to inappropriately upregulated Shh signaling. Although many key players involved in the regulation of the Hh response are identified, many details remain unresolved, and are in fact subject to much debate, some of which we will discuss below.

2. Hedgehog signal transduction

The biology of the Hh proteins is unique and remarkably complex. The ligands are the only known sterolated proteins in the animal kingdom. The addition of the cholesterol moiety is linked to an autocatalytic cleavage event mediated by the carboxy-terminal part of the precursor protein [5]. Subsequently yet another lipophilic group is added to the amino-terminal fragment, resulting in a mature protein that is both sterolated and palmitoylated (Figure 1) [6]. These additions obviously render the protein hydrophobic, and as a consequence obligatorily membrane-bound [7]. This in turn poses a significant challenge for the distribution of Hh in a gradient. As a morphogen, Shh is able to travel at least several cell diameters from its sites of synthesis [8]. The diffusion capacity of Shh bears relevance not only in developmental processes, but also in the progression of tumors that rely on Shh for their growth. Consistent with its nature as a membrane-bound molecule, a dedicated mechanism exists to mediate the distribution of Hh protein through the hydrophilic inter-cellular environment. In particular, the membrane protein Dispatched-1 (Disp1) [9], is required at the sites of Hh synthesis for proper distribution of the Hh ligands. Disp1 is thought to mediate the formation of Shh multimers in which the lipid attachments are sequestered in the interior of the complex, resulting in a soluble particle that bound both by lipophilic interactions via its lipid anchors as well as Shh protein–protein interactions [10]. Consistent with this model, Shh has been found to be associated with nodal vesicular particles that consist of membrane fragments [11] and lipoprotein particles [12]. Nevertheless, the precise nature and form of Shh moving from cell to cell through a tissue remains unclear.

Another unusual feature of the Hh pathway is the receptor pair that relays the Hh signal from the cell surface to the interior. Unlike most pathways, in which one receptor complex transduces a signal into the cell to the downstream pathway components, the Hedgehog pathway is more intricate and uses an ‘on’ receptor (Smoothened (Smo)) and an ‘off’ receptor (Patched1 (Ptch1)) to manage its activation status (Figure 1). In the absence of Hh ligand, or when the ligand is sequestered by other binding partners (such as the Hedgehog interacting protein Hhip1 [13]), the pathway is kept off through the action of Ptch1 (Figure 1) [14]. Ptch1 inhibits Smo, keeping it and its downstream pathway components in an

inactive state. Upon Hh ligand binding to Ptch1 the resulting internalization of this complex traffics it to late endosomes and lysosomes [15,16]. This trafficking event is associated with a redistribution of Smo allowing it to be activated. The precise mechanism by which Ptch, in the absence of (S)hh, inhibits the activity of Smo is unknown, but it presumably involves the secretion or translocation of small lipophilic molecules by Ptch1 that bind to Smo, thereby inhibiting its activity [17]. In flies, some of these small inhibitory molecules can be carried on lipoprotein particles that also could carry Hh [18]. Regulation of Ptch by a small lipid is supported by the observations that Ptch1 inhibits Smo at sub-stoichiometrical concentrations, indicating a catalytic mechanism [19]. Also, the homology between Ptch1 and the Niemann-Pick C1 (NPC1 [20]) protein, which is involved in cholesterol homeostasis in humans, and various prokaryotic and eukaryotic transporter molecules of the resistance, nodulation and division (RND) proton-driven transporters [21] suggest that a critical aspect of Ptch1 function involves the proton-driven translocation of a substrate inhibitory to Smo. The structure of the natural Smo inhibitor cyclopamine [22], combined with the observation that the genetic loss of 7-dehydrocholesterol (7-DHC) reductase activity causes attenuation of Smo [23], leads to the idea that the substrate for Ptch1 is a sterol-like molecule. This could be a 7-DHC derivative such as (pro-)vitamin D3, which is a substrate for Ptch1 pump activity and which binds to, and inhibits Smo, potentially playing a central role in the Shh response [17]. 7-DHC derivatives are expected to localize in membranes, and consistent with this, the function of Ptch1 has previously been shown to be strictly cell autonomous [24]. Nevertheless, overexpression of Ptch1 *in vitro* results in the accumulation of Smo inhibitors in the supernatant, lending further support to the idea that Ptch1 redistributes a Smo inhibitor.

Smo is thought to be a member of the large family of GPCRs, and it associates with a G_i-protein to achieve downstream signaling [25,26]. However, the downstream elements do not appear to be members of a classical GPCR response [27–30], but instead the complex activated by Smo consists of, among others, the kinase Fused (Fu), Suppressor of Fused (SuFu), which is a negative regulator of Hh signaling, and Gli zinc finger transcription factors [31–34]. This complex has more constituents, in particular those that mediate the association with the microtubule skeleton, a function mediated in flies by Cos2 [32,33]. Functional analogs of Cos2 and their roles in the Hh response in vertebrates will be discussed in more detail below.

There are three Gli homologs in vertebrates (Gli1, −2, and −3), and they all differ in the way they modulate Hh-induced gene expression. Gli2 and Gli3 are subject to a proteolytic event that results in the generation of a highly effective inhibitor of the Hh response. Binding of Hh to Ptc, and the subsequent activation of Smo inhibits this proteolytic event, and in particular the full length Gli2 now acts as an activator of Hh responsive genes (see [29] for an exhaustive overview).

The flow of information from Smo to the Gli proteins, and the way in which the proteolysis is regulated remains unclear, although multiple phosphorylation events appear to be involved, and principal kinases in these events are GSK-3β [35–38], PKA and CK1 [39,40], priming (in conjunction with β-TrCP, a ubiquitin ligase [41]) Glis for proteolysis.

In flies, Cos2 is a required for the Hh response. Cos2, a microtubule binding protein is thought to spatially coordinate the complex containing Fu, SuFu and Ci [32,33]. Functional homologs of Cos2 in vertebrates appear to be monomeric versions of the Kif kinesin engines [42]. As a consequence of their binding to microtubules, it is no surprise that they mediate the accumulation of Hh pathway components to the primary cilium, a structure present on most cells that has a microtubule core [43,44]. Even more striking is the observation that Ptch1 and small molecules regulate the position of Smo in the cilium, suggesting that the activation of the Gli transcription factors also occurs in this structure. This notion is backed up by a study by Kim *et al.* [45]. Mutations that abrogate formation of the cilium also affect Hh signaling capacity and this correlation has lead to the suggestion that the role of ciliary localization of Hh signaling components is a physiologically relevant phenomenon [46]. Not entirely unexpectedly, primary cilia have been found to be involved in tumorigenesis of malignancies that are known to rely on excessive Hh pathway activation [47,48].

Although the Gli transcription factors are directly responsible for all transcriptional activation induced by Hh signaling, the observation that Hh signaling is also involved in cell migration and growth cone guidance suggests that a transcription-independent, local Shh response is present in cells [49]. These events are ligand-dependent, and require Smo function, but rather than resulting in altered Gli processing, the leukotriene synthesis machinery is activated. This in turn regulates cell motility resulting in a migratory response [50,51]. Although these pathways undoubtedly hold great promise for the development of future therapeutics (this pathway could be involved in Hh-induced metastasis), very little is known about their exact functioning and actual relevance, and we will thus not elaborate on this branch of the Shh response.

3. Hedgehog in pathophysiology

Insufficient Hh pathway activity has been thought to underlie several congenital malformations. For instance, Smith-Lemli-Opitz syndrome (cause by an absence of 7-DHC reductase [17,23,52]), fetal alcohol syndrome, holoprosencephaly, and perhaps Down syndrome can be attributed to an insufficiently active Hh pathway [53–55]. On the other hand, a beneficial role for Hh pathway activation in the adult organism has been found in various ischemia models. In these models (hind limb- and myocardial ischemia), the Hh response was found to be upregulated and the addition of exogenous Shh aided in salvage of damaged tissue through activation of angiogenic and anti-apoptotic pathways [56–58].

However, the intervention options that this review will address focus on those circumstances where the Shh response is activated inappropriately, which is a frequent cause in the formation of many common and often particularly heinous tumors. The inappropriate activation of the Shh response that causes tumor growth is either caused by excessive Shh ligand production, or defective repression of Smo by mutations in either Ptch1 or Smo itself, or downstream signaling components that result in a defective pathway regulation.

The role of Ptch1 as the inhibitor of Smo provides the explanation why it is a tumor suppressor protein. *Ptch1* heterozygotes suffer from nevus basal cell carcinoma syndrome (NBCCS), which is characterized by the frequent occurrence of basal cell carcinomas

(BCCs), as well as medulloblastomas and rhabdomyosarcomas [59]. Sporadic forms of such tumors are often characterized by the loss of Ptch1 as well. Activation of the pathway by activating mutations in Smo rendering it insensitive to inhibition by Ptch1 is also commonly associated with BCCs [60]. Other pathway components are not known to function in tumor progression, but mutations in SuFu have been detected in medulloblastomas [61]. Tumors arising in the derivatives of the foregut, such as esophageal, gastric, hepatic and in particular pancreatic cancers as well as prostate cancer metastases often have an inappropriate activation of Shh ligand expression [62–64]. These two distinct ways of activating the Hh response, cell autonomously via the loss of Ptch1 or the activation of its downstream actors on one hand, and the excessive production of the Shh ligand on the other hand underlies an essential difference in these tumors. The two fundamentally different tumor types that involve the activation of the Hh response are thus; i) tumors with a cell autonomous, or autocrine, activation of the pathway, which is usually achieved by Ptch1 inactivation [65–67], or activating mutations of Smo [60], or ii) tumors in which the Shh ligand is inappropriately expressed. Interestingly, this aberrantly expressed Shh signals to the normal surrounding cells, which respond by the synthesis of distinct reciprocal signals that sustain the growth of the Shh producing cells [68,69], together forming a polyclonal tumor consisting of mutated Shh-expressing cells and a non-mutated stromal component.

4. Targets for therapeutic intervention in tumors with cell autonomous activation of the Shh response

Tumors arising due to a cell autonomous activation of the Hh pathway critically rely on the activation of the Ptch1/Smo receptor pair by mutations in either protein. As is the case for many GPCRs, Smo has turned out to be an eminently druggable target. In fact, a highly efficient Smo inhibitor, cyclopamine, is present in the common range plant *Veratrum californicum* [70]. Many derivatives of cyclopamine have been developed that have shown improved efficacy, and large compound screens have identified other potent Smo inhibitors [71].

Nevertheless, no Smo inhibitors are yet in clinical use, although some are in early phases of clinical trials [72]. In two recently published papers, a remarkable efficacy was shown for a Smo inhibitor developed by Genentech, GDC-0449. In patients with locally advanced or metastatic medulloblastoma (a type of tumor well known to depend on mutations in the receptors for the Hh pathway), treatment with the inhibitor GDC-0449 was found to have strong anti-tumor activity as indicated by a high percentage of patients with a partial response or stable disease [73]. In the same issue, a case report was published describing a patient with metastatic medulloblastoma. Treatment of this patient with GDC-0449 resulted in a spectacular but temporary reduction in metastases and morbidity [74]. The relapse seemed to be caused by a mutation in Smo that was found in tumor cells after treatment, and rendered Smo insensitive to GDC-0449 [75]. The development of tumor resistance through mutations in pathway components in various pathways is a common event, and explains why combination therapies are much more effective in treating tumors. Also, as the stroma would be less likely to accumulate mutations that confer resistance, targeting this compartment should prove effective.

Several recent and exhaustive reviews have been written about these Smo inhibitors [76,77], so we will not elaborate on those. These inhibitors are likely to become clinically relevant, in particular in those tumors with a cell-autonomous activation of the Shh response. However, they offer a possible remedy to only one aspect of the signaling thought to support the polyclonal, Shh expressing tumors.

Other candidates for therapeutic intervention in tumors dependent on Hh pathway activation would be the Gli transcription factors. As the Gli proteins are the most downstream effectors of the Hh pathway, their inhibition should provide the most effective and arguably specific therapeutic option. There are however two problems with this strategy; the first is that there are only few Gli-inhibitory molecules available, and these have been described fairly recently, casting doubt on the clinical use of a Gli-inhibitor anytime soon [78]. Second, it is potentially hard to devise drugs specifically targeting the transcription activator activity of Glis, while sparing the highly efficient inhibitory activities of Gli3 [79,80].

5. Targets for therapeutic intervention in paracrine signaling tumors

5.1 Introduction

Tumors arising due to excessive Shh ligand production are characterized by the extensive infiltration of non-tumorigenic stromal tissue responding to the Shh produced. This stromal compartment is critical for the maintenance of these tumors. The Shh-expressing cancer cells support the infiltration and growth of stromal cells, which as a consequence release signals that support the growth of the Shh-expressing cells. Such tumors thus consist of two mutually dependent groups of cells supporting each other's growth via distinct factors, one of which is Shh. This of course has significant consequences for potential therapeutic interventions. Whereas many potential targets are known in cancer cells that rely on cell autonomous activation of the Hh pathway, this is not the case in tumors that rely on paracrine signaling. It is likely that not only the Shh response in the stromal cells, but also the reciprocal signal needs to be inhibited for successful inhibition of tumor growth. The relatively disappointing results of the Smo inhibitors might be explained to an extent by the fact that these inhibitors are not expected to act on the mutated, Shh-producing cells, but instead only on the stromal cells. It is possible that the expected reduction of the amount of reciprocal signal produced is insufficient to initiate apoptosis required for tumor shrinkage.

The exact nature of the reciprocal signal returning to the cancer cell compartment is unknown, and might actually vary, or be multiple signals, complicating the treatment of these tumors with designed, target-specific molecules. In a recent study by Shaw *et al.* a species-specific microarray approach was used in a xenograft model for prostate cancer to show that the Shh produced by the tumor cells elicits the activation of pathways usually associated with embryonic development [64].

We expect that blocking the action of the reciprocal signal, in addition to blocking the Shh response, will be much more effective in treating Shh-induced tumors. The resistance to GDC-0449 observed in the above mentioned case of medulloblastoma emphasizes the requirement for combination therapy. This approach, however, complicates treatment, as the nature of the reciprocal signal is not yet known. On the other hand, targeting at least two

paracrine signals opens several options to prevent the maturation and exchange of these factors. Here we focus on some of the events required for productive reciprocal signaling and discuss some attractive targets for drug development.

5.2 Shh production and release into the extracellular space

Targeting the production, processing, distribution or stability of ligands in paracrine signaling tumors should provide specific intervention. The combination of lipophilic modifications on Shh is unique, and the resulting hydrophobic properties have a significant consequence on the distribution and the signaling efficacy of Shh. Although there are no therapeutic strategies that purposely address this possibility, either preventing the events resulting in the sterolation or palmitoylation of Shh could prove valuable in inhibiting its action. Both the sterolation and acylation are highly specific processes.

A possible way to confer specificity might be to specifically block protein sterolation. The sterolation of Shh is directly coupled to an autocatalytic cleavage event [81]. This cleavage is required for Shh activity and might, therefore, prove to be an excellent target for drug development. Similarly, it appears that a dedicated acyltransferase (Hhat) is required for the modification of Shh [82]. Nevertheless, there are several acyltransferases present in cells [83], making it uncertain if the desired specificity can easily be obtained through pharmacological means.

Another well-characterized molecule absolutely required for Shh secretion is Disp1, a member of the RND family of proton-driven pumps (Figure 2). The relative ease with which these membrane proteins can be targeted by small molecules makes Disp1 a potentially attractive target for therapeutic intervention (reviewed in [84]). The unusual post-translational modifications, in combination with the presence of molecules specifically dedicated to the secretion of Shh renders the maturation of Shh a promising, under-explored avenue to prevent paracrine Shh signaling.

The form of Shh that travels from cell to cell remains unresolved, and the topic of much debate. Several molecules are know to bind to Shh during this leg of its transport. There are both genetic and biochemical indications that Shh binds relatively tightly to the extracellular matrix, like most signaling molecules. This general role of the extracellular matrix makes it less attractive as a specific target for Shh signaling. More promising are two other molecules that bind Shh in the extracellular space, Hhip, as well as the Shh co-receptors Cdo and Boc.

5.3 Receptor functioning

Soon after the characterization of the Shh protein, blocking antibodies were developed, and these antibodies are possibly still the most efficient and specific inhibitors of the Shh response. This also demonstrates that during transport between cells, Shh is susceptible to inactivation by binding proteins that probably act by interfering with Shh binding to Ptch1. Under normal signaling conditions several molecules can be present in the extracellular space that bind to Shh and interfere with productive Ptch1 binding. These proteins are often part of regular negative feedback mechanisms, and point the way to the development of Shh-inactivating agents. Recently, a pharmacological method of intervening in excessive ligand production was described. It was demonstrated that robotnikinin, a small molecule

specifically binding to Shh blocks its action on Ptch1 [85]. In addition to antibody or robotnikinin inhibition of Shh binding to Ptch1, one could envision other strategies to target this interaction. A suggestion for one such strategy comes from the naturally occurring Hh-inhibitory protein1 (Hhip1) that complexes with Shh thereby inhibiting the Shh response [13]. The crystal structure of Hhip and Shh has been resolved [86,87]. It appears that Shh binds via the same domain to Ptch1 and Hhip. This information should be very useful to design the minimally required protein motif for this Shh-inhibitory effect. In general, small molecules are easier to produce and deliver than proteins, so it is more likely that robotnikinin-like molecules will successfully find their way to clinical use rather than the Shh-blocking antibodies or proteins such as Hhip1 derivatives.

Ptch1 itself is at first glance not the ideal target to inhibit the Shh response, since the loss of Ptch1 causes a dramatic upregulation of the Shh response pathway [88]. However, an interesting Ptch1 mutant Ptch1ΔL2 (extracellular loop 2 deleted) does not bind Shh, but is still inhibits the activity of Smo [24]. It might therefore be an effective strategy to develop drugs that interfere with the Shh binding to extracellular loop 2.

An interesting idiosyncrasy in Shh signaling is the prominent role played by two related members of the RND family, Disp1 and Ptch1. Whereas Disp1 is required for the secretion of Shh, Ptch1 is required in the uptake of Shh. Whereas the loss of Disp1 prevents the paracrine Shh response, loss of Ptch1 cell-autonomously activates the Shh response. Based on their RND family memberships, it is predicted that both Disp1 and Ptch1 require a proton gradient for at least part of their functions. Importantly, preventing acidification of endosomal compartments with drugs like concanamycin A and chloroquine prevents the normal trafficking of Ptch1 into these compartments, and as a consequence, the Shh response [15]. This indicates that a proton gradient is required both in the Shh-producing cells and in the Shh-responding cells. Although it is currently unclear how the putative proton-driven pump activity of Disp1 is implicated in the secretion of Shh, Disp1 proton channel mutants are unable to secrete active Shh, and pharmacologically perturbing the proton gradient probably inhibits secretion of Shh by Disp1.

These two observations actually point to a possible Achilles’ heel in Shh signaling. The reliance on acidified vesicles for both the secretion and internalization of Shh might point to an underexplored target. V-ATPases, which acidify endocytic vesicles, are required at multiple steps in the Shh secretion as well as the response [15,89]. Since several specific inhibitors of V-ATPases are in clinical use, it would be interesting to test their ability to limit the growth of Shh-induced tumors.

6. Conclusion

The Hh signaling pathway, as well as its prominent role in tumor formation, is unusually complex, and from a therapeutic point of view, this might be blessing in disguise. The rather disappointing efficacy of Smo antagonists in inhibiting Shh-induced tumors might not be such a great surprise, given the mutual dependency of Shh-producing tumor cells and stromal cells that involve one or more alternative signaling pathways. In such Shh-induced tumors, which rely on non-cell-autonomous Shh signaling, there are several proteins that

have dedicated and specific roles in maturation, secretion and internalization of Shh, and most of these proteins appear to be druggable targets. However, it is possible that both Shh signaling as well as reciprocal signaling by the unknown ligands need both to be inhibited to get the desired therapeutic effects. Such a requirement for a synergistic treatment strategy is similar to the way classical cytostatic compounds are now used in conjunction with other drugs for the treatment of tumors.

7. Expert opinion

7.1 Current state of the topic under discussion

Most of the research effort so far has focused on Smo inhibitors. The logic behind this is obvious, Smo is critically required for the Shh response, and very good natural and man-made Smo inhibitors were identified relatively long ago. The preponderance of Shh-induced polyclonal tumors that involve paracrine Shh signaling as well as unknown reciprocal signals, not only provides some explanation why the Smo inhibitors appear relatively ineffective, but also suggests the need for different research strategies, that are not solely focused on Smo inhibition.

7.2 Where the field is going in the next 5 – 10 years

Blocking the Shh response can efficiently be achieved with small-molecule Smo inhibitors, so the problem that needs to be addressed is why these inhibitors fail to effectively treat tumors that rely on the activation of Smo for their growth. Answering this question should involve the identification of the other inappropriately activated signals that support tumor growth in conjunction with Shh. If these signals activate a well-understood intracellular response, mediated by well-characterized ligands then combinatorial use of Smo inhibitors with the appropriate inhibitors blocking the reciprocal signal might finally yield clinically promising results. If the nature of the reciprocal, stromally-derived signal remains obscure or highly variable, then progress will be critically dependent on the development of methods to quickly map tumor transcriptomes and genomes.

7.3 How this will be achieved

The search to identify the cocktail of growth factors that in addition to Shh support the growth and metastasis of polyclonal tumors is ongoing, and likely to yield clear results. Since it is expected that these factors are true tumor-inducing proteins, it is quite likely that several small-molecule inhibiters affecting their response pathway already exist. In the best scenario, the reciprocal signals are constant, and the appropriate combination of small molecules will be effective. However, transcriptional profiling in paracrine signaling tumors has implicated Wnt pathway components, IGF-related signaling molecules, Notch, FGFs, Timp3 and CXCL14 as possible candidates to mediate the reciprocal signaling, complicating this approach [64,68]. For many of these signals, no specific inhibitors exist.

It is expected that a molecular analysis of each individual tumor needs to be performed to determine the optimal combination of small-molecule inhibitors to inhibit the responses elicited by combinations of these signaling molecules. A complicating factor is that similar combination of signaling molecules are likely to play important roles in the maintenance

of stem cell niches, and the combinatorial use of small-molecule inhibitors might have significant side effects.

Declaration of interest

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Article highlights.

- Hedgehog proteins are important in embryonic development, but also for tissue maintenance in adults.
- The signaling mechanisms of the Hedgehog pathway are complicated and poorly understood.
- Excessive Hedgehog production and pathway activity is associated with tumorigenesis and progression.
- Roughly two types of Hedgehog-dependent tumors exist; one in which the tumor cells respond to the ligand they make themselves, and one in which the tumor cells rely on the signals provided to them by the surrounding cells in response to the ligand.
- Good inhibitors exist to target Hedgehog pathway activity on cells that rely on pathway activation themselves, but not all tumors can be efficiently targeted this way.
- Identification of the reciprocal signals from the surrounding cells to the tumor should provide us with targets to more effectively combat certain Hedgehog-dependent malignancies such as pancreatic cancer.

This box summarizes key points contained in the article.

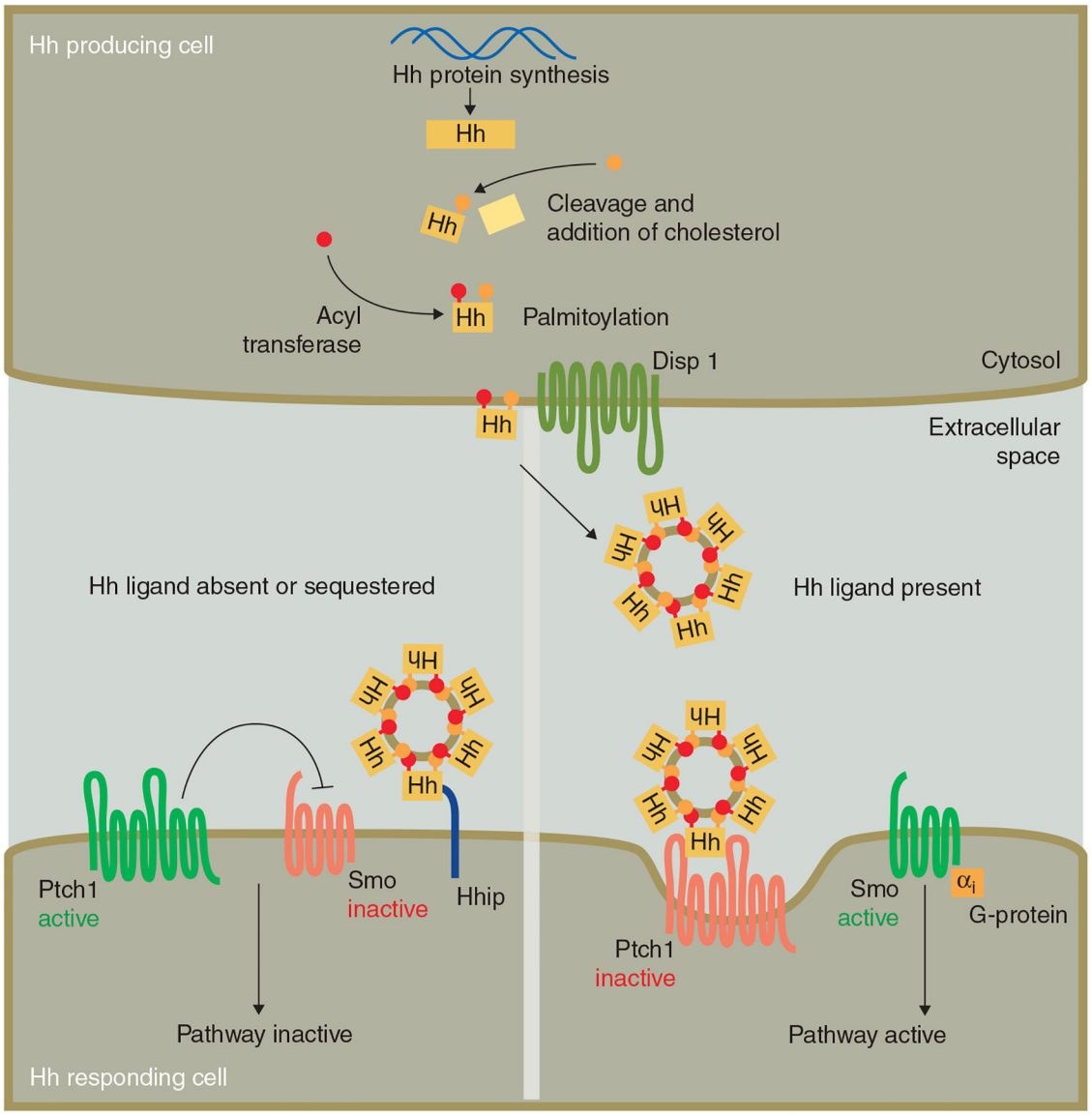

Figure 1. Hedgehog production, transport and signaling.

Following translation, the full length Hh protein autocatalytically cleaves and a cholesterol moiety is added in the process. A palmitoyl group is added by a dedicated acyl transferase. Secretion into the extracellular space in the form of multimers is mediated by the action of Disp1. In the absence of Hh ligand, or when Hh is sequestered by an inhibitory protein like Hhip, Ptch1 represses Smo and the downstream pathway is inactive. In the presence of Hh that is free to bind to Ptch1, the repression of Ptch1 on Smo is released and Smo activates downstream pathway components through G-proteins.

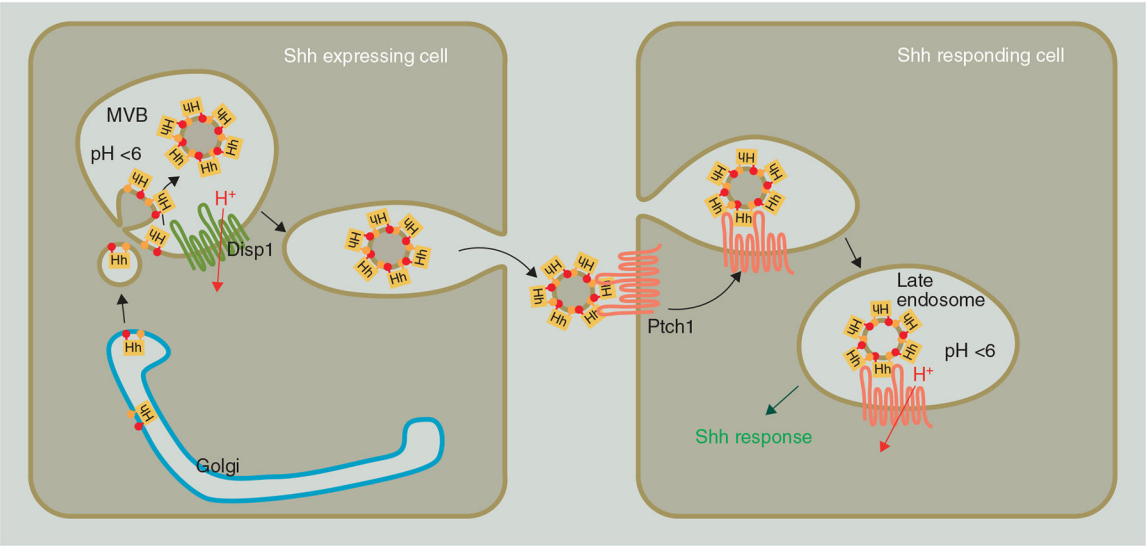

Figure 2. Hedgehog secretion and endocytosis.

Fully processed Shh is trafficked from the Golgi into acidified vesicles where they are complexed into multimers by the proton driven pump activity of Disp1. The complexes are subsequently released in the extracellular space, and subsequently bound and internalized by the action of Ptch1 in adjacent cells. Trafficking of the complex to late endosomes exposes Ptch1 to a lower pH, which is required for the activation of the downstream pathway components.