

Signalling Pathways Driving Cancer Stem Cells: Hedgehog Pathway

Vanessa Medina Villaamil¹, Guadalupe Aparicio Gallego¹,
Silvia Díaz Prado^{1,3} and Luis Miguel Antón Aparicio^{2,3}

¹INIBIC, A Coruña University Hospital, A Coruña

²Medical Oncology Service, A Coruña University Hospital, A Coruña

³Medicine Department, University of A Coruña, A Coruña
Spain

1. Introduction

The hedgehog (Hh) pathway is one of the fundamental signal transduction pathways in animal development and is also involved in stem-cell maintenance and carcinogenesis. The *hedgehog* (hh) gene was first discovered in *Drosophila*, and members of the family have since been found in most metazoan. Hh proteins are composed of two domains, an amino-terminal domain HhN, which has the biological signal activity, and a carboxy-terminal autocatalytic domain HhC, which cleaves Hh into two parts in an intramolecular reaction and adds a cholesterol moiety to HhN. HhC has a sequence similarity to the self-splicing inteins, and the shared region is termed Hint. HhN is modified by cholesterol at its carboxyl terminus and by palmitate at its amino terminus in both flies and mammals. The modified HhN is released from the cell and travels through the extracellular space. On binding its receptor Patched, it relieves the inhibition that Patched exerts on Smoothed, a G-protein-coupled receptor. The resulting signalling cascade converges on the transcription factor Cubitus interruptus (Ci), or its mammalian counterparts, the Gli proteins, which activate or repress target genes. The Hh family of morphogens plays important instructional roles in the development of numerous metazoan structures (Ingham & McMahon, 2001). The Hh ligands, Sonic, Indian and Desert Hh in vertebrates and Hh in *Drosophila*, signal through binding to the membrane receptor Patched (Ptc) (Chen & Struhl, 1996), to reverse the Ptc-mediated inhibition of signalling by the trans-membrane protein Smoothed (Smo) (Alcedo et al., 1996). This allows Smo to activate the intracellular signalling components, resulting in stabilization of down-stream transcriptional activator(s) and activation of target genes (Hooper & Scott, 1989). Transcription activation is facilitated through the Gli family of transcription factors in vertebrates (Ingham & McMahon, 2001). Hh signalling can initiate cellular growth, division, lineage specification, axon guidance and function as a survival factor (Cohen, 2009). Given this range of biological functions, it is not surprising that mutations in components of the Hh pathway are associated with both developmental defects and tumor progression (Cohen, 2009). Disruption of PTC, which functions as a negative regulator of the pathway, is implicated in cancer development in both inherited and sporadic cancers. Mutations in PTC and/or SMO trigger inappropriate activation of the Hh pathway, and have been identified in tumor types including basal cell carcinoma,

rhabdomyosarcoma and medulloblastoma (Taipale & Beachy, 2001). Other studies also implicate activated hedgehog signalling as a mediating factor in small-cell lung cancer, pancreatic cancer and various digestive tract tumors (Kimberly et al., 2010; Brabletz et al., 2009). For the increasing types of cancer that are associated with Hh signalling, understanding signal transduction will be crucial for identifying potential drug targets and devising new therapies. The first purpose of this chapter is review the Hedgehog signalling pathway, analyze its components and describe mutations and gene overexpression that involve Hh signalling network. The last section addresses the study of Hh pathway as a pathological player in the growth of a group of human cancers.

2. Description of the signalling network

EVOLUTIONARY ORIGINS

Hh signal transduction has startling parallels with Wnt signalling, despite the different structures of the ligands and the largely distinct components that are dedicated to the separate pathways (Nusse, 2003). As both pathways are found throughout the animal kingdom, a common ancestral pathway must have been present in the earliest Metazoans. Ptc and Hh have distinct evolutionary origins. The Hh protein is comprised of a N-terminal signalling domain and a C-terminal catalytic domain. The N-terminal domain is structurally related to zinc hydrolases (Hall et al., 1995). The C-terminal catalytic domain of Hh is related to inteins, a family of self-splicing proteins (Hall et al., 1995). Hh protein probably arose when an intein was appended to the signalling domain; release of the signalling domain requires cleavage from the intein and is therefore subject to tight control. In animals, the gene for the NPC1 pump was probably duplicated and then diverged to affect the activity of a Smo ancestor. The acquisition of loops that bind Hh converted the pump into a signal-regulated pump. All of these threads woven together indicate that the Hh pathway emerged by integration of primordial pathways that are involved in protein splicing, vesicular trafficking and nuclear entry.

SIGNALLING IN VERTEBRATES

Hh signalling in vertebrates shares many features with that in *D. melanogaster* (McMahon et al., 2003), although clear distinctions have emerged. First, mammalian gene families take the place of single genes in *D. melanogaster*. There are three *hh* genes in mammals, sonic, Indian and desert hedgehog (*Shh*, *Ihh* and *Dhh*); two *ptc* genes (*Ptc1* and *Ptc2*); and three *ci* homologues (*Gli1*, *Gli2* and *Gli3*). The three *hh* genes are expressed in different tissues and at different stages of development, and might also have different biological activities. The expression and function of *Ptc1* is similar to that of *D. melanogaster ptc* whereas *Ptc2* expression is more restricted and few phenotypes are associated with its loss (Rahnama et al., 2004). The post-translational regulation of Ci (*D. melanogaster*) and the GLI proteins is similar. Each resides in a cytoplasmic pool. In the absence of Hh, each is retained in the cytoplasm by Cos2 (KIF7 in vertebrates) and Sufu to limit transcriptional activation (Merchant et al., 2004; Rahnama et al., 2004; Paces-Fessy et al., 2004). Ci, GLI3, and probably also GLI2, require PKA and a SCF E3 ubiquitin ligase for processing to a transcriptional repressor. However, each GLI protein also has unique roles: GLI3 functions mainly as a transcriptional repressor, GLI2 is mainly a transcriptional activator and GLI1 functions only as a transcriptional activator. The transcription of *Gli1* is induced by Hh signals, which creates a positive-regulatory loop that heightens Hh responses.

The most important differences between the *D.melanogaster* and vertebrate Hh pathways centre on Smo: its regulators and its effectors. The sequence of the cytoplasmic tail of Smo is highly divergent between vertebrates and *D. melanogaster*. The entire KIF7 protein from zebrafish has some sequence similarity to Cos2, and KIF7 can bind GLI1. Like Cos2, KIF7 is required for repression of SHH responses, although it might differ from Cos2 in the degree to which it is required for full activation of Hh responses. Another kinesin and two ciliary proteins (KIF3a and the intraflagellar transport proteins IFT88 and IFT172) also mediate Cos2-like functions in vertebrates, participating in both full repression and full activation of Hh responses (Huangfu et al., 2003). Although it is likely that some, or all, of these four proteins fulfil the biochemical role(s) of Cos2, this remains to be tested. Some vertebrate Hh pathway genes have no known orthologues in *D. melanogaster*; some have orthologues, the role of which in Hh signalling has not been explored; and some have known orthologues with other functions. Missing in metastasis (MIM), which is also known as BEG4, is an actin-binding protein that potentiates GLI dependent transcriptional activation (Callahan et al., 2004). Positive vertebrate regulators of the Hh signalling pathway that have no known orthologues in flies include megalin, which belongs to the low-density lipoprotein (LDL)-receptor-related family and binds SHH41, and iguana, a zinc-finger protein that promotes the nuclear localization of GLI1 (Wolff et al., 2004). Negative regulatory factors distinguish vertebrate Hh signalling as well: FKBP8 is a transcription factor that antagonizes SHH action in the nervous system (Bulgakov et al., 2004), whereas SIL is a cytosolic protein that seems to function downstream of PTC (Izraeli et al., 2001). Rab23 is a regulator of vesicular trafficking and a negative regulator of the Hh response (Eggenchwiler et al., 2001). Shifted (Shf) is a secreted protein and is the *D. melanogaster* orthologue of human Wnt inhibitory factor (WIF). Shf facilitates Hh signalling by binding Hh and heparansulphate proteoglycans, whereas WIF binds WNT proteins and facilitates Wnt signalling (Glise et al., 2005). At least some of the apparent differences between phyla are the result of the functional convergence of non-homologous genes and proteins. The mammalian membrane glycoprotein Hh-interacting protein (HIP) and *D.melanogaster* Pxb have no sequence similarity, but they might fulfil the same function. Each is a transcriptional target of Hh and each participates in a negative- feedback loop that attenuates Hh responses (HIP through direct binding to SHH) (Inaki et al., 2002). The larger question of whether the core of the signal transduction apparatus works in the same manner in the two phyla remains to be elucidated.

Hh acts to regulate the three Gli proteins in different ways. Gli1 appears to act as an activator to mediate and/or amplify the Hh response and is transcriptionally induced by Hh signalling in all context studied. The situation with Gli2 and Gli3 is more complex. Hh signalling represses both the transcription of Gli3 and the proteolytic formation of Gli3 repressors. However, the function of Gli2, and probably Gli3, can be positive or negative in relation to Hh signalling in different situations (Ingham & McMahon, 2001). Therefore, Hh pathway function relies both on Gli activating function and on inhibiting Gli repressor formation (Ruiz i Altaba, 2002).

HEDGEHOG AS A CONCENTRATION-DEPENDENT SIGNAL

One of the most intriguing questions regarding Hh signaling is how Smo signalled to the HSC to regulate Ci. Although Smo shares homology with G-Protein Coupled Receptors (GPCRs), current evidence argues against the involvement of a traditional G-protein. For example, the Smo mutants SmoC and FFS, which lack the domains one would expect to

Properties

Activator/repressor of Hh targets.
Hh signaling inhibits repressor formation.

Activator/repressor of Hh targets.
Hh signal may induce the production of a strong activator.

Activator of Hh targets.

Modulator of Gli function?
Repressor of Hh targets.

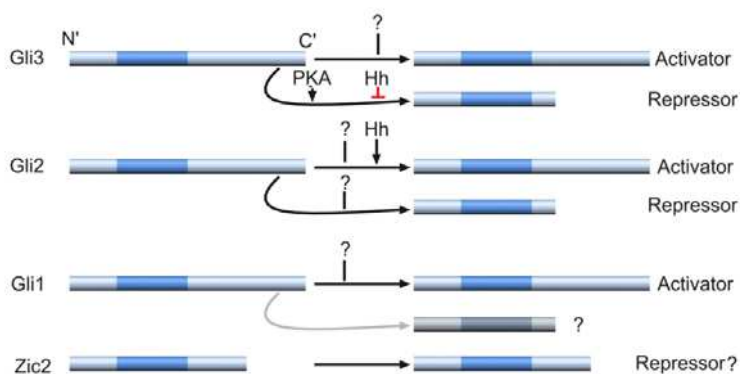


Fig. 1. Diagram schematizes the generalized regulation of the Gli proteins by proteolysis. Zic2 encodes a small protein with a GLI-type zinc-finger domain that may act as a repressor of transcription. Variations of these subjects might occur in different organs.

interact with a G-protein, are still capable of propagating Hh signalling (Hooper, 2003). Additionally, reducing the expression of all known *Drosophila* heterotrimeric GTP binding proteins, through use of RNA interference (RNAi), had little effect on Hh responses in cultured cells (Lum et al., 2003). This lack of compelling evidence for G-protein involvement in the Hh pathway led multiple groups to look for direct interactions between Smo and HSC components. Several recent publications, demonstrating an interaction between the carboxyl terminal tail of Smo and the cargo domain of Cos2, have begun to shed light onto the mechanistic events involved in Smo-mediated signaling to the HSC (Lum et al., 2003; Ruel et al., 2003; Jia et al., 2003; Ogden et al., 2003). In spite of there are some differences in the published studies, there seems to be a consensus on the following points: (1) Smo binds Cos2 directly; (2) the interaction is necessary for functional Hh signaling; (3) Cos2 appears to tether significant amounts of Fu to Smo, while Ci and Su(fu) binding are not as obvious. A requirement for direct Smo–Cos2 binding in signal transduction is most obvious when examining target gene expression following loss of interaction. The Smo carboxyl-terminal tail was demonstrated to contain the Cos2 interaction domain (Jia et al., 2003; Ogden et al., 2003). Over-expression of this domain appears to have a dominant negative effect, resulting in a dose dependent loss of reporter gene expression. Similarly, over-expression of Smo proteins lacking the Cos2 binding domain and/or Cos2 constructs lacking the Smo binding domain demonstrate compromised Hh responses (Lum et al., 2003; Ruel et al., 2003; Jia et al., 2003). These results clearly demonstrate a requirement for Cos2–Smo interaction for proper Hh signal transduction. Additionally, the amount of membrane associated Hedgehog Signalling Complex (HSC) appears to decrease in response to Hh (Ruel et al., 2003). This observation is seemingly inconsistent with the model whereby the Smo/Cos2 association remains constant or even increases. To explain this apparent paradox was proposed (Ogden et al., 2004) that there may be two distinct HSCs, one involved in converting Ci into its repressor form, HSC-R, and one involved in converting Ci into its activated forms, HSC-A. In the absence of Hh, HSC-R is on and HSC-A is off, while in response to maximal Hh, HSC-A is turned on and HSC-R is turned off. In between this two switch system, numerous intermediates exist.

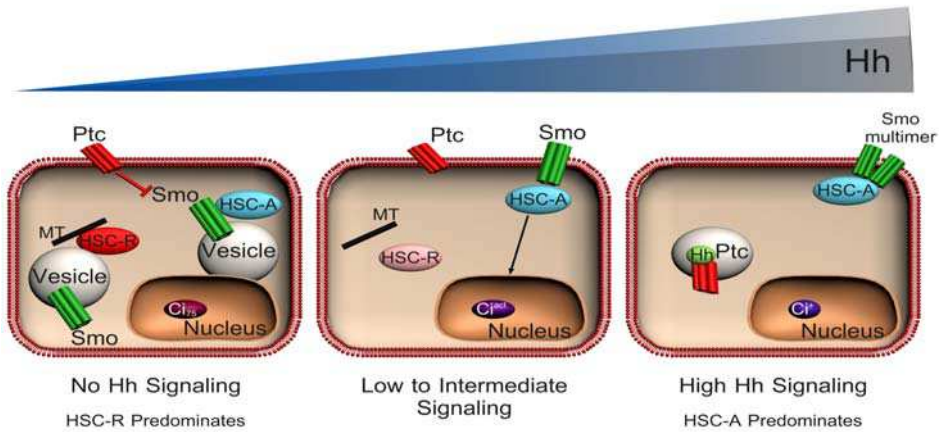


Fig. 2. Diagram shows how the Hh gradient regulates HSC activator and repressor functions. In the without Hg signalling HSC-R is producing Ci_{75} and HSC-A is inactive. In the central cell, HSC-A is producing Ci^{act} , which may be acting in presence of lower Ci_{75} . In the right cell, the greatest amount of Hh is received, HSC-A is maximally activated by Smo and HSC-R is completely silence.

It is known that graded sonic hedgehog activity patterns the ventral neural tube. Five distinct neuronal cell fates are specified in the ventral half of the neural tube in response to a gradient of SHH (Jessell, 2000). The cells of the floor plate respond to the highest concentration of SHH that is secreted by the notochord by becoming glial cells, which, in turn, begin to secrete SHH themselves. The remaining neural tube cells choose between various ventral neural fates that are specified by different thresholds of SHH signalling.

LOCALIZATION AND FUNCTION

Hh proteins are synthesized as precursor proteins (about 400-460 amino acids long) and comprise several different motifs and domains: a signal peptide for protein export, a secreted amino-terminal HhN (Hedge) domain that acts as a signalling molecule, and an autocatalytic carboxy-terminal HhC (Hog) domain that contains a Hint module. Multiple sequence alignments of the HhN and HhC domains defining the conserved residues and features have been presented in (Bürglin, 2008). HhC binds cholesterol in the sterol recognition region (SRR) (Beachy et al., 1997). The catalytic activity of the Hint module cleaves Hh into two parts and adds the cholesterol moiety to the carboxyl terminus of HhN. Figure 2 outlines the Hh signalling pathway.

In the figure 2 Hh is targeted to the endoplasmic reticulum by its signal peptide, is palmitoylated at its amino terminus by Rasp/Skinny Hedgehog (Ski), and autoprocessed. Lipidated HhN (M-HhN) is released by Dispatched (Disp) and forms multimers or associates with lipoproteins (LP) in the extracellular environment (Cohen, 2003). Several molecules can interact with M-HhN and propagate or modulate its trafficking: the Dally-like protein (Dlp) that is modified by the heparan sulfate (HS) polymerases Tout-velu (Ttv), Sister of tout-velu (Sotv), and Brother of tout-velu (Botv), all members of the EXT family; the Hedgehog-interacting protein (Hip); and the Growth-arrest specific1 (Gas1) protein. Hip and Gas1 are not present in *Drosophila*. Megalin (Meg) is most probably involved in the

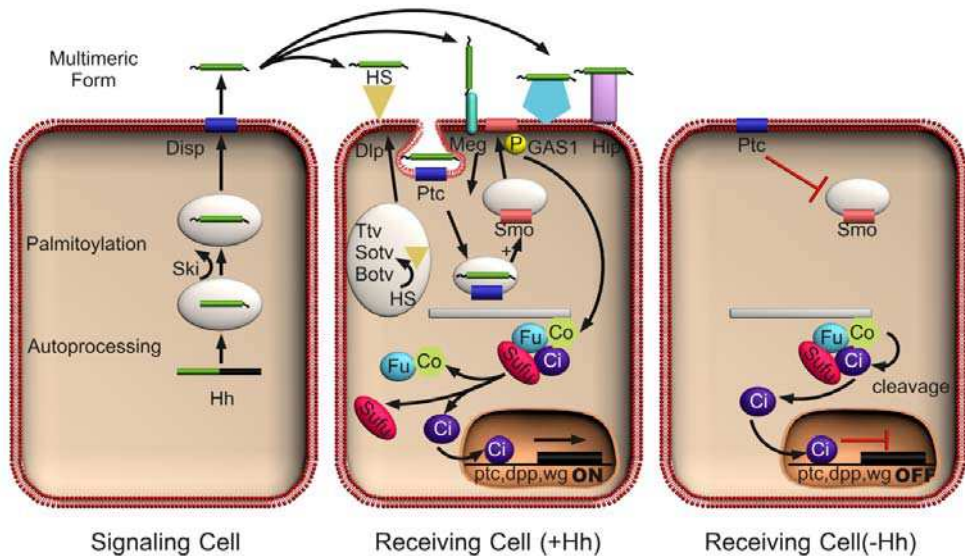


Fig. 3. A schematic Hh signalling pathway, obtained from combined *Drosophila* and mammalian data.

recycling of M-HhN. Ihog is thought to function as co-receptor for M-HhN. M-HhN acts as an antagonistic ligand that represses the function of the receptor Patched (Ptc), a 12-transmembrane protein related to Disp. Binding of M-HhN to Ptc results in internalization. Smoothened (Smo) is a seven-pass membrane receptor, which is the key for the transmission of the signal to the nucleus in the Hh pathway. Smo is inhibited by Ptc when not bound by M-HhN. If the inhibitory function of Ptc is released by M-HhN, Smo can translocate to the plasma membrane or - in mammals - to the primary cilium, and active Smo is phosphorylated. Ptc may secrete pro-vitamin D3 or related compounds (D3) to inhibit Smo. Conversely, oxysterols (Oxy) can indirectly activate Smo (Eaton, 2008; Rohatgi et al., 2007). The Hh pathway downstream of Smo displays some important differences between *Drosophila* and mammals. In *Drosophila*, when Smo is active, the signal passes through a complex comprising the kinesin-like molecule Costal 2 (Cos2), Fused (Fu), Suppressor of fused (Su (fu)) and Cubitus interruptus (Ci), leading to the release of Ci, which can then enter the nucleus to promote transcription. When Smo is inhibited, the Cos2/Fu/Su (fu)/Ci complex remains associated with microtubules, Ci is phosphorylated and is cleaved by Cos2. The Ci fragment now acts as a transcriptional repressor. In mammals, the targeting of Smo to primary cilia is essential for signal transduction. No obvious equivalents of Cos2 and Fu exist in mammals. Instead, Su (fu) has a more prominent role in inhibiting the pathway. Gli1, Gli2, and Gli3 are the mammalian homologs of Ci; Gli1 and Gli2 activate transcription when Smo is active, whereas Gli3 is processed and becomes a repressor when Smo is inhibited.

In animal development, the secreted M-HhN moiety functions as a morphogen. The Hh signalling pathway plays many important roles in development, including conferring segment polarity on the body segments and patterning the wing in *Drosophila*, and

patterning the neural tube in mammals (Dessaud et al., 2008; Varjosalo & Taipale, 2008). Hh is also required for stem-cell maintenance, and mutations in the pathway lead to cancer. Increased activity of the pathway causes basal cell carcinoma and medulloblastoma (Beachy et al., 2004; Jacob & Lum, 2007). For example, insufficient Ptc function leads to Gorlin syndrome in humans, one feature of which is an increased risk of basal cell skin cancer. In mammals, Shh, Dhh, and Ihh have partially redundant functions. Shh is the most widely expressed of the three paralogs, and regulates development from embryo to adult. Key roles are in patterning the neural tube: Shh is first expressed in the notochord, and later in the floor plate of the neural tube, where it produces a gradient of activity in the ventral neural tube. Shh is also expressed in the zone of polarizing activity of the limb buds and is important for limb and digit formation. Other roles of Shh include inner ear, eye, taste bud, and hair follicle development. Ihh is expressed in the primitive endoderm and is required for bone growth and pancreas development. Shh and Ihh both play roles in cardiovascular development. Dhh is expressed in the gonads, and Dhh-mutant males are sterile (Bijlsma et al., 2006; Dessaud et al., 2008; Varjosalo & Taipale, 2008).

SECRETION, TRAFFICKING AND SPREADING OF HEDGEHOG

Dispatched is necessary for Hh secretion (Burke et al., 1999). Dispatched contains 12 transmembrane domains and is related to the resistance-nodulation division (RND) family of bacterial proton-driven pumps (Ma et al., 2002). Bacterial proteins of the RND family use a proton gradient to transport multiple small lipophilic molecules across the membrane bilayer. The two other metazoan members of this family include the Hh receptor Patched, and the protein encoded by the Niemann–Pick type C1 (*NPC1*) disease gene, which promotes cholesterol efflux from late endosomes. Members of the RND family: Patched, Dispatched and NPC1, contain two related copies of a signature domain with six transmembrane-spanning regions. Mutations in Dispatched which disturb conserved residues that are important for the function of bacterial transporters, also prevent Hh release (Ma et al., 2002), consistent with the hypothesis that Dispatched can transport a small molecule across the bilayer. A fragment of the signature RND domain, called a sterol-sensing domain, is also shared with other proteins that are involved in sterol metabolism. The sterol-sensing domain of HMGCoA reductase (the rate-limiting enzyme in cholesterol biosynthesis) regulates its stability in response to cholesterol. The sterol-sensing domain of SCAP [sterol-regulatory-element-binding protein (SREBP) cleavage-activating protein] responds to cholesterol levels by altering membrane trafficking and the cleavage of the membrane-associated transcription factor SREBP, which regulates the transcription of genes that are involved in sterol metabolism (Chang et al., 2006). Whether Dispatched itself responds to sterol levels is not known, and its precise function in Hh release has not yet been determined. The mechanism of Dispatched to respond to the levels of sterol and how Hh is released has not yet been determined.

Biochemical fractionation of imaginal discs from *D. melanogaster* larvae shows that, although most lipid-modified Hh will form pellets with cell membranes, Hh molecules that remain in the supernatant are almost entirely associated with lipoprotein particles (Panáková et al., 2005). It will be interesting to determine whether the cholesterol-dependent Hh multimers that are secreted by tissue-culture cells might reflect the association of Hh with serum-derived lipoproteins, or whether multimer formation is a completely distinct mechanism for Hh release. Lipoproteins comprise a phospholipid monolayer that surrounds a core of esterified cholesterol and triglyceride. Lipid modifications, such as the addition of

cholesterol, palmitate and glycosyl phosphatidylinositol (GPI), that target proteins to the exoplasmic face of the plasma membrane should fit equally well into the outer phospholipid monolayer of lipoproteins. Indeed, *D. melanogaster* lipophorin particles also bind to the morphogen molecule Wingless, which is palmitoylated twice and to several GPI-linked proteins (Panáková et al., 2005; Eugster et al., 2007).

Two mechanisms are hypothesized for Hh release in wing discs: a long-range mechanism that depends on lipophorin and a short-range mechanism that is lipophorin independent. Whether any of the mammalian Hh proteins bind to low-density lipoprotein (LDL) or high-density lipoprotein (HDL)-type particles is unknown, although this would be interesting to investigate. Cholesterol modification clearly has an important influence on Hh trafficking. The 19 kDa N-terminal Hh domain can be artificially generated in the absence of cholesterol modification by the simple expedient of stop codon insertion or C-terminal domain deletions (Porter et al., 1996). This altered protein, termed HhN, is secreted in a Dispatched-independent manner (Burke et al., 1999), does not form multimeric complexes (Gallet et al., 2006; Chen et al., 2004; Feng et al., 2004), and is distributed differently in both producing and receiving cells (Gallet et al., 2003; Callejo et al., 2006). Although HhN has been reported to spread further, it does not seem to signal as efficiently as cholesterol modified Hh (Porter et al., 1996; Gallet et al., 2006; Li et al., 2006). The anchors probably interact, either with each other (when Hh is organized as micellar multimers) or with the outer phospholipid monolayer of a lipoprotein. Interaction with heparan sulphate proteoglycans (HSPGs) provides a likely explanation for the continuing association of Hh micelles or Hh-

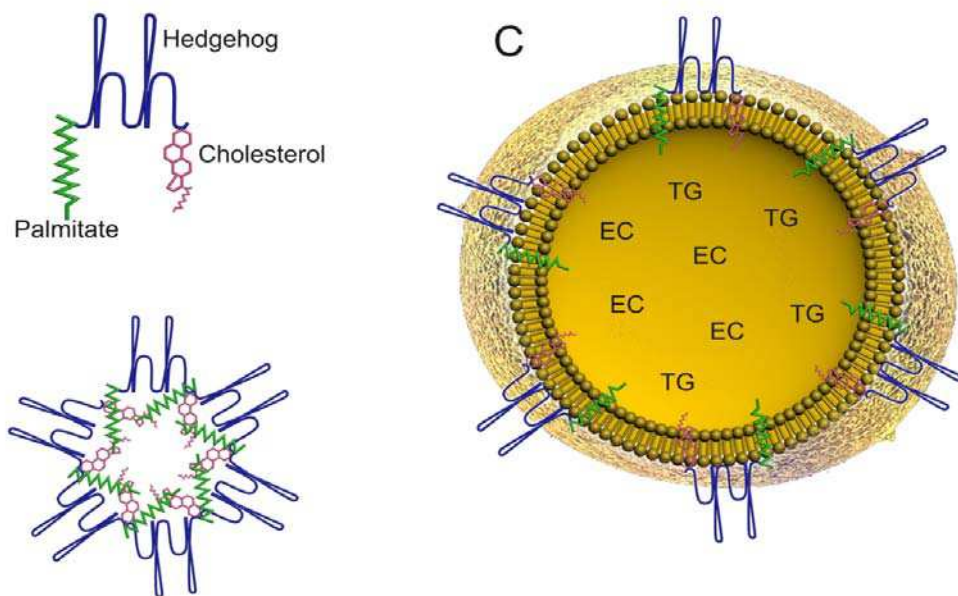


Fig. 4. Possible carriers for Hedgehog release. Hh (blue) is covalently linked to cholesterol (red) and palmitate (green). The interaction of the lipid moieties with each other promotes the formation of Hh multimers. Finally, a lipoprotein consists of an outer phospholipids monolayer (brown) that surrounds a core of sterified cholesterol (EC) and triglyceride (TG).

lipoprotein complexes with tissue. Lipid-modified Hh does not enter tissue that cannot synthesize heparan sulphate (Han et al., 2004). Recent work suggests that lipoproteins interact physically with HSPGs in *D. melanogaster* wing discs (Eugster et al., 2007). Hh that has interacted with lipoproteins through lipid anchors might therefore be restricted to tissue through these lipoprotein–heparan sulphate interactions. This would be consistent with the observation that only lipid modified Hh is dependent on HSPGs in orders to associate with tissue. Direct binding of Hh to HSPGs might also provide tissue affinity. In this case, Hh multimerization might also promote HSPG binding by increasing the local concentration of heparan sulphate-binding sites on Hh.

TRANSCRIPTIONAL REPRESSION OR ACTIVATION IN HEDGEHOG RESPONSE

Hedgehog responsive changes in gene expression are mediated by the zinc finger transcription factor Ci, which can assume repressing and activating forms. The repressing form, CiR, represents an N-terminal proteolytic fragment that retains the zinc finger-mediated DNA binding specificity of Ci but lacks nuclear export signals, a cytoplasmic anchoring sequence, and a transcriptional activation domain. For some genes, such the universal Hh pathway target *ptc*, expression requires loss of CiR and the positive action of Ci. So the expression of Hh pathway targets depends on regulation of Ci processing and localization.

Formation of CiR requires phosphorylation of specific serine-threonine residues by cyclic adenosine monophosphate (cAMP) dependent protein kinase. The phosphorylated form of Ci appears to be a substrate for a proteolytic processing reaction that requires function of the proteasome and of Slimb (Slimb), an F-box-containing E3 ubiquitin ligase component. Ci phosphorylation and processing may be mediated by Cos2 scaffolding of kinases with Ci, although direct associations of these kinases with Cos2 or Ci have not yet been reported. (Lum & Beachy, 2004).

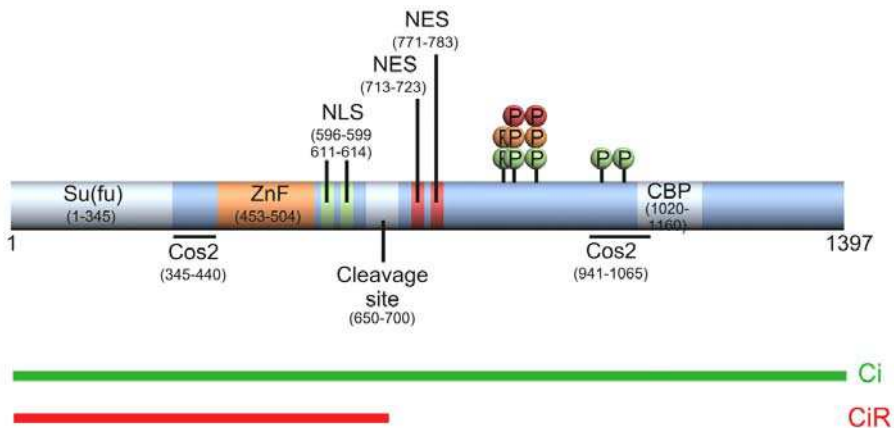


Fig. 5. Representation of Ci functional domains and motifs. They are labelled by amino acid numbers (in parenthesis). Phosphorylation sites are indicated, they are required for initiation of Ci proteolytic processing. Pathway activating functions of Ci are associated with full-length Ci (green line) whereas repressor functions are associated with the proteolytically processed form CiR (red line).

The kinase(s) that phosphorylate Fu and Cos2 remain to be identified and the significance of their phosphorylation remains to be determined. Hh also facilitates the association of a small population of Sufu molecules with the Cos2-Fu-Smo complex. A genetic analysis indicates that Fu phosphorylates Sufu, but biochemical evidence for this lacking. One possibility is that high concentrations of Hh promote Smo phosphorylation and dimerization. This activates the Fu kinase that is associated with Complex I, which then phosphorylates Sufu to release CiA. Curiously, the full spectrum of Hh responses is seen in *D.melanogaster* in the absence of Sufu, if Cos2 regulation is normal. So there must be an alternative pathway to CiA that involves Cos2 rather than Sufu that in vertebrates remain to be determined (Lum et al., 2003; Stegman et al., 2000).

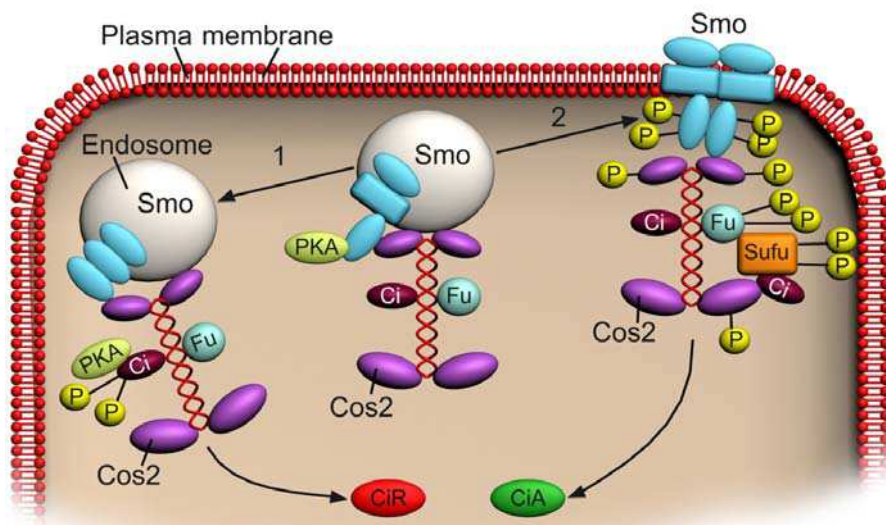


Fig. 6. Illustration of a model for signalling by Smoothed (Smo) and Costal-2 (Cos-2). Smo can assume three distinct states. Under Patched influence, Smo adopts an inactive conformation. This form of Smo distributes between endosomes, where it can associate with Cos-2, and lysosomes, where it is degraded. The remaining Cos2 recruits protein kinase A (PKA), casein kinase I and glycogen synthase kinase-3 to Ci, which enables phosphorylation of Cubitus interruptus (Ci) and its subsequent processing to the transcriptional repressor form (CiR). When the influence of Ptc decreases, the transmembrane helices of Smo are rearranged, which exposes a new surface in its cytoplasmic tail. This surface causes PKA, CKI and GSK3 to dissociate from Cos2, so that Ci is no longer phosphorylated or processed to CiR. Smo is phosphorylated instead, and it assumes its third state. Phosphorylated Smo traffics to the surface, rather than to lysosomes for degradation. Smo concentrations rise and Smo assembles a modified signalling complex that promotes the phosphorylation of Fused (Fu) and Cos2. Phosphorylated Cos2 dissociates from membranes and recruits Fu to Sufu (Suppressor of Fused), which produces the activated form of Ci (CiA), probably through phosphorylation of Sufu phosphorylated (Ogden et al., 2004).

3. Hedgehog signalling in human disease

In addition to functioning in the embryo, Hh proteins and Hh signal-transduction components are expressed in postnatal and adult tissues, suggesting that they function in the mature organism. Defects in Hh signalling could, therefore, affect both the human embryo and the adult (Ruiz i Altaba, 2002).

THE CANCER STEM CELL THEORY

A great deal of interest has focused on mutation or aberrant regulation in stem cells as a key factor in carcinogenesis. A link between stem cells and cancer is not a new concept (Sell, 2004). Subsequently it was widely accepted that the initiation and progression of malignancy is a multi-step process, driven by numerous genetic changes that result in the transformation of normal cells into malignant cells. Environmental factors apply evolutionary pressure on the tumor, which leads to selection of clones with a greater capacity to survive, grow and metastasise. In this theory, any normal cell undergoing sufficient genetic alterations to result in its unregulated proliferation may become a tumor-initiating cell. The observed heterogeneity of many tumors is due to the development and expansion of numerous subclones. This clonal evolution theory is believed to explain the ultimate insensitivity of many tumors to chemotherapy, as clones with the ability to export the drug, or which lack key components of metabolic pathways targeted by the drug, are positively selected for their ability to evade death. The identification of stem cells in a range of tissues and organs and a greater understanding of their biology has again focused attention on the "stem cell theory of cancer" which proposes that malignancy arises from the transformation of a normal tissue stem cell. The cancer stem cell theory hypothesises that, analogous to stem cells in normal tissues, there are a small proportion of cells within tumors that have stem cell properties giving rise to progeny which may show heterogeneous patterns of differentiation and form the bulk of the tumor mass. The existence of cancer stem cells is thought to explain the failure of chemotherapy and other treatments to eradicate metastatic disease. With the continuing identification of stem-like cells within increasing numbers of malignancies, it is obviously that a new approach to treatment is required, one which directly targets the cancer stem cells in association with more traditional approaches that affect tumor bulk. These highly tumorigenic cells, also known as cancer stem cells, have the ability to self-renew and differentiate into multiple lineages. Cancer stem cells have been isolated from human tumors involving the breast, lung, colon, pancreas, prostate, skin, head/neck and brain. Experimental and clinical research indicates that cancer stem cells, as well as cells from solid tumors, are resistant to chemotherapy and radiation therapy. Therapeutic approaches are in development to block embryonic pathways that play a role in cancer stem cells, including Notch, sonic hedgehog and Wnt.

HEDGEHOG IN CANCER

Defects in Hh signalling pathway have long been known to be associated with human congenital disease with the loss of one copy of Shh resulting in holoprosencephaly (Roessler et al., 1996). More recently has been documented that aberrant Hedgehog signalling is associated with the development and progression of a wide range of human malignancies. Mutations such as Ptch1 and Smo are associated with medulloblastoma, basal cell carcinoma and rhabdomyosarcoma. Aberrant activation of Hh signalling is also suggested to play a role in other cancers that have no known mutational basis, such as glioma, breast, esophageal, gastric, pancreatic, prostate, chondrosarcoma and small-cell lung carcinoma. In

these tumors the Hh pathway abnormalities are called ligand-dependent and were described first in lung (Watkins et al., 2003) and then in gastrointestinal tract and pancreatic carcinoma (Berman et al., 2003), which show no mutation in Hh pathway genes but are characterised by upregulation of the expression of Hh ligand which is also thought to include autocrine and paracrine mechanism of activation. There are two proposed models to explain how Hh ligands promote tumor growth: one postulates that Hh ligands produced by cancer cells and/or their stromal environment maintain secreted stem cell renewal within the tumor (Jiang & Hui, 2008); the second proposes that Hh ligands secreted by the tumor may act on nearby stromal cells, resulting in pathway activation in the stromal microenvironment that promotes tumor growth (Yauch et al., 2008).

HEDGEHOG SIGNALLING IN CANCER STEM CELLS

Data from many human tumors including glioblastoma, breast cancer, pancreatic adenocarcinoma, multiple myeloma, and chronic myeloid leukemia (CML) have suggested that Hh signaling moderates cancer stem cells (CSC). Self-renewal of CSC is required for maintenance of the malignant clone, and reports studying mouse models of CML have provided evidence that Hh signaling regulates this property (Dierks et al., 2008; Zhao et al., 2009). Active Hh signalling pathway has also been identified in glioblastoma CSCs, and pathway inhibition with cyclopamine or siRNA directed against pathway components results in the loss of tumorigenic potential (Clement et al., 2007). In breast cancer, pathway activation in CSCs using Hh ligand and GLI1 or GLI2 expression or inhibition with cyclopamine or siRNA directed against GLI1 or GLI2 alters the expression of BMI-1 (Liu et al., 2006). In multiple myeloma, CSCs that phenotypically resemble normal memory B cells have been found to display relatively higher levels of Hh signalling than the mature plasma cells constituting the tumor bulk (Peacock et al., 2007). So, HH signalling may dictate CSC fate decisions that include self-renewal and differentiation possibly by generation of a malignant niche (LaBarge, 2010). Data from multiple myeloma demonstrate that Hh signaling can act through multiple signaling modes within the same cancer and can mediate interactions between CSCs, differentiated tumor cells and the microenvironment. Additionally to tumor formation, CSCs have been implicated in disease progression and the development of metastasis in solid tumors (Rasheed et al., 2010), and Hh signaling may play a critical role in this process similar to the Notch and Wnt pathways in cancer. In colon carcinomas, Hh signaling has been found to be preferentially activated within CSCs as evidenced by relatively higher expression of GLI1, GLI2, PTCH1, and HIP within this cellular compartment (Varnat et al., 2009). Moreover, the relative expression of these pathway components as well as the target gene SNAIL1, which is associated with the epithelial-to mesenchymal transition (EMT) and implicated in metastasis, increases in CSCs with disease stage.

INHIBITORS OF HEDGEHOG SIGNALLING

It has been accepted that altered Hh signalling contributes to the development of up to one third of all human malignancies (Beachy et al., 2004) and so there is a great interest in therapeutic inhibition of the pathway, with a number of inhibitors in clinical trials. The first inhibitor of the Hedgehog pathway identified was cyclopamine (Cooper et al., 1998), Cyclopamine is a small molecule inhibitor of Smoothened, and a number of compounds have been identified or synthesized which have similar mechanisms of action. To inhibition of the pathway several approaches have been developed:

1. Prevention of Hh ligand binding to Ptch receptor.
2. Inhibition of Smoothed via cyclopamine and related compounds. Much of the preclinical and clinical trial work on Hh inhibitors undertaken to date focuses on inhibitors of Smo.
3. Inhibitors of Gli transcription.

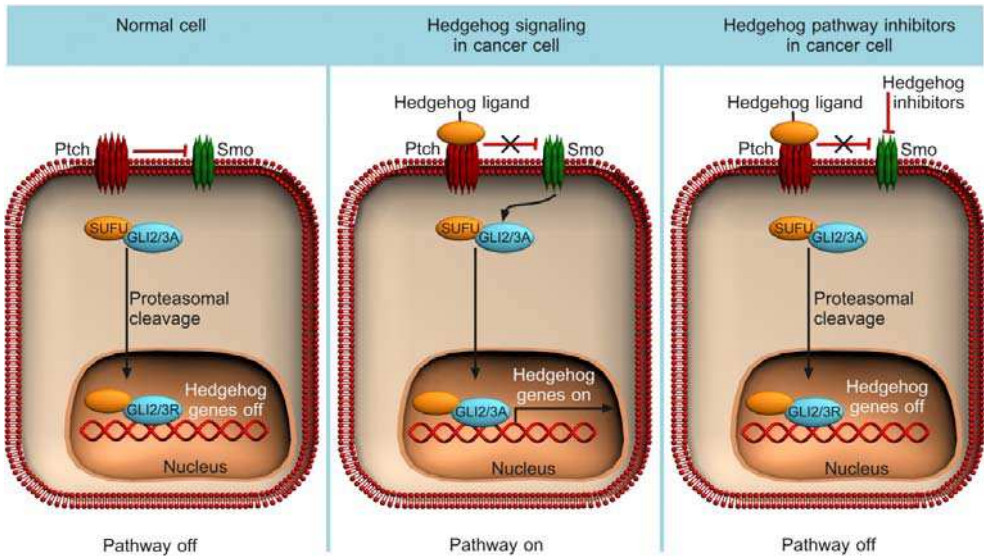


Fig. 7. Illustration of a schematic representation of the pathway in a normal cell, a cancer cell and the action of inhibitors over the Hedgehog pathway.

DEVELOPING IMPROVED HEDGEHOG THERAPIES

In adulthood, the Hh signalling pathway is silenced in the great majority of cells. However, there have been an increasing number of reports over the past decade documenting the implication of the Hh pathway in human diseases, such as cancer. For these reasons, Hh pathway antagonists have been sought after as potential new treatments for cancer. The theory that many tumors arise from a small number of cancer stem cells (CSCs) has recently gained strength with some landmark studies that point to the existence of a discrete population of slowly dividing tumor cells capable of self-renewal and differentiation along multiple lineages. The control of these processes in cancer stem cells is thought to be regulated by a small number of signaling pathways as Hh, Wnt, and Notch, and growing evidence suggests that some of these pathways are deregulated, allowing for their abnormal expansion and the formation of cancer.

These Hh, Wnt, and Notch pathways might be interconnected and ultimately lead to the regulation of stem-cell self-renewal via Bmi-1 transcription factor (Liu et al., 2006). These data suggest additional possible uses for inhibitors of pathway such as Hh. The outcome of Hh signaling varies according to the receiving cell type, but it can include expression of a variety of cell-specific transcription factors mediating different developmental fate response: upregulation of D-type cyclins, resulting in cell proliferation; upregulation of anti-apoptotic proteins such as Bcl-2, mediating cell survival; production of vascular endothelial growth

factor (VEGF) and angiopoietins regulating angiogenesis; and transcription of SNAIL, initiating the epithelial-mesenchyme transition (EMT) in metastasis. It is, therefore, not surprising that deregulated Hh signalling can lead to a variety of cancers.

Three basic models have been proposed for Hh pathway activity in cancer (Rubin & de Sauvage, 2006). The type I cancers, which harbour pathway-activating mutations, such as basal carcinomas (BCCs), medulloblastomas, and rhabdomyosarcomas. Type II cancers, are ligand dependent and autocrine/juxtacrine, meaning that Hh is both produced and responded to by the same/neighbouring tumor cells, including breast, upper gastrointestinal tract, colorectal, prostate and lung tumors. Type III cancer, are also ligand dependent but paracrine, in that Hh produced by the tumor epithelium is received by cells in the stroma, which feed other signals back to the tumor to promote its growth or survival.

The clinical reality is that the majority of cancer patients present with locally or distant metastatic, surgically inoperable disease. Therefore, the development of more potent therapies for advanced/metastatic human cancers mandates great urgency. Multiple lines of evidence support the idea that Hedgehog signalling has a role in the maintenance and progression of many human cancers. First, studies involving global sequencing analysis have identified the Hh pathway as one of the core signalling pathways of human cancers; second, the inhibition of Hh enhanced survival in genetically engineered mouse models of cancers; third, blockade of the Hh pathway eliminates cancer stem cells.

Intervention of the Hh pathway has provided a therapeutic opportunity for treatment of malignancies. Effective inhibition of the Hh pathway can be achieved at the level of ligands by using anti-Hh antibodies, or through downstream effector molecules, such as Smo, with small-molecule antagonists (Evangelista et al., 2006).

4. References

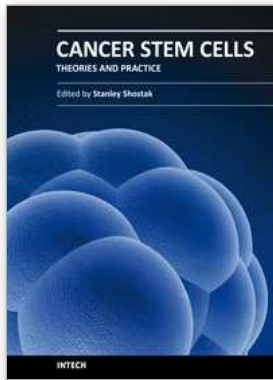
- Alcedo J.; Ayzenzon M.; Von Ohlen T. et al. (1996) The *Drosophila* smoothened gene encodes a seven-pass membrane protein, a putative receptor for the hedgehog signal. *Cell* 86(2):221-32
- Beachy P.A.; Karhadkar S.S. & Berman D.M. (2004) Tissue repair and stem cell renewal in carcinogenesis. *Nature* 432(7015):324-331
- Beachy P.A.; Cooper M.K.; Young K.E. et al. (1997) Multiple roles of cholesterol in hedgehog protein biogenesis and signaling. *Cold Spring Harb Symp Quant Biol* 62:191-204
- Berman D. M.; Karhadkar S. S.; Maitra A. et al. (2003) Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* 425: 846-851
- Bijlsma M.F.; Peppelenbosch M.P. & Spek C.A. (2006) Hedgehog morphogen in cardiovascular disease. *Circulation* 114:1985-1991
- Bulgakov O. V.; Eggenschwiler J. T.; Hong D. H et al. (2004) FKBP8 is a negative regulator of mouse sonic hedgehog signaling in neural tissues. *Development* 131, 2149-2159
- Bürglin T.R. (2008) Evolution of hedgehog and hedgehog-related genes, their origin from Hog proteins in ancestral eukaryotes and discovery of a novel Hint motif. *BMC Genomics* 9:127.
- Burke, R. et al. (1999) Dispatched, a novel sterol-sensing domain protein dedicated to the release of cholesterol-modified hedgehog from signaling cells. *Cell* 99: 803-815
- Callejo A.; Torroja C.; Quijada L. et al. (2006) I. Hedgehog lipid modifications are required for Hedgehog stabilization in the extracellular matrix. *Development* 133, 471-483

- Chang T. Y.; Chang C. C.; Ohgami N. et al (2006) Cholesterol sensing, trafficking, and esterification. *Annu. Rev. Cell Dev. Biol.* 22, 129–157
- Chen Y. & Struhl G. (1996) Dual roles for patched in sequestering and transducing Hedgehog. *Cell* 87(3):553–63
- Chen M. H.; Li Y. J.; Kawakami, T. et al. (2004) Palmitoylation is required for the production of a soluble multimeric Hedgehog protein complex and long-range signaling in vertebrates. *Genes Dev.* 18, 641–659
- Clement V.; Sanchez P.; de Tribolet N. et al. (2007) HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol* 17:165–72
- Cohen Jr M.M. (2003) The hedgehog signaling network. *Am J Med Genet* 123A(1):5–28.
- Cooper M.K.; Porter J.A.; Young K.E. et al. (1998) Teratogen-mediated inhibition of target tissue response to Shh signalling. *Science* 280 (5369):1603–1607
- Dessaud E.; McMahon A.P. & Briscoe J. (2008) Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. *Development* 135:2489–2503
- Dierks C.; Beigi R.; Guo G.R., et al. (2008) Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell* 14:238–49
- Eaton S. (2008) Multiple roles for lipids in the Hedgehog signalling pathway. *Nat Rev Mol Cell Biol.* 9(6):437–45
- Eggenchwiler J. T.; Espinoza E. & Anderson K. V. (2001) Rab23 is an essential negative regulator of the mouse Sonic hedgehog signalling pathway. *Nature* 412, 194–198
- Eugster C.; Panakova D.; Mahmoud A. et al. (2007) Lipoprotein-heparan sulfate interactions in the Hedgehog pathway. *Dev. Cell* 13, 57–71
- Evangelista M.; Tian H. & de Sauvage F.J. (2006) The hedgehog signaling pathway in cancer. *Clin Cancer Res* 12:5924
- Feng J. et al. (2004) Synergistic and antagonistic roles of the Sonic hedgehog N- and C-terminal lipids. *Development* 131, 4357–4370
- Gallet A.; Rodriguez R.; Ruel L. et al. (2003) Cholesterol modification of Hedgehog is required for trafficking and movement, revealing an asymmetric cellular response to Hedgehog. *Dev. Cell* 4, 191–204
- Gallet A.; Ruel L.; Staccini-Lavenant L. et al. (2006) Cholesterol modification is necessary for uncontrolled planar long-range activity of Hedgehog in *Drosophila* epithelia. *Development* 133, 407–418
- Glise B. et al. (2005) Shifted, the *Drosophila* ortholog of wnt inhibitory factor-1, controls the distribution and movement of hedgehog. *Dev. Cell* 8, 255–266
- Hall T. M.; Porter J. A.; Beachy P. A. et al. (1995) A potential catalytic site revealed by the 1.7-Å crystal structure of the amino-terminal signalling domain of Sonic hedgehog. *Nature* 378, 212–216
- Han C.; Belenkaya T. Y.; Wang B. et al. (2004) *Drosophila* glypicans control the cell-to-cell movement of Hedgehog by a dynamin-independent process. *Development* 131, 601–611
- Hooper J.E. & Scott M.P. (1989) The *Drosophila* patched gene encodes a putative membrane protein required for segmental patterning. *Cell* 59(4): 751–65
- Hooper J. E. (2003) Smoothened translates Hedgehog levels into distinct responses. *Development* 130(17):3951–63

- Huangfu D. et al. (2003) Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* 426, 83–87
- Inaki M.; Kojima T.; Ueda R. et al. (2002) Requirements of high levels of Hedgehog signaling activity for medial region cell fate determination in *Drosophila* legs: identification of *pxb*, a putative Hedgehog signalling attenuator gene repressed along the anterior-posterior compartment boundary. *Mech. Dev.* 116, 3–18
- Ingham P.W. & McMahon A.P. (2001) Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 15: 3059–87
- Izraeli, S. et al. (2001) Genetic evidence that *Sil* is required for the Sonic Hedgehog response pathway. *Genesis* 31, 72–77
- Jacob L. & Lum L. (2007) Deconstructing the hedgehog pathway in development and disease. *Science* 318:66–68
- Jessell T.M. (2000) Neuronal specification in the spinal cord: inductive signals and transcriptional codes *Nature Rev. Genet.* 1:20–29
- Jia J.; Tong C. & Jiang J. (2003) Smoothed transduces Hedgehog signal by physically interacting with Costal2/Fused complex through its Cterminal tail. *Genes Dev* 17(21):2709–20.
- Jiang J. & Hui C.C. (2008) Hedgehog signalling in development and cancer. *Dev Cell* 15:801–812.
- LaBarge M. (2010) The difficulty of targeting cancer stem cell niches. *Clin Cancer Res* 16:3121–9.
- Li Y.; Zhang H.; Litingtung Y. et al. (2006) Cholesterol modification restricts the spread of Shh gradient in the limb bud. *Proc. Natl Acad. Sci. USA* 103, 6548–6553
- Liu S.; Dontu G.; Mantle I.D.; et al. (2006) Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res* 66:6063–71
- Lum L.; Yao S.; Mozer B. et al. (2003) Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells. *Science* 299(5615):2039–45
- Lum L.; Zhang C.; Oh S. et al. (2003) Hedgehog signal transduction via Smoothed association with a cytoplasmic complex scaffolded by the atypical kinesin, Costal-2. *Mol Cell* 12(5):1261–74
- Lum L. & Beachy A. (2004) The hedgehog response network: sensors, switches, and routers. *Science* 304: 1755–1759
- Lum L.; Zhang C.; Oh S.; et al. (2003) Hedgehog signal transduction via Smoothed association with a cytoplasmic complex scaffolded by the atypical kinesin, Costal-2. *Mol Cell.* 12(5):1261–74.
- Ma Y. et al. (2002) Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of Dispatched. *Cell* 111, 63–75
- McMahon A. P.; Ingham P. W. & Tabin C. J. (2003) Developmental roles and clinical significance of hedgehog signaling. *Curr. Top. Dev. Biol.* 53, 1–114
- Merchant, M. et al. (2004) Suppressor of fused regulates Gli activity through a dual binding mechanism. *Mol. Cell. Biol.* 24, 8627–8641
- Nusse R. (2003) Wnts and Hedgehogs: lipid-modified proteins and similarities in signaling mechanisms at the cell surface. *Development* 130, 5297–5305
- Ogden S.K.; Ascano M. Jr.; Stegman M.A. et al. (2004) Regulation of Hedgehog signaling: a complex story. *Biochem Pharmacol.* 67(5):805–14

- Ogden S.K.; Ascano Jr M.; Stegman M.A. et al. (2003) Identification of a functional interaction between the transmembrane protein Smoothed and the kinesin related protein Costal2. *Curr Biol* 13(22):1998–2003
- Ogden S.K.; Ascano M.Jr.; Stegman M.A., et al. (2004) Regulation of Hedgehog signaling: a complex story. *Biochem Pharmacol.* 67(5):805-14
- Paces-Fessy M.; Boucher D.;Petit E. et al. (2004) The negative regulator of Gli, Suppressor of fused (Sufu), interacts with SAP18, Galectin3 and other nuclear proteins. *Biochem.J.* 378: 353–362
- Panáková D.; Sprong H.; Marois E. et al. (2005) Lipoprotein particles carry lipid-linked proteins and are required for long-range Hedgehog and Wingless signalling. *Nature* 435, 58–65
- Peacock C.D.; Wang Q.; Gesell G.S., et al. (2007) Hedgehog signalling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci U S A*104:4048–53
- Porter J. A. et al. (1996) Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxyterminal autoprocessing domain. *Cell* 86, 21–34
- Rahnama F.; Toftgard R. & Zaphiropoulos P. G. (2004) Distinct roles of PTCH2 splice variants in Hedgehog signalling. *Biochem.J.* 378, 325–334
- Rasheed Z.A.; Yang J.; Wang Q., et al. (2010) Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. *J Natl Cancer Inst* 102:340–51.
- Roessler E.; Belloni E.; Gaudenz K. et al. (1996) Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat Genet.* 14:357-360
- Rohatgi R.; Milenkovic L. & Scott M.P. (2007) Patched1 regulates hedgehog signalling at the primary cilium. *Science* 317:372-376
- Rubin L.L. & de Sauvage F.J. (2006) Targeting the hedgehog pathway in cancer. *Nat Rev Drug Discov* 5:1026
- Ruel L.; Rodriguez R.; Gallet A. et al. (2003) Stability and association of Smoothed, Costal2 and Fused with Cubitus interruptus are regulated by Hedgehog. *Nat Cell Biol* 5(10):907–13
- Ruiz i Altaba A.; Sánchez P. & Dahmane N. (2002) Gli and hedgehog in cancer: tumours, embryos and stem cells. *Nat Rev Cancer.* 2(5):361-72.
- Sell S. (2004). Stem cell origin of cancer and differentiation therapy. *Crit. Rev. Oncol. Hematol.* 51:1-28.
- Stegman M.A.; Vallance J.E.; Elangovan G. et al. (2000) Identification of a tetrameric hedgehog signaling complex. *J Biol Chem.* 275(29):21809-12.
- Taipale J. & Beachy P.A. (2001) The Hedgehog and Wnt signalling pathways in cancer. *Nature* 411 (6835):349–54.
- Varjosalo M. & Taipale J. (2008) Hedgehog: functions and mechanisms. *Genes Dev* 22:2454–2472
- Varnat F.; Duquet A.; Malerba M, et al. (2009) Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. *Embo Mol Med* 1:338–51
- Watkins D. N.; Berman D. M.; Burkholder S. G. et al. (2003) Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* 422: 313-317.

- Wolff, C. et al. (2004) *iguana* encodes a novel zinc-finger protein with coiled-coil domains essential for Hedgehog signal transduction in the zebrafish embryo. *Genes Dev.* 18, 1565-1576
- Yauch R.L.; Gould S.E.; Scales S.J. et al. (2008) A paracrine requirement for hedgehog signalling in cancer. *Nature* 455: 406-410
- Zhao C.; Chen A.; Jamieson C.H.; et al. (2009) Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature* 458:776-9.



Cancer Stem Cells Theories and Practice

Edited by Prof. Stanley Shostak

ISBN 978-953-307-225-8

Hard cover, 442 pages

Publisher InTech

Published online 22, March, 2011

Published in print edition March, 2011

Cancer Stem Cells Theories and Practice does not 'boldly go where no one has gone before!' Rather, Cancer Stem Cells Theories and Practice boldly goes where the cutting edge of research theory meets the concrete challenges of clinical practice. Cancer Stem Cells Theories and Practice is firmly grounded in the latest results on cancer stem cells (CSCs) from world-class cancer research laboratories, but its twenty-two chapters also tease apart cancer's vulnerabilities and identify opportunities for early detection, targeted therapy, and reducing remission and resistance.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Vanessa Medina Villaamil, Guadalupe Aparicio Gallego, Silvia Díaz Prado and Luis M. Antón Aparicio (2011). Signalling Pathways Driving Cancer Stem Cells: Hedgehog Pathway, Cancer Stem Cells Theories and Practice, Prof. Stanley Shostak (Ed.), ISBN: 978-953-307-225-8, InTech, Available from: <http://www.intechopen.com/books/cancer-stem-cells-theories-and-practice/signalling-pathways-driving-cancer-stem-cells-hedgehog-pathway>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](#), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.