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Hedgehog signaling pathway

Anil Reddy Marasani [1*], Radhika Anakonti [1], Deepthi Rudrapati [2], Rosi reddy Jonnavarapu [2], Basani Gavaskar[3]

[1]Department of Pharmacology, St. Peter's Institute Of Pharmaceutical Sciences, Hanamkonda, Warangal, A.P.
[2]Department of Pharmacology,Sri Venkateswara university, Tirupati, Chittoor, A.P.
[3] Department of Pharmaceutics, Vagdevi college Of Pharmacy, Hanamkonda, Warangal, A.P.

ABSTRACT

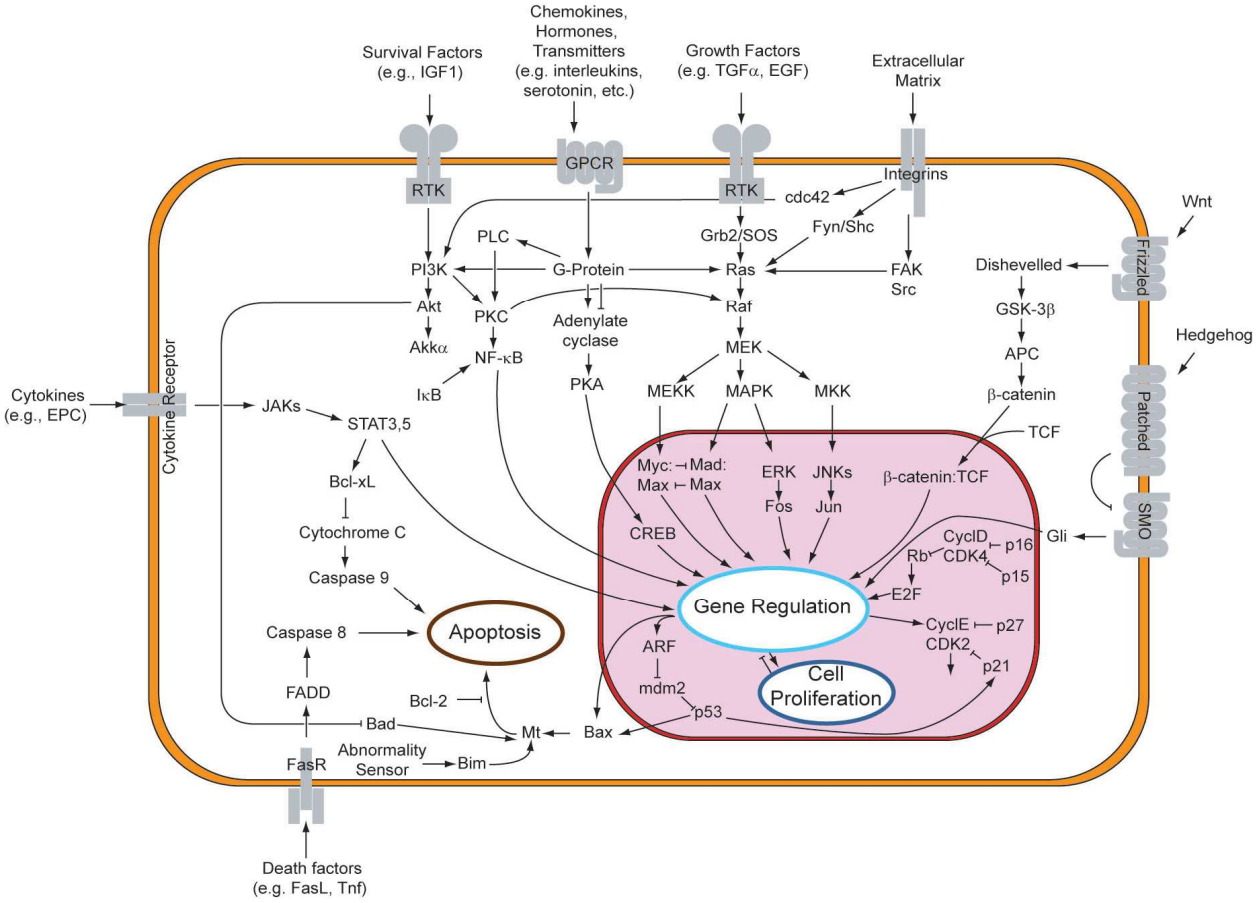
The control and mediation of the cell cycle is influenced by cell signals. Different types of cell signaling molecules: Proteins (growth factors), peptide hormones, amino acids, steroids, retenoids, fatty acid derivatives, and small gases can all act as signaling molecules. The hedgehog signaling pathway is one of the key regulators of animal development conserved from flies (wing development in Drosophila) to humans (development of the brain, GI tract, fingers and toes in mammals). The pathway name is from its polypeptide ligand, an intercellular signaling molecule called Hedgehog (Hh) found in fruit flies of the genus Drosophila. Mutations or other sorts of regulatory errors in the hedgehog pathway are associated with a number of birth defects as well as some cancers. In this report, we will review the role of Hedgehog in cell signaling and impact of it in clinical medicine.

Key words: Hedgehog, Metamorphosis, SMO, PTCH, SHH, Gorlin syndrome.

INTRODUCTION

Cell signaling is a complex system of communication, which controls and governs basic cellular activities and coordinates cell actions [1]. In biology point of view signal transduction refers to any process through which a cell converts one kind of signal or stimulus into another. Signal transduction involves sequences of biochemical reactions inside the cell, which are carried out by enzymes, activated by second messengers, resulting in a *signal transduction pathway* [Figure 1].

Figure 1.Overview of signal transduction pathways. Hedgehog is at center right**.**



In a growing embryo, cells develop differently in the head or tail end of the embryo, the left or right, and other positions. They also form segments which develop into different body parts. The hedgehog signaling pathway gives cells this information that they need to make the embryo develop properly. Different parts of the embryo have different concentrations of hedgehog signaling proteins. The pathway also has roles in the adult. When the pathway malfunctions, it can result in diseases like basal cell carcinoma.

The hedgehog signaling pathway is one of the key regulators of animal development conserved from flies to humans. The pathway takes its name from its polypeptide ligand, an intercellular signaling molecule called Hedgehog (*Hh*) found in fruit flies of the genus Drosophila. *Hh* is one of Drosophila's segment polarity gene products, involved in establishing the basis of the fly body plan. The molecule remains important during later stages of embryogenesis and metamorphosis.

Mammals have three Hedgehog homologues, of which Sonic hedgehog is the best studied. The pathway is equally important during vertebrate embryonic development. In knockout mice lacking components of the pathway, the brain, skeleton, musculature, gastrointestinal tract and lungs fail to develop correctly.

Discovery:

In 1995 Edward B.Lewis, Christiane Nusslein-Volhard and Eric F.Wieschaus were awarded a Nobel Prize for their work producing and studying genetic mutations in Drosophila embryogenesis. Nüsslein- Volhard and Wieschaus attempted to isolate mutations in genes that control development of the segmented anterior-posterior body axis of the fly; [2] their "saturation

mutagenesis" technique resulted in the discovery of a group of genes involved in the development of body segmentation.

Some hedgehog mutants result in abnormally-shaped embryos that are unusually short and stubby compared to wild type embryos. The function of the hedgehog segment polarity gene has been studied in terms of its influence on the normally polarized distribution of larval cuticular denticles as well as features on adult appendages such as legs and antennae [5]. Rather than the normal pattern of denticles, hedgehog mutant larvae tend to have "solid lawns" of denticles [Figure 2].

Figure 2. Normal and Hedgehog mutant larvae

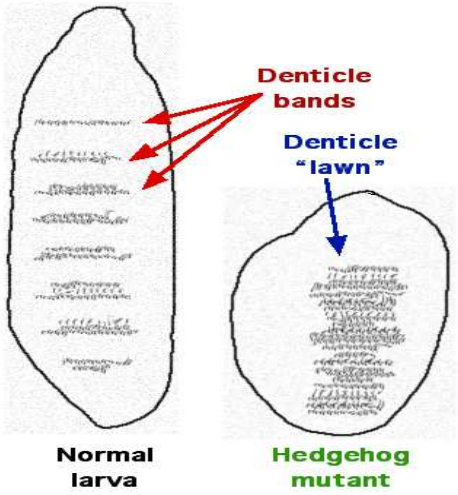
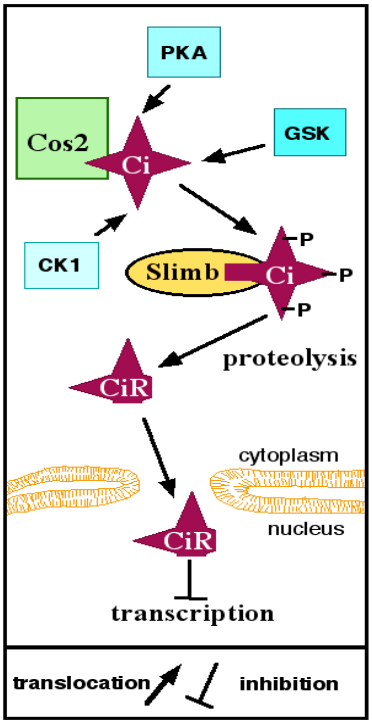


Figure 3. Production of the CiR transcriptional repressor when Hh is not bound to Patched. In the diagram, "P" represents phosphate.



Fruit fly:

Mechanism

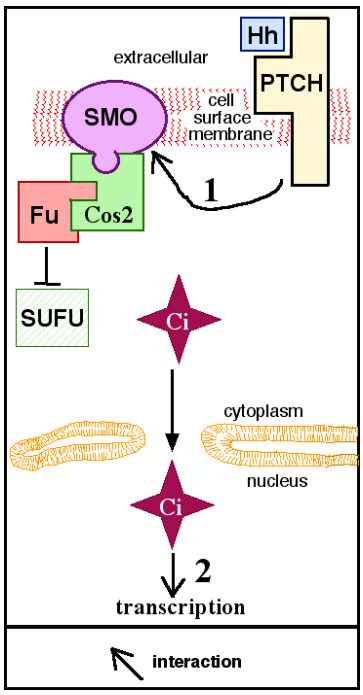
Insect cells express a full size zinc-finger transcription factor Cubitus interruptus (Ci), which forms a complex with the kinesin- like protein Costal-2 (Cos2) and is localized in the cytoplasm bound to cellular microtubules (Figure 3). The complex targets the Ci protein for proteosome-dependent cleavage, which generates a fragment (CiR) that functions as a transcriptional repressor. CiR builds up in the cell and diffuses into the nucleus, where it acts as a co-repressor for Hh target genes [4]. The steps leading to Ci protein proteolysis include phosphorylation of Ci protein by several protein kinases; PKA, GSK-3β and CK1 [Figure 3] [5]. The *Drosophila* protein Slimb is part of an SCF complex that targets proteins for ubiquitylation. Slimb binds to phosphorylated Ci protein.

In the absence of Hh (Figure 3), a cell-surface transmembrane protein called Patched (PTCH) acts to prevent high expression and activity of a 7 membrane spanning receptor [6] called Smoothened (SMO). Patched has sequence similarity to known membrane transport proteins.

Mechanism

When extracellular Hh is present [Figure 4], it binds to and inhibits Patched, allowing Smoothened to accumulate and inhibit the proteolytic cleavage of the Ci protein. This process most likely involves the direct interaction of Smoothened and Costal-2 and may involve sequestration of the Ci protein-containing complex to a microdomain where the steps leading to Ci protein proteolysis are disrupted [6]. The mechanism by which Hh binding to Patched leads to increased levels of Smoothened is not clear [Step 1 in Figure 4]. Following binding of Hh to Patched, Smoothened levels increase greatly over the level maintained in cells when Patched is not bound to Hh [7]. It has been suggested that phosphorylation of Smoothened plays a role in Hh-dependent regulation of Smoothened levels [8].

Figure 4. When Hh is bound to Patched (PTCH), Ci protein is able to act as transcription factor in the nucleus.

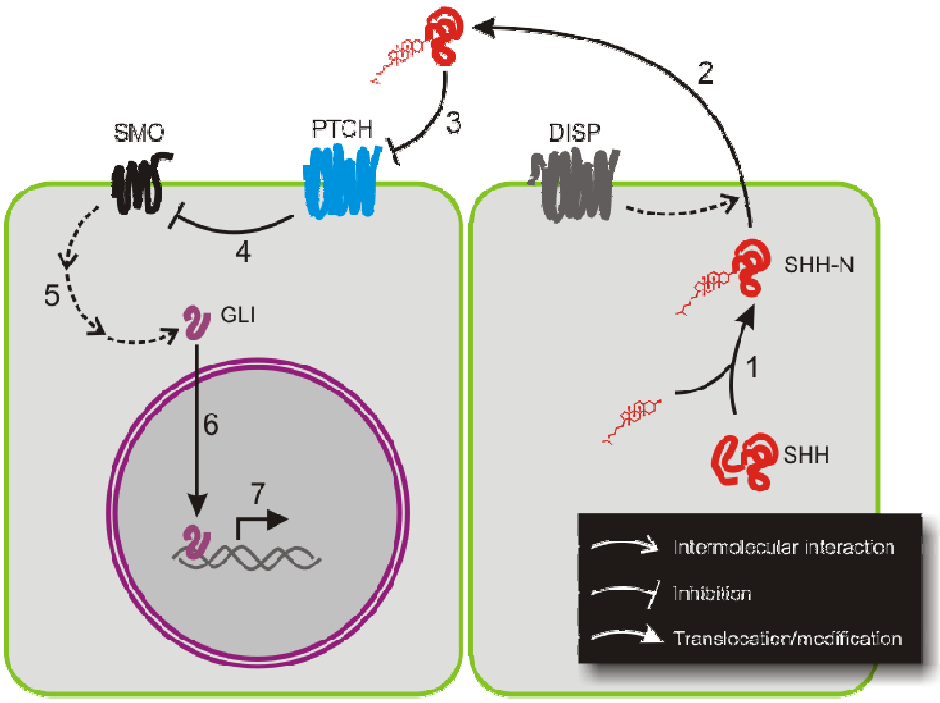


In cells with Hh-activated Patched [Figure 4], the intact Ci protein accumulates in the cell cytoplasm and levels of CiR decrease, allowing transcription of some genes such as decapentaplegic (dpp, a member of the BMP growth factor family). For other Hh-regulated genes, expression requires not only loss of CiR but also the positive action of uncleaved Ci acting as a transcriptional activator [5]. Costal-2 is normally important for holding Ci protein in the cytoplasm, but interaction of Smoothened with Costal-2 allows some intact Ci protein to go to the nucleus. The *Drosophila* protein Fused [Fu in Figure 4] is a protein kinase that binds to Costal-2. Fused can inhibit Suppressor of Fused (SUFU), which in turn interacts with Ci to regulate gene transcription in some cell types[9].

Vertebrates:
Receptor:
Patched (**Ptc**) — a 12-pass transmembrane protein embedded in the plasma membrane.
Ligands:
Secreted **hedgehog** proteins (**Hh**) that diffuse to their targets. Mammals have three hedgehog genes encoding three different receptors. However, hedgehog was first identified in Drosophila, and the bristly phenotype produced by mutations in the gene gave rise to the name.

Mechanism:
Sonic hedgehog(SHH) is the best studied ligand of the vertebrate pathway. It is translated as a ~45kDa precursor and undergoes autocatalytic processing to produce an ~20kDa N-terminal signaling domain (referred to as SHH-N) and a ~25kDa C-terminal domain with no known signaling role [step1 on figure 5]. During the cleavage, a cholesterol molecule is added to the carboxyl end of the N-terminal domain, which is involved in trafficking, secretion and receptor interaction of the ligand. SHH can signal in an autocrine fashion, affecting the cells in which it is produced. Secretion and consequent paracrine hedgehog signaling require the participation of Dispatched protein [step 2].

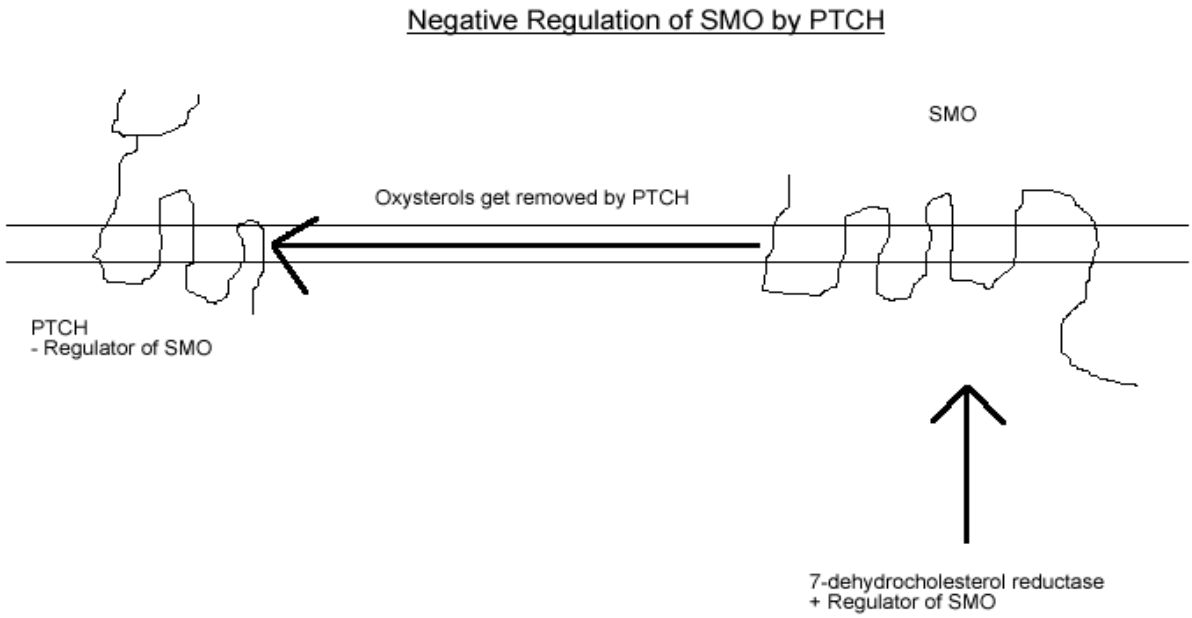
Figure 5. Overview of Sonic hedgehog signaling.



When SHH reaches its target cell, it binds to the Patched-1 (PTCH1) receptor (step 3). In the absence of ligand, PTCH1 inhibits Smoothened (SMO), a downstream protein in the pathway [step 4]. It has been suggested that SMO is regulated by a small molecule, the cellular localization of which is controlled by PTCH [10]. PTCH1 has homology to Niemann-Pick disease, type C1 (NPC1) that is known to transport lipophilic molecules across a

Membrane [11].PTCH1 has a sterol sensing domain (SSD), which has been shown to be essential for suppression of Smo activity[12].A current theory of how PTCH regulates SMO is by removing oxysterols from SMO. PTCH acts like a sterol pump and remove oxysterols that have been created by 7-dehydrocholesterol reductase.(figure6)[13]

Figure 6. Overview of PTCH/SMO signaling



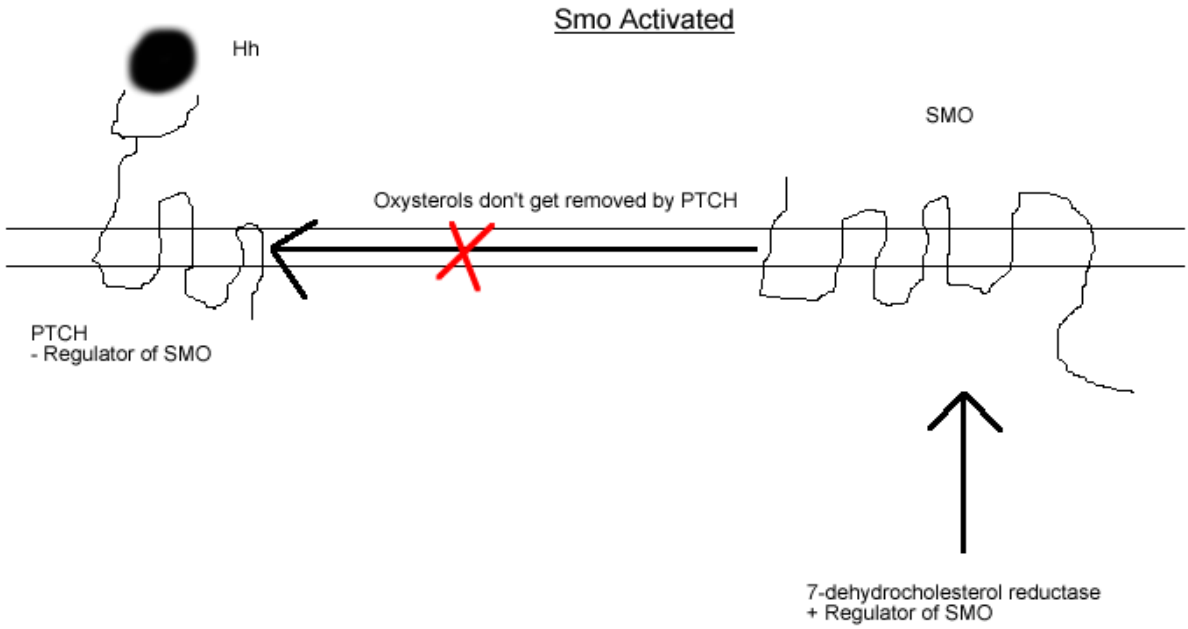
Upon binding of a Hh protein or a mutation in the SSD of PTCH the pump is turned off allowing oxysterols to accumulate around SMO [figure7].

This accumulation of sterols allows SMO to become active or stay on the membrane for a longer period of time. This hypothesis is supported by the existence of a number of small molecule agonists and antagonists of the pathway that act on SMO. The binding of SHH relieves SMO inhibition, leading to activation of the GLI transcription factors [step 5]: the activators Gli1and Gli2 and the repressorGli3. The sequence of molecular events that connect SMO to GLIs is poorly understood. Activated GLI accumulates in the nucleus [step 6] and controls the transcription of hedgehog target genes [step 7]. PTCH1 has recently been reported to repress transcription of hedgehog target genes through a mechanism independent of Smoothened [14].

In addition to PTCH1, mammals have another hedgehog receptor PTCH2 whose sequence identity with PTCH1 is 54 % [15]. All three mammalian hedgehogs bind both receptors with similar affinity, so PTCH1 and PTCH2 cannot discriminate between the ligands. They do, however, differ in their expression patterns. PTCH2 is expressed at much higher levels in the testis and mediates desert hedgehog signaling there [15]. It appears to have a distinct

downstream signaling role from PTCH1. In the absence of ligand binding PTCH2 has a decreased ability to inhibit the activity of SMO [16].Furthermore, over expression of PTCH2 does not replace mutated PTCH1 in Basal cell carcinoma [17].

Figure 7. Overview of PTCH/SMO signaling.



In mammals, when there is no hedgehog protein present, the patched receptors bind a second transmembrane protein called smoothened (Smo). However, when Hh protein binds to patched, the Smo protein separates from Ptc, enabling Smo to activate a zinc-finger transcription factor designated GLI. GLI migrates into the nucleus when it activates a variety of target genes.

In invertebrates, just as in *Drosophila*, the binding of hedgehog to PTCH leads to internalization and sequestration of the ligand [18]. Consequently *in vivo* the passage of hedgehog over a receptive field that expresses the receptor leads to attenuation of the signal, an effect called ligand-dependent antagonism (LDA). In contrast to *Drosophila*, vertebrates possess another level of hedgehog regulation through LDA mediated by Hh-interacting protein 1 (HHIP1). HHIP1 also sequesters hedgehog ligands, but unlike PTCH, it has no effect on the activity of SMO [19].

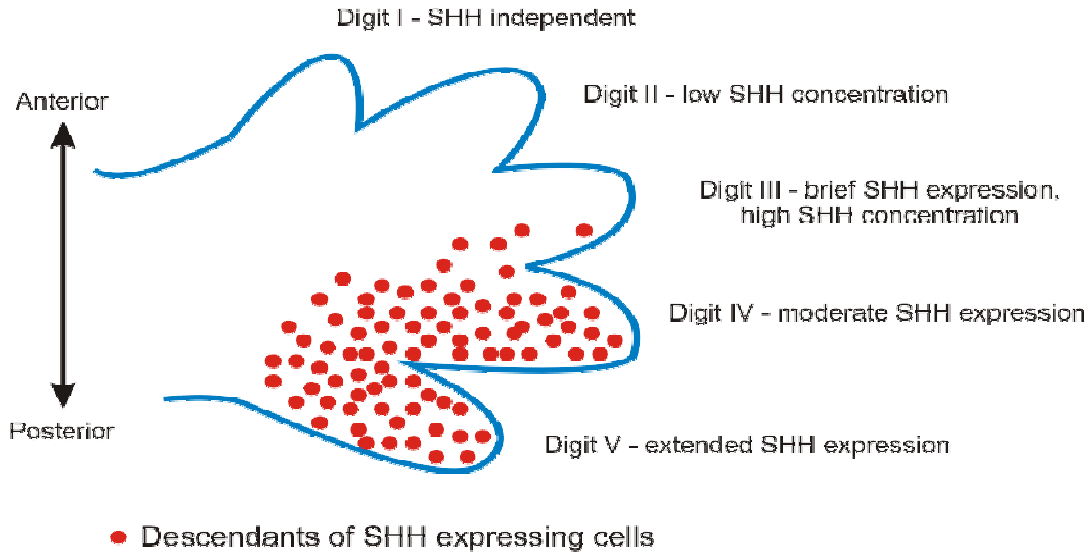
Role:

The most widely found hedgehog homolog in vertebrates is sonic hedgehog (Shh). Shh participates in the development of the neural plate and neural tube. Shh binds to a transmembrane protein encoded by the *patched* gene and suppresses transcription of genes encoding members of the Wnt and TGF-β families and inhibits cell growth. Mutation of the patched homolog in humans (*PTC*) causes the Gorlin syndrome (rib abnormalities, cyst of the jaw, and basal cell carcinoma, a form of skin cancer).

Members of the hedgehog family play key roles in a wide variety of developmental processes [20]. One of the best studied examples is the action of Sonic hedgehog during development of the vertebrate limb. The experiments of Saunders and Gasseling in 1968 on the development of

the chick limb bud formed the basis of the morphogen concept. They showed that identity of the digits in the chick limb was determined by a diffusible factor produced by the zone of polarizing activity (ZPA), a small region of tissue at the posterior margin of the limb. Mammalian development appeared to follow the same pattern. This diffusible factor was later shown to be Sonic hedgehog. The current model, proposed by Harfe *et al*.[21], states that both the concentration and the time of exposure to SHH determines which digit the tissue will develop into in the mouse embryo [figure 8].

Figure 8. Sonic hedgehog specifies digit identity in mammalian development.



Digits V, IV and part of III arise directly from cells that express SHH during embryogenesis. In these cells SHH signals in an autocrine fashion and these digits develop correctly in the absence of DISP, which is required for extracellular diffusion of the ligand. These digits differ in the length of time that SHH continues to be expressed. The most posterior digit V develops from cells that express the ligand for the longest period of time. Digit IV cells express SHH for a shorter time, and digit III cells shorter still. Digit II develops from cells that are exposed to moderate concentrations of extracellular SHH. Finally, digit I development does not require SHH. It is, in a sense, the default program of limb bud cells.
Hedgehog signaling plays many important developmental roles in the animal kingdom. For example,

- Wing development in Drosophila
- Development of the brain, GI tract, fingers and toes in mammals.

Mutations or other sorts of regulatory errors in the hedgehog pathway are associated with a number of birth defects as well as some cancers. Basal-cell carcinoma, the most common skin cancer (and, in fact, the most common of all cancers in much of the world), usually reveals mutations causing

- Extra-high hedgehog or
- Suppressed patched activity (both leading to elevated GLI activity).

Hedgehog signaling remains important in the adult. Sonic hedgehog has been shown to promote the proliferation of adult stem cells from various tissues; including primitive hematopoietic cells

[22], mammary [23] and neural stem cells. Activation of the hedgehog pathway is required for transition of the hair follicle from the resting to the growth phase [24].

Curis Inc. together with Procter & Gamble are developing a hedgehog agonist to be used as a drug for treatment of hair growth disorders. This failed due to toxicities found in animal models.

Human disease:
Disruption of hedgehog signaling during embryonic development, either through deleterious mutation or consumption of teratogens by the gestating mother, can lead to severe developmental abnormalities. Holoprosencephaly, the failure of the embryonic prosencephalon to divide to form cerebral hemispheres, occurs with a frequency of about 1 in 16,000 live births and about 1 in 200 spontaneous abortions in humans and is commonly linked to mutations in genes involved in the hedgehog pathway, including *SHH* and *PTCH* [25] Cyclopia, one of the most severe defects of Holoprosencephaly, results if the pathway inhibitor Cyclopamine is consumed by gestating mammals [26].

Activation of the hedgehog pathway has been implicated in the development of cancers in various organs, including brain, lung, mammary gland, prostate and skin. Basal cell carcinoma, the most common form of cancerous malignancy, has the closest association with hedgehog signaling. Loss-of-function mutations in Patched and activating mutations in Smoothened have been identified in patients with this disease [27]. Abnormal activation of the pathway probably leads to development of disease through transformation of adult stem cells into cancer stem cells that give rise to the tumor. Cancer researchers hope that specific inhibitors of hedgehog signaling will provide an efficient therapy for a wide range of malignancies [28].

Targeting the Hedgehog Pathway:
The most common way to target this pathway is modulate SMO. Antagonist and agonist of SMO have already shown to effect the pathway regulation downstream. PTCH [29] and Gli3 (5E1) [30] antibodies are also a way to regulate the pathway. A downstream effector and strong transcriptional activator siRNA Gli1 has been used to inhibit cell growth and promote apoptosis [31].

Hedgehog Pathway and Metastasis:
Activation of the Hedgehog pathway leads to an increase in Snail protein expression and a decrease in E-cadherin and Tight Junctions [32]. Hedgehog signaling also appears to be a crucial regulator of angiogenesis and thus metastasis [33].

Hedgehog Pathway and Tumor Regulation:
Activation of the Hedgehog pathway leads to an increase in Angiogenic Factors (angiopoietin-1 and angiopoietin-2) [34], Cyclins (cyclin D1 and B1)) [35] anti-apoptotic genes and a decrease in apoptotic genes (Fas)[36].

Clinical Trials:
GDC-0449 in Treating Patients With Locally Advanced or Metastatic Solid Tumors. A Study of Systemic Hedgehog Antagonist with Concurrent Chemotherapy and **Bevacizumab** as First-Line Therapy for Metastatic Colorectal Cancer.

Evolution
Hedgehog-like genes, 2 Patched homologs and Patched-related genes exist in the worm *C. elegans*[37,38] . These genes have been shown to code for proteins that have roles in *C. elegans*

development [37]. The hedgehog-like and Patched-related gene families are very large and function without the need for a Smoothened homolog, suggesting a distinct pattern of selection for cholesterol modification and sensing mechanisms in coelomate and pseudo-coelomate lineages[38].

Various vertebrate lineages have adapted hedgehogs to unique developmental processes. For example, a homologue of the *X.laevis banded hedgehog* is involved in regeneration of the salamander limb [39]. *Shh* has undergone accelerated evolution in the primate lineage leading to humans [40].

The frizzled family of WNT receptors has some sequence similarity to Smoothened [41]. However, G proteins have been difficult to link to the function Smoothened. Smoothened seems to be a functionally divergent member of the G protein coupled receptor super family.

CONCLUSION

Hedgehog signaling plays many important developmental roles in the animal kingdom. For example, wing development in Drosophila, development of the brain, GI tract, fingers and toes in mammals. Mutations or other sorts of regulatory errors in the hedgehog pathway are associated with a number of birth defects as well as some cancers.Recent studies point to the role of hedgehog signaling in regulating adult stem cell involved in maintenance and regeneration of adult tissues. Drugs that specifically target hedgehog signaling to fight this disease are being actively developed by a number of pharmaceutical companies. Neverthless, significant progress has been made in the last few years by many investigators. Further clarification of the precise role of Hedgehog in different biological context will likely lead to new and novel therapeutic strategies for various diseases such as cancer, Gorlin syndrome, etc… In addition, further insights into Hedgehog biology may reveal novel, unexpected therapeutic targets that influence the Hedgehog signalling.

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