

REVIEW

Hedgehog signalling in endocrine development and disease

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Abstract

The hedgehog (Hh) pathway is an evolutionarily conserved signalling pathway that is required for many essential tissue and cellular properties such as patterning fields of cells or regulating cell differentiation and proliferation. Disruption of the pathway results in serious pathologies. In this review, we

provide an update on recent findings in the field of vertebrate Hh signalling and also describe contributions of Hh signalling to the development, maintenance and pathology of endocrine tissues.

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Hedgehog signalling

The *hedgehog* (*Hh*) gene was originally identified in the seminal work of Nusslein-Volhard & Wieschaus (1980) in which they performed mutagenesis screens of *Drosophila* and isolated and characterised mutants defective in embryonic cuticle formation. One of these they named *Hh* because mutation of the gene resulted in the disorganised denticles forming a spiky pattern. Subsequent investigations demonstrated that *Hh* encodes an intercellular signal with multiple critical functions as a regulator of embryonic development. *Hh* homologues have since been identified in many invertebrates and vertebrates, and found to play similar vital roles controlling tissue patterning, and cellular differentiation and proliferation both during embryonic development and in the control of stem cell behaviour and homeostasis in the adult.

Ligands

The three mammalian homologues of *Drosophila Hh* are *Sonic Hh* (*Shh*), *Indian Hh* (*Ihh*) and *Desert Hh* (*Dhh*). Each of the Hh proteins is processed post-translationally, and the precursor forms undergo multiple covalent modifications to produce mature signalling molecules. Hh proteins undergo autocatalytic cleavage that removes the carboxyl-terminal domain (Lee *et al.* 1994, Bumcrot *et al.* 1995) and simultaneously modifies the newly exposed carboxyl-termini of the amino-terminal signalling molecules by the addition of cholesterol (Porter *et al.* 1996). These proteins are further modified into their mature forms by signal peptide cleavage and palmitoylation at

amino-terminal cysteine residues (Pepinsky *et al.* 1998) by the acyltransferase Skinny Hh (Chamoun *et al.* 2001). This dual lipidation makes Hh proteins highly hydrophobic, and the release of processed Hh from the plasma membrane is thus an active process. Secretion is mediated by 12 pass transmembrane protein Dispatched (Disp), which is required for signalling to non-adjacent cells (Burke *et al.* 1999). The majority of secreted Hh found in soluble multimeric forms thought to assume micelle-like structures (Chen *et al.* 2004, Goetz *et al.* 2006). Hhs are the only known proteins that are covalently linked to cholesterol in this way. The requirement for cholesterol modification partially underlies the spectrum of developmental disorders observed in Smith-Lemli-Opitz syndrome (SLOS, OMIM 270400), a condition in which defects in 7-dehydrocholesterol reductase (DH7CR), an enzyme required for the formation of cholesterol from 7-dehydrocholesterol, leads to abnormalities that overlap those seen in cases of impaired Hh signalling (Kelley & Hennekam 2000).

Signal transduction pathway

Hh proteins bind 12 pass transmembrane receptors Patched1 (Ptch1) and Ptch2 on responding cells (Fig. 1). Binding to these receptors promotes Ptch internalisation and derepresses the activity of Smoothed (Smo), a seven pass transmembrane protein of the G protein-coupled receptor family that is related to the frizzled class of Wnt receptors. Following Hh binding to Ptch, Smo is phosphorylated on its intracellular carboxyl-terminal tail (Chen *et al.* 2004) and undergoes a reciprocal translocation from intracellular vesicles to the plasma membrane (for a review, see Huangfu & Anderson 2006).

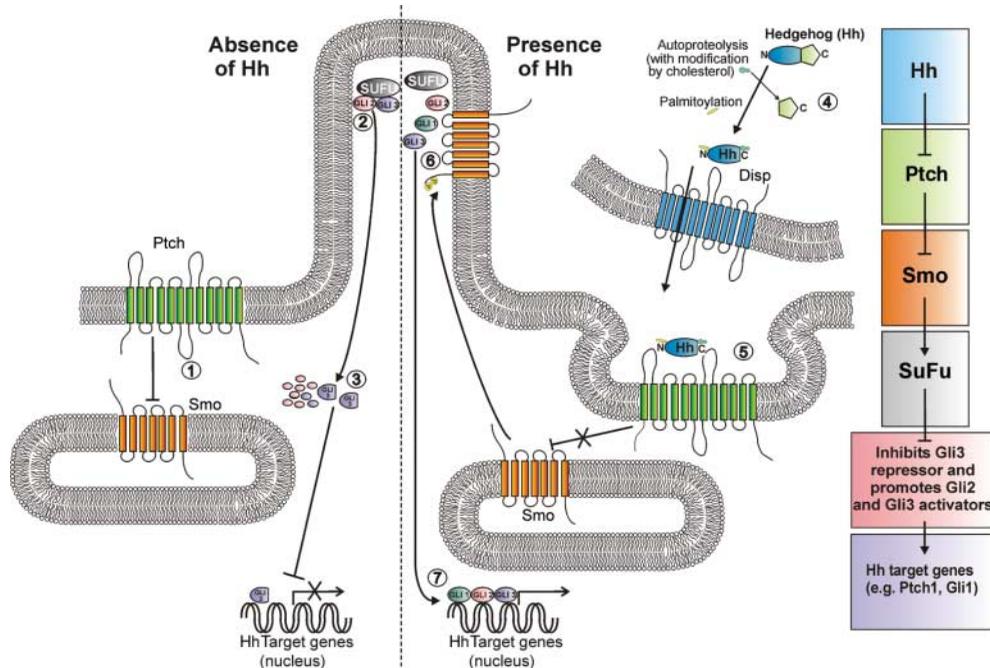


Figure 1 A scheme for Hh signalling. In the absence of Hh ligand, Ptch, located at the base of the cilium, inhibits Smo, possibly via a small molecule (1). Gli2 and 3, along with SuFu, are located at the tip of the cilium (2). IFT is required for movement of these molecules in and out of the cilium and they are proteasomally processed outside the organelle (3). Processing cleaves Gli3 (and Gli2) to produce a transcriptional repressor that inhibits the expression of Hh target genes and degrades Gli2. Hh is synthesised in signal-producing cells as a precursor molecule that is cleaved to remove the C terminus and is simultaneously modified by cholesterol addition. Following palmitoylation at the N terminus, it is secreted via Disp (4). Hh binding to Ptch on target cells promotes internalisation and relieves the repression Ptch exerts on Smo (5). Smo then translocates from endosomes to the tip of the cilium (6) resulting in the inhibition of processing of the Gli's which are then able to activate transcription of target genes, including Gli1 (7). A simplified pathway is shown on the right-hand side of the panel. For details see text.

The inhibition of Smo by Ptch is sub-stoichiometric, and the two proteins do not appear to physically interact, which raises the still unanswered question of how Ptch inhibits Smo activity. Both Ptch and Disp have homology to bacterial small molecule pumps and the Niemann-Pick C1 cholesterol transporter (Carstea *et al.* 1997, Loftus *et al.* 1997), which has led to the suggestion that, in an Hh binding-modulated fashion, Ptch might transport small molecule regulators of Smo activity. Adding support to this idea, the sterol-related alkaloid cyclopamine and the purine homologue purmorphamine can bind Smo and inhibit or activate it respectively. One candidate for an endogenous negative regulator of Smo is 7-dehydrocholesterol ((pro-) vitamin D3), which can be transported out of the cell by Ptch1, and accumulates in the plasma of SLOS patients (Bijlsma *et al.* 2006). Interestingly, oxysterols, which are cholesterol oxidation products, can activate the Hh signalling pathway by binding to Smo, raising the additional possibility that oxysterols or related molecules are natural intracellular Smo agonists (Corcoran & Scott 2006, Dwyer *et al.* 2007). These data, together with the modification of Hh and the presence of sterol-sensing domains in Ptch and Disp

(Kuwabara & Labouesse 2002), underscore the importance of cholesterol synthesis both in cells that produce Hh and those which receive Hh signals.

Ptch and Smo act through a signal transduction cascade that culminates in the modulation of the activity of the Gli family of zinc finger transcription factors. There are three Gli genes in mammalian cells, *Gli1*, *Gli2* and *Gli3*, which have partially overlapping functions. In the absence of Hh signalling, *Gli2* and *Gli3*, but not *Gli1*, are expressed at significant levels. In uninduced cells, these factors are phosphorylated by protein kinase A (PKA), casein kinase 1 (CK1) and glycogen synthase kinase 3 β (GSK3 β) (Sheng *et al.* 2006, Tempe *et al.* 2006, Wang & Li 2006) and ubiquitinated via interaction with the E3 ligase β transducin repeat containing protein (β TrCP) (Wang & Li 2006). These modifications lead to proteasomal processing that degrades the majority of Gli2, while limited proteolysis of Gli3 and Gli2 removes a carboxyl-terminal activation domain, producing truncated repressor forms that inhibit the expression of Hh target genes.

Activation of Smo via Hh-mediated inhibition of Ptch activity inhibits proteolytic processing of Gli2 and Gli3, and leads to the production of full-length transcriptional activator

forms of these proteins. This changes the ratio of activator and repressor Gli isoforms in the nucleus, and thus alters the expression of Hh target genes by a combination of transcriptional derepression and activation. *Gli1* transcription is induced as a primary response to Hh signalling, and Gli1 acts solely as a transcriptional activator to prolong or increase Hh target gene expression. Gli1 thus acts in conjunction with Gli2 and Gli3, although the loss of *Gli1* alone does not cause Hh signalling defects (Bai *et al.* 2002). *Gli3* mRNA expression is also regulated by Hh signalling, although in a negative fashion (Marigo *et al.* 1996). Thus, pathway activation leads not only to the induction of positively acting transcription factors, but also to the inhibition of negatively acting ones.

Among the Hh targets are several components of the Hh signalling system, including *Ptch1* and *Hh interacting protein (Hip)*. Hip is a transmembrane glycoprotein that binds all forms of Hh with similar affinity to Ptch1 and acts to sequester Hh and inhibit signalling in a negative feedback loop (Chuang & McMahon 1999, Bak *et al.* 2001). The expression of these genes, in particular *Gli1*, which is not observed in the absence of Hh, is therefore a useful marker of cells actively transducing an Hh signal (Ahn & Joyner 2004, Vokes *et al.* 2007).

Signalling and cilia

Recent work has highlighted the importance of primary cilia, which had previously been thought to be functionless vestigial organelles, for Hh signalling (for a recent review, see Eggenschwiler & Anderson 2007). Primary cilia are non-motile and are found on the surface of most, if not all, vertebrate cells as solitary microtubular protrusions extending from the centriole or basal body. Defects in intraflagellar transport (IFT), which is required for the formation and function of primary cilia, present with a spectrum of defects that overlaps those seen in Hh signalling mutants (see below). Examples of ciliopathies with *Hh* mutant markers include Bardet–Biedl syndrome (BBS, OMIM 209900) and Meckel–Gruber syndrome (OMIM 249000), which are complex disorders involving multiple developmental defects including polydactyly, situs inversus and hallmarks of holoprosencephaly (for reviews see, Davis *et al.* 2006, Tobin & Beales 2007).

A rationale for these observations was provided in a series of studies that revealed that IFT proteins and other ciliary components are required for Hh signalling. In the absence of IFT, there is no Gli activator or repressor activity with the result that there is no response to ligand (Huangfu *et al.* 2003, Haycraft *et al.* 2005, Huangfu & Anderson 2005, Liu *et al.* 2005, May *et al.* 2005). IFT mutations do not completely phenocopy mutations that inactivate the Hh pathway because the contribution of repression and activation of gene expression varies between cell types and both processes are affected in most ciliopathies. IFT is required for the transport of Gli1, Gli2 and Gli3, along with Suppressor of Fused (SuFu), into the cilium where they localise at the tip (Haycraft *et al.* 2005) and are modified in a poorly understood manner by a complex that includes SuFu (Kogerman *et al.* 1999,

Svard *et al.* 2006). Fused (Fu) is crucial for *Hh* signalling in *Drosophila* but in mammalian cells the importance of its role is as yet unclear (Chen *et al.* 2005, Merchant *et al.* 2005). In the absence of ligand, Gli2 and Gli3 are processed by the proteasome at the base of the cilium whereupon they can translocate to the nucleus and repress target gene transcription.

Upon binding Hh, Ptch1 is internalised from its position at the base of the cilium in uninduced cells and Smo becomes localised to the cilium tip (Corbit *et al.* 2005, May *et al.* 2005, Rohatgi *et al.* 2007) where it promotes the formation of activators and inhibits formation of repressors in an as yet unexplained manner. Part of the inhibition of Smo by Ptch1 is thus mediated by preventing entry of Smo into the cilium. This organelle is utilised apparently as a centre for accumulating pathway components that link Smo to Gli either in order to promote efficient signal transduction, or perhaps to allow for an ordered and vital sequence of protein interactions and modifications (Caspary *et al.* 2007).

Hh signalling during development, in the adult and in disease

Hh signalling plays many crucial roles during embryonic development, with the Gli family of transcription factors activating and repressing transcription of target genes in a multitude of settings. These include the notochord and floor plate which are required for neural tube patterning and the zone of polarising activity which is involved in limb development, and in many other developing organs and tissues (for review see Ingham & McMahon 2001). Hh signalling also has importance beyond development and is required for tissue maintenance and differentiation in the adult, for example in the gastrointestinal tract (Parkin & Ingham 2008), T-cell activation (Outram *et al.* 2000), haematopoiesis (Bhardwaj *et al.* 2001) and skin (Athar *et al.* 2006), with the control of stem cell behaviour being a common observation (Lai *et al.* 2003, Machold *et al.* 2003, Palma *et al.* 2005, Trowbridge *et al.* 2006, Zhou *et al.* 2006, Peacock *et al.* 2007).

Hh can act as a morphogen, a simple inductive signal, a mitogen, a survival factor or a chemoattractant, with the relevant modality depending on the specific tissue and developmental context. When Hh patterns a tissue as a morphogen, such as during ventral neural tube development, a gradient of ligand is established and cells respond to variations in the local concentration of ligand by differentially regulating target gene expression (Ingham & McMahon 2001, Ashe & Briscoe 2006). It is thus perhaps not surprising that many genes are involved in establishing and shaping the Hh ligand gradient. The Hh lipid modifications lead to interaction with the extracellular matrix and thus restrict diffusion (for review, see Guerrero & Chiang 2007). Several extracellular Hh-binding proteins such as Gas1, Cdo and Boc facilitate Hh signalling by increasing sensitivity to low concentrations of ligand, while others, such as Hip, sequester Hh and reduce signalling (Tenzen *et al.* 2006). Hh signalling

regulates the expression of many of these Hh-binding factors, including *Ptch1* itself, and thus activation of the pathway can lead to subsequent modification in the shape and steepness of the gradient (Martinelli & Fan 2007a,b). Modulation of the extracellular concentration of Hh might similarly be important for establishing chemoattractant Hh gradients, such as that which is sensed by the growing axons of dorsal commissural neurons as they extend towards the ventral midline of the neural tube (Charron *et al.* 2003).

The differential response of cells to variations in the Hh ligand gradient appears to be exhibited through graded alterations in the activity of the Gli transcription factors. Thus, a low level of Hh response might be achieved through reduced production of Gli repressor protein, while a higher level might be achieved by producing Gli activator from Gli repressor, or inducing additional activator expression *de novo*. By modulating both the type of Gli protein produced as well as utilising multiple Gli genes, cells can achieve a wide range of Hh responses. It is now clear that these responses vary with tissue context. Thus, for example, in the spinal cord, Gli2 appears to be the most significant family member, while in the limb Gli3 is most significant (Ahn & Joyner 2004, Bai *et al.* 2004).

Developmental disorders of Hh signalling can be separated into those that result primarily from either inactivation (constitutive repression) or overactivation of the pathway. Holoprosencephaly (HPE) results from inactivation of the pathway, with defects observed in *Shh* (HPE3, OMIM 142945) (Nanni *et al.* 1999), *Ptch1* (HPE7, OMIM 610828) (Ming *et al.* 2002) and *Gli2* (HPE9, OMIM 610829) (Roessler *et al.* 2003), as well as *DH7CR* (SLOS, OMIM 270400). The commonly used Smo binding molecule and Hh pathway inhibitor cyclopamine, a teratogenic alkaloid derived from the corn lily (*Veratrum californicum*), was discovered after it was observed that the offspring of ewes that grazed on the lilies during pregnancy were born with holoprosencephaly (Keeler & Binns 1968). Pallister–Hall syndrome (PHS, OMIM 146510), which encompasses a number of symptoms including polysyndactyly, hypothalamic hamartoma and internal organ malformations (see below), is caused by truncations of the *GLI3* gene which mimic the repressor form of the transcription factor resulting in constitutive inhibition of Hh signalling (Bose *et al.* 2002, Hill *et al.* 2007).

Inappropriate activation of Hh signalling contributes to several cancers. Gorlin syndrome (basal cell nevus syndrome, OMIM 109400) is a disorder with predisposition to basal cell carcinoma (BCC), medulloblastoma and rhabdomyosarcoma (Gorlin 1995), as well as skeletal and other abnormalities. Inactivating mutations in *PTCH1* are responsible for this syndrome (Hahn *et al.* 1996, Johnson *et al.* 1996) and inactivating mutations in *PTCH1* (Gailani & Bale 1997), or activating mutations in *SMO* (Lam *et al.* 1999) and other pathway components (Reifenberger *et al.* 2005) are estimated to be present in up to 76% of sporadic BCC. Hh pathway mutations have also been described in 25% of sporadic medulloblastomas (Zurawel *et al.* 2000). Adult reactivation of

the Hh pathway is associated with lung cancer (Watkins *et al.* 2003), glioma (Dahmane *et al.* 2001), gastric tumours (Berman *et al.* 2003) and others discussed below (see Table 1).

Hh signalling in endocrine tissues

The pancreas

The developing gut is formed from a primitive endodermal tube that gives rise to the entire digestive system and associated organs from the pharynx to the colon. During development organs such as the pancreas, lungs and liver are derived from endodermal buds that grow out of the walls of the gut tube (for review see, Kiefer 2003). Studies on Hh signalling during gut development have elucidated a general mechanism whereby endodermal Hh signals act on the surrounding mesenchyme via an epithelial–mesenchymal inductive mechanism. One example of this is seen in pancreatic development, and although space precludes the discussion of similar crosstalk mechanisms that control the lung and liver development, these are well covered in other reviews (Warburton *et al.* 2000, Shannon & Hyatt 2004, Watkins & Peacock 2004).

The pancreas contains two primary tissues, comprising the exocrine and endocrine pancreas. The exocrine pancreas is made up of acinar cells, which constitute the majority of cells in the pancreas, and ductal cells. Acinar cells produce digestive enzymes that drain into the duodenum via the ductal tissue. The islets of Langerhans are clusters of cells found within the exocrine pancreas and constitute the endocrine pancreas. These endocrine islets are themselves composed of five different cell types: α -cells (which produce glucagon), β -cells (insulin), δ -cells (somatostatin), ϵ -cells (ghrelin) (Prado *et al.* 2004) and PP-cells (pancreatic polypeptide), and the hormones these cells produce regulate blood glucose levels (for reviews, see Slack 1995, Docherty 2001, Murtaugh 2007).

The pancreas develops from two buds forming on the dorsal and ventral sides of the posterior foregut. These buds come into contact and fuse after stomach rotation (for review, see Murtaugh 2007). The gut and pancreatic development proceeds under the control of pancreatic and duodenal homeobox 1 (*Pdx1*), a homeodomain-containing transcription factor essential for the development and function of the gland (Jonsson *et al.* 1994, Offield *et al.* 1996). *Shh* and *Ihh* are expressed initially throughout the gut including within the presumptive pancreas region during early mouse development (Bitgood & McMahon 1995, Ramalho-Santos *et al.* 2000, Spence & Wells 2007), but both are repressed in this region by activin B and Fgf2 signals derived from the adjacent notochord (Hebrok *et al.* 1998). Ectopic activation of Hh signalling in the pancreas, achieved by the overexpression of *Shh* under the control of the *Pdx1* promoter (Apelqvist *et al.* 1997) or targeted deletion of *Ptch1* or *Hip* (Kawahira *et al.* 2003), results in severely disrupted endocrine and exocrine pancreatic development. By contrast, disruption of the *Shh* or

Table 1 Hedgehog family members expressed in, or responsible for the development of, the endocrine organs discussed in the text are shown together with the role these factors play in the development and function of the organ and their contribution to endocrine pathologies

| Organ | Hedgehogs | Function | Pathology |
|--------------|-------------|--|--|
| Prostate | Shh | Required for bud formation and subsequent branching morphogenesis during development | Shh and pathway component expression predictive of pancreatic cancer and metastasis |
| Adrenal | Shh | Required for normal adrenal development | Adrenal insufficiency observed in Hh-associated conditions Pallister–Hall syndrome and holoprosencephaly ^a |
| Testes | Dhh | Required for organogenesis, Leydig cell formation and sex cord formation | <i>DHH</i> mutations cause testicular dysgenesis |
| Ovary | Dhh Ihh | Hh pathway activation increases granulosa cell proliferation and inhibition increases progesterone production | Overexpression contributes to ovarian tumours? |
| Pituitary | Shh | Shh signal from oral ectoderm required for development. Shh promotes proliferation and differentiation of gonadotrophs and thyrotrophs. Possible post-natal expression and signalling in corticotrophs | Agenesis observed in holoprosencephaly ^a . Loss of Shh expression may contribute to adenoma in the adult |
| Hypothalamus | Shh | Patterning ventral neural tissue | <i>Shh</i> null mice show hypothalamic dysgenesis |
| Bone | Ihh | Controls chondrocyte proliferation and progression to hypertrophy both directly and via secondary signalling in the growth plate; controls osteogenesis during bone formation | <i>Ihh</i> null mice have malformed limbs |
| Adipocytes | Ihh Dhh | Hh signalling inhibitory to adipogenesis | Ptch1 mutant mice have decreased white adipose tissue. BBS patients exhibit truncal obesity |
| Pancreas | Shh Ihh Dhh | Repression of Shh and Ihh in the developing gut is required for pancreas formation. Ihh and Dhh are expressed in islets at later stages | Disruption of Hh expression causes annular pancreas-like condition. Activation of Hh signalling in adults is associated with chronic pancreatitis and cancer |

^aHoloprosencephaly phenotypes are possibly secondary to ventral midline patterning defects independent of specific activity in endocrine tissue. See text for details.

Ihh genes in mice (Ramalho-Santos *et al.* 2000) or inhibition of Hh signalling in chicks with cyclopamine (Kim & Melton 1998) leads to extension of the pancreatic anlagen. This latter case is similar to the rare annular pancreas condition in which a ring of pancreatic tissue encircles the duodenum, and can inhibit intestinal transit in severe cases. Taken together these data indicate that the pattern of *Shh* and *Ihh* expression in the developing gut creates boundaries between the pancreatic anlagen and those of the stomach and the duodenum, and that continuous Hh family gene expression within the anlagen is inhibitory to pancreatic development. Indeed, a recent report describing the differentiation of embryonic stem cells into insulin-secreting islet cells for treatment of diabetes used Shh pathway inhibitors to direct the differentiation of endoderm precursors towards a pancreatic lineage (D'Amour *et al.* 2006).

Ihh and *Dhh*, along with *Ptch1* and *Smo*, are expressed later in the developing murine pancreas, by 13.5 days post coitum (dpc). While the cell types that express these genes are undefined, expression increases throughout gestation (Hebrok *et al.* 2000) and persists in the islet cells of the adult organ (Thomas *et al.* 2000). Studies on islet cells using the rat β -cell line INS-1 have found that insulin production is controlled by Hh signalling. Cyclopamine treatment inhibits insulin secretion and Shh administration activates the insulin promoter indirectly by increasing *Pdx1* levels (Thomas *et al.*

2000, 2001). Reduced *Pdx1* transcription results in adult hyperglycaemia in mice and *PDX1* has been identified as a maturity onset of diabetes in the young (OMIM 606391) gene in humans, raising the possibility that impaired Hh signalling could be a cause of type 2 diabetes in humans.

Chronic pancreatitis (CP) is an inflammatory condition in which, the first exocrine and later endocrine tissue is compromised, with both a reduction in islet cell number and changes in their morphology. In CP, expression of *IHH*, *HIP* and *PTCH1* is upregulated in the islet cells and is also detectable in the exocrine cells (Kayed *et al.* 2003). CP is a risk factor for pancreatic cancer, and aberrant Hh signalling has been observed in the majority of pancreatic tumours (Berman *et al.* 2003, Thayer *et al.* 2003). Sonic hedgehog homolog (*SHH*), which is undetectable in the normal adult gland, is expressed in 70% of adenocarcinomas (Thayer *et al.* 2003). In pancreatic tumours, *IHH* expression is elevated in the islets, as in CP, and displays both a more diffuse pattern of expression than in normal islet cells, and ectopic expression in surrounding tissue, as do *PTCH1* and *SMO*. *SHH* is strongly expressed in pancreatic cancer cells but is not expressed in the islet cells (Kayed *et al.* 2004). Inhibition of Hh signalling is inhibitory to growth of pancreatic tumours and cells derived from them (Thayer *et al.* 2003, Feldmann *et al.* 2007), indicating that Hh signalling might be required for the development of some pancreatic cancers.

The prostate

The prostate gland is specified by the prostatic anlagen of the urogenital sinus (UGS) from which it develops in the form of epithelial buds, under the control of androgen produced by foetal Leydig cells. These buds elongate and canalise during branching morphogenesis to form ductal structures that contain the main prostate cell types, neuroendocrine, basal and secretory cells (Cunha *et al.* 1987). Studies on prostate development in mice have shown that increased expression of *Shh* in the UGS is observed at the onset of prostate ductal budding and remains elevated during the formation of the main prostatic ducts (15 dpc to post-natal day 5). *Dhh* and *Ihh* are either undetectable or present at very low levels. Equivalent increases in *Shh* are not observed in the analogous regions in female mice and it has been determined that *Shh* upregulation in the UGS is dependent upon androgen (Podlasek *et al.* 1999). Initial experiments in which embryonic UGS tissues were grafted into host animals demonstrated that anti-*Shh* antibodies block prostate development (Podlasek *et al.* 1999). However, in organ culture experiments using UGS explants from *Shh* null mice, prostate buds formed but subsequent branching morphogenesis was disrupted (Freestone *et al.* 2003, Berman *et al.* 2004). Further studies have suggested that these contradictory data can be reconciled by functional redundancy with *Ihh*, which is upregulated in the absence of *Shh*, maintaining the developmental pathway (Doles *et al.* 2006). Because combined *Shh* and *Ihh* null embryos do not survive to the time of prostate budding, this issue has not been formally resolved. Nonetheless, Hh signalling is clearly important for prostate development and differentiation. Examination of human prostate cancers has shown upregulation of *GLI1* and *PTCH1* expression indicating active Hh signalling, and high *SHH* levels in prostatic epithelial tissue are correlated with tumours with metastatic potential (Karhadkar *et al.* 2004, Sanchez *et al.* 2004). The possibility of targeting the Hh pathway for therapeutic benefit has also been demonstrated, as anti-*Shh* antibodies and cyclopamine inhibit the growth of both prostate cell lines and tumours (Karhadkar *et al.* 2004, Sanchez *et al.* 2004).

The pituitary

Pituitary organogenesis begins with an invagination of the anterior pituitary placode within the oral ectoderm to form Rathke's pouch. The dorsal region of this structure makes direct contact with a region of the ventral diencephalon, the infundibulum, and this interaction, along with regions within Rathke's pouch and the surrounding mesenchyme, is required for the specification of the pituitary endocrine cell types from progenitor cells within the pouch (for reviews, see Dasen & Rosenfeld 1999, Scully & Rosenfeld 2002). These cell types are defined by the particular hormones they produce, which in the anterior pituitary are the corticotrophs (adrenocorticotrophin, ACTH secreting), somatotrophs (growth hormone,

GH), lactotrophs (prolactin), thyrotrophs (thyrotrophin) and gonadotrophs (luteinizing hormone, LH and follicle-stimulating hormone), and in the intermediate lobes the melanotrophs (melanocyte-stimulating hormone, MSH).

Shh is expressed throughout the ventral diencephalon and the oral ectoderm at 8 dpc in the mouse. Its expression is downregulated in the region of the oral ectoderm that gives rise to the invaginating pituitary placode by 9 dpc (Treier *et al.* 1998, 2001). As in the early stages of pancreas development, *Shh* expression thus demarcates a molecular boundary that defines the developing pituitary. *Pitx1* is expressed throughout Rathke's pouch indicating that these cells are receiving Hh signals. *Shh* null mice (Chiang *et al.* 1996), and mutations in human *SHH* or the Hh pathway that cause holoprosencephaly (Odent *et al.* 1999, Roessler *et al.* 2003), display pituitary agenesis. While this might reflect an indirect requirement for *Shh*, as *Shh* null mice have a disrupted ventral diencephalon, which is required for pituitary development (Kimura *et al.* 1996, Pabst *et al.* 2000), other experiments indicate Hh signalling is directly required for the pituitary development. Attenuation of *Shh* signalling by forced expression of the Hh antagonist *Hip* under the control of the *Pitx1* enhancer, which is active in the oral ectoderm and Rathke's pouch, results in pituitary agenesis with the formation of only a rudimentary pouch, and normal development of the ventral diencephalon (Treier *et al.* 2001). Overexpression of *Shh* in the pituitary under the control of the α glycoprotein (α GSU) promoter, which is expressed throughout Rathke's pouch, leads to pituitary hyperplasia and premature appearance of *LH β* expression, indicating that *Shh* promotes proliferation and differentiation of the ventral pituitary cell types (gonadotrophs and thyrotrophs) (Treier *et al.* 2001). It is unclear as yet whether *Shh* induces different ventral cell types in a dose-dependent manner. Some evidence suggests that *Shh* might regulate ventral pituitary cell specification and proliferation at least partially through a secondary bone morphogenetic protein (BMP)2 signal induced at the *Shh* expression boundary in the oral ectoderm (Treier *et al.* 1998).

Studies of the adult human pituitary suggest possible roles for *Shh* signalling in the mature organ. *SHH* is apparently expressed almost exclusively in corticotrophs in normal tissue, which also express *PTCH2* and *GLI1* (Vila *et al.* 2005a). However, *SHH* is not expressed in corticotrophin-secreting adenomas (Vila *et al.* 2005b). Treating humans corticotrophinoma- (Vila *et al.* 2005a), somatotrophinoma- or prolactinoma- (Vila *et al.* 2005b) derived cell cultures with recombinant *Shh* led to the increased production of ACTH, GH and prolactin respectively. In rodent pituitary cell cultures, *Shh*-stimulated ACTH secretion occurs via Gli-dependent transcriptional activation of pro-opiomelanocortin (*POMC*), which is enhanced by co-stimulation with corticotrophin-releasing hormone (CRH). *Shh* also increased the expression of the CRH receptor *CRH-R1*, and CRH was shown to stimulate Gli1-dependent transcription (Vila *et al.* 2005a). It was also reported that *Shh* treatment of the mouse

corticotrophinoma AtT-20 cell line could inhibit its proliferation (Vila *et al.* 2005b). These data indicate that the loss of *SHH* expression in the adult pituitary could play a role in tumour formation and represent a novel avenue of investigation for the treatment of pituitary adenomas.

Shh null mice lack a hypothalamus as well as a pituitary (Chiang *et al.* 1996). Studies in zebrafish and chicks indicate that Hh signalling promotes the development of the anterior dorsal hypothalamus and inhibits the development of the posterior ventral hypothalamus (Mathieu *et al.* 2002, Manning *et al.* 2006). Thus, the importance of *Shh* for hypothalamic development can further compound the defects in the pituitary and endocrine organs under its control.

The gonads

The initial phase of gonad development is the formation of the bipotential or indifferent gonads, which occurs in both sexes. The bipotential gonads arise from the mesonephric mesenchyme and coelomic epithelium of the urogenital ridge, and the mesenchyme also produces the Wolffian and Mullerian ductal structures. The primordial germ cells migrate from the base of the allantois along the hindgut and then into the developing gonads, and remain bipotential until 13 dpc in the mouse (for a review, see Wilhelm *et al.* 2007). Testis development is dependent upon the expression of *Sry* from the Y chromosome at around 10.5 dpc in the mouse, which triggers all the subsequent steps of testis formation including Sertoli cell differentiation. Sertoli cell precursors express *Dhh* from 11.5 dpc in the mouse testis and *Dhh* null male mice exhibit defects in spermatogenesis, abnormal sex cord organisation and steroid-producing Leydig cell differentiation (Bitgood *et al.* 1996, Clark *et al.* 2000, Pierucci-Alves *et al.* 2001, Yao *et al.* 2002). Leydig cells express elevated levels of *Ptch1* indicating that it is these cells within the testes that are the primary target of Dhh signalling. In the absence of Dhh signalling, the size of the precursor Leydig cell population is unaffected but these cells have reduced expression of the transcription factor steroidogenic factor 1 (*SF-1*). This is the likely cause of impaired expression of steroidogenic enzymes and ultimately testosterone, which is required for virilisation and spermatogenesis (Yao *et al.* 2002). While this work has been performed solely in the mouse, in humans disrupted *DHH* genes are also correlated with gonadal dysgenesis (Umehara *et al.* 2000, Canto *et al.* 2004, 2005). Interestingly, reduced testis size and function observed in offspring exposed to maternal smoking during pregnancy might be linked to significantly decreased *DHH* expression in foetal Leydig cells (Fowler *et al.* 2008).

In the absence of *Sry*, the gonads develop into ovaries, accompanied by the production of granulosa and subsequently theca cells, which are required for steroidogenesis and gametogenesis (Wilhelm *et al.* 2007). *Dhh* null female mice are fertile and viable (Bitgood *et al.* 1996), suggesting that Hh signalling is not important for female gonad development. However, shortly after birth, granulosa cells

in mouse ovaries express both *Dhh* and *Ihh*, while *Ptch1* and *Gli1* mark the differentiating theca cells in the surrounding stroma. Both *Dhh* and *Ihh* expression in follicles and *Ptch1*/*Gli1* expression in surrounding theca cells are lost during ovulation (Wijgerde *et al.* 2005). A specific role for this signalling in ovarian development or function has not been defined, although *Ihh* produced by the granulosa cells may complement *Dhh* function, and thus explain the normal phenotype of *Dhh* null animals (Wijgerde *et al.* 2005). Recombinant *Shh* increased the rate of proliferation of murine granulosa cells in culture and their progesterone production was increased by cyclopamine treatment, indicating that granulosa cells may be a target for Hh signalling (Russell *et al.* 2007). Epithelial ovarian tumours develop from the surface epithelium, which does not normally express Hh signalling pathway ligands or target genes. However, *SHH* and *DHH* are frequently upregulated in ovarian neoplasias. The level of expression of these genes, as well as *PTCH1*, *GLI1* and *SMO*, correlates well with the aggressiveness of the tumour, with *DHH* expression levels providing the best prognosis. Cell lines derived from these tumours were growth inhibited by blocking Hh signalling, indicating that Hh pathway activation is likely to be critical for their growth (Chen *et al.* 2007).

The adrenal cortex

The adrenal cortex and the gonads share a common primordium and this cell population, derived from the mesonephric mesenchyme and overlying coelomic epithelium of the urogenital ridge (see above), is referred to as the 'adrenogenital primordium' (AGP) (Hatano *et al.* 1996). At around 10 dpc in the mouse, these cells begin to express the transcription factor *SF-1*, which is essential for both adrenal and gonadal development (Luo *et al.* 1994). At 11 dpc, the anterior end of the AGP splits into the bipotential gonad and the adrenocortical primordium, which is located between the gonad and the dorsal aorta. These cells are then invaded by a group of migrating sympathetic neural crest cells that ultimately form the adrenal medulla (Hatano *et al.* 1996), and the entire cortical plus medullary unit becomes encapsulated by mesenchymal cells. The majority of factors known to affect adrenal development also affect gonadal development and are thus required for the formation of the AGP. These include the transcription factors *SF-1*, Wilms tumor 1 (*WT-1*) and *Dax-1* (Luo *et al.* 1994, Muscatelli *et al.* 1994, Zanaria *et al.* 1994, Morohashi 1997). Surprisingly, little is known about the signals that specifically control adrenal primordium specification and development or the subsequent zonation of the cortex, but some data implicate a role for the Hh pathway. *In situ* hybridisation experiments showed *Shh* mRNA expression restricted to subcapsular adrenocortical cells after segregation of the adrenal and gonadal anlagen in mice (Bitgood & McMahan 1995). In humans, holoprosencephaly frequently presents with adrenal hypoplasia and hypoadrenalism was noted in the original description of PHS (Hall *et al.* 1980),

which is caused by a protein-truncating mutation in *GLI3* (Hall *et al.* 1980). The introduction of a similar *Gli3* mutation into mice results in adrenal agenesis at late gestation (Bose *et al.* 2002). In addition, SLOS is also associated with adrenal insufficiency (Andersson *et al.* 1999, Chemaitilly *et al.* 2003).

These observations are all consistent with a requirement for Shh in early adrenal development. However, as described above, defective Shh signalling also causes pituitary agenesis and thus can lead to the loss of POMC-derived pituitary hormones that are required for the trophic support of adrenal growth in post-natal animals. While the adrenal insufficiency observed in these cases might thus be secondary to defective pituitary function, adrenal glands develop normally in POMC null mice (Karpac *et al.* 2006) indicating that pituitary function is likely to be dispensable for prenatal adrenal development. Our unpublished experiments also indicate that adrenal glands form in *Shh* null mice but their development is already defective by 12.5 dpc, when pituitary POMC expression begins (Liu *et al.* 2001). Taken as a whole, these data indicate the likelihood of a direct role for Shh in controlling adrenal development. Thus, it is possible that disruption of the Shh signalling pathway is a cause of unassigned cases of adrenal hypoplasia.

Osteogenesis and adipogenesis

During embryonic development, endochondral bone formation is initiated by the condensation of clusters of mesenchymal cells that differentiate into chondrocytes to form the cartilage anlagen. This is followed by chondrocyte proliferation to expand the cartilage and the subsequent differentiation, growth arrest and hypertrophy of the more medial chondrocytes. Osteoblasts are recruited from the perichondrium and promote ossification at the medial extent of the growth plate. The rates of chondrocyte proliferation and hypertrophy govern the elongation rate of the bone and are processes under complex control, including systemic hormonal signals from the GH–insulin-like growth factor system, glucocorticoids, thyroid hormone and sex hormones and local signals that include Ihh- and parathyroid-related peptide (PTHrP) (for reviews, see van der Eerden *et al.* 2003, Kronenberg 2003). *Ihh* is expressed in prehypertrophic chondrocytes (Bitgood & McMahon 1995), whereas *Ptch1*, the *Gli* genes and *Smo* are expressed in the distal proliferating chondrocytes as well as in the perichondrium, marking these as the signal receiving cells. Ihh signals directly to less mature chondrocytes where it promotes their proliferation (Long *et al.* 2001) indirectly to these cells by inducing *PTHrP* expression in distal periarticular chondrocytes near the ends of the growing bone. PTHrP signalling then acts to delay the differentiation of the proliferating chondrocytes. *Ihh* and *PTHrP* together establish a negative feedback circuit as cartilage growth increases the distance between the distal periarticular *PTHrP*-expressing cells and the more medial proliferating chondrocytes, which leads to the latter cells differentiating to a pre-hypertrophic state and upregulating

Ihh expression (Karaplis *et al.* 1994, Lanske *et al.* 1996, Vortkamp *et al.* 1996, St Jacques *et al.* 1999). In addition to controlling chondrocyte proliferation and hypertrophy, Ihh is also required independently of PTHrP for proper formation of the perichondrium, and hence osteoblast formation, and for vascularisation of the bone (Colnot *et al.* 2005). The crucial roles Ihh plays in chondrocyte proliferation and osteogenesis are dramatically demonstrated in *Ihh* null mice, which have markedly malformed long bones that are one-third normal size at birth (St Jacques *et al.* 1999).

Mesenchymal stem cells are pluripotent cells that can differentiate into osteoblasts, osteoclasts, chondrocytes, adipocytes and other cell types (Tocci & Forte 2003). The control of these alternative differentiation pathways is crucial for both bone and fat formation and recent data suggest that Hh signalling plays an important role in these processes. Shh, in concert with BMPs, can promote osteoblastic differentiation of the murine mesenchymal C3H10T1/2 (Zehentner *et al.* 2000, Spinella-Jaegle *et al.* 2001) and KS483 (van der Horst *et al.* 2003) cell lines, as well as primary calvaria cell cultures (Spinella-Jaegle *et al.* 2001). They also block adipogenesis in C3H10T1/2 cells, primary calvaria cells (Spinella-Jaegle *et al.* 2001), the model adipogenesis cell line 3T3-L1 (Suh *et al.* 2006) and human mesenchymal stem cells (Fontaine *et al.* 2008). Furthermore, a *Shh* transgene blocks fat body formation in *Drosophila* (Suh *et al.* 2006). Taken together, these studies provide support for a model in which Hh provides a pro-bone and anti-fat signal. Further evidence comes from mouse obesity models in which Hh signal components are reduced in the fat deposits (Suh *et al.* 2006). BBS patients can present with increased truncal obesity (Tobin & Beales 2007), and recent data indicate that mice with an inactivating mutation in *Ptch1*, and hence activated Hh signalling, have significantly less white adipose tissue than wild-type litter mates (Li *et al.* 2008). However, some data show a positive relationship between Hh signalling and adipogenesis. For example, toxicology studies showed that an Shh fusion protein increased fat mass in a reversible fashion (Martin *et al.* 2002), while signal blocking anti-Shh antibodies can protect mice from diet-induced weight gain (Buhman *et al.* 2004).

It should be noted, however, that Shh signalling has effects at several levels of metabolic control which may confound efforts to see specific effects on adipogenesis *in vivo*. Nevertheless, there is an inverse relationship between osteogenesis and adipogenesis in several mesenchymal stem cell models that are controlled, at least *in vitro*, by Hh signalling. This suggests a possible role for this signalling pathway in human conditions of imbalance between osteogenesis and adipogenesis, such as the increase in marrow adipose tissue and decrease in bone seen in osteoporosis (Meunier *et al.* 1971, Verma *et al.* 2002, Nuttall & Gimble 2004). Furthermore, Hh pathway activation in human mesenchymal stem cells undergoing adipogenesis leads to insulin resistance, suggesting a potential role for Hh signalling in this pathology (Fontaine *et al.* 2008).

Summary

We have described the current understanding of Hh signalling and highlighted its role in several endocrine settings. Hh signalling is required for the development of all the endocrine organs derived from the foregut, as well as the development of the hypothalamo-pituitary-gonadal and hypothalamo-pituitary-adrenal axes, and other organs not discussed here. Defective Hh signalling during embryogenesis is the underlying cause of many endocrine malformation syndromes, and Hh signalling is important in the aetiology of several adult endocrine disorders. These include diabetes, with effects on adiposity, islet β -cell function and potentially insulin resistance, and osteoporosis, as a consequence of its effect on bone formation. The identification of Hh signalling in an increasing number of adult stem cells suggests the potential for therapeutic interventions in some or all of these processes in the future. In summary, the Hh pathway is a fascinating signalling mechanism controlling many fundamental cell processes that are likely to become increasingly studied in the search for the causes of common endocrine disorders and may be explored as a novel avenue for clinical management.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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