HORMONES 2015, 14(1):5-18

Review

The role of Notch and Hedgehog signaling pathways in pituitary development and pathogenesis of pituitary adenomas

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ABSTRACT

Pituitary adenomas are usually benign tumors that cause symptoms by compression of surrounding structures or impaired hormone secretion. Treatment, whether surgical or medical depends, on the tumor subtype and degree of compression; however, a significant proportion of patients do not achieve optimal control of mass effects or hormonal hypersecretion. Unraveling the pathogenesis of pituitary adenomas is a critical step in the quest for new subcellular treatment targets that will decrease morbidity and mortality related to these tumors. A large diversity of pathogenetic mechanisms has been described so far including deregulation of cell cycle, molecular pathways and angiogenesis. Major signaling pathways such as Notch, Wnt and Hedgehog, which are mainly active in the early phase of pituitary organogenesis and are essential for the development of somatotrophs, lactotrophs thyrotrophs and corticotrophs, have been implicated in the pathogenesis of pituitary adenomas. In this review we present novel data regarding the role of Notch and Hedgehog regulatory networks in pituitary development and pathogenesis of pituitary adenomas.

Key words: Hedgehog signaling, Notch signaling, Pituitary adenomas, Pituitary development

1. INTRODUCTION

Pituitary adenomas are among the most frequent intracranial tumors. Series of radiological and autopsy studies suggest a prevalence as high as 22.5%.[1] Although the majority of pituitary adenomas are benign and malignancy rarely occurs, they usually behave as an expanding mass causing compression and excessive hormone secretion or insufficiency.[2] Surgical resection remains the first-line treatment for most pituitary tumors, with the exception of prolactinomas, which respond successfully to medical treatment. Despite the recent advances in molecular genetics, the molecular pathogenesis of pituitary adenomas remains largely unknown, while the current classification of clinically functional and nonfunctional tumors does not reflect possible molecular distinctions between the subtypes.

Over the past 30 years, developmental biology studies have identified the role of evolutionarily highly

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Received: 30-06-2014, Accepted: 28-01-2015

conserved intracellular signaling pathways such as Wnt, Notch and Hedgehog in pituitary development and disease. Through these pathways, specific secreted proteins control differentiation and function of recipient cells in a paracrine and/or autocrine manner. Tight concerted actions of these signals lead to specific cell fates and the formation of distinct cell populations in the developing pituitary, while in the adult pituitary they regulate proliferation and hormonal secretion of pituitary cells.

In this review, we outline current knowledge regarding the role of Notch and Hedgehog signaling pathways in the development of the pituitary and their involvement in the pathogenesis of pituitary adenomas.

2. NOTCH SIGNALING

Notch signaling regulates cell fate through lateral inhibition and formation of boundaries, both of which represent patterning processes of critical importance in the regulation of spacing of different cell types within tissues.[3,4] During "lateral inhibition", Notch signaling, having mainly a permissive role, contributes to binary cell fate choices in populations of developmentally equivalent cells by inhibiting one of the fates in some cells and allowing them to later adopt an alternative one. Notch may also regulate the adoption of a third cell fate at the border of neighbouring populations of different cell types.[5,6]

The formation of boundaries takes place between distinct cell populations and promotes the exhibition of discrete physiological functions in specific organs and tissues.[7] Embryogenesis undergoes a variety of boundary formations and Notch signaling is a critical regulator of this process.[8]

2.1 Ligands and Receptors

There are four Notch receptors in mammals (Notch1, Notch2, Notch3, Notch4) and five classic DSL (Delta/Serrate/Lag-2) ligands: Jagged 1, Jagged 2, Delta-like 1, Delta-like 3 and Delta-like 4 (Figure 1).

Notch receptors are single-pass transmembrane

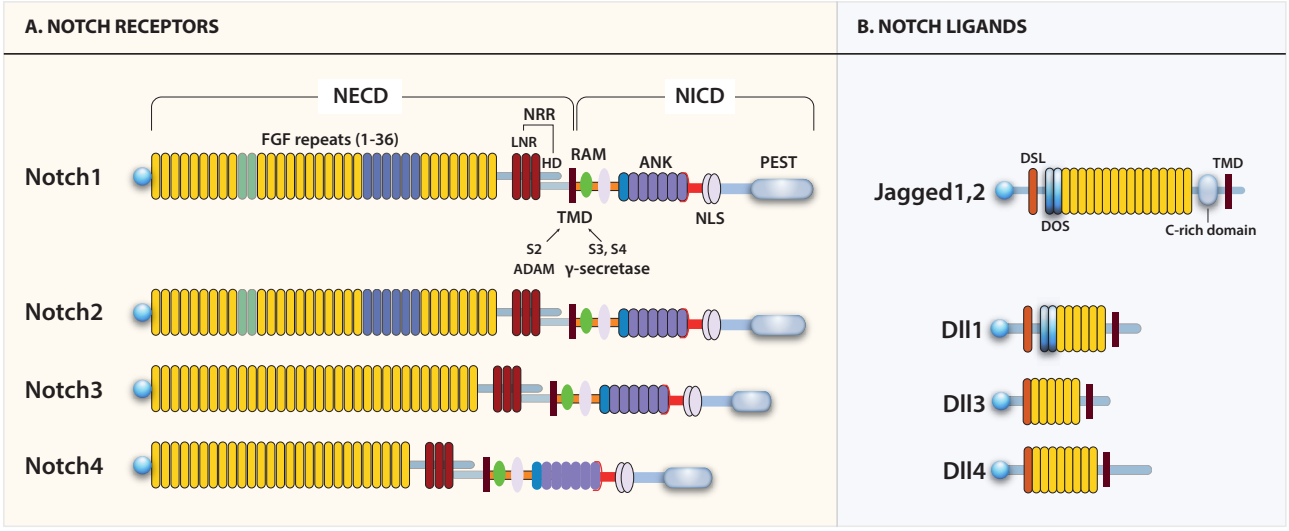

Figure 1. Notch receptors and ligands. (A) Notch receptors are type I proteins that contain multiple extracellular EGF (epidermal growth factor) repeats. There are four mammalian Notch variations (Notch1-4) that differ in the number of repeats (29-36). EGF repeats 11-12 (green) and 24-29 (blue) mediate ligand interactions. EGF repeats are followed by the negative regulatory region (NRR), which is composed of three cysteine-rich Lin repeats (LNR) and a heterodimerization domain (HD). Notch also contains a transmembrane domain (TMD), a RAM domain, nuclear localization sequences (NLS), a seven ankyrin repeats (ANK) domain and a transactivation domain (PEST). Mammalian Notch proteins are cleaved by furin-type convertases, which convert the Notch polypeptide into an NECD-NICD (Notch extracellular domain-Notch transmembrane and intracellular domain) heterodimer that is connected by noncovalent interactions between the halves of the heterodimerization domain. After ligand binding, Notch is cleaved at site 2 by metalloproteases. γ-secretase can cleave multiple bonds at sites 3 and 4. (B) The ligands of Notch receptors can be divided into several groups on the basis of their domain composition. Classical DSL ligands contain the DSL (Delta/Serrate/LAG-2), DOS and EGF motifs. Non-canonical ligands lacking DSL and DOS domains have been reported to activate Notch in some contexts.

proteins composed of a functional extracellular domain (NECD), a transmembrane domain and an intracellular domain (NICD). Notch ligands are also transmembrane proteins in the signal-sending cells and both ligands and receptors require a catalytic process to become active. Some of the Delta-like ligands can undergo cleavage in the membrane by a specific metalloprotease and thus act not only in neighbouring cells but in distant tissues as well. In this way, both the signal sending and receiving cell are affected through the ligand-receptor crosstalk.

2.1.1 Ligand endocytosis and processing

Endocytic transfer and processing of the Notch ligands are considered a critical step for their signaling activity. Ligand endocytosis is initiated by ubiquitination that is mediated by E3 ubiquitin ligases. After endocytosis, a largely unknown process takes place leading to a more active cell surface ligand. Current views as to the nature of ligand modification include clustering of the ligand, posttranslational modifications to the ligand and recycling of the ligand into specific membrane microdomains.[9,10] Interestingly, certain protein members, which negatively regulate ligand activity and thus reduce the efficiency of Notch activation, are themselves Notch target genes.[11] This notch-mediated regulation of ligand proteins constitutes a negative feedback loop that is further regulated by micro RNAs (miRNAs) that target specific mRNAs.[12] In addition, miRNAs can also regulate Delta ligands expression.[13]

2.1.2 Non-canonical Notch ligands

Apart from the canonical ligands, a multitude of non-canonical ligands have also been reported to activate or suppress Notch signaling. The most well studied non-canonical ligand is Delta-like homolog 1 (DLK1), which is structurally similar to the DLK ligands but lacks the DSL domain. In the anterior pituitary, DLK1 is localized in all hormone-producing cells supporting several regulatory functions.[14] The observed expression pattern of DLK1 in the developing pituitary suggests that DLK1 may have a role in the regulation of differentiation of pituitary cell types by altering Notch signaling activity. Corroborating such a role, the pituitary of adult knockout mice contains a significantly lower number of somatotrophs and a reduction in cell-specific gene expression in gonadotrophs compared to wild-type mice.

2.2 The intracellular cascade

Ligand binding leads to the cleavage of Notch receptors by a TNF-a converting enzyme (TACE), which belongs to the ADAM family of disintegrin and metalloproteases. The clipping of the extracellular domain creates a membrane–tethered intermediate called Notch extracellular truncation (NEXT), which is a substrate for an intramembrane cleaving protease, γ-secretase. After the γ-secretase-induced cleavage, NICD is free to enter into the nucleus where it interacts with the CSL (CBF1/Suppressor of Hairless/LAG-1) DNA-binding protein (RPBJ in Drosophila) and activates transcription of target genes. The CSL protein is bound to transcriptional co-repressors in the absence of NICD. The formation of the NICD-CSL complex displaces transcriptional co-repressors and recruits co-activators such as MAML (mammalian Mastermind/Lag-3) and MED8 (Mediator of RNA polymerase II transcription subunit 8), promoting the transcription of target genes. The downstream effectors of canonical Notch signaling are the transcription factors Hairy Enhancer of Split (HES) 1, 5, 6 and 7 and HES-related with YRPW motif (HEY) 1, 2 and HEY L (Figure 2).

HES belong to a family of basic helix-loop-helix (bHLH) genes and participate in the regulation of cell fate decision and boundary formation. Some of them have been implicated in specific disorders in humans. In particular, *HES1* is essential for Notch-induced T-cell acute lymphoblastic leukemia.[15] On the other hand, *HES7*, which is both a direct target of the Notch signaling pathway and part of a negative feedback mechanism required to attenuate Notch signaling,[16] was found mutated and non-functional in a family with autosomal recessive spondylocostal disorder.

Co-activators and co-repressors that are recruited during activation of Notch signaling are shared with other signaling pathways, and thus overexpression of NICD can affect the transcription of genes that are regulated by proteins apart from the Notch pathway. Elucidation of the nuclear environment is the key that will determine which targets are available to Notch-CSL-induced activation (many recent and comprehensive reviews are available for further reading).[17-19]

Since the protracted function of NICD can have serious adverse effects, optimal signal strength is primarily

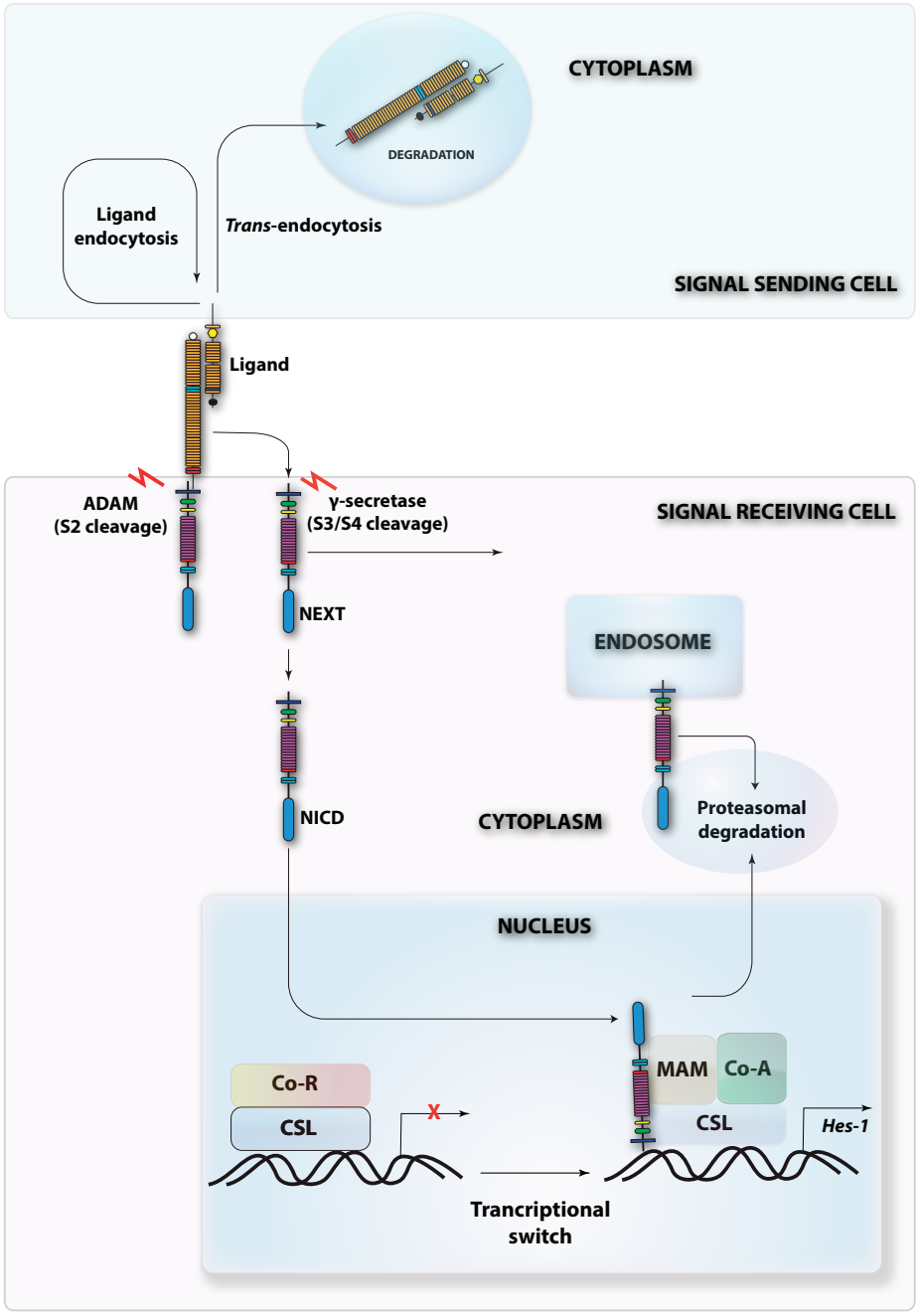

Figure 2. The intracellular cascade of Notch signaling.

regulated by ensuring that NICD half-life is short. Most Notch-mediated processes require a transient pulse of activity. In most cases the transcriptional activation process is followed by NICD phosphorylation within the PEST domain by cyclin-dependent kinase 8 (CDK8) and targeted for proteasomal degradation by E3 ubiquitin ligases.[20] This process eliminates NICD, disassembles the transcription of activation of the ternary complex and resets the cell for the next round of signaling. Other as yet uncharacterized kinases and E3 ubiquitin ligases are also likely to participate in NICD regulation. For every new round of signaling synthesis an assembly of new receptor molecules is a prerequisite.

3. NOTCH SIGNALING AND THE PITUITARY GLAND

3.1 Development

The development of the pituitary gland is based on the interaction between the neural ectoderm, which becomes the posterior pituitary, and the oral ectoderm fated to become Rathke's pouch. [21,22] The definitive Rathke's pouch initially contains undifferentiated proliferative progenitors. Gradually, these will differentiate and give rise to the five endocrine cell types present in the anterior pituitary.

During pituitary development, Notch signaling components are expressed in the invaginating pouch. They are then quickly down-regulated from the differentiating zone ventrally but are maintained dorsally around the lumen of Rathke's pouch.[23,24] *NOTCH 2* is expressed in the periluminal cells of Rathke's pouch that are undergoing rapid proliferation but not in the differentiated cells that express αGSU (α-subunit of glycoprotein) and are able to secrete glycoprotein hormones, such as TSH, FSH and LH.[25] *NOTCH 2* expression decreases as pituitary development proceeds, indicating that it may need to be absent for cell differentiation to occur. The time and location of *NOTCH 2* expression appears coincident with the expression of *PROP-1* (prophet of pituitary transcription factor), which precedes and is required for the expression of *PIT-1* (Pou domain transcription factor 1), the master transcription factor of pituitary development and hormone expression.[26] Mutations in the *PROP-1* gene have been associated with deficiencies in luteinizing hormone, follicle-stimulating hormone, growth hormone, prolactin and thyroid-stimulating hormone.[27] Expression of *NOTCH 2* is not detectable in *PROP-1* mutant pituitaries.[23] On the other hand, in mice with early embryonic conditional loss of *NOTCH 2, PROP-1* expression is affected, whereas early embryonic pituitary proliferation remains unaltered.[28]

There are several Notch family members expressed in the pituitary in similar spatiotemporal patterns to NOTCH 2, although their expression is maintained in *PROP-1* mutants. These include NOTCH 3, DELTA-LIKE 1 and HES 1. *HES1* expression overlaps with expression of *NOTCH 2* in undifferentiated cells in Rathke's pouch.[23] The normal location of *NOTCH 2* expression at the juncture between highly proliferating and differentiating cells, and the lack of expression in *PROP-1* mutants, make it a plausible candidate for explaining the profound organ disorganization characteristic of *Prop-1* mutant mice.[25]

According to recent data, Notch signaling in the pituitary controls the transition from proliferation to differentiation and potentially, through lateral inhibition, influences the differentiation of cells into the five hormone producing cell types. *Notch-2* knockout mice display early embryonic lethality and there is potential for redundancy of NOTCH 2 and NOTCH 3 function because of their overlapping expression patterns. Thus, it is difficult to discern the function of NOTCH 2 in the development of the pituitary using simple loss-of-function approaches. Certain studies have shown that persistent expression of activated NOTCH 2 interferes with the development of gonadotropes and, to a lesser extent, of thyrotropes (Figure 3).

Moreover, fully differentiated gonadotropes extinguish *NOTCH 2* transgene expression, suggesting that NOTCH 2 can inhibit cell differentiation in the pituitary gland similarly to what it does in the nervous system and pancreatic endocrine cells. In contrast, the proliferation of pituitary cells expressing activated NOTCH 2 does not seem to be affected.

In the adult gland, components of the Notch signaling pathway are expressed by cells in the same locations suggested as containing progenitor/stem cells.[29]

Exclusive deletion of R*bp-j* (Rbp-J-/-) in Rathke's pouch results in premature differentiation of corticotropes. Such a phenotype is also observed when the *HES1* gene is deleted[30] and is correlated with decreased proliferation, although one study also reported increased cell death.[31] Mice deficient for Hes1 and Hes5 exhibit severe pituitary hypoplasia due to increased differentiation of progenitor cells and also fail to form neurohypophysis because of incomplete evagination of the diencephalon,[30] pointing to the crucial role of Notch effectors together with ligands and receptors in development of both the posterior and anterior lobe of the pituitary.

It appears that Notch signaling is required to prevent early (corticotrope) differentiation and maintain undifferentiated progenitors fated for a later

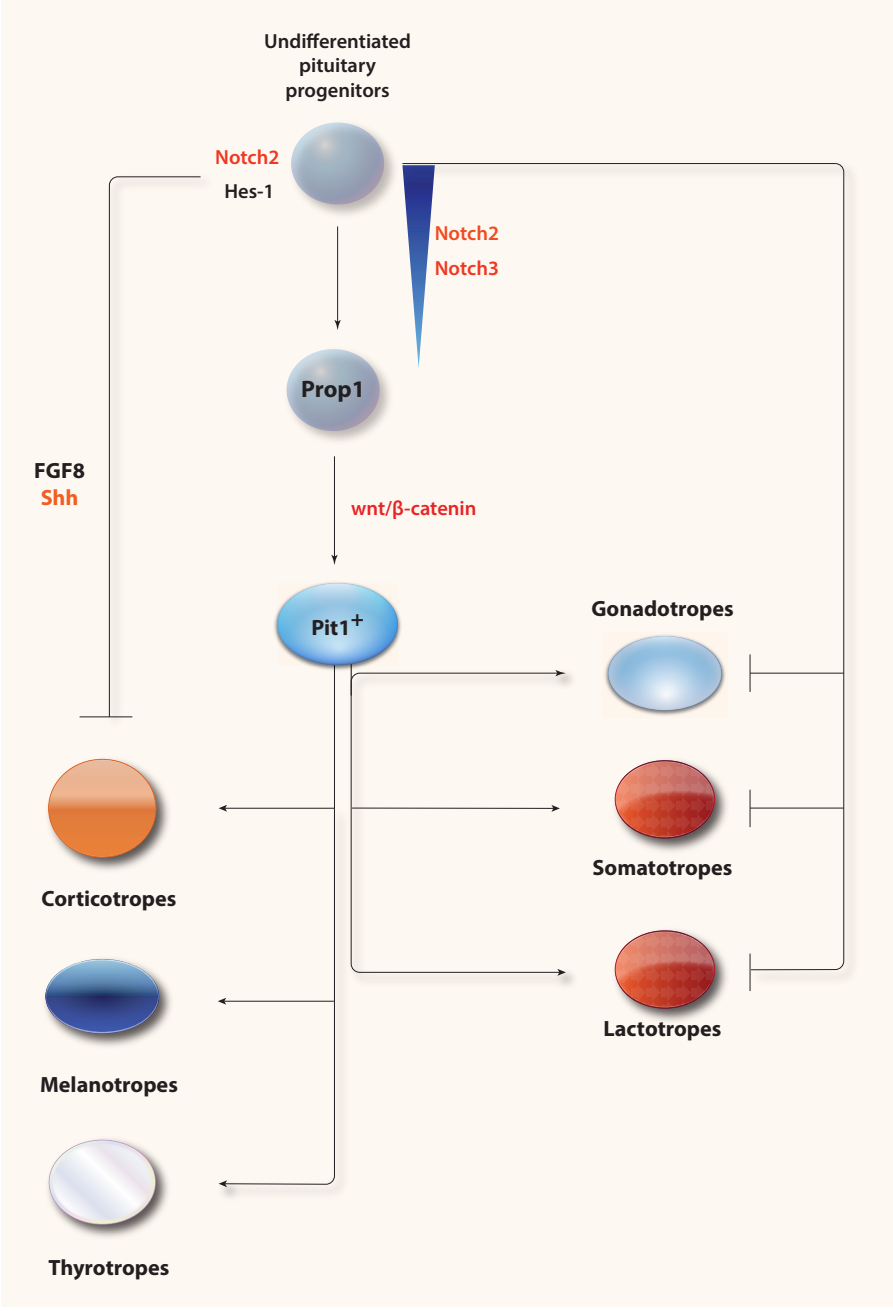

Figure 3. The main sites of action of the Notch, Wnt and sonic Hedgehog (Shh) during pituitary development. Notch2 and Notch3 are present in pituitary progenitors with diminishing presence as Prop1 increases its expression, allowing the beginning of differentiation. Shh controls mainly the corticotroph differentiation in co-ordination with members of the fibroblast growth factor (FGF) family. Wnt signaling controls the expression of Pit1.

Pit-1-dependent fate. In this way, Notch signaling enables the generation of different endocrine cell types by controlling the time and the context in which they differentiate. The failure of multiple cell types to differentiate in *PROP-1* mutants is probably attributed to lack of *NOTCH 2* expression.

3.2 Pituitary adenomas

Hypogonadotrophic hypogonadism (HH) is characterized by low levels of serum gonadotropins, delayed puberty and infertility. Although the genetic causes of HH are heterogeneous, activation of NOTCH 2 has been found sufficient to delay gonadotrope dif-

ferentiation and it has been proposed that some cases of gonadotrope dysfunction in HH might be due to aberrant Notch 2 signaling.[25]

NOTCH3 and JAGGED1 expression was found significantly increased in non-functioning pituitary adenomas, compared to normal human pituitary tissue.[32] In prolactinomas there was also a 1.5 to 5.3-fold increase in *NOTCH 3* mRNA expression, while in somatotropinomas there was a significantly reduced expression of *NOTCH 3*.[33] Although data on pituitary adenomas are scarce, activation of Notch seems to be associated with more undifferentiated and aggressive tumors.

4. HEDGEHOG SIGNALING AND ITS COMPONENTS

The Hedgehog (Hh) signaling pathway is comprised of a family of evolutionary conserved signaling molecules that play a significant role during embryonic development and remain active in adult life regulating a variety of cellular processes in both vertebrates and invertebrates. In adult organisms, it is implicated in the maintenance and homeostasis of stem cells and therefore a variety of skin (i.e. basal carcinomas) muscle and brain cancers develop when this pathway is impaired.

4.1 Ligands

There are three mammalian homologs of the Drosophila Hh gene: Sonic Hh (Shh), Indian Hh (Ihh) and Desert Hh (Dhh). Sonic Hedgehog is coded by the *SHH* gene, which is located on the long (q) arm of chromosome **7** at position 36 and is the most studied ligand.

The precursor forms of Hedgehog signaling proteins have a molecular weight of 45kDa; they undergo an autocatalytic internal cleavage of the C-terminal leading to a 19-kDa N-terminal domain, which displays Hedgehog signaling activity and a 25-kD C-terminal domain, which is active in precursor processing. This post-translational auto-processing consists of a simultaneous peptide bond cleavage and attachment of a lipophilic moiety to the newly exposed carboxyl-terminal of the amino-terminal molecule. The lipophilic modification is critical for the spatially restricted localization of the Hedgehog proteins on plasma membranes. Further modification to mature forms includes signal peptide cleavage and amino-terminal palmitoylation.[34]

This dual lipidation makes mature Hh proteins highly hydrophobic, and thus their release from the plasma membrane is an active process that is mediated by two transmembrane proteins: the 12 pass transmembrane protein Dispatched (Disp), which is required for release of membrane anchored Hedgehog protein and initiation of signaling to non-adjacent cells, and the Tout-velu (Ttv) protein, a homolog of the mammalian *EXT* tumor suppressor gene family, that regulates the synthesis of proteoglycans enabling the movement of Hedgehog ligands after their release and promoting long-range signaling.

Due to their lipophilic nature, the majority of secreted Hh are found in soluble multimeric forms reminiscent of intestinal micelle.[35,36]

4.2 Receptors

In vertebrates, Hh proteins bind to12 pass transmembrane receptors Patched 1 (Ptch1) and Ptch2 on responding cells. The transmembrane loops of Patched proteins are divided into two groups. Five loops compose the sterol sensing domain (SSD) of the receptor, which is important for the internalization of Ptch and the repression of Hh target genes. This sterol-sensing domain is found in HMGCoA (3-Hydroxy-3-methylgluatryl coenzyme A) reductase, Niemann-Pick C1 protein (NPC1) and SCAP (sterol regulatory element binding protein)—all proteins involved in cholesterol metabolism—and is considered to be an important regulator of the activity and stability of the proteins that contain them, in response to local sterol concentrations.[37] In the absence of Hedgehog proteins, Ptch receptors repress the activity of Smoothened (Smo), a seven-pass trans-membrane protein, which is attached to membranes of cytoplasmic microsomes that belong to G-protein-coupled receptors and are related to the Frizzled class of Wnt receptors. In contrast to the Frizzled receptors family, however, Smo is not connected to the trimeric complex of G-proteins and does not bind directly to the ligand. Moreover, it is constitutively active and is associated with lipid rafts, biochemically defined fractions of membranes enriched in cholesterol, sphingo-lipids and certain proteins, which are related to intracellular trafficking events.[38,39]

Mutant Ptch-SDD leads to inappropriate activation of the Smo protein and downstream target genes.[40]

Movement of Smo on the cell surface leads to interactions with elements of the cellular membrane and is protected by destabilization from protein phosphatases.

Loss-of-function mutations in Ptch receptors are associated with Gorlin syndrome which predisposes to basal cell carcinomas, medulloblastomas and rhabdomyosarcomas.[41] In addition, activating mutations of Smo have been implicated in the development of basal cell carcinomas.[42]

4.3 The intracellular cascade

In the absence of the Hedgehog ligand, the Patched receptor represses activity of Smo by preventing its membrane incorporation from endosomes. Downstream of Smo is a multi-protein complex, the Hedgehog signaling complex (HSC), which includes the Gli (glioblastoma proteins) family of zinc finger transcription factors in mammals, the serine/threonine kinase Fused (Fu), the kinesin-like molecule, Costal 2 (Cos2) and the Suppressor of Fused (Sufu). Cos2 is a docking protein that also binds to protein kinase A (PKA), casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3).[43]

The HSC is bound to microtubules/membranes and associates with Smo through Cos2. There are three Gli genes in mammalian cells, Gli1, Gli2 and Gli3, which have partially overlapping functions. Gli1 and Gli2 are transcriptional activators, while Gli3 functions as a transcriptional repressor. When the Hedgehog signaling is inactive, the Sufu and Cos2 allow phosphorylation of the microtubule-bound pool of Gli transcription factors, leading to their degradation through ubiquitination.[44-46]

In the un-stimulated cells, Gli2 and Gli3 are expressed at significant levels, while Gli1 transcription is increased in the presence of Hedgehogs and activates expression of Hh target genes. Binding of Hedgehog signaling proteins to Ptch receptors promote their internalization and de-represses the activity of Smo. Phosphorylation of the intracellular carboxylterminal part of Smo[35] promotes its translocation from intracellular vesicles to plasma membrane. In turn, activation of Smo inhibits the proteolytic processing of Gli2 and Gli3 and enhances the production of full-length EXP transcription-activating forms of these proteins that translocate to the nucleus and activate transcription of target genes (Figure 4). Gli1 acts in conjunction with Gli2 and Gli3, although the loss of Gli1 alone does not cause Hh signalling defects.[47] Hh target genes include several components of the pathway itself including Gli1, which is considered the earliest response of Hh activation, Gli3 which is regulated by a negative manner,[48] Ptch1 and Hedgehog interacting protein (Hip). Hip is a transmembrane glycoprotein that functions as a putative antagonist of the pathway binding to Hh proteins with similar affinity to Ptch1 and sequesters them in a negative feedback loop.[49,50] Among the Hh target genes, the expression of Gli1 particularly, which is not observed in the absence of Hh, is a very useful marker of Hh signaling activation.[51,52]

5. THE ROLE OF SONIC HEDGEHOG HOMOLOG IN PITUITARY DEVELOPMENT

During embryogenesis, *Shh* is expressed in the early oral ectoderm and ventral diencephalon at 8 dpc in the mouse embryo. The pituitary placode, which arises from a patch of oral ectoderm by 9 dpc is negative for *Shh* expression, reflecting the role of Shh signaling as the molecular boundary that defines the area of the developing pituitary.[53,54] The *PTC1* gene is expressed in the Rathke pouch, demonstrating that these cells can receive Hh signaling. Expression of *SHH*, however, is excluded from the pouch as soon as it becomes morphologically visible, allowing the proliferation of the anterior pituitary cells.[53]

Shh null mice display pituitary agenesis but is not clear whether *Shh* is directly required for pituitary development since these mice also have disrupted ventral diencephalon.[49] Expression of *SHH* in ventral diencephalon is necessary for restriction of pituitary expansion to the middle brain and attenuation of the Shh signaling leads to pituitary enlargement.[55] However, it has been shown that increased expression of *Hip* under the control of the *Pitx1* enhancer, which is active in the oral ectoderm and Rathke's pouch, leads to pituitary agenesis but normal ventral diencephalon,[54] indicating a direct role of Hh signaling. On the other hand, overexpression of *Shh* in the pituitary under the control of a GSU promoter leads

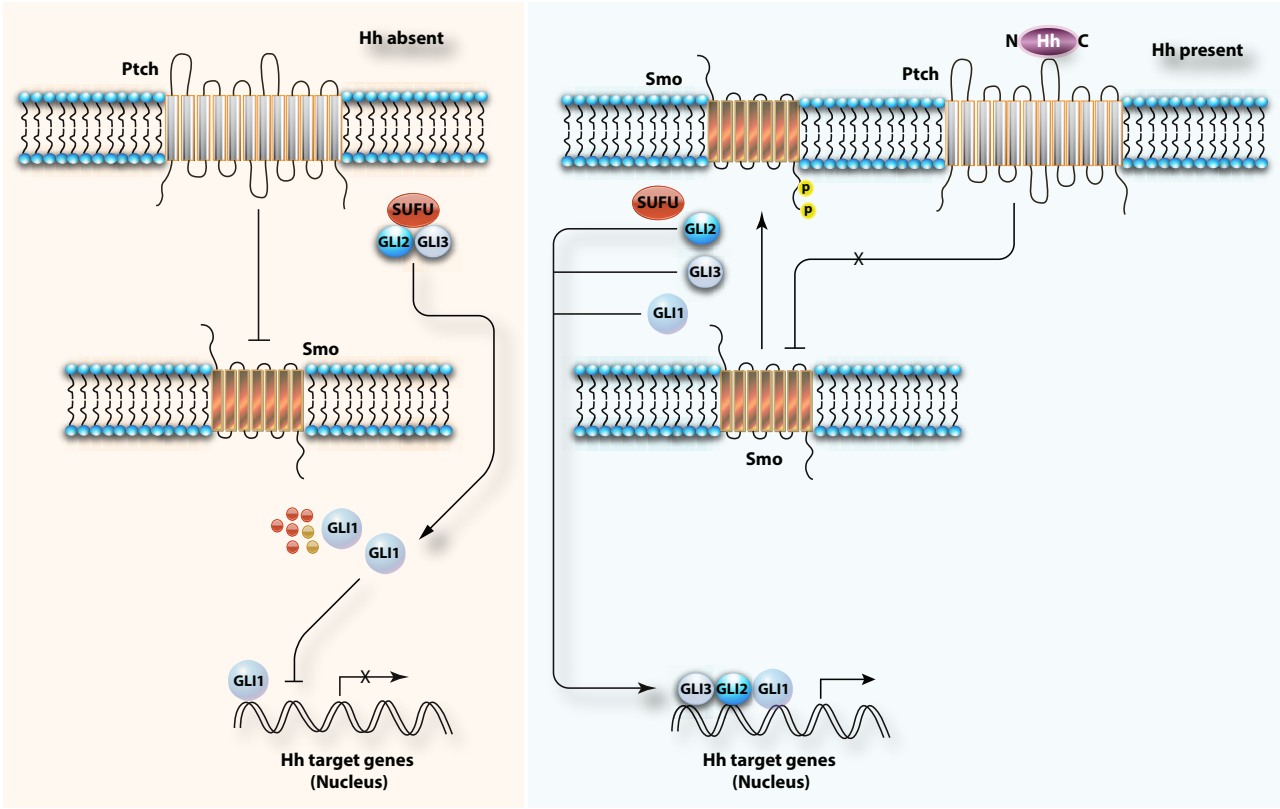

Figure 4. The intracellular cascade of Hedgehog signaling.

to pituitary hyperplasia, particularly of the ventral pituitary cell types (gonadotrophs and thyrotrophs).[54] It has been shown that Shh regulation of ventral pituitary cell specification and proliferation is mediated, at least in part, through bone morphogenetic proteins (BMPs). Gli2 null embryos have reduced expression of BMP4 and fibroblast growth factor expression 8 (FGF8), demonstrating the essential role of the Gli family of transcription factors in proliferation of early progenitors and induction of transforming factors during embryogenesis.[56] In line with the mouse model, inactivating *GLI2* germline mutations in humans is associated with severe pituitary developmental defects and holoprosencephaly.[57]

6. THE ROLE OF SONIC HEDGEHOG HOMOLOG IN THE ADULT PITUITARY AND PATHOGENESIS OF PITUITARY ADENOMAS

Expression of SHH, PTCH2, but not PTCH1, and GLI1 at the level of RNA and protein has been detected in normal anterior pituitary gland in corticotroph cells.[58] Cells that express other pituitary hormones did not show SHH expression. Therefore, since GLI-1 expression requires activation of Shh signaling, this study demonstrated that Shh/Pth2/Shh signaling is active in the corticotrpoph cells of the adult pituitary.[58] PTCH1 expression was found in thyreotrophs and gonadotrophs, and PTCH2 was also expressed in somatotrophs and, at a lower percentage, in lactotrophs.[59] In addition, Shh administration in a rat primary pituitary cell culture and mouse corticotropinoma cell line (AtT20) induced ACTH and pro-opiomelanocortin secretion through a Gli1-dependent transcriptional activity.[58] Co-stimulation of mouse corticotropinoma cells with Shh and CRH showed an additive effect on ACTH and POMC secretion. Moreover, Shh increased the expression of the CRH receptor, while CRH stimulated Gli1-transcriptional activity, demonstrating a crosstalk between CRH and Shh pathways that converge at the Gli-1 transcription factor.

Although Shh was found to be selectively produced

by corticotrophs, addition of Shh in rat pituitary primary cultures increased secretion of growth hormone (GH) and prolactin (PRL).

In pituitary adenomas, PTCH1 and PTCH2 proteins are expressed at variable levels.[59] Unlike data concerning the normal pituitary, the Shh protein is not expressed in corticotropinomas. However, in primary cell cultures established from pituitary adenomas, exogenous Shh increased secretion of GH, PRL and ACTH from somatotropinomas, lactotropinomas and Cushing tumors, respectively.[59] In addition, administration of Shh in pituitary tumor cell lines exerted antiproliferative effects, thus its significant downregulation in pituitary adenomas could play a pathogenetic role in their development. Collectively, the data reported so far are indicative of a functional role of Shh signalling in pituitary tumorigenesis.

7. NOTCH AND HEDGEHOG SIGNALING CROSSTALK

Interaction between the Notch and Hedgehog signaling pathways is of critical importance during organogenesis where their concerted actions modulate survival, proliferation and differentiation of target cells ensuring the appropriate development of organs and the specialized functions of tissue-specific cells.

Recent data have demonstrated the importance of this crosstalk in cancer biology and in the development of chemotherapy-resistant cancer stem cells.[60] In addition, Notch and Hedgehog interaction is implicated in the regulation of the fate of embryonic stem cells,[61] neural progenitors in zebrafish[62] and eye precursors in Drosophilla.[63]

In adult tissues, Notch and Hedgehog crosstalk stimulates hepatic stellate cells to become myofibroblasts through activation of epithelial-to-mesenchymal-like transition.[64] When Hedgehog signaling is suppressed in liver myofibrolasts/hepatic stellate cells, Notch is also blocked inducing a mesenchymal-like-to-epithelial transition. In this way, Notch/Hedgehog interaction regulates adult liver repair of damaged cells by modulating mesenchymal-like-to-epithelial and epithelial-to-mesenchymal-like transition.[64] In particular, regulation of Notch signaling by Hh proteins in liver cells is downstream of Jagged 1 and is mediated, at least in part, through modulation of NOTCH 2 expression.[64]

In the central nervous system, neural progenitor cell proliferation is regulated by Hes1 independent of Notch signaling and is activated by Shh through transcriptional activation of Gli2.[65] The stability of Gli proteins[66] enhances Gli2 binding to Hes1 promoter at two Gli consensus sites only in the presence of Shh signaling, suggesting the mechanistic link between Shh-Gli2 signaling and Hes1 in regulating the proliferation of neural progenitor cells.[65] On the other hand, in brain tumors such as medulloblastoma and glioblastoma, Notch signaling via Hes1 suppresses Shh activity by directly inhibiting expression of Gli1.[67] Primary human-derived glioblastomas presented a dramatic response to co-inhibition of Notch and Hedgehog signaling. However, these inversed expression levels between Hes1 and Gli1 were not found in all subtypes of glioblastomas in humans,[68] implying that other factors, such as binding partners, heterochromatin structure or methylation, may also contribute.

The exact molecular mechanisms that underlie this feedback mechanism in cancer remain unknown and require further investigation. Co-treatment with Hedgehog and Notch inhibitors in advanced breast cancer (NCT01071564) is currently underway and may pave the way for examining the safety of such co-treatment potential.

In pituitary adenomas this interaction has not been studied yet. However, it seems that the increased expression of NOTCH 3 and JAGGED 1 reported in some studies[32,33] and the low expression or absence of Shh and Patched 1 and 2 found by others[59] could be more than coincidental and reflect an underlying feedback regulation between the two that is implicated in the pathogenesis of pituitary adenomas.

8. CONCLUDING REMARKS

During embryonic development, the question remains as to how Notch, Wnt and Hh signaling integrate their activity with other cell inputs to control specific developmental events (Figure 5). Another important issue is the mechanisms by which these signaling molecules activate distinct target genes according to cell type and time. Genomic studies will undoubtedly increase the number of target genes and

facilitate development of a systematic approach for understanding these different responses in the future.

Impaired signaling activity of these pathways is implicated in the pathogenesis of cancer and disease, but it remains to be determined whether modulation of such pleiotropic and cross-talking pathways could be a promising target for therapeutic approach.

Notch 3 and Jagged 1 have been implicated in the pathogenesis of human non-functioning pituitary adenomas thereby providing a potential therapeutic target for the medical treatment of these tumors. Several elements of the Notch pathways have also been identified in the transcriptome of prolactinomas and multihormonal pituitary adenomas.[69]

In addition, Notch and Wnt signaling are important regulators of cell to cell communication which is used by pituitary stem cells during self-renewal and differentiation into multiple types of specialized cells.[70] Evidence from studies in humans and in mice has shown that the effector of Wnt signaling, β-catenin, exerts a dramatic effect on the expansion of the pituitary progenitor pool and the decision to differentiate.[71] Moreover, a very common intracranial tumor in children, craniopharyngiomas, seems to arise from activation of β-catenin in pituitary progenitors during embryogenesis, this shedding more light on the pathophysiology of pituitary adenomas.

Hh signaling also exerts differential effects on pituitary cell growth (with stimulating effects on

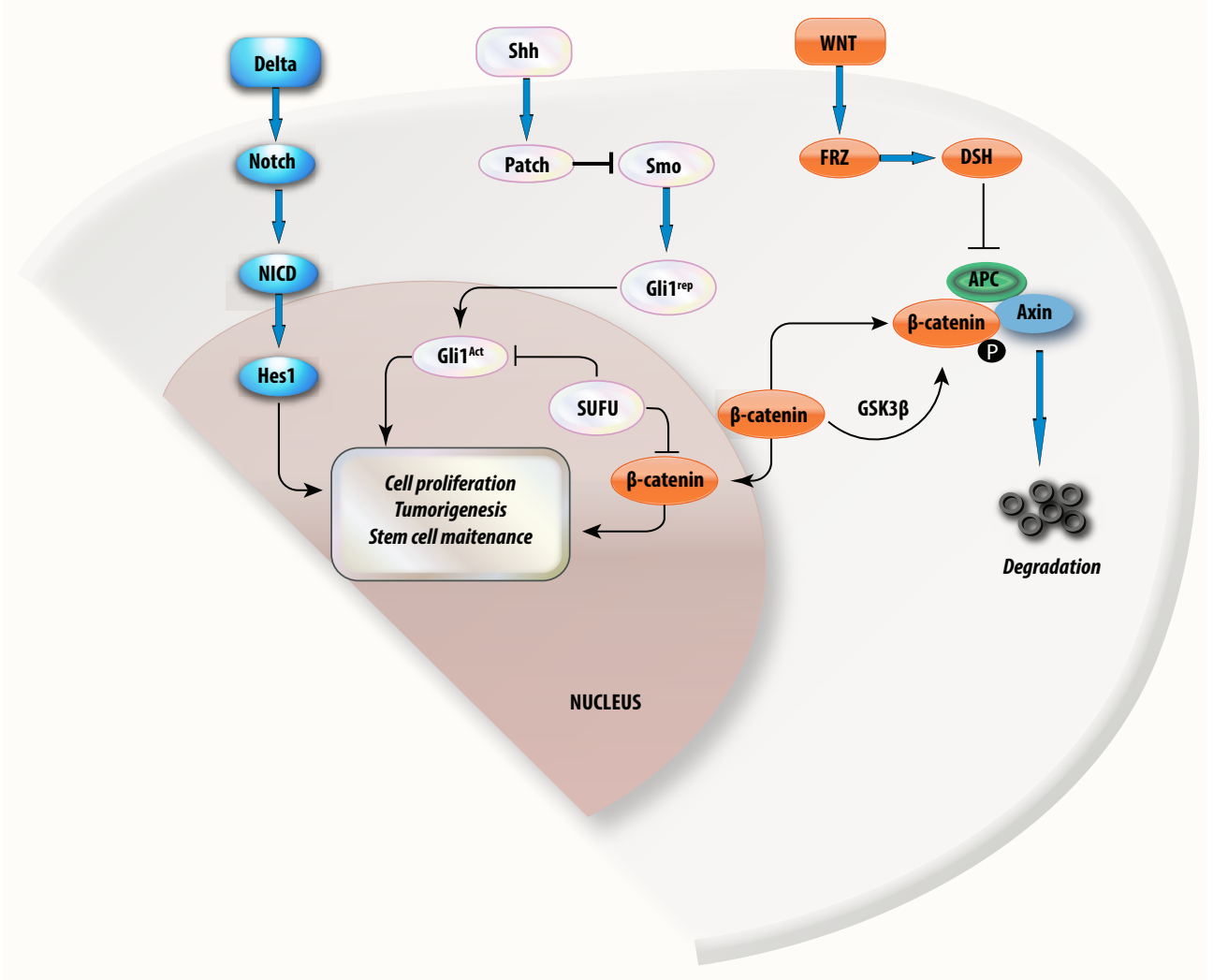

Figure 5. An integrative diagram of Notch, Hedgehog and Wnt signaling crosstalk in the regulation of normal development and tumorigenesis.

progenitor cells and inhibiting effects in differentiated cells) and is able to modulate hormone secretion and proliferation in pituitary adenomas. Moreover, exogenous treatment with Hh proteins in cell cultures has been shown to increase hormone secretion from pituitary tumor cells, reflecting their role as hypophysiotropic factors that regulate pituitary hormone release in normal and tissue cells.

Taken together, Hh and Notch signaling pathway crosstalk appears to play significant roles in pituitary development and tumorigenesis, this evidence pointing to novel targets for therapeutic interventions. Ongoing research will definitely increase our understanding of the role of these pathways in the development of pituitary adenomas and pave the way for their potential therapeutic targeting in humans.

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