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REVIEW

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Role of Ihh — a progesterone-responsive gene in mammalian reproduction: a review

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Abstract

Indian hedgehog (Ihh) is a member of the developmentally regulated morphogens, the hedgehog gene family. The Hh protein family was initially discovered in *Drosophila* and has since been widely investigated in both *Drosophila* and higher animals. Ihh exhibited a dynamic spatiotemporal expression pattern in the mammalian uterus and ovaries. The downstream targets of the Ihh signaling pathway include PTCH-1, SMO, and COUP-TFII. Ihh is a progesterone-responsive gene that plays an important function in the female reproductive system; conditional ablation results in infertility due to failed embryo implantation. The literature addressing Ihh's functions and ways of action is expanding, as is the number of processes that use it in cell signaling as well as physiology. Even while our grasp of the path has expanded tremendously, we still have many gaps in our knowledge. This review will address the discovery, evolution, mechanisms, and manifestations of Ihh especially in mammalian reproduction.

Keywords Ihh, Progesterone-responsive gene, Discovery, Downstream targets, Significant role

Background

During implantation, the embryo binds to the receptive uterine epithelium, resulting in pregnancy [1]. Later, the embryo invades the underlying endometrial stroma, where the stromal cells are converted into decidual cells that promote embryonic growth and survival. The steroid hormone progesterone (P) is important during pregnancy establishment and maintenance because it has a significant impact on endometrial functions. In preimplantation phase, P acts in concert with 17β estradiol (E) to orchestrate changes in the uterine epithelium rendering it competent for embryo implantation [2]. In mice, ovarian E on day 1 and day 2 of pregnancy stimulates proliferation of uterine epithelium. During this E-dominated phase, the epithelium has a unique columnar phenotype and makes cell–cell interactions via intracellular tight and adherens junctions. The uterine epithelium stops proliferating and begins to differentiate in response to increased P levels, beginning in the middle of day 2 of pregnancy. Upon differentiation, the luminal epithelium undergoes structural remodeling that includes the breakdown of tight and adherens junctions, allowing for embryo attachment and invasion [3]. On day 4 of pregnancy, as the embryo adheres to the luminal epithelium, the surrounding fibroblastic stromal cells differentiate into distinct secretory decidual cells. P is the primary driver of this differentiation process, termed decidualization, which is a prerequisite to successful implantation [2]. There are numerous genes which elucidate the molecular mechanisms by which P regulates the early steps leading to the acquisition of uterine receptivity for implantation and successful establishment of pregnancy.

Ihh is one of those P-regulated genes which is expressed during the time of implantation and has role in uterine receptivity and establishment of a successful pregnancy [4]. Ihh signaling has been shown to be important for the development of multiple tissues including the limbs, cerebellum, bone cartilage, gonads, and heart [5]. Deregulation of hedgehog signaling has been implicated

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in cancers, such as basal cell carcinoma, medulloblastoma, pancreatic cancer, prostate cancer, and lung cancer [3]. Ihh is expressed in the mouse uterine luminal epithelium in the preimplantation period, with its highest expression on day 3 (D3), whereas its downstream target genes, patched 1 (PTCH-I), smoothened (SMO), and chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) are expressed in the uterine stroma, with their highest expression on D4 during the "window of receptivity" [6]. In endometrium, Ihh expression significantly decreases during the transition from the early to the mid-secretory phase, which is associated with a downregulation of cellular division.

The discovery of hedgehog

The embryonic development is organized by a small set of secreted signaling molecules that together mediate the inductive interplay between cell populations that carve the form of an animal. Among these signaling molecules, the members of the hedgehog (Hh) protein family are the prominent one [7]. The Hh gene family is a member of the developmentally regulated morphogens. In the 1970s, the understanding of how a very basic egg can give birth to a complicated segmented body plan was a major topic in developmental biology. Using a saturation mutagenesis technique, Christiane Nusslein-Volhard and Eric Wieschaus discovered a set of genes involved in the development of body segmentation in the late 1970s. These mutations control the development of the segmented anterior–posterior body axis of the fly. For their research on genetic alterations in *Drosophila* embryogenesis, Christiane Nusslein-Volhard, Eric Wieschaus, and Edward B. Lewis received the 1995 Nobel Prize [8].

The hedgehog (hh) gene family is named after a mutant phenotype that occurs when *Drosophila* embryos missing hedgehog (hh) gene activity are covered with denticles, which are tiny, pointed projections that resemble hedgehog spikes. Dr. Clifford J. Tabin, a developmental biologist at Harvard Medical School, advocated naming each newly found gene after a certain hedgehog species. This method worked for the first two genes, i.e., the Indian hedgehog (Ihh) and desert hedgehog (Dhh). However, Dr. Robert Riddle, a postdoctoral fellow in Tabin's lab, disobeyed the system and named the third homolog sonic hedgehog (Shh), after the protagonist of a Sega video game. This was because he had uncovered the most fascinating hedgehog gene yet known [9] (Table 1).

Hh genes have been found in various invertebrate species, including the leech and sea urchin, as well as the cephalochordate amphioxus [7]. The nematode worm *Caenorhabditis elegans* lacks Hh homologs but does have numerous genes that encode proteins related to the Hh downstream target patched (PTCH) [10]. Vertebrate hedgehog genes were discovered in 1993 as a result of a coordinated effort between three groups (fish, chick, and mouse) [11]. The following year, Chang et al. published an additional report on hh homologs. These preliminary results contain a number of surprises. Unlike the drosophila, which has a single hh gene, vertebrate species have many related genes. Three hh genes have been found in mice: Shh, Dhh, and Ihh. Dhh is the most closely linked Hh homology to *Drosophila*, but Shh and Ihh are more similar to one another [9]. The phylogenetic tree (Fig. 1) depicts the evolutionary connections between various homologs.

Table 1 History of the hedgehog family

Year	Events	References
1970	Discovery of Hh gene in *Drosophila*	[8]
1993	First vertebrate Hh gene reported	[11]
1994	Nomenclature of Hh homologies	[9]
1995	Nobel Prize for research on genetic alterations in *Drosophila* embryogenesis	[8]

All Hh family homologs are involved in actions that are critical to the development, patterning, and morphogenesis of many different areas within the body plans of vertebrates, insects, and most likely other invertebrates. In some cases, Hh signals function as morphogens, inducing diverse cell fates within a target field in a dose-dependent manner; in others, they serve as mitogens, regulating cell proliferation or initiating factors, determining the shape of a growing organ (Table 2). Furthermore, in recent years, Hh proteins have been implicated in a wide range of processes in the developing embryo. Indeed, a Hh signal influences almost every aspect of a vertebrates' body layout [12].

The structure of Ihh protein

Ihh is a 45.251-kDa protein yielded by signal peptide cleavage between Gly^{65} and Cys^{39}. The protein has a highly conserved core region of around 411 amino acids. The Ihh protein is composed of two domains: an amino-terminal domain (HhN) called the Hedge domain and a carboxy-terminal autocatalytic domain (HhC) called the Hog domain [23]. The HhN domain has the biological signal activity, whereas HhC domain deals with cholesterol moiety. The HhC domain cleaves Hh into two parts in an intramolecular reaction and adds a cholesterol moiety to HhN. The Hog domain is again separated into two regions; the first two-thirds shows resemblance with self-splicing inteins, and the module has been named Hint, whereas the carboxy-terminal third binds cholesterol in Hh protein and has

been named as sterol-recognition region (SRR). The tip of the whole structure consists of the signal peptide sequence for protein export (SS) [24] (Fig. 2).

Terminology

- *Hedge domain*: Comprehensive term for the amino-terminal domain of Hh proteins

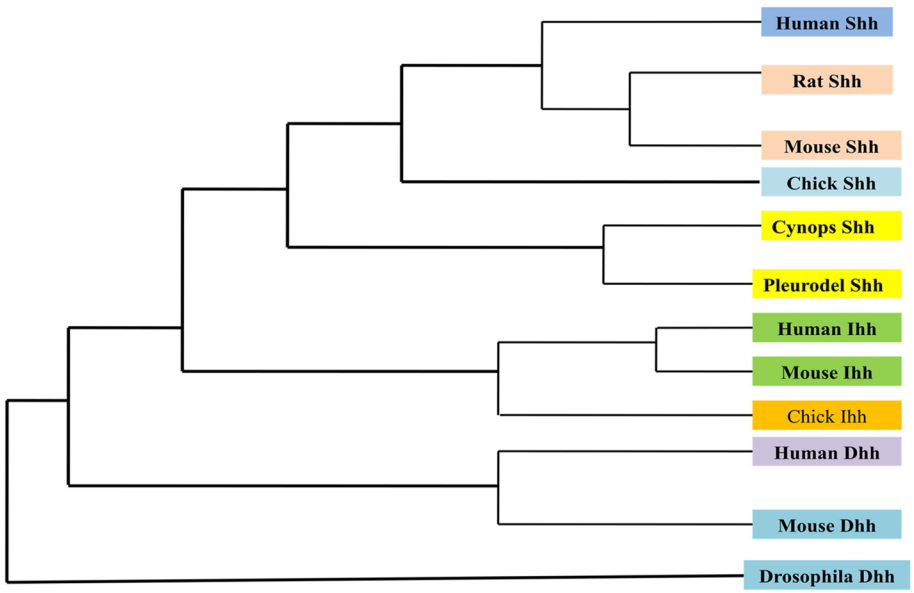

Fig. 1 Phylogenetic relationships between members of the Hh protein family from various species

Table 2 Functions of the hedgehog genes in vertebrates

Cell type/organ	Ligand	Nature of role	References
1. Blood cells	Ihh	Activation of hematopoiesis	[13]
2. Bone and cartilage	Ihh	Differentiation of endochondral skeleton	[14]
3. Gonads	Dhh	Maturation of testes, Sertoli-Leydig cell interactions	[15]
4. Heart	Ihh	Cardiac morphogenesis	[16]
5. Limbs	Shh	Outgrowth of limb bud	[17]
6. Lungs	Shh	Branching epithelium	[18]
7. Muscle	Shh	Regulation smooth muscle differentiation	[19]
8. Pituitary	Shh	Cell type determination	[20]
9. Pancreas	Shh	Insulin production	[21]
10. Eye	Dhh	Retinal precursor proliferation	[22]

Fig. 2 Structural features of Ihh protein, adapted from [23]

- *Hog domain*: Comprehensive term for the carboxy-terminal of Hh proteins. The Hint and SRR region together comprises the Hog domain.
- *Hint module*: A autoproteolytic module found in hedgehog protein and self-splicing inteins
- *SRR module*: The cholesterol-binding site of HhC.

The signaling pathway

Indian hedgehog (Ihh) is a known component of the Hh signaling pathway and a progesterone receptor target gene [25]. Ihh signaling begins in the uterine epithelium compartment and progresses from epithelial to stromal cells within the uterus. Patched-1 (PTCH 1) is the transmembrane receptor responsible for signal transmission [26]. The major function of PTCH-1 is to inhibit smoothened (SMO) activation, which is another transmembrane receptor. The SMO is activated as soon as the Hh ligand attaches to PTCH-1, stopping its attempt to suppress it. The chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) is a significant factor that is activated when SMO is activated [27]. After the uterine stroma's COUP-TFII is activated, the downstream target receives a signal that establishes the Ihh-COUP-TFII axis inside the two uterine compartments. During the postimplantation phase, COUP-TFII is a crucial effector of decidualization and pregnancy maintenance [28] (Fig. 3).

COUP-TFII

COUP-TFII is a member of nuclear receptor superfamily and has been identified as a crucial regulator in proliferation, decidualization, cell survival, and progesterone sensitivity. Research have showed that ablation in COUP-TFII results in embryonic lethality due to defects in vascular development [29]. Mice heterozygous for the ablation of COUP-TFII show reproduction defects including the inability of the uterus to undergo the events required to support a successful pregnancy [30]. Therefore, even the loss of one allele of COUP-TFII can impair reproduction. On day 3 and day 4 of pregnancy, Ihh is expressed in uterine epithelium, followed by COUP-TFII expression in the stromal cells [25]. The expression of COUP-TFII is just prior to the window of receptivity giving further support for a function of this signaling axis in preparing the uterus for embryo implantation.

The Ihh-COUP-TFII *axis*

The establishment of Ihh-COUP-TFII axis within the uterus is very crucial for both implantation and decidualization. The absence of this axis results in embryonic inability for attachment to the uterine lumen leading to implantation failure [31]. This axis acts concurrently between both uterine compartments to carry out successful progesterone receptor (PR) function in early pregnancy. Thus, this axis plays a very critical role for the proper development and preparation of the uterus for the implanting embryo.

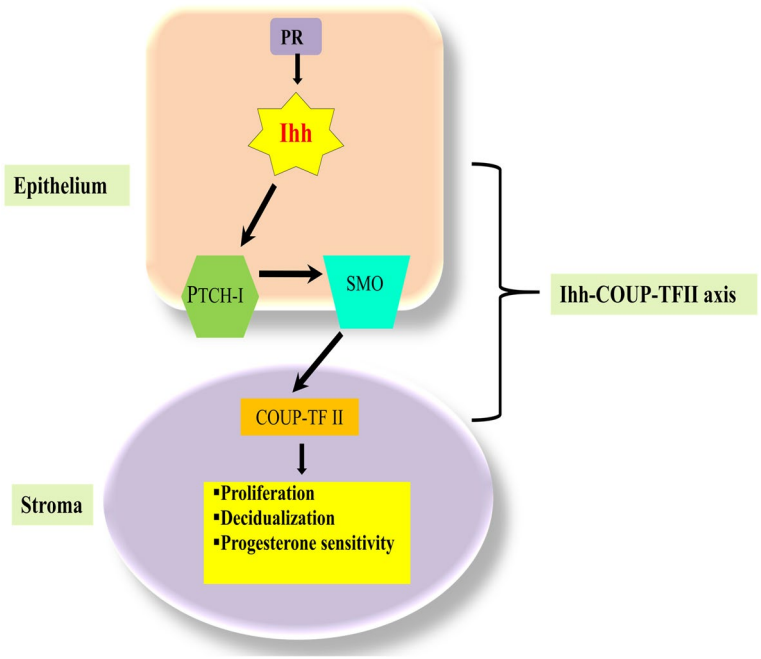

Fig. 3 The progesterone-regulated Ihh signaling model

In human endometrium, the Ihh was found to be expressed at the secretory phase [32]. Increased level of Ihh protein and mRNA levels during proliferative phase upon treatment with selective progesterone receptor modulator (SPRM) determines the dependency of the gene on PR. Another research work demonstrated that Ihh expression is dysregulated in patients with endometriosis [33], while COUP-TFII was found to be expelled within endometriotic and eutopic endometrial stromal cells [34]. Consequently, it has been observed that the Ihh-COUP-TFII axis, which was shown to be significant in the murin system, is retained in humans as well.

Expression in uterus

Progesterone (P) act through its cognate receptors plays crucial role in regulating uterine processes essential for embryo implantation. The progesterone receptor (PR) signals are critical regulators for crosstalk between the epithelial and stromal compartments of the uterus. In mouse, uterine Ihh was shown to be induced by PR [35]. Ihh expression is restricted to the epithelium, whereas its established effectors, PTCH-1 and COUP-TFII, are coordinately expressed in the endometrial stroma [25]. Ihh signaling pathway underlies inter-compartmental cellular communication that is obligatory for the establishment and maintenance of the maternofetal interface in the uterus [4].

In uterus during preimplantation period, the uterine stroma undergoes P-mediated increase in cell proliferation and vascularization, and this becomes the preparatory stage for the decidual cell reaction [36]. Ihh is a very decisive factor for cellular proliferation and vascularization, which are two distinct cellular responses that prepare the uterine stroma for the induction of the decidual response. Research on molecular effects of Ihh ablation showed that the expression of PR in uterus remains unaffected, but the expression of PTCH-1 and COUP-TFII significantly decreases [37]. Ihh ablation does not have affect on overall P signaling but does regulate a crucial subset of genes that are necessary for uterine function [38].

In uterus, for an appropriate cellular response, the communication between the epithelial and stromal compartments is mandatory. Ihh is a pathway which act as a molecular bridge between the two uterine compartments through which P projects its effects on cell growth, differentiation, and angiogenesis. Ihh has evolved specifically as a uterine mediator of the P signal. Ihh is not induced by P in other progestin target-tissues like ovary, pituitary, or mammary gland [39]. Ihh pathway is the cardinal signaling cascade downstream of PR, and that other P uterine molecular targets fail to act as alternative pathways. Moreover, uterine Ihh signaling pathway spans the epithelial-stromal cleave.

IHH expression in ovary

In the postnatal mammalian ovary, androgens are primarily produced by the theca cells. These theca cells subsequently differentiate into granulosa cells which serves as the major source of estradiol 17β [40]. During the postnatal period, follicle recruitment and development commence from the pool of primordial follicles, aligning with the initiation of the female reproductive cycle and ovulation. This process is regulated by the feedback effects of LH and FSH from the pituitary, leading to steroidogenesis, specifically the production of estradiol-17β by granulosa cells of the Graafian follicle and progesterone by lutein cells of the corpus luteum [41].

During the adult reproductive life, the recruitment of follicles from the primordial pool is an uninterrupted process which led to the formation of primary follicles and sets the basis for subsequent follicle development [42]. In a healthy developing follicle, the growth of the oocyte and the proliferation and differentiation of the somatic granulosa and thecal cell compartments are highly coordinated events. This demands intercellular communication between these cell types and compartments.

The mammalian ovary acts as a novel site of active Ihh signaling. Granulosa cells of growing follicles serve as a source of Ihh signaling [43]. Initiation of follicular growth in ovary can be defined as the transition of a nongrowing primordial follicle to a primary follicle. During this transition, granulosa cells increase in number and change its morphology from a squamous to a cuboidal cell. Granulosa cells of primordial follicles do not express Ihh [44]. Ihh mRNAs were first detected when granulosa cells take up the cuboidal morphology and attain the primary follicular stage. Induced expression of Ihh downstream target gene PTCH-1 is detected in mesenchymal cells adjacent to granulosa cells [45]. Ihh signaling does not play part in the initiation of follicle growth but rather starts to act early after the transition from the primordial to the primary follicle stage. So we can deduce that expression of Ihh mRNAs initiates in granulosa cells at the primary follicle stage, while the induced expression of hedgehog target gene PTCH-1 was found in the surrounding pre-theca cell compartment. The thecal cell compartment remains a target of Ihh signaling throughout follicle development manifesting induced expression of the downstream hedgehog target genes [35]. The important role of Ihh signaling in ovary is to communicate between granulosa cells and developing theca cells (Endocrinology 146: 3558–3566, 2005) (Fig. 4).

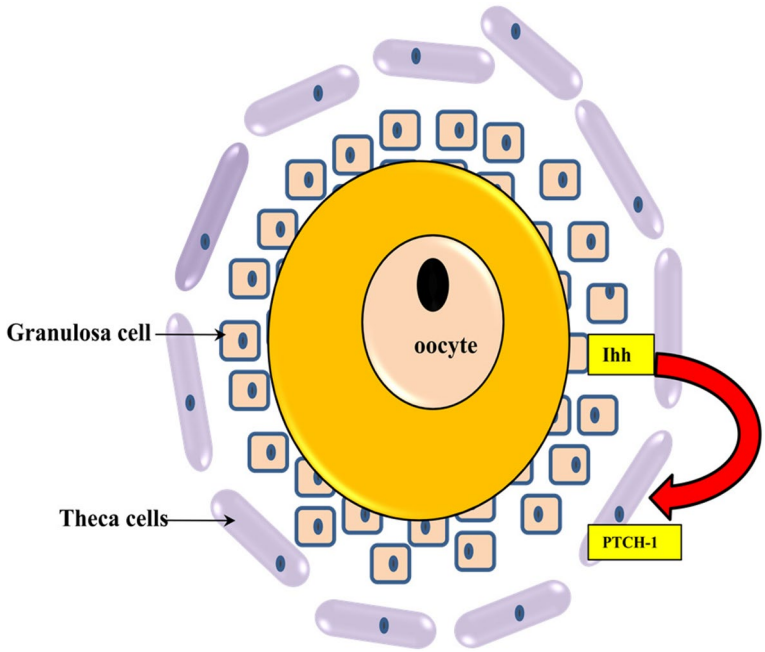

Fig. 4 Ihh signaling in granulosa cell-induced expression of PTCH-1 in theca cells

Cross-linking of Ihh and other transcriptional factors during early gestational period in mice

Ihh is critical for proper adult uterine function since conditional ablation of Ihh in the uterus causes infertility in mice due to poor embryo attachment and decidualization. Microarray study of the Ihh target genes at their greatest expression level revealed 863 Ihh-regulated genes. Ihh influences embryo implantation by regulating stromal cell proliferation, inhibiting epithelial E signaling, and triggering steps required for effective embryo implantation [46]. Leukemia inhibitory factor (LIF) is a cytokine of the interleukin-6 family and is a major mediator for action of E. LIF is secreted mainly from the uterine gland by nidatory E on the fourth day of pregnancy and is expressed in the subluminal stroma at the implantation site [47]. Secreted LIF activates signal transducer and activator of transcription 3 (STAT3) via heterodimerization of LIF receptor. LIF can be substituted for E action in terminating artificial delayed implantation and reinitiation of embryo implantation in mice. Both of these two factors, i.e., LIF and Ihh, are expressed in uterine epithelium during implantation [47]. Uterine Ihh mRNA was not detectable on day 1, but rose on days 3–4, and then reduced on day 5 of pregnancy. The expression of PR mRNA and protein in the luminal epithelium matched that of Ihh, but unlike the epithelium, progesterone receptor levels increased in the stroma after implantation [8]. However, the LIF mRNA in mice did exhibit the same expression patterns like Ihh and PR mRNAs. This suggests that LIF might have a cross-link, regulating the expression of Ihh and progesterone receptor mRNA in the luminal epithelium. Expression of Ihh mRNA increased after LIF injection in wild-type mice. Administration of E induces LIF mRNA, but not Ihh mRNA in ovariectomized mice without P treatment. This indicates that P is required for upregulation of Ihh mRNA mediated by LIF. The peak expression of PR mRNA was preceded by that of Ihh mRNA after LIF injection. LIF increases Ihh mRNA and other P-related factors by upregulation of PR in luminal epithelium. Uterine LIF is indued by E surge on day 4 which results in high expression of Ihh mRNA on day 4 [48]. These findings imply that LIF has an influence on upregulating Ihh levels.

In another study, it has been reported that coadministration of LIF and P leads to a synergistic stimulation of Ihh expression in luminal epithelium during early pregnancy. The group of Demayo and colleagues had shown that Ihh produced by the luminal epithelium acts on its receptor PTCH1 on the stromal cells to induce COUP-TFII, an essential factor for decidualization. These findings provide a plausible mechanistic pathway linking glandular production of LIF to its paracrine action in the luminal epithelium to induce Ihh, which then acts on the stroma to promote decidualization. LIF exhibits a biphasic pattern of expression in the preimplantation uterus [49]. During the first phase, glandular LIF production is high at proestrus/estrus near the time of ovulation in response to the preovulatory surge of E and continues on

day 1 and day 2 of pregnancy. The LIF level then declines on day 3. The second phase involves its rise again on day 4 concomitant with the transient surge of nidatory E [50]. During the entire preimplantation phase spanning days 1–4 of pregnancy, the LIF receptor is constitutively expressed in uterine luminal epithelium, consistent with the view that this tissue is the primary target of LIF action during this preparatory period [51]. Comparison of the uterine expression profiles of LIF and LIF receptor with that of Ihh during days 1–3 of pregnancy indicated that the first phase of LIF expression and signaling temporally overlaps with the induction of Ihh, which peaks during days 2–3 of pregnancy [25]. The expression of Ihh drops gradually from day 5 onwards when the second surge of glandular LIF expression occurs [25]. Based on these results, it has been postulated that the first phase of LIF expression influences the expression of Ihh in preimplantation uterus. Ihh then acts on the stromal cells via the PTCH-1 receptor to set in motion a cascade of pathways that prepare the uterus to fully respond to the decidual stimulation provided by the attachment of the embryo to the receptive uterus at day 4.5. The second peak of LIF expression occurs prior to implantation and plays an important role in inducing signaling pathways that modulate uterine luminal epithelial junctional complexes, thereby facilitating embryo attachment [51].

Signaling by LIF is initiated when it binds to the LIF receptors on the target cell. The LIF receptor is known to signal through distinct downstream pathways: JAK-STAT3 or Ras/ERK or AKT [52]. Studies have shown that a transient surge of LIF on day 5 of gestation induces embryo attachment by activating the JAK-STAT3 pathway [53]. The induction of Ihh in response to LIF signaling remained unaffected in uteri lacking epithelial STAT3; instead, the active form of ERK1/2 is present in the luminal epithelium on days 2–4 of gestation, and they exhibit a similar temporal expression pattern as that reported for Ihh. Collectively, these findings are consistent with the concept that the first phase of LIF expression activates the ERK1/2 pathway in luminal epithelium to induce Ihh expression in the preimplantation uterus, which then acts on the stromal cells to promote decidualization. The second surge of LIF on day 4 activates JAK-STAT3 pathway in the luminal epithelial cells and regulates a distinct set off genes that promote epithelial remodeling, uterine receptivity, and embryo attachment [54].

Conditional deletion of Ihh in the uterus caused infertility due to a flaw in embryo implantation [37]. In mice lacking Ihh ($Ihh^{d/d}$), the epithelium failed to reach the receptive condition. $Ihh^{d/d}$ microarray analysis revealed upregulation of several E-regulated genes, including mucin 1 (Muc1), lactotransferrin (Ltf), and wingless-type MMTV integration site (WNT) family member 4 (Wnt4), implying that Ihh may be involved in regulating estrogen receptor (ER) activity during the peri-implantation period. Mice with COUP-TFII uterine deletion exhibit elevated expression of epithelial ERα and its targets, including Ltf and Muc1, leading to reduced uterine receptivity and implantation failure [31]. The PR-IHH-COUP-TFII axis is thus critical during implantation because it regulates epithelial function.

Another research found that Ihh depletion in the uterine epithelium is related with altered gene expression in the stroma, indicating that Ihh modulates stromal function through paracrine pathways [31]. $Ihh^{d/d}$ mice did not commence the P-induced stromal cell proliferation that occurs before decidualization in the peri-implantation period [37]. The cell cycle regulatory factor CCND1 and the minichromosome maintenance family member MCM3, both of which are required for stromal cell proliferation, were not detected in Ihh-null uteri [46]. Further work demonstrated that Ihh deletion reduces epidermal growth factor receptor (EGFR) expression in stromal cells, identifying it as another downstream target of the Ihh signaling cascade. Microarray analysis revealed that Ihh regulates other members of the EGF receptor family, such as Erbb/Her2, Erbb3/Her3, and Erbb4/Her4 [46]. These findings suggested that Ihh, via modulating downstream EGF-EGFR signaling, may play a significant role in stromal proliferation and differentiation. Thus, P-induced Ihh activates several signaling pathways in epithelial and stromal compartments, regulating uterine receptivity and decidualization during implantation.

Conclusion

The Ihh signaling system is a crucial regulator of metazoan development that was first identified by its involvement in patterning the *Drosophila* larval epidermis. The spatially limited production of Ihh ensures that the *Drosophila* Wnt1 orthologue remains wingless in neighboring cells. Ihh's significance in developmental processes has served as a model for classical morphogens. During the uterine remodeling stage, the Ihh gene was expressed in the mouse uterus (Table 3). It works as a facilitator of the endometrium's P4-dependent activity and is critical in initiating uterine reconstruction in preparation for embryo implantation, not just in rodents but also in other mammalian species.

Dysfunction of Ihh pathway underlies a number of human developmental abnormalities and diseases, making it a crucial therapeutic target. Studies from many laboratories reveal activation of this pathway in a variety of human cancer which includes basal cell carcinomas (BCCs), medulloblastomas, leukemia, gastrointestinal, lung, ovarian, breast, and prostate cancers. Targeted

Table 3 Importance of the Ihh gene

Function	Description	References
1. Regulation of endometrial function	Involved in the regulation of endometrial epithelial cell proliferation and differentiation. It modulates the receptivity of the endometrium to embryo implantation	[25]
2. Establishment of pregnancy	COUP-TFII and IHH are part of a coordinated signaling network that ensures that the endometrium is appropriately prepared for embryo implantation, and any disruption in this crosstalk can lead to implantation failure and early pregnancy loss	[31]
3. Skeletal development	Functions through Ihh signaling pathway which involves interaction with its receptors, patched 1 (PTCH 1), and the downstream transcriptions Gli 1 and Gli2. This pathway coordinates chondrocyte proliferation, differentiation, and endochondral ossification	[55]
4. Gonadal development	Regulates the development of ovarian follicles. Ihh influences the differentiation and function of granulosa cells, which are essential for folliculogenesis and oocyte maturation	[56]
5. Ovarian follicle development	Affects the transition of ovarian follicle through various stages of development, influencing the selection and maturation of dominant follicles and the timing of ovulation	[57]
6. Embryonic development	Controls the development of structures such as limbs, kidneys, and central nervous system by influencing cell fate decisions and tissue morphogenesis	[58]
7. Decidualization of stromal cells	PR (progesterone receptors) are essential for decidualization, and Ihh signaling modulates PR expression. Another factor GATA2 is important for regulation of genes necessary for decidualization and interact with Ihh signaling	[59]
8. Formation and function of uterine glands	Ihh interact with FOXA2 to regulate the development of the development of uterine glands and their functional maturation	[60]
9. Trophoblast differentiation	GATA3 is crucial for trophoblast lineage specification and differentiation into trophoblast giant cells. Ihh modulate GATA3 expression and activity which in turn impacts the differentiation process and overall development of the placenta	[61]
10. Support early pregnancy	Ihh signaling helps regulate HAND2 expression in stromal cells, which in turn inhibits excessive stromal proliferation and promotes decidualization for supporting early pregnancy	[62]

inhibition of hedgehog signaling may be effective in treatment and prevention of human cancer. Specific signaling antagonists for the Ihh sinaling pathway were discovered. Optimized use of these antagonists will make the novel cancer therapeutics feasible.

Ihh signaling has emerged as one of the leading pathways regulating cell fate specification, differentiation, and tissue homeostasis. The record of processes involving in Ihh pathway continues to grow as well as its functions and mechanism of action. Despite having enormous knowledge about the pathway, there are still many areas where understanding remains incomplete. Major unresolved questions concern how Ihh is mediating PTCH-1 and how PTCH-1 regulates Smo activity, and the significance of the dynamic distributions of pathway components and the release and transport of Ihh proteins are physiologically important. Future biochemical and structural analysis will help to resolve these puzzles.

Abbreviations

Ihh	Indian hedgehog
Hh	Hedgehog protein
PTCH-1	Protein patched homolog 1
SMO	Smoothened
COUP-TFII	Chicken ovalbumin upstream promoter
P	Progesterone
E	Estrogen
Dhh	Desert hedgehog
Shh	Sonic hedgehog
HhN	Amino-terminal domain of hedgehog protein
HhC	Carboxy-terminal domain of hedgehog protein
SRR	Sterol-recognition region
SS	Signal peptide sequence
SPRM	Selective progesterone receptor modulator
PR	Progesterone receptor
LIF	Leukemia inhibitory factor
STAT3	Signal transducer and activator of transcription 3
JAK-STAT3	Janus kinase/signal transducers and activators of transcription
Ras/ERK	Rat sarcoma virus/extracellular-signal-regulated kinase
ERK ½	Extracellular signal-regulated kinase 1/2
EGFR	Epidermal growth factor receptor
MCM3	Minichromosome maintenance family member
CCND1	Cell cycle regulatory factor
ERα	Estrogen receptor alpha
ER	Estrogen receptor
$Ihh^{d/d}$	Ihh null mice
Muc1	Mucin 1
Ltf	Lactotransferrin

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Authors' contributions
AS and HNS contributed to the conception and design of the manuscript. Materials preparation and analysis were performed by AS. The first draft of the manuscript was written by AS. Both the authors read and approved the final draft.

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Availability of data and materials
The data supporting this review are from previously reported studies which have been cited in the manuscript. The relevant information can be accessed through the references provided.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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