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CM Nishanthika Jesslyn
Postgraduate Research Scholar, Department of Veterinary Physiology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

G Suganya
Assistant Professor, Department of Veterinary Physiology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

V Leela
Professor and Head, Department of Veterinary Physiology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Sabiha Hayath Basha
Professor and Head, Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Corresponding Author:
G Suganya
Assistant Professor, Department of Veterinary Physiology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Indian hedgehog: Role in skeletal development

CM Nishanthika Jesslyn, G Suganya, V Leela and Sabiha Hayath Basha

Abstract
The Hedgehog (HH) family of proteins consists of Indian hedgehog (IHH), Sonic hedgehog (Shh) and Desert hedgehog (Dhh). These proteins serve as essential regulators in a variety of developmental events. Within the hedgehog family IHH is considered the master regulator of endochondral bone formation. Normal endochondral bone development requires the coordination of chondrocyte proliferation and differentiation. IHH is a morphogen produced by chondrocytes in the early stages of terminal differentiation and plays several key roles in this process. In pathological and degenerative processes, such as osteoarthritis or cartilaginous tumour formation, hedgehog signalling is dysregulated. Pharmacological strategies targeting this pathway could lead to development of novel therapeutic approaches. Thus, regulation of hedgehog signalling could be manipulated to safeguard skeletal health.

Keywords: Hedgehog, role, skeletal development

Introduction
During embryonic development signalling molecules play a key role in providing positional information and specifying cell fates within a forming organ [8]. Cells responding to these morphogens substantially alter their gene expression patterns and start to perform specialized tasks during organogenesis, growth and homeostasis of an organ. Starting from a single cell, the fertilized egg, all the diverse cell types of the body are produced and organized into tissues and organs. During development, signalling pathways specify cell fates by activating transcriptional programmes in response to extracellular signals [9]. Extensive studies have revealed that only few pathways exist to regulate developmental programmes which falls into 11 main classes defined by the ligand/ signal transducers involved namely Notch,Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Wnt/ Wingless, Hedgehog (Hh), Transforming Growth Factor β (TGFβ) /Bone morphogenetic proteins (BMPs), Cytokine, Hippo, Jun Kinase, Nuclear Factor Kappa Light chain enhancer of activated B-cells and Retinoic acid receptor [22].

The Hedgehog signalling: A brief history
Mutations in the Hh gene were identified by Nusslein-Volhard and Wieschaus in their large-scale screen for mutations that impair or change the development of the fruit fly larval body plan [35]. Drosophila Hh DNA was cloned in the early 1990s [15, 21, 32]. In addition to Drosophila, Hh genes have also been found in a range of other invertebrates including *Hirudo medicinalis* (leech) and *Diadema antillarum* (sea urchin) [3, 29]. Hh orthologs from vertebrates including *Mus musculus* (mouse), *Danio rerio* (zebrafish), and *Gallus gallus* (chicken) were cloned in 1993 [3, 7, 12, 24]. Cloning of the first *Rattus rattus* (rat) and human Hh genes were reported shortly thereafter, in 1994 and 1995, respectively [9, 26]. The vertebrate genome duplication has resulted in expansion of the Hh genes, which can be categorized into three subgroups: the Desert Hedgehog (Dhh), Indian Hedgehog (IHH) and Sonic Hedgehog (Shh) groups [7]. Desert Hedgehog is concerned with the development of peripheral nerves and spermatogenesis. Sonic hedgehog establishes lateral symmetry, anterior posterior limb axis and aids in CNS development. Indian hedgehog is the master regulator of endochondral bone development (26).

HH secretion
Hedgehog (HH) proteins are synthesized as precursors of about 45 kDa. Autoproteolytic cleavage probably occurs in the endoplasmic reticulum (ER). The sterol recognition region of the HH carboxy- terminal peptide (HH-C) recruits cholesterol, which then acts as a nucleophile to attack an intramolecular thioester intermediate of HH [2].

This results in the covalent attachment of cholesterol to the amino terminal peptide (HH-N) and the dissociation of HH-C14. The acyltransferase skinny hedgehog (SKI) modifies HH-N by attaching a stable amide-linked palmitic acid group to the most N- terminal Cys residue, whereas HH-C translocates out of the ER and undergoes proteasomal degradation. Once at the outer surface of the plasma membrane, dually lipid-modified HH-N is associated with the lipid bilayer as a monomer until it is released by one of four key mechanisms. The cholesterol-modified HH-N monomer is released by the cooperative action of the transmembrane protein Dispatched (DISP) and the secreted SCUBE2 protein. Both proteins bind directly to a different part of the cholesterol moiety of HH-N. Monomeric cholesterol-modified HH-N can also self-associate to form large soluble multimers that are released from the membrane. HH-N oligomers can interact with the heparan sulphate chains of glypicans, which enables them to recruit lipophorin apolipoproteins and assemble into lipoprotein particles. The glycosylphosphatidylinositol (GPI) anchor of glypican might be cleaved by the phospholipase C-like protein Notum, promoting the release of the HH-N-associated lipoprotein particles. Alternatively, HH-N may be released at the surface of exovesicles [10, 27].

HH signalling

There are three mammalian hedgehog-regulated GLI transcription factors (GLI1–GLI3). Binding of Hedgehog proteins to the transmembrane receptor patched homologue (PTC) releases the inhibition of smoothened homologue (SMO) and leads to activation of GLI transcription factors, which translocate to the nucleus [4, 33, 34]. Although GLI2 does contain an enzyme processing site, its other structural features render this protein relatively resistant to proteolytic cleavage. GLI2 and GLI3 contain both activator and repressor domains, and can be proteolytically processed to either an activator or repressor form during development of many organ systems. In the absence of hedgehog ligand binding to PTC, SMO is inhibited and maintains GLI3 in the transcriptional repressor form. In the presence of hedgehog ligand binding to PTC, SMO is activated and induces intracellular proteins including SUFU and KIF7 to process GLI proteins to the transcriptional activator form. SMO, SUFU and KIF7 also facilitate nuclear translocation of GLI proteins. GLI target genes include the hedgehog pathway members GLI1 and PTCH1. Primary cilia are present in almost all cell types, including chondrocytes, and have a critical role in hedgehog signalling. Since cilia act as a site for integrating the various proteins that mediate processing of GLI transcription factors, they can aid in either activation or inactivation of GLI transcriptional activity, depending on the status of hedgehog signalling in the specific cell context [32].

Mechanism of skeletal development

Bones develop from mesenchymal cells through two different process namely intramembranous and endochondral ossification. Intramembranous ossification involves direct differentiation of mesenchymal progenitor cells into bone forming osteoblasts. During this process new bone matrix is synthesized and mineralized by osteoblasts. Facial bones and cranium are formed by intramembranous ossification. Endochondral ossification involves the formation of a cartilage primordium and growth plate, where chondrocytes initially undergo proliferation and a series of differentiation steps secreting a cartilage template that is eventually replaced by bone [23].

Chondrocyte differentiation in endochondral ossification

During endochondral bone development, committed mesenchymal pre-chondrogenic cells undergo condensation and secrete a number of matrix proteins that form the cartilaginous template. Cells in the centre of the condensate will differentiate into mature chondrocytes, while cells on the periphery becomes perichondrium forming the boundary of the cartilage. Mature chondrocytes at the centre of the cartilage actively undergo proliferation, forming columns of proliferating cells whereas those at the epiphyseal ends divide at a much slower rate, becoming the reserve chondrocytes. Chondrocytes express specific array of gene products as they differentiate into postmitotic hypertrophic cells [13]. The appearance of these gene products demarcates the various stages of chondrocyte development. Mature chondrocytes express collagen type II and Sox9, and as they reach the pre-hypertrophic stage, they express PTH/PTHrP receptors (PPR) and Indian hedgehog (IHH) [6]. When they become hypertropic chondrocytes the cells express type X collagen and vascular endothelial growth factor (VEGF).

Functions of IHH in endochondral bone development

IHH and PTHrP form a feedback loop that regulates chondrocyte differentiation at different stages [13, 18]. IHH and parathyroid hormone related peptide (PTHrP) participate in a feedback loop, which coordinates chondrocyte proliferation and differentiation in fetal developing bones. IHH is expressed and secreted by pre-hypertrophic chondrocytes preceding and overlapping with expression of PPR. IHH, either directly or indirectly induces PTHrP production from the periarticular perichondrium. PTHrP is able to diffuse to the PPR expressed by proliferative and pre-hypertrophic chondrocytes [14, 18]. Activation of PPR in these cells delays their rate of differentiation into hypertrophic chondrocytes thus shutting off the supply of IHH by keeping chondrocyte in the proliferative state. This feedback loop between IHH and PTHrP is clearly important for regulation of normal endochondral bone development as disruption of any component of the system results in abnormal limb development [13].

IHH promotes chondrocyte proliferation

The mechanism by which IHH promotes chondrocyte proliferation has been linked to cell-cycle regulators. Cyclin D1 promotes cell-cycle progression through G1/S phase transition and since cyclin D1 is expressed at low levels in slowly dividing reserve chondrocytes, but at high levels in rapidly dividing columnar chondrocytes, cyclin D1 mediates IHH-dependent proliferative effects [1, 2, 30, 31].

IHH regulates perichondrial development and angiogenesis

Recent studies exploring new functions of IHH in endochondral bone development identified IHH as a regulator of perichondrial differentiation and development [17, 25]. The effect of IHH on early perichondrial development affects vascular invasion, which occurs at a later stage of endochondral bone development. IHH, Ptc1, and Gli1 are all expressed in the perichondrium during early chondrogenic development, and endothelial cells normally develop from cells adjacent to that area. IHH signalling during the early developmental stage affects subsequent endothelial cell development and retention in the cartilage matrix.

Regulation of IHH signalling in growth plate

Heparan sulfate proteoglycans (HSPG) are cell surface or secreted extracellular molecules that regulate the activities and distribution of various signalling molecules, including Hh proteins. HSPG consist of a core protein to which heparan sulfate glycosaminoglycan chains are attached. Based on the core protein structures, they are divided into different families including glypicans, syndecans, and the secreted perlecans. They can facilitate long-range diffusion of signalling proteins, present them to target cells, or restrict the activity range of these molecules [5, 28]. IHH proteins, despite their lipid modifications, have been demonstrated to migrate over a long distance.

IHH signalling in osteoarthritis

Osteoarthritis (OA) is a degenerative condition of articular cartilage [11]. As the cartilage degrades, the articular chondrocytes undergo changes resulting in a phenotype similar to that of hypertrophic chondrocytes in the growth plate [16]. Proteins expressed during growth plate chondrocyte differentiation are also upregulated in OA. These include IHH, PTHrP and type X collagen. Terminal differentiation of articular chondrocytes in OA leads to apoptosis, which is also the fate of hypertrophic chondrocytes in the growth plate. Bone changes also occur: osteophytes develop, and the density of subchondral bone increases [36, 37, 38]. These changes are reminiscent of endochondral bone formation by growth plate chondrocytes, in which cartilage is replaced with bone, suggesting that hedgehog signalling has similar roles in both OA and in the growth plate [39, 40].

In OA, expression of hedgehog target genes in cartilage is increased versus that in healthy individuals and the level of expression correlates positively with the severity of OA. Thus increased activation of hedgehog signalling in OA, and that the use of hedgehog signalling inhibitors could attenuate the severity of OA or even prevent its development.

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