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Review

The many lives of SHH in limb development and evolution

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ABSTRACT

The SHH signaling pathway is essential for proper formation of the limb skeleton, as is required for the survival and expansion of distal chondrogenic progenitor cells. At the same time, SHH is important to specify digit identities along the anterior–posterior axis. Upon gain or loss of activity of the SHH pathway, bones are gained, lost or malformed, and such deregulation underlies the aetiology of various human congenital limb defects. Likewise, accumulating evidence suggests that evolutionary tampering with SHH signaling underlies the morphological diversification of the tetrapod appendicular skeleton. This review summarizes the roles of the SHH pathway in the context of limb development and evolution and incorporates recent evidence into a mechanistic view of how the positioning of digit condensations is integrated with the specification of distinct bone morphologies.

Contents

"On the other hand, you have different fingers"

Steven Wright

1. Basic concepts in mouse limb development

Forelimb primordia emerge from the flank around mouse embryonic day E9.0, while hindlimb development is delayed for about half a day. The early limb bud, composed of undifferentiated mesenchyme encapsulated by ectoderm, grows out and elongates along the proximal–distal (PD) axis. Around embryonic day E10.75, the distal part of the forelimb bud starts to expand along the anterior–posterior (AP) axis, forming the handplate, which contains the progenitors that will give rise to the digits. All pre-chondrogenic condensations that prefigure the definitive limb skeleton are already distinguishable in E12.5 forelimbs. The proximal segment of the forelimb, the stylopod, will give rise to the humerus (femur in the hindlimb), the middle segmentor zeugopod will form the ulna and radius (fibula and tibia), while the distal part, the autopod, contains the progenitors for the carpal bones of the wrist (tarsal bones of the ankle), the metacarpal bones of the palm (metatarsals in the feet) and the phalanges of the digits (Fig. 1A).

Growth and patterning of the limb skeleton are coordinated along the three anatomical axes through the concerted interaction of two signaling centers or organizers (reviewed in [1,2]). The AP organizer is called the polarizing region or zone of polarizing activity (ZPA), which is a small group of SHH-producing cells located in the posterior limb bud mesenchyme (Fig. 1B). The PD organizer

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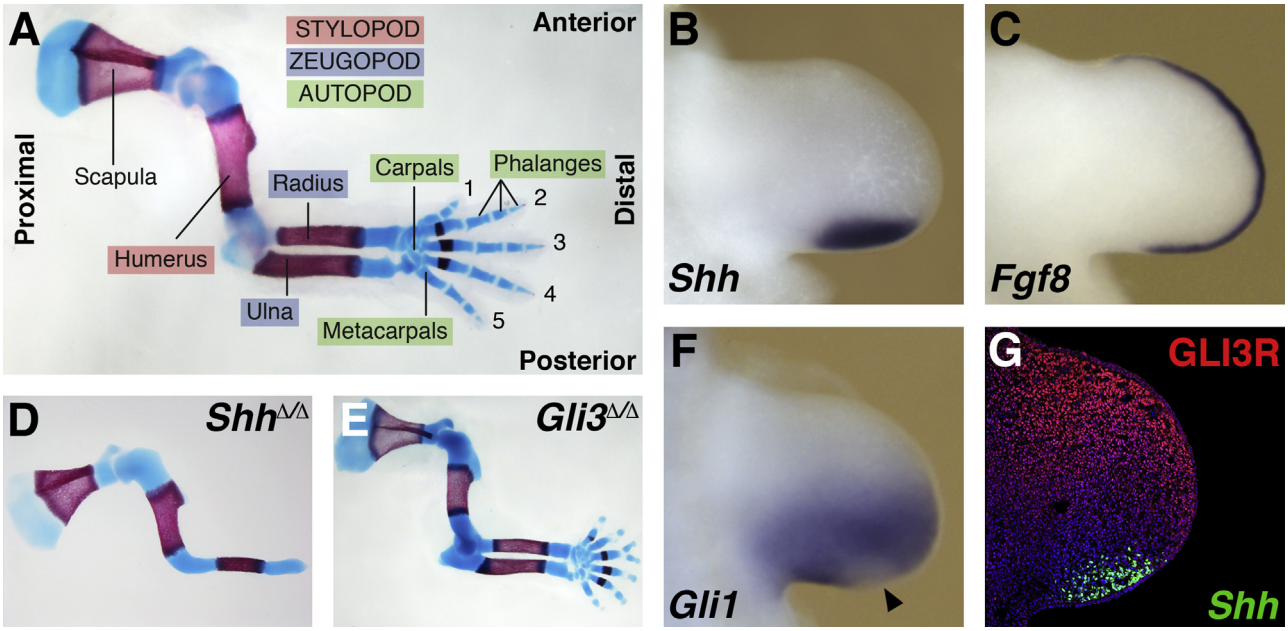

Fig. 1. Mouse limb development and the SHH pathway. (A) Mouse forelimb skeletal elements. Digits are numbered 1–5 along the anterior–posterior axis. (B, C) Expression of *Shh* and *Fgf8* in mouse forelimbs. (D, E) Forelimb skeletal phenotypes of *Shh* and *Gli3*-deficient embryos. (F) *Gli1* expression is a sensitive transcriptional readout of SHH signaling. The arrowhead points to the domain that becomes insensitive to SHH signaling. (G) GLI3R is cleared from the posterior limb bud mesenchyme under the influence of SHH signaling. *Shh*-expressing cells are labelled in green, while GLI3R (in red) is detected with antibodies that primarily recognize the repressor isoform on tissue sections [8]. Stages shown correspond to embryonic days E16.5 (A, D, E), E11.0 (B, C, F) and E10.25 (G).

is the apical ectodermal ridge (AER), a specialized ectoderm that runs along the limb bud margin and produces several factors, most importantly FGFs (AER–FGFs; Fig. 1C). Both signaling centers maintain each other's activity via a series of interlinked feedback loops that converge on the regulation of the BMP-antagonist GREM1 [1]. Decades of research have uncovered the essential roles of SHH signaling in the growth and AP patterning of the limb, as evidenced by the phenotypes resulting from loss or gain of function of the pathway (Fig. 1D and E) [3,4]. This review summarizes the current knowledge on the functions and mechanisms of SHH signaling during mouse limb development and puts them in the context of human congenital defects and morphological evolution of the tetrapod limb. For more general accounts on limb development or on mechanistic aspects of Hedgehog signal transduction that are out of the scope of this article, the reader is referred to other recent reviews [1,2,5–7].

2. Early AP polarization of the limb bud upstream of *Shh* expression

Recent studies have provided strong evidence for a mechanism that polarizes the early limb bud mesenchyme along the anterior-posterior axis prior to the onset of *Shh* expression [8,9]. In the limb field, *Hox9* paralogous genes in the forelimb and *Isl1/Sall4* in the hindlimb contribute to position *Gli3* and *Hand2* domains in the anterior and posterior limb bud, respectively [10–12]. In turn, GLI3 and HAND2 proteins control a gene regulatory network of transcription factors that specifies abutting anterior and posterior mesenchymal compartments and enables *Shh* activation in the polarizing region [8,12–14]. Several transcriptional activators and repressors such as HAND2, HOX, PBX, ETS, TWIST1, ALX4, PLZF or GATA6 proteins are required to delimit the *Shh* domain [15–26]. Many of them do so by interacting with a distant conserved enhancer referred to as the ZRS/MFCS1, whose genetic inactivation phenocopies the limb defects observed in *Shh*-deficient embryos [27,28]. In contrast, point mutations affecting the ZRS are associated to polydactyly in humans and other species due to ectopic *Shh* expression in the anterior limb bud [27,29]. In addition, FGF and WNT signals produced by the AER and surface ectoderm are required for *Shh* expression, while the BMP pathway negatively regulates it [30–37]. Moreover, a self-regulatory system operates in the polarizing region, as *Shh* is downregulated after increasing SHH pathway activity and *vice versa* [38,39]. Nevertheless, it remains to be mechanistically defined how the downstream effectors of all these morphogenetic pathways impact, directly or indirectly, on *Shh* expression.

3. Shaping and sensing the SHH gradient

The release of SHH by the polarizing region results in graded distribution of the ligand in the posterior half of the distal limb bud [40]. This pattern fits well with the expression domains of the transcriptional targets *Gli1* and *Ptch1*, which are sensitive readouts of pathway activation (Fig. 1F) [41,42]. PTCH1 is the main SHH receptor and constitutively represses the pathway by preventing the accumulation of another multi-pass membrane protein, Smoothened (SMO), in the primary cilium [5,6,43–45]. This ligand-independent antagonism ensures that the pathway is silenced in cells not exposed to SHH. In addition, *Ptch1* is a transcriptional target of the pathway, which leads to high levels of PTCH1 receptor being produced close to the source of SHH, which results in ligand sequestration [43,46,47]. This negative feedback loop is referred to as ligand-dependent antagonism, and is essential to limit the range of SHH signaling.

Additional mechanisms are important to shape the SHH gradient. The active SHH ligand is modified by the covalent addition of palmitoyl and cholesterol groups, which are required to target the protein to lipid rafts and for the assembly of soluble multimeric complexes [48–50]. Lack of cholesterol modification in SHH proteins induces the formation of ectopic digits in the anterior margin, which indicates that the cholesterol moiety is required to restrict the ligand to the posterior half [51]. In contrast, mice producing SHH proteins missing the palmitic acid group display loss and fusions of central digits, suggesting that the palmitoyl group is important for

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