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REVIEW

Roles for Hedgehog signaling in adult organ homeostasis and repair

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ABSTRACT

The hedgehog (HH) pathway is well known for its mitogenic and morphogenic functions during development, and HH signaling continues in discrete populations of cells within many adult mammalian tissues. Growing evidence indicates that HH regulates diverse quiescent stem cell populations, but the exact roles that HH signaling plays in adult organ homeostasis and regeneration remain poorly understood. Here, we review recently identified functions of HH in modulating the behavior of tissue-specific adult stem and progenitor cells during homeostasis, regeneration and disease. We conclude that HH signaling is a key factor in the regulation of adult tissue homeostasis and repair, acting via multiple different routes to regulate distinct cellular outcomes, including maintenance of plasticity, in a context-dependent manner.

KEY WORDS: Adult stem cells, Hedgehog signaling, Homeostasis

Introduction

During vertebrate development, hedgehog (HH) signaling plays an essential role in orchestrating the complex cell specification programs and extensive cell division required to form an organism. In the adult, HH continues to signal to discrete populations of stem and progenitor cells within various organs, including the brain (Ahn and Joyner, 2005; Ihrie et al., 2011; Machold et al., 2003), skin (Brownell et al., 2011), prostate (Peng et al., 2013) and bladder (Shin et al., 2011), among others. Whether HH signaling in the adult functions to control proliferation, specification and/or plasticity, and the mechanism by which this occurs, remain unclear. Given that a role for HH signaling in both the maintenance of adult resident stem cells and the progression of various diseases, including cancer, has emerged in recent years, it is important to determine how this major signaling pathway can regulate processes that lie on either side of the life-death spectrum.

The HH signaling pathway in mammals

The three mammalian HH proteins, called sonic (SHH), Indian (IHH) and desert (DHH) hedgehog, are homologs of the *Drosophila* segment polarity gene bearing the same name (Briscoe and Therond, 2013; Echelard et al., 1993). HH proteins undergo extensive post-translational modifications, after which they are released by the secreting cell with the help of dispatched, a membrane transporter protein. SHH is the most broadly expressed vertebrate HH and its paracrine activity on adjacent cells is the most common mode of pathway transduction, although HH has also been proposed to signal in an autocrine manner. HH signaling is propagated by a receptor complex that includes the G-protein-coupled receptor smoothened (SMO) and the twelve-pass membrane protein patched 1 (PTCH1) (Fig. 1). In the absence of HH ligand, PTCH1 inhibits SMO activation, but when HH is present this repressive action is released. In addition to PTCH1, HH interacts with PTCH2 and the cell-surface proteins growth arrest specific (GAS), cell adhesion molecule-related/downregulated by oncogenes (CDO) and brother of CDO (BOC), which function as co-receptors. This interaction is crucial for signal propagation and for establishing a HH gradient (Briscoe and Therond, 2013).

Downstream of SMO, the GLI (glioma-associated oncogene family members) transcription factors mediate HH signal transduction in a process referred to as canonical signaling (reviewed extensively by Briscoe and Therond, 2013; Hui and Angers, 2011). In the absence of HH ligand, GLI2 and GLI3 undergo limited proteasomal degradation, resulting in the cleavage and removal of the GLI C-terminal activator domain, which leads to the conversion of GLI3, and to a lesser extent GLI2, into transcriptional repressors ($GLI3^R$ and $GLI2^R$) (Fig. 1). GLI transcriptional activators (GLI^A), primarily $GLI2^A$, are formed only in response to HH stimulation. Thus, HH signaling functions through modulating the balance between GLI^A and GLI^R. GLI^A then triggers expression of HH target genes such as *Gli1*, the protein product of which functions only as a transcriptional activator and thus amplifies HH signaling. SMO and PTCH1 base level expression, much like that of GLI2 and GLI3, is independent of pathway activity. However, PTCH1 production is upregulated with increasing HH levels, thus forming a negative-feedback loop in the canonical signaling pathway (Ribes and Briscoe, 2009). Recent analysis of the *cis*-regulatory modules of HH-regulated genes has revealed that cells interpret the levels of HH signaling through differential affinity GLI-binding sites in target genes, whereas tissue specificity is achieved through the participation of co-activators (Balaskas et al., 2012; Oosterveen et al., 2012, 2013). Although the required receptors PTCH1/PTCH2 and SMO are committed to propagating canonical HH signaling, in some processes pathway activation does not result in GLI-induced transcriptional changes and this is referred to as non-canonical HH signaling (Brennan et al., 2012; Briscoe and Therond, 2013; Jenkins, 2009) (see Box 1).

A major distinction between canonical HH signaling in vertebrates and flies is the role that the primary cilium plays in vertebrate HH signaling (Fig. 1). Most components of the HH pathway transit through the cilium, and depending on whether PTCH1 or SMO is present, the GLI proteins are processed into transcriptional activators or repressors, respectively (Goetz and Anderson, 2010). Whereas the primary cilium plays a central role in the canonical pathway, no evidence has been found to suggest the same applies to non-canonical HH signaling.

HH signaling: a master regulator of development

Extensive genetic analyses of HH/GLI signaling mutants have helped to establish that each of the core components of the canonical pathway, except GLI1, play a crucial role in mouse embryonic

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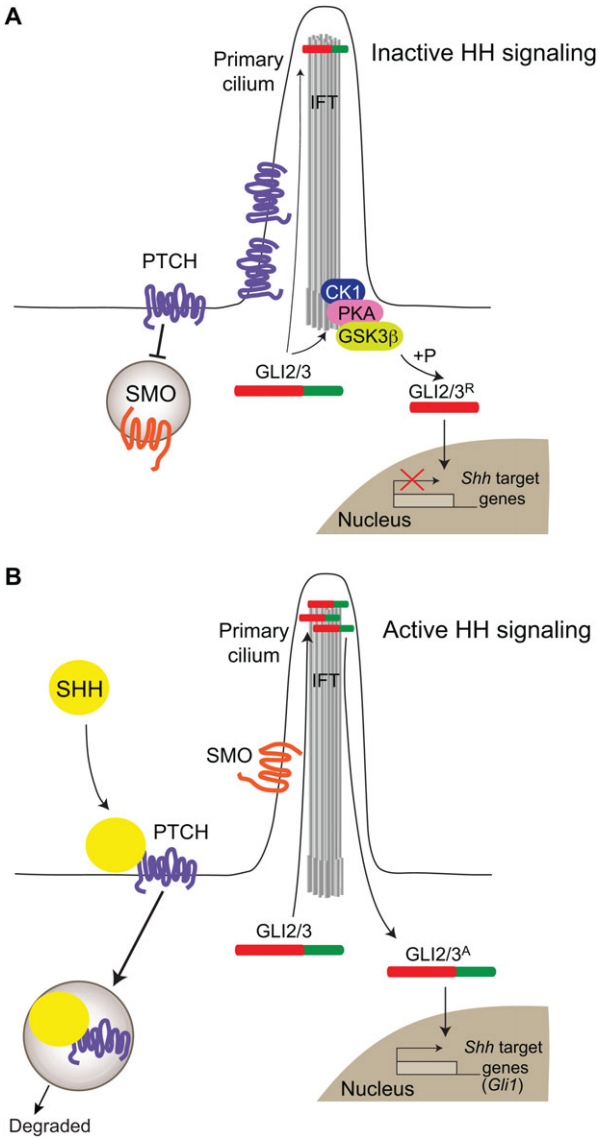

Fig. 1. Mechanism of canonical HH signal transduction in vertebrates. (A) In the absence of hedgehog (HH) ligand, patched 1 (PTCH) localizes to the primary cilium where it prevents activation of smoothened (SMO), which is sequestered into endocytic vesicles (circle). Microtubule motors within the cilium form the intraflagellar transport (IFT) machinery responsible for shuttling components of the HH signaling pathway, including small amounts of the glioma-associated oncogene proteins (GLIs), in and out of the cilium. At the base of the cilium, the GLI proteins (GLI2 and GLI3) are phosphorylated by protein kinase A (PKA), casein kinase 1α (CK1) and glycogen synthase kinase 3β (GSK3β), which results in their proteolytic cleavage and removal of the C-terminal 'activator' domain (green), generating $GLI2^R$ and $GLI3^R$ (red), which then suppress transcription of HH target genes in the nucleus. (B) HH signaling is activated upon binding of the ligand to PTCH proteins, which leads to their exiting the cilium and SMO subsequently entering. With the help of the IFT, the GLIs accumulate in the ciliary tip and then exit the cilium as full-length transcriptional activators ($GLI2^A$ and $GLI3^A$). GLI^A isoforms translocate to the nucleus, where they induce expression of HH target genes, including the transcriptional activator *Gli1*. The PTCH-bound HH ligand is internalized and degraded.

development (Hui and Angers, 2011). $Shh^{-/-}$ mutant embryos survive to birth but exhibit a multitude of developmental defects, including malformation of the central nervous system (CNS) starting at embryonic day E8.5, which is later accompanied by severe abnormalities in the skeletal system as well as defective limb,

Box 1. Non-canonical HH signaling

There are a few examples where a subset of the components of the canonical hedgehog (HH) signaling pathway regulate various basic cellular processes seemingly independently of the full pathway. For example, patched 1 (PTCH1) has been implicated in cell cycle regulation through interaction with cyclin B1, which acts at the G2/M checkpoint and is required for mitotic progression (Barnes et al., 2001). PTCH1 has also been shown to induce apoptosis independently of the GLI (glioma-associated oncogene family members) proteins when HH ligand is absent (Thibert et al., 2003). Smoothened (SMO), however, was recently found to function as a G-protein-coupled receptor (GPCR) (Riobo et al., 2006), which allows it to control axon guidance possibly through monomeric G proteins (Yam et al., 2009). In terms of the function of SMO as a GPCR, second messengers such as calcium (Ca^{2+}) have also been implicated (Belgacem and Borodinsky, 2011). Finally, although not commonly described as non-canonical HH signaling, a role for the GLI transcription factors independent of the traditional HH/SMO-signaling cascade has also been reported, particularly in cancer, where other signaling pathways appear to directly regulate the GLIs (Stecca and Ruiz, 2010). It is therefore possible that the progression of oncogenic disease is somewhat dependent on hijacking GLI activity to override the limiting step in ligand/receptor-induced HH signaling.

foregut and lung development (Chiang et al., 1996; Litingtung et al., 1998; Pepicelli et al., 1998; Varjosalo and Taipale, 2008). These defects are a result of the role of SHH in multiple vertebrate patterning centers and its rather broad pattern of expression. One of the major phenotypes associated with developmental loss of SHH is cyclocephaly (cyclopia) – a form of holoprosencephaly resulting in the formation of a single eye and the development of a proboscis instead of mouth and nose (Chiang et al., 1996). Ablation of *Smo*, and thus the ability of cells to propagate all canonical HH signaling during embryogenesis, results in early embryonic lethality associated with arrested somitogenesis, disrupted heart and gut development, and cyclopia (Zhang et al., 2001). In contrast to $Shh^{-/-}$ mutants, *Smo* knockout embryos exhibit more severe defects overall and do not develop to term. This is due to the fact that, during development, SMO plays a role not only in the transduction of SHH-induced signaling but also that of IHH (Zhang et al., 2001). Inactivating mutations of *Ptch1*, which result in HH pathway upregulation, are also embryonic lethal when homozygous, and $Ptch1^{-/-}$ mouse embryos have open and overgrown neural tubes (Goodrich et al., 1997), which is possibly a result of GLI-dependent upregulation in cyclin levels (Kenney and Rowitch, 2000). Furthermore, in the absence of *Ptch1*, HH signaling target genes such as *Gli1* become upregulated in ectodermal and mesodermal tissues but not in the endoderm, suggesting that HH signaling might not play a major role in the endoderm during early development (Goodrich et al., 1997). Unlike SHH, which is required for the development of seemingly all organs, the role of IHH and DHH is restricted to a more limited number of tissue-specific developmental events, e.g. bone morphology and gonadal differentiation, respectively (Bitgood et al., 1996; St-Jacques et al., 1999).

The requirement for HH signaling components downstream of the ligand-receptor complex is perhaps most extensively studied in CNS development (Fuccillo et al., 2006), where SHH acts initially as a morphogen to pattern the dorsal-ventral axis of the neural tube and to establish distinct ventral neuron populations in a concentration-dependent manner (Dessaud et al., 2008). Work from a number of different labs has shown that $GLI2^A$ function is crucial for the specification of the ventral-most neuronal types, whereas the medial spinal cord neurons require the correct level of $GLI3^R$ (Bai et al., 2004; Ding et al., 1998; Matise et al., 1998; Park et al., 2000; Persson et al.,

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2002). In contrast to spinal cord development, anterior regions of the CNS that give rise to the forebrain and the midbrain show less requirement for GLI^A function. Instead, SHH primarily functions by inhibiting $GLI3^R$ activity to prevent the dorsalization of ventral domains and maintain normal proliferation (Park et al., 2000), whereas in the midbrain, both $GLI3^R$ and $GLI2^A$ functions are important for patterning (Blaess et al., 2006, 2008; Rallu et al., 2002).

Apart from tissue patterning, SHH signaling also regulates cell expansion in the developing neural tube (Rowitch et al., 1999). Here, SHH stimulates cell division in E12.5 embryos, whereas at later developmental stages SHH inhibits the differentiation of neural progenitors, suggesting that HH signaling plays a role in maintaining stem/progenitor cells in a naïve state. Stimulation of cell division and inhibition of differentiation are both consistent with the role of HH signaling in promoting cancer (Jiang and Hui, 2008), as well as in maintaining stem cell functions. A recent study established that SHH also regulates the expansion of multipotent progenitors in the cerebellar white matter that give rise to astrocytes and inhibitory neurons in the postnatal brain (Fleming et al., 2013). *In vitro* studies of cerebellar granule neuron precursor proliferation have helped to determine that SHH signaling functions through the upregulation of MYCN and cyclin D1 to further cell cycle progression (Kenney et al., 2003; Kenney and Rowitch, 2000). These developmental studies raise the possibility that HH signaling in the adult could regulate multiple stem cell properties, including proliferation, specification and maintenance of the undifferentiated state.

HH signaling in the adult central nervous system

Neural stem cells

Given the role of HH in embryonic CNS development, it is perhaps not surprising that HH signaling persists as a key regulator of adult neurogenesis (Traiffort et al., 2010). In the adult mammalian brain, new neurons are generated from short-lived transit-amplifying cells (TACs) that derive from self-renewing and largely quiescent neural stem cells (NSCs) located mainly in the subventricular zone (SVZ) of the lateral ventricles (Fig. 2A) and in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) (Fuentealba et al., 2012). Stem cell populations in most adult tissues similarly consist of rare long-lived quiescent stem cells that both maintain the stem cell pool and give rise to TACs, which are committed progenitors that transiently expand the cell population as needed. In self-renewing tissues like the adult forebrain and skin, TACs are continuously produced, whereas in most other organs the quiescent stem cells appear mainly to respond to natural death of cells in the organ or to injury. Neurogenesis in the adult brain persists throughout the life of mice and is central to maintaining aspects of brain structure and function.

Conditional genetic loss-of-function studies have provided *in vivo* evidence that SHH is required for the establishment of the stem and progenitor cell populations in both the SVZ and SGZ. Midgestation removal of *Shh*, *Smo* or *Kif3a* – a crucial component of the primary cilium – results in a severe depletion of progenitors in the neurogenic regions (Balordi and Fishell, 2007a; Han et al., 2008; Machold et al., 2003). In these mutant mice, the stem cell compartments suffer from extensive early postnatal cell apoptosis, resulting in severely perturbed olfactory bulb (OB) interneuron and DG neuronal production, indicating that early SHH signaling is required for the survival of NSCs.

Once the SVZ and SGZ are formed, SHH is required for the continuous maintenance of neurogenesis. Early fate-mapping studies showed that a population of *Gli1*-expressing cells self-renew and contribute to neuronal production throughout life in both NSC compartments (Ahn and Joyner, 2005). Complimentary

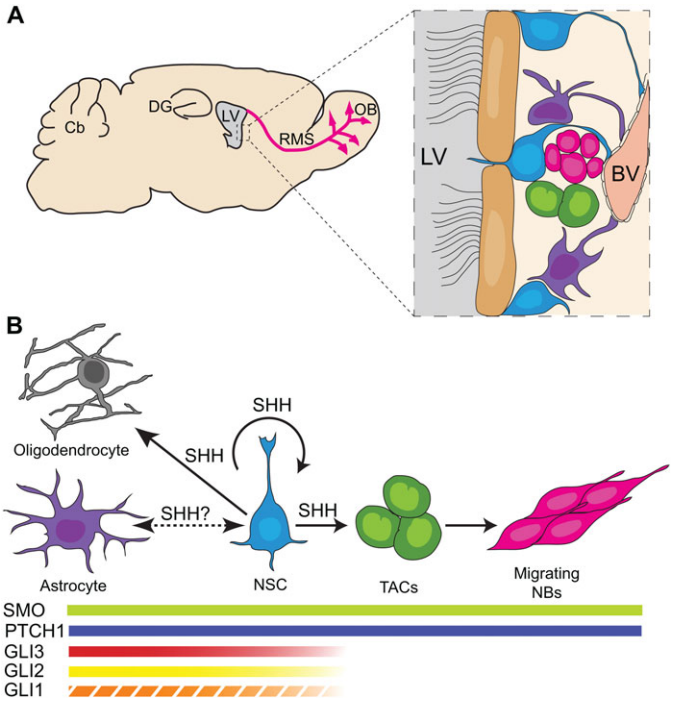

Fig. 2. Neural stem cells in the mouse forebrain SVZ, like other adult stem cells, produce lineage-restricted progenitors and respond to SHH. (A) In the subventricular zone (SVZ) lining the lateral ventricles (LVs), neural stem cells (NSCs; blue) self-renew or divide asymmetrically to generate transit-amplifying cells (TACs, green), progenitors that proliferate and give rise to proliferating neuroblasts (NBs, magenta) that migrate away from the SVZ via the rostral migratory stream (RMS) to the olfactory bulb (OB). The end feet of NSCs and astrocytes (purple) often contact blood vessels (BVs), which are an essential component of the neurogenic niche. Multiciliated ependymal cells (brown) form the immediate boundary between the cerebrospinal fluid-filled ventricle and the SVZ. Cb, cerebellum; DG, dentate gyrus. (B) Mature astrocytes and NSCs share many molecular and morphological characteristics, and both cell types respond to sonic hedgehog (SHH) signaling. Whether one cell type can be transformed into the other (dashed double-headed arrow), as appears to occur during injury (Sirko et al., 2013), and what role HH signaling may play in this process remain to be determined. NSCs also produce a small number of oligodendrocytes (gray), which is augmented by SHH signaling (Loulier et al., 2006). Smoothened (SMO) and patched 1 (PTCH1) are thought to be expressed at all the stages of NSC lineage progression. Activation of the canonical HH signaling pathway, however, occurs only at the NSC stage, where it is important for maintaining the undifferentiated and proliferative state of the NSCs (circular arrow). Expression of the glioma-associated oncogene proteins (GLIs) ends as the lineage progresses from NSCs to TACs. In addition, whereas GLI2 and GLI3 are expressed in mature astrocytes and NSCs throughout the SVZ and the rest of the brain, GLI1 is present in only a subset of NSCs and astrocytes (hatched orange line), possibly in regions where HH signaling is the highest (Balordi and Fishell, 2007a; Garcia et al., 2010; Petrova et al., 2013).

studies using small molecules showed that SHH gain or loss of function augments or inhibits proliferation, respectively, in the adult neurogenic regions *in vivo*, as well as in neural stem/progenitor cells cultured *in vitro* (Lai et al., 2003; Machold et al., 2003; Palma et al., 2005). Furthermore, SMO removal in the majority of adult SVZ NSCs results in reduced SVZ neurogenesis (Balordi and Fishell, 2007b; Petrova et al., 2013). This failure to achieve normal levels of neurogenesis occurs without an obvious increase in cell death or differentiation, and thus it is likely that SMO removal instead induces a state in which quiescent stem cells cannot generate TACs. Thus, after the initial 'expansion' phase of setting up the stem/progenitor cell pool in the two neurogenic niches, SHH may

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function to maintain the undifferentiated and proliferation-capable state of NSCs in the adult forebrain. Consistent with this, it was recently found that overactivation of the SHH pathway in SZV NSCs (by deletion of *Ptch1*) promotes NSC self-renewal at the expense of TAC and neuron production, through inducing NOTCH signaling and symmetric cell divisions (Ferent et al., 2014).

In contrast to neural tube development, a role for SHH as a morphogen is yet to be established in the adult brain. Perhaps the only example of a patterning-like function for SHH is the recently proposed involvement of the signaling pathway in influencing the OB fate of NSC-derived progenitors in the adult SVZ (Ihrie et al., 2011; Merkle et al., 2013; Petrova et al., 2013). SVZ NSCs preferentially produce specific subtypes of cells in the OB, depending on their dorsal-ventral and medial-lateral coordinates within the SVZ (Merkle et al., 2013, 2007). Genetic SHH conditional loss- and gain-of-function experiments revealed that the proportions of different OB interneurons and periglomerular cells are sensitive to the level of SHH signaling (Ihrie et al., 2011; Petrova et al., 2013). Although the ventral enrichment of *Gli1* expression in the adult SVZ (Ahn and Joyner, 2005; Ihrie et al., 2011) indicates a ventral source of ligand, which is similar to the developing neural tube, there is no proof for the establishment of a SHH gradient along the dorsal-ventral axis of the SVZ. A recent examination of *Gli* transcription revealed that SHH signals primarily to the slow-cycling NSCs in the adult SVZ, as expression of *Gli1*, *Gli2* and *Gli3* is downregulated as NSCs differentiate into progenitor cells (Petrova et al., 2013) (Fig. 2B). Using conditional mouse genetic techniques, it was found that, whereas GLI2 and GLI3 are mostly not required for SVZ NSCs, precise titration of GLI^R levels, primarily $GLI3^R$, is crucial for the long-term maintenance of adult SVZ neurogenesis and for proper OB interneuron production (Petrova et al., 2013). Thus, the manner in which $GLI3^R/GLI2^A$ are used by SHH signaling appears to be largely conserved between embryonic and adult forebrain progenitor and stem cell populations.

In addition to neurons, adult SVZ NSCs can also give rise to oligodendrocyte progenitors (Capilla-Gonzalez et al., 2013; Menn et al., 2006). Augmentation of SHH signaling by *in vivo* ligand infusion was shown to increase oligodendrocyte production in the adult forebrain and spinal cord (Bambakidis et al., 2003; Loulier et al., 2006), although the identity of the SHH-responding cells in the latter case is unclear. The role for SHH signaling in oligodendrogenesis is particularly intriguing in light of a recent study indicating that oligodendrocyte progenitor cells can act as the cell of origin for glioblastomas (Liu et al., 2011). Such observations raise the possibility that atypical activation of SHH signaling could be sufficient to transform the ability of the brain to repair itself into a malignant process.

Adult NSCs have ultrastructural characteristics and a molecular profile similar to that of mature parenchymal astrocytes (Doetsch et al., 1997), which is the other major population that responds to high level SHH signaling in the normal adult brain (Garcia et al., 2010). Both *Gli2* and *Gli3* are expressed in astrocytes throughout the adult brain but only select populations of astrocytes are exposed to high levels of SHH signaling (i.e. express *Gli1*). Postnatal removal of *Smo* in astrocytes results in a partial reactive astrogliosis-like phenotype (Garcia et al., 2010), which would normally occur only after CNS injury or in disease (Sofroniew, 2009). Recently, the precise level of SHH signaling was shown to be crucial for the induction of reactive astrogliosis in response to invasive brain injury, as both inhibition and stimulation of the pathway resulted in reactive astrocytes (Sirko et al., 2013). Interestingly, however, only SHH augmentation was able to trigger proliferative and neurosphere-forming abilities in cortical astrocytes. This result is particularly intriguing as a separate study has shown that reactive astrocytes can be directly converted to functional neurons *in vivo* (Guo et al., 2013; Sirko et al., 2013). Thus, although the precise role of SHH signaling in maintaining parenchymal astrocyte function is unclear, SHH seems to act as a modulator of brain plasticity and regeneration. SHH pathway activation in response to injury could function to recruit distinct resident cell populations to achieve tissue repair by coordinately generating new neurons, astrocytes and oligodendrocytes.

Sources of SHH in the CNS

A particularly intriguing question is the identity of the ligand source for neurogenic regions and astrocytes throughout the forebrain. SHH is the only member of the HH family that continues to be expressed in the adult brain (Traiffort et al., 2010). Under normal conditions, neurons appear to be the main SHH-secreting cell type (as opposed to SHH-responding cells, which are primarily of glial nature) (Garcia et al., 2010; Sirko et al., 2013; Traiffort et al., 1999). Following injury, however, SHH expression has been reported in reactive astrocytes (Amankulor et al., 2009; Sirko et al., 2013). *In situ* hybridization and genetic fate-mapping techniques to detect *Shh* expression have helped identify neurons in the medial and ventral septum of the adult forebrain as a possible ligand source for the SVZ (Fig. 3A) (Ihrie et al., 2011). Such basal forebrain structures also project to the adult DG (Amaral and Kurz, 1985), and thus could be a source of SHH for the postnatal SGZ. Dispersed SHH-positive neurons have been detected throughout the adult cortex and could also be the source of HH ligand for astrocytes (Garcia et al., 2010). Alternatively or perhaps in addition to these cells, a population of Shh^{gfpCre} fate-mapped calretinin+ neurons in the DG hilus region has also been proposed to serve as a local source of ligand for the postnatal SGZ region (Li et al., 2013); however, it remains to be determined whether these cells continue to express SHH in adulthood. Finally, delivery of HH ligand through the cerebrospinal fluid (CSF) in the brain ventricular system has been reported in the developing brain (Huang et al., 2010), and increased levels of SHH protein are detected in the adult CSF following brain injury (Sirko et al., 2013). These observations raise the possibility of an extraneural source of SHH, as well as a potential novel method of SHH delivery.

SHH was recently implicated in the reciprocal signaling between *Shh*-expressing midbrain dopaminergic neurons and striatal neurons in the adult forebrain, and was shown to be crucial for the maintenance of the nigrostriatal circuit (Gonzalez-Reyes et al., 2012). Indeed, SHH signaling has previously been demonstrated to protect dopaminergic neurons from neurotoxic effects (Dass et al., 2005; Hurtado-Lorenzo et al., 2004; Suwelack et al., 2004). As midbrain dopaminergic neurons also project to the SVZ (Lennington et al., 2011), along with other neuronal populations (Berg et al., 2013), it is possible that anterograde movement of SHH protein along axons allows the delivery of the protein to both the SVZ and striatum (Fig. 3A). Release of SHH from both the dendrites and axons of dopaminergic neurons would allow signaling to two distinct cell populations, as was recently proposed to be the case for Purkinje cells in the developing cerebellum (Fleming et al., 2013). However, whether such spatial regulation of SHH release exists for dopaminergic neurons remains to be determined.

In summary, the apparent dominant relationship between neurons and glia as ligand-releasing and signal-transducing cells, respectively,

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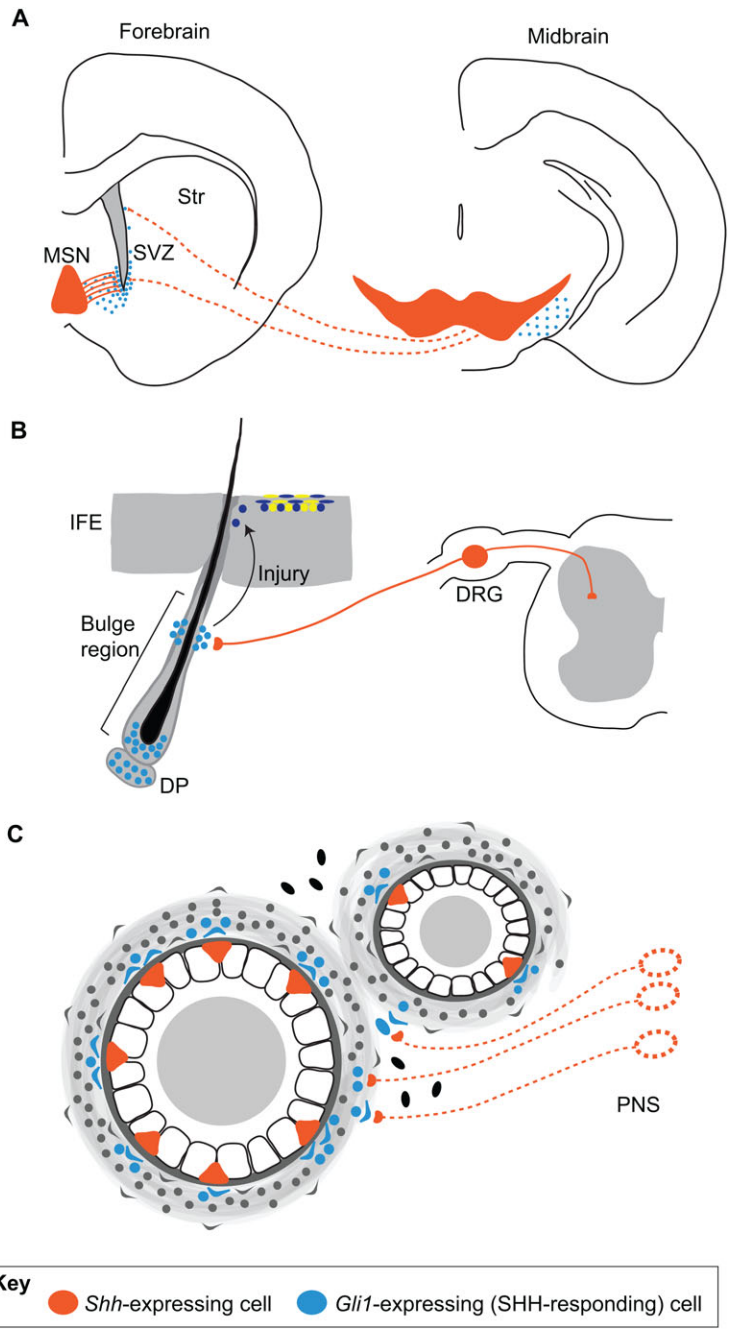

Fig. 3. Roles of nerve-derived HH signaling in adult organs. (A) (Left) Schematic of the adult rodent forebrain where *Shh*-expressing neuronal populations (orange) in the medial septal nucleus (MSN) are thought to deliver sonic hedgehog (SHH) to the subventricular zone (SVZ) neural stem cells (NSCs) and adjacent astrocytes (both depicted in blue). (Right) Dopaminergic neurons in the midbrain (orange) also express *Shh* (Gonzalez-Reyes et al., 2012) and are located in close proximity to a midbrain population of GLI1+ astrocytes (blue). As these neurons project to the forebrain lateral striatum (Str) and SVZ lining the lateral ventricles, it is possible that SHH might travel in an anterograde direction along the axons to be released in the SVZ (orange dashed lines), thus serving as an alternative distant source of ligand. (B) In adult mouse skin, stem cell domains in the upper and the lower bulge are exposed to SHH ligand during telogen phase. An unidentified source induces *Gli1* in the lower bulge and dermal papilla (DP), whereas the GLI1+ stem cell domain below the isthmus receives SHH from cutaneous nerves (orange line) originating from dorsal root ganglia (DRG) adjacent to the spinal cord (Brownell et al., 2011). A nerve-derived factor sustains the plasticity of the latter stem cell population, which after injury can transform into interfollicular stem cells and contribute to the regeneration of the interfollicular epidermis (IFE). Upon doing this, these cells downregulate *Gli1* expression (dark-blue cells; yellow cells are resident interfollicular cells). (C) An adult mouse prostate duct in which basal epithelial cells (orange) express SHH and signal to the surrounding stroma that consists of GLI1+ (blue) and GLI1– (gray) subepithelial (round) and wrapping (crescent) cells surrounding a smooth muscle layer (circled in gray). Interductal fibroblasts (black ovals) are located between the two ducts and also express *Gli1*, enabling them to respond to SHH. Nerves from the peripheral nervous system (PNS) innervate the prostate and signal to stromal cells via neurotransmitters (Magnon et al., 2013). Although SHH expression has not been reported in peripheral nervous system (PNS) nerves (dashed orange lines), these nerves might serve as an exogenous source of HH ligand for the interductal prostate stroma.

indicates a novel function for HH signaling in neuron-astrocyte communication. Exploring how such HH-dependent neuroglial relationships change in the context of injury, neurological disorders and neurodegeneration might prove particularly useful in identifying putative entry points for therapeutic treatments.

Hedgehog signaling in other adult tissues of ectodermal origin

Apart from a role in the CNS, HH signaling has been shown to regulate the long-term maintenance of other tissues derived from the ectoderm, such as the skin and teeth. Consistent with what is observed in the CNS and indeed in other tissues, an increasing number of scientific reports have implicated HH primarily in maintaining the stem/progenitor cell compartments in both of these tissues, as well as in the onset of tumorigenesis.

Hair and skin

Multiple populations of phenotypically distinct and normally lineage-restricted stem cells exist within the adult mammalian skin (reviewed by Solanas and Benitah, 2013). Mitotically active cells in the epithelial basal layer of the skin constantly produce new interfollicular skin cells that are later shed as dead squamous keratinocytes. Bulge stem cells reside in the lower bulged region of the hair follicle and are responsible for cyclic regeneration of the follicle, whereas other stem cell populations located above the bulge contribute to epidermal compartments such as the sebaceous gland and the infundibulum. Similar to the adult brain, SHH is the main HH ligand present in postnatal skin. During the expansion (anagen) phase of the hair cycle, *Shh* expression is readily detectable in the epithelial cells of the lower end of the hair follicle, while the downstream effectors *Gli1* and *Ptch1* are more broadly expressed

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(Brownell et al., 2011; Oro and Higgins, 2003). Treatment of adult mice with an anti-SHH antibody blocks anagen progression and hair regrowth (Wang et al., 2000), indicating that SHH is required for the regenerative function of adult bulge stem cells. Conversely, exogenously administered SHH triggers anagen onset in resting hair follicles and stimulates hair growth (Sato et al., 1999). Consistent with these early reports, recent evidence of *Shh* expression by the committed progeny of stem cells within the anagen follicle has revealed a feedback mechanism whereby SHH+ progenitor cells signal to their parental quiescent stem cells in the bulge region to trigger stem cell activation and proliferation (Hsu et al., 2014). Whereas removal of *Shh* expression in the hair germ caused marked proliferative defects throughout the hair follicle, genetic abrogation of *Smo* or *Gli2* alone in the stem cells reduced their proliferative abilities but did not affect anagen progression, indicating that SHH is required to maintain the function of hair follicle stem cells but not that of the progenitor cells in which it is expressed. The same study revealed a secondary effect of progenitor-secreted SHH, namely to stimulate the expression of factors such as FGF7 (fibroblast growth factor 7) and NOG (noggin) by the dermal papilla, which in turn help to maintain the expansion of the progenitor pool (Hsu et al., 2014). Exactly how this feed-forward mechanism is regulated and ultimately extinguished remains to be determined.

Although significant *Shh* mRNA expression has not been detected in the resting (telogen) hair follicle, *Gli2* and *Gli3* continue to be broadly expressed both in the follicle and in the surrounding dermis. By contrast, *Gli1* and *Ptch1* expression during the telogen phase is restricted to two distinct epithelial stem cell domains: a keratin 15 (K15)-negative domain mainly in the upper bulge; and a domain that overlaps with K15 and LGR5 (leucine-rich G protein-coupled receptor 5) within the lower bulge, as well as in the dermal papilla (Brownell et al., 2011). Cells in the upper GLI1+ domain were found to receive SHH ligand from the sensory nerves that wrap around the upper hair follicle (Fig. 3B) (Brownell et al., 2011). Some of these GLI1+ stem cells might overlap with a subset of LGR6+ stem cells recently identified in the isthmus, as the expression of *Lgr6* in these cells has also been shown to depend on cutaneous nerves (Liao and Nguyen, 2014). Pathway abrogation by back skin denervation was found to result in loss of *Gli1* expression specifically in the upper GLI1+ domain, and more importantly, to abolish the ability of these normally follicular stem cells to become interfollicular stem cells after contributing to wound healing (Brownell et al., 2011). Thus, in contrast to the anagen follicle where epithelial SHH stimulates hair follicle renewal, neural-derived SHH might enable the upper bulge GLI1+ stem cells to remain plastic, allowing them to contribute to an alternative cell lineage during regeneration. Whether SHH is the only nerve-derived signal required for such plasticity, what the requirement for the downstream $GLI^{A/R}$ effectors is and what the key SHH target genes are still remain to be determined.

Consistent with a role for HH in stimulating cell proliferation, SHH signaling gain-of-function mutations are found in human basal cell carcinoma (BCC) (Hahn et al., 1996; Johnson et al., 1996; Reifenberger et al., 1998; Unden et al., 1996). SMO gain-of-function and PTCH1 loss-of-function mutations give rise to BCC-like lesions when induced either in mouse interfollicular epidermis, or in hair follicle stem cells that move into the interfollicular skin after wounding (Kasper et al., 2011; Wong and Reiter, 2011; Youssef et al., 2012, 2010). Hence, it is likely that two different stem cells can function as tumor-initiating cells for BCC. Transcriptional profiling of tumor-initiating cells in the interfollicular epidermis has revealed that the tumorigenic cells assume an identity similar to that of embryonic hair follicle progenitor cells (Youssef et al., 2012). Furthermore, continuous GLI2-dependent SHH signaling appears to be required for the full establishment of BCC (Hutchin et al., 2005). Thus, SHH signal upregulation is the driving factor behind the transformation of interfollicular and/or hair follicle stem cells into tumor-initiating cells, a process likely to be dependent on their transition to a more immature cell state (Youssef et al., 2012). Beyond BCC, the precise mechanism of HH signaling in other tumorgenic processes in the adult is unclear, and is likely to be highly context dependent (see Box 2).

Box 2. HH signaling in cancer

Three major cancers have been identified that involve cell-autonomous hedgehog (HH) pathway over-activation: medulloblastoma (MB), rhabdomyosarcoma (RMS) and basal cell carcinoma (BCC) (Ng and Curran, 2011). Whereas BCC likely can arise from multiple adult skin stem cells gone rogue under the influence of aberrant HH (Wong and Reiter, 2011; Youssef et al., 2010), the other two cancer types have embryonic origins and are triggered by developmental defects in HH signaling (Onishi and Katano, 2011). The SHH subtype of MB, a highly prevalent childhood brain tumor originating in the cerebellum, is characterized by activation of the HH pathway, which accounts for one quarter of all MB cases (Remke et al., 2013). This may be via inactivation of patched 1 (*PTCH1*) or suppressor of fused (*SUFU*), which encodes a negative regulator of the canonical HH pathway, or by activating mutations in smoothened (*SMO*). Both granule neuron progenitor cells, as well as more primitive stem-like cells in the young cerebellum have been deemed the cell of origin for MB (Manoranjan et al., 2012). Similar HH gain-of-function mutations were found to be the driving force behind the progression of RMS, a common type of soft tissue neoplasia in children (Roma et al., 2012). In both cases, the exact mechanism underlying HH-mediated malignant transformation remains largely unclear. A reciprocal paracrine mode of HH signaling is thought to be the basis of tumor growth regulation in many epithelial tumors (carcinomas). Here, the concept is that the tumors express HH ligand that induces changes in the surrounding stromal cells, which in turn triggers the expression of other cancer-altering ligands by the cancer-associated stroma (Teglund and Toftgard, 2010). Determining whether mesenchymal adult stem cells play a role in this epithelial-mesenchymal reciprocal paracrine signaling and their subtype identity are important issues for further investigation.

Teeth

Rodent incisors are an example of an organ that continues to grow throughout the life of the animal and that requires constant repair. The proximal end of the rodent incisor, known as the cervical loop region, is a stem cell hub that generates progenitors that migrate towards the distal tip of the incisor to produce enamel-depositing amelioblasts and renew the incisor epithelium (Harada et al., 1999). During development, SHH signaling plays a key role in early tooth germ initiation, as well as in tooth growth and morphogenesis (Dassule et al., 2000). More recently, it was shown that during the growth of the adult rodent incisor, SHH is secreted by the differentiating pre-amelioblasts and signals back to their parental *Gli1*-expressing amelioblast stem cells at the proximal end of the incisor (Seidel et al., 2010). Blocking SHH signaling resulted in decreased amelioblast production and tooth growth, but did not deplete the GLI1+ stem cell pool (Seidel et al., 2010). Thus, SHH signaling in the incisor epithelium appears not to be required for stem cell survival but instead for maintaining the ability of stem cells to expand the amelioblast lineage, perhaps similar to the function of SHH in the adult forebrain SVZ in producing TACs.

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SHH signaling was also recently shown to play an important role in the maintenance of dentin, the mesenchymal compartment of the incisor, which is located under the outer enamel surface (Zhao et al., 2014). The study showed that dentin turnover is dependent on HH-responding periarterial mesenchymal stem cells, which also contribute to dentin repair after injury. Much like in the skin, the HH ligand that maintains the GLI1+ stem cell population is secreted by nerves in the neighboring neurovascular bundle. HH inhibitor administration revealed that, as in the incisor epithelium, the pathway is not required to support stem cell maintenance, survival or progenitor proliferation, but is necessary for the differentiation of odontoblasts (Zhao et al., 2014).

Although human teeth do not grow continuously, stem cells from human dental pulp have been isolated (Gronthos et al., 2000) and it remains to be determined whether they respond to canonical SHH signaling. A multipotent human stem cell population within the periodontal ligament, which is the connective tissue surrounding the tooth, was shown to express *SHH* as well as *GLI1* and *PTCH1*, and to respond to exogenous stimulation with recombinant SHH and to inhibition with the SMO inhibitor cyclopamine (Martinez et al., 2011). Understanding how SHH and other signaling pathways regulate the function of adult stem cells associated with tooth homeostasis might prove beneficial for the development of dental implants and improved dental repair (Nakashima and Iohara, 2014; Nakashima et al., 2009).

HH signaling in adult tissues of mesodermal origin

Unlike the skin and brain, there is less evidence for HH signaling in homeostasis and repair of adult tissues of mesodermal origin. The mesoderm forms tissues such as bone, cartilage and muscle, as well as the circulatory system. As bona fide adult stem or progenitor cell populations have yet to be identified in some of these tissues, we focus this section of our review on the reported roles of HH signaling in tissue repair following injury.

Bone

IHH is one of the main regulators of chondrocyte proliferation and osteoblast differentiation during skeletal development (Long and Ornitz, 2013). In developing long bones, IHH together with the parathyroid hormone-related protein (PTHrP) regulate chondrocyte behavior within the bone growth plate. Disruption of the IHH-PTHrP pathway and upregulation of HH signaling leads to the formation of childhood cartilaginous neoplasms, such as enchondromas and osteochondromas (Tiet and Alman, 2003). Throughout adulthood, HH signaling continues to help maintain bone structure as systemic administration of the SMO inhibitor cyclopamine to adult mice was shown to result in bone mass reduction. By contrast, adult *Ptch1*$^{+/-}$ mutant mice exhibit an increase in bone mass density and osteoblast differentiation associated with loss of GLI3^{R} activity (Ohba et al., 2008). Consistent with this, upregulation of HH signaling, either by transient adenovirus-mediated overexpression of SHH or by conditional deletion of *Ptch1* in mature osteoblasts, not only causes increased osteoblast production but also an indirect increase in osteoclast activity leading to bone resorption and decreased bone strength (Kiuru et al., 2009; Mak et al., 2008). By contrast, HH signaling inhibition by partial conditional ablation of *Smo* in mature osteoblasts results in protection from bone loss in 1-year-old mice (Mak et al., 2008). Taken together, these studies indicate a role for HH signaling in bone homeostasis through regulating the balance between bone formation and bone resorption in a concentration-dependent manner. In addition to bone homeostatic regulation, HH signaling has also been implicated in disease processes, such as osteoporosis, as well as during bone repair and revascularization after injury (Fuchs et al., 2012; Wang et al., 2010). Upregulation of both *Ihh* and *Shh* transcription, as well as *Ptch1*, has been observed immediately after bone fracture (Ito et al., 1999; Miyaji et al., 2003), indicating that HH signaling functions in the adult bone to modulate cell behaviors following disruption of homeostasis to ensure tissue repair. However, no bona fide stem cell population responsible for adult bone repair has been identified to date.

Muscle

Skeletal muscle is one of the few mammalian organs in the adult capable of almost complete regeneration after injury. This is possible due to the presence of satellite cells, the *in situ* muscle stem cell population (Lepper et al., 2011). These cells remain mostly inactive under normal conditions but in response to injury they can give rise to myogenic cells that reconstitute the myofibers of the muscle (Yin et al., 2013). During embryogenesis, canonical HH signaling to somites plays a role in the direct induction of myogenic factors such as MYOD1 (myogenic differentiation 1) and MYF5 (myogenic factor 5), which are essential for skeletal myogenesis (Pownall et al., 2002). In adult mouse satellite cells, HH signaling continues to function as a pro-survival and proliferation factor (Koleva et al., 2005). Intriguingly, upregulation in *Shh* and *Ptch1* transcription has also been detected in adult fully differentiated muscle upon the induction of regeneration following ischemic injury. During this process, HH plays a crucial role in promoting angiogenesis and increasing satellite cell number at the affected site (Pola et al., 2003, 2001; Straface et al., 2009). By contrast, SMO inhibition by cyclopamine treatment in injured animals results in muscle fibrosis and increased inflammation (Straface et al., 2009). Furthermore, although the regenerative ability of skeletal muscle declines in aging mice, intramuscular injection of a *Shh*-expressing vector was shown to successfully boost muscle repair to levels comparable with those found in much younger mice (Piccioni et al., 2013).

The existence of a HH-responding stem cell population in adult mammalian cardiac muscle has not yet been demonstrated; however, HH signaling is nonetheless required for the proper function of the adult heart. Conditional ablation of *Smo* in adult smooth muscle cells surrounding the blood vessels results in loss of coronary blood vessels, heart failure and even lethality (Lavine et al., 2008). By contrast, *Shh* gene transfer in an adult mouse myocardial ischemia model reduces fibrosis and augments angiogenesis, thus aiding heart repair (Kusano et al., 2005).

A crucial role for HH in smooth muscle development has been observed in many different organs, including gut, bladder (Mao et al., 2010; Tasian et al., 2010) and kidney. During kidney smooth muscle development, HH functions through interacting with members of the bone morphogenic protein family (Yu et al., 2002). Although the role of HH signaling in the adult kidney remains unclear, upregulation of HH and *Gli* expression, as well as an expansion of the α-smooth muscle actin-expressing myofibroblast population has been reported in a mouse model of kidney fibrosis (Fabian et al., 2012). Although the role of HH in mediating muscle repair and fibrosis is context dependent, it is clear that this pathway is an important player in disease progression and therefore represents a possible therapeutic target for the treatment of muscular disorders and possibly for heart failure.

Hematopoiesis

Hematopoietic stem cells (HSCs) are long-lived, largely quiescent stem cells that reside in the bone marrow and constantly replenish the myeloid (monocytes, macrophages, neutrophils, basophils,

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eosinophils, erythrocytes, platelets) and lymphoid (T, B and natural killer) cell lineages (Jagannathan-Bogdan and Zon, 2013). Whereas HH has been shown to play a role in the induction of vasculogenesis and hematopoiesis at embryonic stages (Lim and Matsui, 2010), reports on the role of the canonical signaling pathway in the adult are controversial. Ligands of the pathway are expressed in the hematopoietic niche and have been shown to function as survival signals for leukemia, lymphoma and myeloma cancer stem cells, whereas inhibition of the pathway appears to suppress disease progression (Dierks et al., 2008, 2007; Zhao et al., 2009). Despite being implicated in hematopoietic malignancies, the precise role of HH signaling during adult homeostasis remains unclear. Much of the controversy surrounding HH signaling in hematopoietic homeostasis concerns the varying results obtained from loss-of-function studies. *Ptch1*$^{+/-}$ adult animals have been shown to undergo accelerated recovery after hematopoietic damage but this is accompanied by reduced long-term grafting potential when *Ptch1*$^{+/-}$ adult HSCs are transplanted into irradiated adult animals (Trowbridge et al., 2006). Another study using transplantation of fetal liver-derived HSCs from *Ptch1*$^{+/-}$ embryos reported an enhancement in the long-term self-renewing potential of *Ptch1*$^{+/-}$ HSCs, whereas *Smo*$^{-/-}$ fetal liver-derived HSCs were shown to have normal regeneration capacity when placed in an adult wild-type host (Dierks et al., 2008). By contrast, CRE-mediated conditional ablation of *Smo* in both HSCs and their niche from embryonic stages onwards was shown to result in a profound loss of long-term grafting potential of the HSCs *in vivo* (Zhao et al., 2009). Yet another set of independent studies based on the conditional ablation of *Smo* in adult hematopoietic tissues concluded that SMO-mediated HH signaling is not required to maintain normal adult HSC function (Gao et al., 2009; Hofmann et al., 2009). In summary, the long list of discrepancies with regard to the requirement for HH in HSC function might result from a differential requirement for HH signaling in the niche versus the HSCs themselves, and/or from a different role for HH at different stages of development.

HH signaling in adult tissues of endodermal origin

The embryonic endoderm contributes to tissues of the respiratory, gastrointestinal and genitourinary systems. During development, canonical HH signaling is involved in the epithelial-mesenchymal communication that regulates the early formation of these systems, during which ligand-releasing epithelial cells signal to the GLI1+ mesoderm (Haraguchi et al., 2007; Motoyama et al., 1998; Ramalho-Santos et al., 2000). In the adult, HH continues to signal to the mesenchymal stromal cells but the effect of HH on tissue-specific stem cell populations within these tissues is only starting to be defined.

Respiratory and gastrointestinal systems

During early mammalian embryonic development, both the respiratory and digestive tubes arise from the primitive gut, also known as the archenteron – a cavity within the gastrula. Even at this early developmental stage, by instructing mesodermal Hox gene expression, HH regulates the specification and subdivision of the gut (Sheaffer and Kaestner, 2012). Therefore, due to their commonality of origin, we review the roles of HH signaling in the respiratory and gastrointestinal systems together.

Much like in the adult bone and muscle, localized upregulation of HH signaling occurs in response to injury in adult lung airways. In the normal adult mouse lung, only a few *Gli1*-expressing fibroblasts are detected around the airways. However, HH signaling is upregulated upon bleomycin-induced lung fibrosis or airway injury after treatment with naphthalene, as evidenced by an increase in stromal GLI1+ cells (Liu et al., 2013; Watkins et al., 2003). A similar increase in HH signaling has been detected in human lung fibrotic tissue (Stewart et al., 2003), whereas in adult mice *Shh* overexpression augments collagen deposition and lung fibrosis following airway injury (Liu et al., 2013). Consistent with a role for SHH in lung tissue repair, overexpression of *Shh* in normal adult mouse airway epithelium can induce cell proliferation and lung tissue modifications similar to those seen in injury (Krause et al., 2010).

Shh and *Ihh* expression continues to be detected throughout the adult gastrointestinal tract of both humans and rodents where it signals to the *Gli*-expressing mesenchyme (Kolterud et al., 2009; van den Brink et al., 2002, 2001; van Dop et al., 2010). In the adult murine stomach, HH signaling is thought to be responsible for inhibiting proliferation and stimulating the differentiation of the gastric epithelium (van den Brink et al., 2002, 2001). By contrast, upregulation of HH signaling by conditional removal of *Ptch1* in adult colonic mesenchyme results in the depletion of the epithelial precursor cell pool due to premature differentiation (van Dop et al., 2009). Furthermore, HH signaling has been found to be downregulated during repair following gastric ulcer induction, whereas inhibition of SMO via cyclopamine treatment of injured mice further inhibits gastric progenitor cell differentiation (Kang et al., 2009). In mouse models of HH pathway inhibition, atrophy of the small intestinal villi is observed resulting from the loss of villus smooth muscle cells, which is also accompanied by inflammation and an increase in proliferation in the epithelial compartment (van Dop et al., 2010; Zacharias et al., 2010). Conversely, upregulation of IHH expression in adult intestine promotes villus smooth muscle differentiation (Zacharias et al., 2011), indicating that HH signaling in the adult murine intestine regulates tissue homeostasis in a concentration-dependent manner.

The liver also forms part of the GI system and has the greatest regenerative capacity of any other endoderm organ. Here also, HH signaling activation is one of the steps towards tissue reconstruction following injury (Omenetti et al., 2007). After partial hepatectomy, canonical HH signaling is required for hepatocyte proliferation; blocking of the HH pathway with the SMO inhibitor cyclopamine decreases post-operative survival rates in mice (Ochoa et al., 2010). Upregulation of the pathway has been observed in the livers of individuals with primary biliary cirrhosis (Jung et al., 2007), also implicating HH signaling in the response to liver damage in humans. These studies together suggest that the role of HH signaling in cells of the gastrointestinal system is strongly dependent on the context of tissue injury or disease state.

Deconstructing the exact mechanism of HH signaling in normal and injured respiratory and gastrointestinal tissues might prove more complex than previously imagined. Results from several new studies in the adult trachea, stomach and liver have indicated that differentiated non-mitotic cells in these tissues can fully replace resident stem cells if the latter are selectively ablated (Stange et al., 2013; Tata et al., 2013; Yanger et al., 2013). These findings challenge the importance of an adult resident stem cell population, given that committed cells can replace the stem cells under specific conditions. Whether HH/GLI activity is required to maintain the function of endogenous putative stromal stem cell populations or plays a role in the dedifferentiation of mature cells remains to be explored.

Genitourinary system

One component of the adult genitourinary system that has great regenerative capacity is the adult prostate. Normally dormant like the

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liver, the prostate is capable of multiple rounds of androgen-induced regeneration following castration-induced involution (degeneration) of the ductal structures (Isaacs and Coffey, 1989). The complete regeneration of the prostate following injury and presence of label-retaining cells (Tsujimura et al., 2002) suggests the presence of quiescent stem cells in the prostate. A possible role for HH signaling in prostate regeneration was suggested by an experiment in which HH signaling was blocked during regeneration following castration and androgen stimulation in adult mice, which resulted in the failure of the tissue to regenerate (Karhadkar et al., 2004). However, the experiment has not been repeated and the cell type that responded to HH was not identified. SHH was recently found to be secreted by basal cells within the epithelial compartment of the prostate, which are likely to be the main source of HH within the prostate ducts (Peng et al., 2013). In the same study, *Gli1* was shown to be expressed in four subtypes of stromal cells, each possibly maintained by a distinct unipotent progenitor. Following multiple rounds of involution and regeneration, GLI1+ stromal cells were shown to continuously self-renew (Peng et al., 2013), indicating that epithelial SHH signals to bona fide stem cells in the prostate stroma in a paracrine fashion, much like during prostate development (Shaw and Bushman, 2007). Determining the identity of SHH-responding stem cells in the prostate is a priority, as SHH signaling has been implicated in prostate cancer (Chen et al., 2011), and overexpression of SHH ligand in the adult prostate is sufficient to induce neoplasia (Chang et al., 2011). A recent report on prostate cancer development revealed that nerve fibers innervating the prostate act as a positive regulator of cancer progression (Magnon et al., 2013). It will be interesting to determine whether any HH proteins are delivered to the prostate through peripheral nerves as is the case in the adult skin and rodent incisors (Brownell et al., 2011; Zhao et al., 2014) (Fig. 3C).

Similar to the prostate, SHH is also involved in the epithelial-mesenchymal interaction between ligand-secreting basal stem cells and the underlining GLI1+ mesenchyme in the adult murine bladder (Shin et al., 2011). Upon tissue regeneration following bladder injury, HH signaling becomes upregulated and participates in reciprocal signaling, leading to the increase in epithelial cell proliferation required for the restoration of normal bladder function. Furthermore, in a mouse model of muscle-invasive bladder cancer, the *Shh*-expressing basal cells were recently demonstrated to function as neoplasia-initiating cells (Shin et al., 2014). After chemical carcinogenesis, individual SHH+ cells could give rise to lesions that quickly progressed to carcinomas, after which *Shh* expression within the tumor was lost. Whether this is the case in humans and whether there are roles for HH in the progression of bladder carcinoma remains to be determined, especially given that constitutive upregulation in HH signaling has been detected in human bladder cancer cell lines (Pignot et al., 2012). There are likely to be interesting parallels between bladder and prostate cancer: in mouse models of prostate cancer, basal cells can also give rise to carcinomas, and the tumor cells also lose their basal cell characteristics (Choi et al., 2012; Wang et al., 2013). These few examples clearly demonstrate that, in the genitourinary system, base levels of HH signaling help maintain homeostasis and participate in tissue repair; however, in a disease context, the contribution of the HH signaling pathway can have detrimental and diverse consequences.

Conclusions

In adult tissue homeostasis, high levels of HH signaling are seen in specific populations of cells, many of which have stem and progenitor cell properties. However, the exact mechanism of canonical HH signal propagation downstream of the GLIs remains largely unknown, as specific HH target genes, stemness-inducing or otherwise, have not been identified for most adult tissues. Recent findings in the developing embryo have indicated that the activating and repressing effects of the GLIs are enforced through collaboration with local master regulators from the SOX (SRY box containing), FGF and HOX (homeobox) families, and that this collaboration allows tissue- or cell-specific interpretation of HH signaling. Whether this is the case in the adult and which factors HH is interacting with in various tissues during homeostasis, injury and regeneration are some of the most exciting and challenging issue the field is facing today.

Following injury, HH signaling can trigger stem and other resident cells to participate in repair, whereas in diseases, including cancer, perturbed levels of HH signaling can contribute to disease progression by different routes. Thus, HH upregulation can be viewed as a natural response to injury and a way to achieve tissue repair by promoting cell survival, proliferation, plasticity or transdifferentiation. If HH levels are reduced with aging, the decrease could prove detrimental to tissue homeostasis and repair, and represents a possible mechanism underlying age-related organ degeneration and poor repair. In an attempt to find treatments for various human cancers where HH is the suspected driving force behind disease progression, a large degree of effort has been spend on developing SMO inhibitors, which seem to be generally well tolerated in pediatric patients (Lin and Matsui, 2012). Although promising, these results are somewhat surprising given the broad regulatory role of HH in multiple tissues during development and in adulthood. Further long-term investigations are required to completely exclude the possibility that HH inhibition pharmacologically results in permanent adverse defects later in life or during aging.

The role of peripheral nerves in delivering signaling factors such as HH to regulate normal and regenerate non-neuronal tissues is only just beginning to emerge (Brownell et al., 2011; Zhao et al., 2014). Given this role of the nervous system in delivering HH ligands and the involvement of HH signaling in the developing enteric system (Liu and Ngan, 2013), it is interesting to speculate that HH released by nerves is also involved in transducing the signaling pathway in the GI system. Thus nerve-derived HH stands out as a putative crucial mediator of organ homeostasis and regeneration with the potential to target stem cell populations located in different organs.

In summary, although the HH signaling pathway was originally discovered over 20 years ago, there remain exciting avenues of exploration, particularly in the stem cell and regeneration fields where the exact roles of HH signaling in different cellular and disease contexts is still unclear. Understanding what regulates HH signaling at the systemic and local levels, as well as how such signals are translated at the transcriptional level in target tissues to allow a context-specific response is likely to be a central challenge for the HH field in the years to come and will ultimately help to uncover new therapeutic targets in multiple disease contexts.

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Competing interests

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