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Review

Sonic hedgehog signaling in the postnatal brain

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ABSTRACT

Sonic hedgehog (Shh) is a pleiotropic factor in the developing central nervous system (CNS), driving proliferation, specification, and axonal targeting in multiple sites within the forebrain, hindbrain, and spinal cord. Studies in embryonic CNS have shown how gradients of this morphogen are translated by neuroepithelial precursors to determine the types of neurons and glial cells they produce [1,2]. Shh also has a well-characterized role as a mitogen for specific progenitor cell types in neural development [3,4]. As we begin to appreciate that Shh continues to act in the adult brain, a central question is what functional role this ligand plays when major morphogenetic and proliferative processes are no longer in operation. A second fundamental question is whether similar signaling mechanisms operate in embryonic and adult CNS. In the two major germinal zones of the adult brain, Shh signaling modulates the self-renewal and specification of astrocyte-like primary progenitors, frequently referred to as neural stem cells (NSCs). It also may regulate the response of the mature brain to injury, as Shh signaling has been variously proposed to enhance or inhibit the development of a reactive astrocyte phenotype. The identity of cells producing the Shh ligand, and the conditions that trigger its release, are also areas of growing interest; both germinal zones in the adult brain contain Shh-responsive cells but do not autonomously produce this ligand. Here, we review recent findings revealing the function of this fascinating pathway in the postnatal and adult brain, and highlight ongoing areas of investigation into its actions long past the time when it shapes the developing brain.

Contents

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1. Introduction

The Hedgehog (Hh) signaling pathway, of which Shh is the major activating ligand in the brain, is central to the development and patterning of the CNS and other organs [4,5]. Signaling is initiated when secreted Shh binds Patched (Ptc) at the cell surface, relieving inhibition of the transmembrane protein Smoothened (Smo) [6,7] and ultimately triggering the activation of the Gli transcription factors. In the absence of Hh signal, Gli3 is proteolytically processed and acts as a transcriptional repressor. Gli2 functions primarily as a transcriptional activator upon Hh stimulation and can initiate transcription of Gli1, a constitutive transcriptional activator that indicates high levels of pathway activity [8–11]. However, in some contexts, Gli3 may act as a weak activator, and Gli2 may act as a weak repressor [12,13]. The relative levels of the Gli transcription factors, and the balance between repression and activation of Hh pathway target genes, are a major mechanism by which cells in the developing neural tube, one of the best-characterized Shh-responsive tissues, translate a gradient of Hh ligand into a pattern of distinct neuronal fates [6,14–22]. Expression of Shh and modulation of downstream target genes is also thought to be critical for establishing the early patterning of the ganglionic eminences, a major source of inhibitory interneurons for the telencephalon [18,23]. Shh also functions in regulating the patterning and proliferation of precursor cells in the postnatal cerebellum, with a concomitant role (when mutated) in cerebellar tumor development [24–28]. More recently, Gli3, and transduction of the Shh signal via the primary cilium, have also been implicated in development of the cortex [29,30]. Finally, Shh appears to act through non-canonical mechanisms, which are likely Gli-independent, to regulate axon guidance and cortical microcircuit formation in the developing brain [31,32].

2. Establishing neurogenic niches – Shh and the primary cilium in maturing brain

In addition to the many functions Hh signaling has in development, this pathway is key for the formation and patterning of brain germinal niches that continue to produce neurons and glial cells throughout the life of most mammals [33–36]. These niches are the ventricular–subventricular zone (V-SVZ), which is found along the walls of the lateral ventricles, and the subgranular zone (SGZ), within the dentate gyrus in the hippocampus (highlighted in Fig. 1) [37,38]. The V-SVZ primarily generates neuroblasts, which migrate anteriorly to the olfactory bulb and differentiate into several types of neurons [39–42]. The V-SVZ also generates oligodendrocytes *in vivo*, and stem cells isolated from each of these regions generate astrocytes, oligodendrocytes, and neurons *in vitro*. The adult V-SVZ NSCs have many characteristics of astrocytes, including expression of glial fibrillary acidic protein (GFAP), and are known as B1 cells. These cells arise from radial glia, the embryonic stem cells that persist in the periventricular region of neonatal mice [43,44].

The SGZ, in contrast, is not located next to the ventricles, but is found deep in the brain parenchyma at the interface of the granule cell layer and hilus in the dentate gyrus of the hippocampus [45]. The NSCs in the SGZ also correspond to cells with astroglial properties: they are radially oriented astrocytes with a cell body in the SGZ and a prominent GFAP-positive process that traverses radially through the granule cell layer to profusely branch in the inner molecular layer [38,45–47]. These cells are known by various names, including radial astrocytes, radial glial-like cells or radial progenitors; since these cells do not retain radial glia morphology and are not located in a VZ or SVZ, we will refer to them here as radial astrocytes (RA), the original name used when they were first identified as the SGZ primary progenitors in the adult brain [46]. Like the B1 cells in the V-SVZ, RA in the SGZ were thought to be derived from embryonic radial glia [48,49]. However, recent work has also shown that some RA precursors undergo a curious and extensive tangential migration, taking them from a unique ventral Hh-responsive location at the temporal pole of the developing hippocampus to ultimately disperse throughout the SGZ [50]. Once established in the SGZ, RAs persist throughout life, generating new granule neurons through intermediate precursors. A number of studies focusing on prenatal removal of Hh pathway components have revealed a stringent requirement for canonical Hh signaling, mediated through the primary cilium, in the patterning and maintenance of both the V-SVZ and SGZ.

Shh knockout mice lack ventral structures in the CNS and die after birth, precluding analyses of postnatal brain development [9,51]. As a consequence, many of the clues indicating that the Shh pathway is central to the establishment of adult germinal zones came from studies using cell type-specific or inducible Cre recombinases to ablate specific pathway members in the mouse. Using the *Nestin-Cre* transgenic mouse, which drives recombination throughout the developing telencephalon, Machold and colleagues ablated either Shh itself or Smo, which is required for cells to transduce the Shh signal [9]. Although early Shh-dependent dorsoventral patterning, such as the establishment of the ganglionic eminences, is largely normal in these animals, striking defects were present in both postnatal neurogenic niches at two weeks after birth, suggesting a requirement for Shh in their establishment or maintenance. Smo-deficient animals exhibit an overall reduction in brain mass, enlarged ventricles, and reduced numbers of progenitor cells in the germinal regions. Specifically, both the V-SVZ and SGZ are thinner, and have decreased BrdU incorporation and increased apoptotic markers at early postnatal timepoints. These data suggest an ongoing requirement for Smo in both neurogenic niches. Following this initial observation, subsequent studies used tamoxifen-inducible Cre recombinase, again driven by the Nestin promoter, to specifically examine the postnatal requirement for Smoothened in the V-SVZ [52,53]. Similar to the effects observed following ablation during embryonic development, deletion of Smo during the immediate postnatal period results in a marked decrease in neurogenesis. However, no increase in apoptosis was observed. These data suggest that Smo, and Shh signaling that occurs in the juvenile brain after embryonic and fetal development, have a specific effect on the proliferation, and possibly on the self-renewal, of NSCs.

The function of Shh in developing stem cell niches is also dependent on the presence of a functional primary cilium. Ablation of the motor protein KIF3A, intraflagellar transport protein IFT88, or the ciliary protein Stumpy, and therefore the removal of functional primary cilia in neural precursors, results in decreased Shh target gene expression and a phenotype similar to that observed in Smo-deficient animals [54,55]. *hGFAP-Cre; Kif3a*$^{fl/fl}$ and *hGFAP-Cre; Smo*$^{fl/fl}$ animals, like *Nestin-Cre; Smo*$^{fl/fl}$ animals, have a hypocellular and disorganized dentate gyrus at birth, accompanied by decreased proliferation and neurogenesis. Removal of primary cilia also blocks the effects of heightened pathway activation via expression of a hypermorphic Smo, SmoM2. Similarly, ablation of primary cilia has significant effects in the postnatal V-SVZ, but here the interpretation is complicated as the promoters used for genetic ablation of primary cilia also affect the function of motile cilia in ependymal cells and therefore cerebrospinal fluid (CSF) flow (unpublished observation). Ependymal cells and CSF are integral components of the adult V-SVZ niche [56–58] and disruption of motile cilia in ependymal cells is likely to indirectly affect V-SVZ progenitors. New approaches to selectively ablate cilia in V-SVZ progenitors, but not ependymal cells, are required to understand the role of primary cilia in these periventricular NSCs.

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