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Sonic hedgehog as a chemoattractant for adult NPCs

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The developmental morphogen Sonic hedgehog (Shh) is well known for its role in modulating the proliferation and survival of neural progenitor cells in the developing mouse brain. A recent report now showed that Shh could regulate the migration of neuroblast in the adult subventricular zone (SVZ) along the rostral migratory stream (RMS) to the olfactory bulb, by functioning as a chemoattractant. Functions of Shh in regulating the migration and survival of neural progenitor cells in the adult central nervous system are suggestive of its potential roles in neural regeneration and CNS oncogenesis.

Key words: cell migration, neural progenitor cells (NPCs), rostral migratory stream (RMS), sonic hedgehog (Shh), subventricular zone (SVZ)

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Sonic Hedgehog (Shh) is the best studied member of the Hedgehog family of secreted developmental morphogens,[1] and has wide ranging roles in the developing central nervous system (CNS).[2,3] In the developing neural tube, Shh is secreted by the notochord and floor plate cells, resulting in a dorsoventral concentration gradient that induces the formation of different ventral cell types (ventral patterning).[4] Shh also plays critical roles in regulating neural progenitor or neural precursor cell (NPC) proliferation of the developing mouse brain,[5] including those of the neocortex[6] and cerebellum.[7,8] Of particular interest in this regard is that conditional knockout of *Shh* and its signaling component *Smoothened* (*Smo*) resulted in a reduction of neural progenitors at the two key neurogenic regions of the adult brain, the subventricular zone (SVZ) and hippocampal dentate gyrus (DG).[9,10] Shh is a known axonal guidance factor, and recent findings indicates that neuronal growth cone chemoattraction occurs through a non-canonical signaling pathway involving Src family kinases.[11] The multifaceted activity of Shh in the adult brain is now further emphasized by recent findings of Angot et al.[12] that other than a growth/survival factor, it could also serve as a chemoattractor for NPCs and modulates their long distance migration.

Shh Protein and its Receptors in the Adult SVZ and RMS System

The SVZ of the lateral ventricles (LV) is one of two major CNS stem cell niches where significant neurogenesis still takes place in the adult mammalian brain. Here, periventricular astrocyte-like neural stem cells (known as B cells) generate actively dividing transit amplifying C cells, which in turn give rise to migratory neuroblast cells (or A cells).[13] The latter then follows the rostral migratory stream (RMS) to the olfactory bulb (OB), where they would differentiate into OB interneurons to continuously replace demised ones. As mentioned above, loss of Shh signaling by conditional knockout resulted in reduced number of proliferating progenitor cells, and increased cell death.[9] A cell migration to OB were also apparently impaired.[10] Results from earlier studies reported by Palma and colleagues indicated that SVZ cells express Shh and its key downstream effector, the transcription factor Gli1.[14] These authors also found that Shh regulates progenitor cell renewal and proliferation of SVZ lineages in mitogenic cooperation with epidermal growth factor.

Angot et al. showed further in their recent report that the active processed Shh N-terminal fragment (ShhN) could be detected in SVZ lysates and the cerebrospinal fluid (CSF), but not in samples of CSF-producing choroid plexus of the

brain's ventricular system. Transcripts of the Shh receptor Patched (Ptc), and its signaling mediator Smo, are also found in SVZ. Co-tracing the expression of a migrating neuroblast marker (polysialated neural cell adhesion molecule (PSA-NCAM) and Ptc expression using a *lacZ* reporter inserted into a deleted allele of $Ptc^{+/-}$ mice, the authors detected Ptc expression in both PSA-NCAM positive A cells in SVZ and the RMS, as well as PSA-NCAM negative (presumably C or even B cells) cells in the SVZ. These data reaffirmed the notion that Shh present within SVZ could affect neural progenitors as well as migrating neuroblasts.

Blocking or Elevating Shh Activity had Reciprocally Opposite Effects on Dividing Progenitor Cell Number in the SVZ Compared to those at the Olfactory Bulb (OB)

Angot et al.[12] investigated the effect of blocking Shh signaling on the migrating neuroblasts in the SVZ-RMS-OB pathway using adenoviral vector expressed Hip, a putative Shh antagonist in vertebrates. Dividing cells were labeled by intraperitoneally administered bromodeoxyuridine (BrdU). Interestingly, with robust Hip expression, the number of BrdU positive nuclei was decreased in the SVZ, but increased in the granular layer of OB. Hip expression did not significantly affect the total number of BrdU positive cells in the entire SVZ-RMS-OB pathway, and did not result in any significant changes in apoptotic rate in either the SVZ or OB, as assessed by TUNEL analysis. Double labeling of BrdU with a neuronal marker (NeuN) and an astrocyte marker (glial fibrillary acidic protein, GFAP) indicated that the ratio of neuronal versus astroglia differentiation tendency is not affected by Hip, and that there is an absence of orientation towards NG2 positive oligdendroglia precursor. The effect of Hip in reducing BrdU positive cells in SVZ and increasing these at OB is faithfully phenocopied by the introduction of hybridoma cells expressing a ShhN neutralizing antibody (5E1).

In performing the reciprocal experiment, the authors injected *Shh* bearing adenovirus and again followed cells labeled by BrdU. Overexpression of Shh in the SVZ-RMS-OB pathway resulted in an effect that is directly opposite to that of Shh blocking, namely an increased in BrdU positive nuclei in the SVZ, accompanied by a corresponding decrease in the granular layer of the OB. Again Shh overexpression did not result in a statistically significant difference in total number of BrdU positive cells. These results, taken together, indicate that modulation of Shh activity affects, in a concordantly opposite and reciprocal manner, the numbers of migrating neuroblast in the proximal and distal ends of the SVZ-RMS-OB pathway. As these phenomena could not be attributed to changes in their rate of generation or survival, a very likely possibility is that the migration properties of these cells were modulated by Shh.

Shh is a Chemoattractant for Migrating Neuroblasts in vitro and in vivo

To test the above notion, the authors co-cultured SVZ explants with COS cells that either transiently express Shh (or does not), and quantified cells migrating out of these explants with relevance from their proximity to the Shh source. When co-cultured together with COS cells expressing Shh, outward migration of βIIItubulin-positive (therefore neuronal precursor in nature) cells become asymmetrical. Significantly more cells migrate into the proximal quadrant compared to the distal quadrant. This asymmetry is abolished by a specific Smo antagonist added to the media. However, quantification of migratory distances revealed no significant changes to neuroblast motility per se.

Extending the above investigations in vivo, the authors grafted Shh-expressing QT6 cells to an area devoid of progenitor cells in the dorsal telencephalon above the RMS. This resulted in a thickening of the RMS, and the appearance of PSA-NCAM positive migrating neuroblasts in the area between the Shh-expressing graft and the RMS. This RMS thickening and deviation of migratory cells from the original RMS track is numerically significant, and is not due to changes in proliferation or survival. The in vitro and in vivo demonstration of Shh's chemoattractant property for SVZ-derived migrating neuroblasts affirms the notion that beyond modulating progenitor cell survival in the adult brain, Shh could also regulate their migration. These interesting results are in some agreement with earlier studies,[9,10] but remain mechanistically unexplored. The notion of Shh being a chemoattractant for migrating neural progenitors has important implications, as outlined below.

Functions of Shh Signaling in Adult Brain Progenitors and Implications of the Chemoattractant Role of Shh

The idea of morphogens serving guidance roles has been around for some time. Other than Shh, boundary defining morphogenic factors like Wnt, transforming growth factor β (TGFβ), and fibroblast growth factor (FGF) could function at later developmental stages to control axon growth.[15] Angot et al.'s observations, on the whole, presented much milder phenotypes compared to those reported using nestin-Cre driven *Smo* conditional knockout from Fishell's laboratory.[9,10] The latter authors observed deterioration of the SVZ postnatally, and increased cell death of perhaps all SVZ cell types. OB migration by A-cells was impaired (probably due to Shh signaling affecting indirectly slit expression by these cells) and the population is eventually depleted by P30. Angot et al. did not observe significant cell death, but it would nonetheless be interesting to check if guidance molecules, such as slit, act downstream of Shh. Shh has been shown to affect neuroepithelial cell adhesion through modulation of surface β1-integrin dispersal and N-cadherin mediated adhesion though a Ptc/Smo-independent mechanism.[16] As Shh's chemoattraction is clearly Smo-dependent and neuroblast motility out of SVZ explant is not apparently altered by a Shh source, this effect is likely to be fundamentally different from that of neuroepithelial cells.

Any survival/migration modulating factor of the neural progenitor population within the adult CNS are potentially relevant to two important aspects of human health, namely neural regeneration and

CNS oncogenesis. Of course, the existence of a rodent equivalent of SVZ-RMS-OB in the adult human brain has been controversial,[17] and the degree of morphological and functional conservation of the pathways between rodents and humans have remained uncertain. In any case, understanding factors modulating progenitor cell migration could provide the foundation to future strategies that aim to guide cells from active adult neurogenic regions (SVZ and DG) to sites of injury or degeneration. On the other hand, it is clear that ectopic or dysregulated Shh expression could affect neural progenitor proliferation and migration, which makes it a potentially useful point of intervention in the treatment of brain tumor invasion or metastasis.

References

1. Jiang J, Hui CC. Hedgehog signaling in development and cancer. Dev Cell 2008; 15:801-12.
2. Martí E, Bovolenta P. Sonic hedgehog in CNS development: one signal, multiple outputs. Trends Neurosci 2002; 25:89-96.
3. Ruiz I, Altaba A, Palma V, Dahmane N. Hedgehog-Gli signaling and the growth of the brain. Nat Rev Neurosci 2002; 3:24-33.
4. Wilson L, Maden M. The mechanisms of dorsoventral patterning in the vertebrate neural tube. Dev Biol 2005; 282:1-13.
5. Lai K, Kaspar BK, Gage FH, Schaffer DV. Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. Nat Neurosci 2003; 6:21-7.
6. Palma V, Ruiz I, Altaba A. Hedgehog-GLI signaling regulates the behavior of cells with stem cell properties in the developing neocortex. Development 2004; 131:337-45.
7. Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron 1999; 103-14.
8. Wallace VA. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. Curr Biol 1999; 9:445-8.
9. Machold R, Hayashi S, Rutlin M, Muzumdar MD, Nery S, Corbin JG, et al. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 2003; 39:937-50.
10. Balordi F, Fishell G. Hedgehog signaling in the subventricular zone is required for both the maintenance of stem cells and the migration of newborn neurons. J Neurosci 2007; 27:5936-47.
11. Yam PT, Langlois SD, Morin S, Charron F. Sonic hedgehog guides axons through a noncanonical, Src-family-kinase-dependent signaling pathway. Neuron 2009; 62:349-62.
12. Angot E, Loulier K, Nguyen-Ba-Charvet KT, Gadeau AP, Ruat M, Traiffort E. Chemoattractive activity of sonic hedgehog in the adult subventricular zone modulates the number of neural precursors reaching the olfactory bulb. Stem Cells 2008; 26:2311-20.
13. Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 2009; 32:149-84.
14. Palma V, Lim DA, Dahmane N, Sánchez P, Brionne TC, Herzberg CD, et al. Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. Development 2005; 132:335-44.
15. Sánchez-Camacho C, Rodríguez J, Ruiz JM, Trousse F, Bovolenta P. Morphogens as growth cone signalling molecules. Brain Res Rev 2005; 49:242-52.
16. Jarov A, Williams KP, Ling LE, Koteliansky VE, Duband JL, Fournier-Thibault C. A dual role for Sonic hedgehog in regulating adhesion and differentiation of neuroepithelial cells. Dev Biol 2003; 261:520-36.
17. Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelso C, et al. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 2007; 315:1243-9.