Mechanisms of Development 138 (2015) 233–245

Contents lists available at ScienceDirect

Mechanisms of Development

journal homepage: www.elsevier.com/locate/mod

Insm1 promotes neurogenic proliferation in delaminated otic progenitors

Sarah M. Lorenzen [a], Anne Duggan [a], Anna B. Osipovich [b], Mark A. Magnuson [b], Jaime García-Añoveros [a,c,d,*]

[a] Department of Anesthesiology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
[b] Center for Stem Cell Biology, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA
[c] Departments of Neurology and Physiology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
[d] Hugh Knowles Center for Clinical and Basic Science in Hearing and Its Disorders, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 2 October 2015
Accepted 2 November 2015
Available online 3 November 2015

Keywords:
Spiral ganglion
Vestibular ganglion
Outer hair cell
Otocyst
Basal progenitors
Subventricular zone

ABSTRACT

INSM1 is a zinc-finger protein expressed throughout the developing nervous system in late neuronal progenitors and nascent neurons. In the embryonic cortex and olfactory epithelium, *Insm1* may promote the transition of progenitors from apical, proliferative, and uncommitted to basal, terminally-dividing and neuron producing. In the otocyst, delaminating and delaminated progenitors express *Insm1*, whereas apically-dividing progenitors do not. This expression pattern is analogous to that in embryonic olfactory epithelium and cortex (basal/subventricular progenitors). Lineage analysis confirms that auditory and vestibular neurons originate from *Insm1*-expressing cells. In the absence of *Insm1*, otic ganglia are smaller, with 40% fewer neurons. Accounting for the decrease in neurons, delaminated progenitors undergo fewer mitoses, but there is no change in apoptosis. We conclude that in the embryonic inner ear, *Insm1* promotes proliferation of delaminated neuronal progenitors and hence the production of neurons, a similar function to that in other embryonic neural epithelia. Unexpectedly, we also found that differentiating, but not mature, outer hair cells express *Insm1*, whereas inner hair cells do not. *Insm1* is the earliest known gene expressed in outer versus inner hair cells, demonstrating that nascent outer hair cells initiate a unique differentiation program in the embryo, much earlier than previously believed.

1. Introduction

During embryogenesis, several different neural epithelia contribute to the generation of central and peripheral nervous system neurons. The primary sensory neurons (auditory and vestibular) of the ear are generated by a largely placode-derived structure known as the otocyst (Anniko and Wikstrom, 1984; Freyer et al., 2011). There is also a controversy as to whether the neural tube might contribute a small number of cells to the otocyst (Freyer et al., 2011; Sandell et al., 2014; Steventon et al., 2014). In the mouse, beginning at embryonic day 8.5 (E8.5), the otic placode begins to invaginate. It then pinches off and separates from the ectoderm to form the epithelial sac known as the otocyst or otic vesicle by E9.5 (Anniko and Wikstrom, 1984; Barald and Kelley, 2004). Within the otocyst, the anteroventral quadrant specializes to produce the neurons, as well as the sensory receptor cells (i.e. hair cells) and their support cells (Appler and Goodrich, 2011).

As in other neural epithelia, uncommitted otocyst progenitors undergo interkinetic nuclear migration whereby mitosis occurs apically within the epithelium but DNA synthesis (S-Phase) occurs basally. Beginning at E9.5, neuronal progenitors migrate basally and delaminate from the otocyst into the adjacent mesenchyme (Raft et al., 2004; Rubel and Fritzsch, 2002) (Fig. 1). There they divide to produce only auditory and vestibular primary neurons. However, it is yet to be determined the extent of proliferation after delamination, whether all delaminated cells divide or some delaminate as postmitotic nascent neurons (D'Amico-Martel and Noden, 1983). In fact, one report has shown that some cells within the otic epithelium may have already sent projections to the hindbrain before delaminating (Fritzsch, 2003; Yang et al., 2011). Delaminated progenitors exit the cell cycle from E9.5–E13.5 (Matei et al., 2005). All glia associated with these neurons derive from the neural crest and migrate into the forming ganglia around E10.5. In this article, we will refer to all cells that delaminate from the otocyst as delaminated progenitors (DPs), including those that will divide to produce neurons and those that may directly differentiate into neurons.

The morphological changes of the developing spiral and vestibular ganglia (SVG) are accompanied by a cascade of transcription factors. First, Neurog1 defines the proneural domain and is necessary for SVG formation (Ma et al., 2000; Ma et al., 1998). After Neurog1 establishes the neuron producing region of the otocyst, it activates NeuroD in the neuronal progenitors, which promotes delamination and neuron survival (Jahan et al., 2010a; Kim et al., 2001; Liu et al., 2000). As DPs migrate to the base of the epithelium they activate Isl1, whose function is not known. Around this time, Gata3 expression is restricted in the otocyst and enriched in the ventral region that will become the organ of Corti (Lawoko-Kerali et al., 2002). DPs that express Gata3 will produce the SpG and, in its absence, no SpG neurons are generated (Duncan

* Corresponding author at: 303 E. Chicago Ave, Ward 10-070, Chicago, IL 60613, USA.
E-mail address: anoveros@northwestern.edu (J. García-Añoveros).

http://dx.doi.org/10.1016/j.mod.2015.11.001
0925-4773/

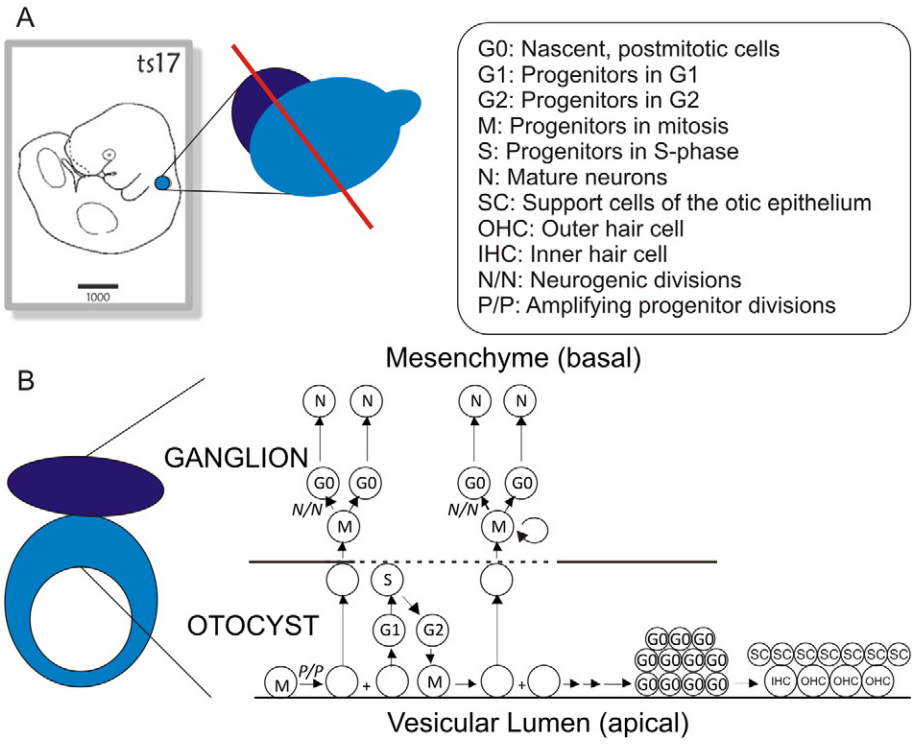

Fig. 1. Schematic of otocyst proliferation and differentiation. (A) Position of the otocyst in an E10.5 mouse embryo (left panel, highlighted in blue), and relationship between delaminated progenitor region (purple) to the otocyst (center panel). (B) Morphological structure through a cross section of the otocyst along the red line in (A) with its arrangement of cell nuclei shown to the right. Within the otocyst uncommitted progenitors undergo interkinetic nuclear migration and divide apically. Neuronally-committed progenitors delaminate into the mesenchyme where some or all of them will divide an undetermined number of times to produce neurons. These neurons will then coalesce to form both spiral and vestibular ganglia. Cells that remain within the otocyst will form auditory and vestibular hair cells as well as support and structural cells. The arrangement of mature cells in the organ of Corti is more complex than we have illustrated in this figure. For a more detailed and accurate representation of the arrangement, ratios and cell types present in the organ of Corti see (Jahan et al., 2015a). Left panel in (A) from emouseatalas.org. See figure for abbreviations.

et al., 2011; Lawoko-Kerali et al., 2004). Gata3 has also been conditionally ablated in SpG neurons after neurogenesis and has been shown to be necessary for their axonal pathfinding and survival (Appler et al., 2013; Duncan and Fritzsch, 2013). After DPs delaminate, they express Pou4f1, which contributes to SpG neuron differentiation by regulating cell size and maintaining TrkC expression (Deng et al., 2014; Huang et al., 2001). Following cell cycle exit, differentiating nascent SVG neurons express Pou4f2, whose function has not yet been determined (Deng et al., 2014; Huang et al., 2001).

While elements of this signal cascade are well understood, there are still many aspects of SVG formation that are not. As of yet, the regulation of DP proliferation is not well understood. Deletion of N-Myc from the mouse ear results in an overall smaller ear beginning as early as E9.0, ultimately including a shortened cochlea and smaller spiral ganglion (Dominguez-Frutos et al., 2011; Kopecky et al., 2011). This phenotype is attributed to a decrease in proliferation in the otocyst, where the effect is global and not specific to delaminating progenitors (Dominguez-Frutos et al., 2011; Kopecky et al., 2011). Likewise, Foxg1 is expressed throughout the developing ear and its deletion leads to global disruptions in the ear including a shortened cochlea with shorter spiral ganglion, though the mechanism for the shortened ganglion was not investigated (Pauley et al., 2006). Tis21, a marker of cortical basal progenitors, was recently proposed to promote neurogenesis of the spiral ganglion, though the specific mechanism and timing of expression were not determined (Yamada et al., 2015). Also, experiments using chick otic explants have shown that IGF-1 can promote proliferation of DPs (Camarero et al., 2003). However, SpG size and number of neurons were unaltered in *Igf1* null mice at postnatal day 5. Hence, IGF-1 has little to no effect on neurogenesis in the mouse, though it is required for neuron survival (Camarero et al., 2001).

INSM1 is a zinc-finger protein whose mRNA is expressed in all examined neurogenic regions of the developing nervous system, including areas of adult neurogenesis (Duggan et al., 2008). In many developing neural epithelia, *Insm1* is expressed in a basal zone that includes neuronally-committed progenitors and nascent neurons. *Insm1* is not expressed in the apical zones, where uncommitted progenitors divide, or in mature neurons (Duggan et al., 2008). In the cortex and olfactory epithelium, deletion of *Insm1* results in fewer basally-dividing, neuronally-committed progenitors and consequently in fewer neurons (Farkas et al., 2008; Rosenbaum et al., 2011).

Here, we examine the expression pattern and function of *Insm1* in the embryonic mouse ear. We find that *Insm1* is transiently expressed during neurogenesis in delaminating, neuronally-committed progenitors and nascent SVG neurons. This pattern of expression is equivalent to that in embryonic olfactory epithelium and cortex, revealing homologies in the neurodevelopment of ear, nose and cortex. Additionally, the absence of *Insm1* results in fewer SVG neurons. This is not due to an increase in apoptosis, or to a decrease in delaminations, but instead it is due to a reduction in divisions of DPs. Unexpectedly, we also find expression of *Insm1* in outer hair cells (OHCs) of the embryonic cochlea beginning as they initiate differentiation and subsiding as they mature.

2. Results

Since previous studies show that *Insm1* is typically expressed transiently by neuronal progenitors (Duggan et al., 2008), we used a lineage tracing technique to determine which inner ear cell types are generated by *Insm1* expressing progenitors (see methods). In postnatal day 7 (P7) mouse ear, all spiral ganglion (SpG) neurons, vestibular ganglion (VG) neurons, and OHCs were positive for β-gal (Fig. 2A and C). Saccular, utricular, and cochlear inner hair cells (IHCs) and support cells of the sensory epithelia were not β-gal positive. Also, no cell was $Insm1^{GFP.Cre}$ positive at P7, limiting *Insm1* expression to prior (embryonic to early