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Original Article

Spatiotemporal expression of Bmi1 in the developing mouse cochlea

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Abstract: Bmi1 is a member of the Polycomb protein family and has been reported to play important roles in regulating the survival of auditory hair cells. However, the detailed expression profile of Bmi1 during mouse cochlear development has not yet been fully investigated. Here, we used C56BL/6J mice to examine the spatiotemporal expression of Bmi1 in the cochlear duct during embryonic and postnatal development. Bmi1 expression was first observed throughout the nuclei of the otocyst at embryonic day 11 (E11). At E14.5 and E15.5, Bmi1 was expressed in the nuclei of differentiating cochlear epithelia from the apical to the basal turns. At E15.5, Bmi1 also began to be expressed in the spiral ganglion. From E18 to postnatal day 0 (P0), the expression of Bmi1 was located in the nuclei of the cochlear epithelium in all three turns and could be distinctly seen in the outer hair cells, inner hair cells, supporting cells (inner phalangeal cells, Deiters' cells, pillar cells, and Hensen's cells), Kölliker's organ, the striae vascularis, and tympanic border cells. Bmi1 continued to be expressed in spiral ganglion cells from E18 to P0. Our results provide the basic expression pattern of Bmi1 and might be helpful for future investigations of the detailed role of Bmi1 during the development of the cochlea.

Keywords: Polycomb protein, Bmi1, inner ear, cochlea, development

Introduction

The mammalian cochlea is a complex sensory organ that is responsible for sound detection, and it contains two types of auditory hair cells (outer hair cells and inner hair cells) and at least four types of supporting cells (Hensen's cells, pillar cells, inner phalangeal cells, and Deiters' cells). Development of the mouse cochlea begins with a thickening of the ectoderm adjacent to the hindbrain-known as the otic placode-on embryonic day 8.5 (E8.5) [1]. The otic placode then invaginates to form the otocyst from E9.5 to E11.5. At this time, the otocyst is already regionalized into different developmental compartments [2]. The progenitors of the hair cells and supporting cells of the prosensory epithelium are still dividing at E12.5 and E13.5 [3, 4]. At E14.5, all progenitor cells of the inner ear have exited the cell cycle [5]. On day E14.5, the cristae medialis and the cristae lateralis in the basal turn of the cochlea form the primordia that develop into Kölliker's organ and the organ of Corti, respectively. Both organs are fully developed by E15.5, and cell differentiation in the organ of Corti into outer hair cells, inner hair cells, and supporting cells is completed between E17 and E18 [2].

Epigenetic regulation plays important roles in the development of the inner ear [6], and a recent report has revealed the epigenetic mechanism behind Atoh1 regulation that underlies hair cell differentiation and subsequent maturation [7]. Dynamic changes in the histone modifications H3K4me3/H3K27me3, H3K9ac, and H3K9me3 correlate with the onset of Atoh1 expression during hair cell differentiation. Bmi1 is the first identified member of the Polycomb group of proteins [8], which form large complex-

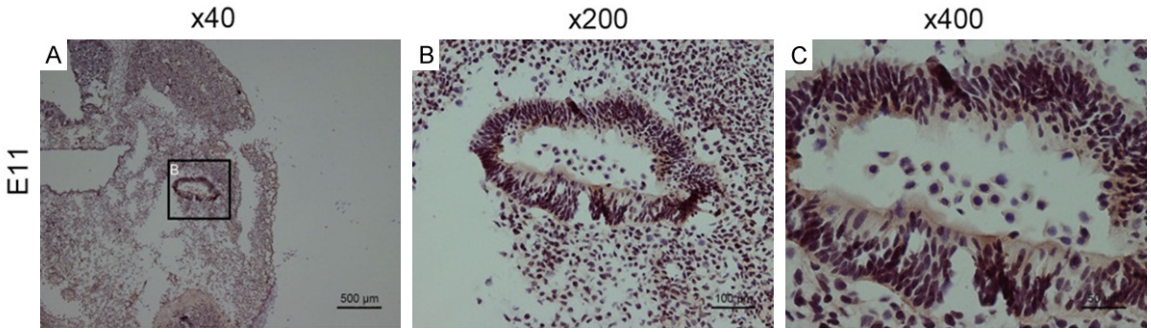

Figure 1. Bmi1 staining in the otocyst on E11, which is shown at three magnifications.

es, including PRC1 (Polycomb repressive complex 1) and PRC2 (Polycomb repressive complex 2). PRC2 methylates H3K27 of the chromatin containing the target gene, and this attracts PRC1, which maintains the silencing effect of H3K27me3 [9].

Bmi1 is a key protein in PRC1 and plays an important role in cell cycle regulation, cell immortalization, and cell senescence [10, 11]. Bmi1 is also involved in the regulation of self-renewal and differentiation of stem cells and in the development of several organs [12]. Our recent findings show that Bmi1 is expressed in the cochlea and plays an important role in hair cell survival by controlling redox balance and ROS level [13]. However, the detailed expression profile of Bmi1 has not yet been fully investigated during mouse inner ear development.

In this study, we performed immunohistochemistry in C56BL/6J mice to characterize the detailed spatiotemporal expression of Bmi1 in the embryonic and postnatal mammalian cochlear duct.

Materials and methods

Animals

Timed pregnancies were established for C57BL/6J wild type mice, and the morning of plug identification was defined as E0.5. P0 was defined as the day of birth. E11, E14.5, E15.5, E18, and P0 mice were used in this experiment. Mice were housed with open access to food and water at the Experimental Animal Center, Shanghai Medical College of Fudan University, China. All experiments were approved by the Shanghai Medical Experimental Animal Administrative Committee (Permit Number: 2009-0082), and all efforts were made to minimize suffering and reduce the number of animals used.

Tissue and frozen section preparation

Pregnant mice were anesthetized by subcutaneous injection of xylazine and ketamine and were coeliotomized to remove the embryos. Embryos were decapitated, and the heads were fixed overnight in 4% paraformaldehyde (PFA) in PBS, pH 7.2. P0 mice were decapitated to dissect out the inner ear, and the cochleae were fixed overnight in 4% PFA in PBS, pH 7.2. P0 cochleae were decalcified in 10% EDTA. Specimens were embedded in sucrose at 4% and cut into 10-μm-thick sections and mounted onto glass slides.

Immunohistochemistry

Epitopes were unmasked by microwave heating (1000 W) in retrieval buffer (Maixin Biotech) for 15 min. Sections were permeated with 0.1% Triton X-100, blocked with 10% donkey serum in 10 mM PBS (pH 7.4) for 30 min at room temperature, and then incubated with the primary anti-Bmi1 antibody overnight at 4°C in a humidified chamber. The following day, the sections were rinsed with PBS and then incubated with secondary antibody (DAKO) for 30 min at room temperature. After washing with PBS, the sections were stained with 3,3'-diaminobenzidine and counterstained with hematoxylin.

Results

Bmi1 was expressed in the otocyst

We first investigated the expression pattern of Bmi1 in the otocyst. At E11, the otic placode has invaginated and closed up to form the otocyst, in which the ventral part will give rise to

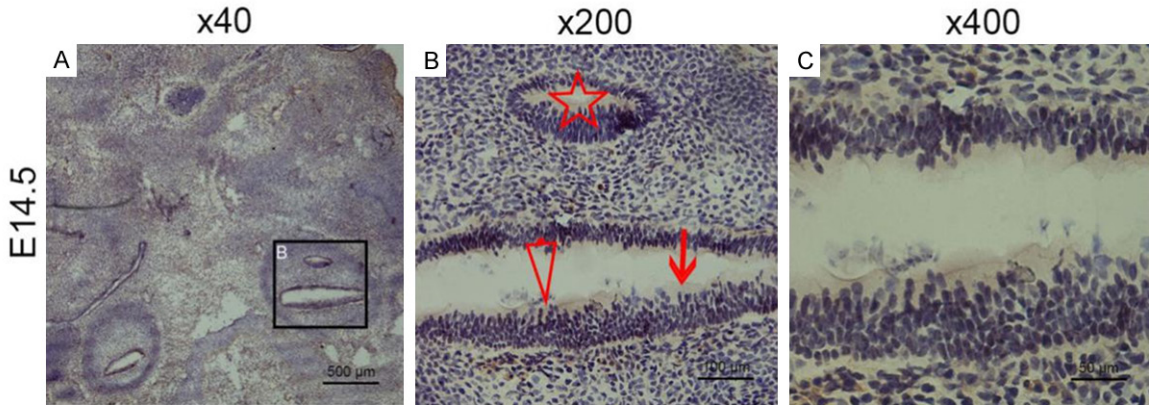

Figure 2. Bmi1 expression was seen in all turns of the cochlear epithelium on E14.5 shown at three magnifications. The red star indicates the apical turn and the red hollow arrowhead and arrow indicate Kölliker primordium and Corti's primordium, respectively.

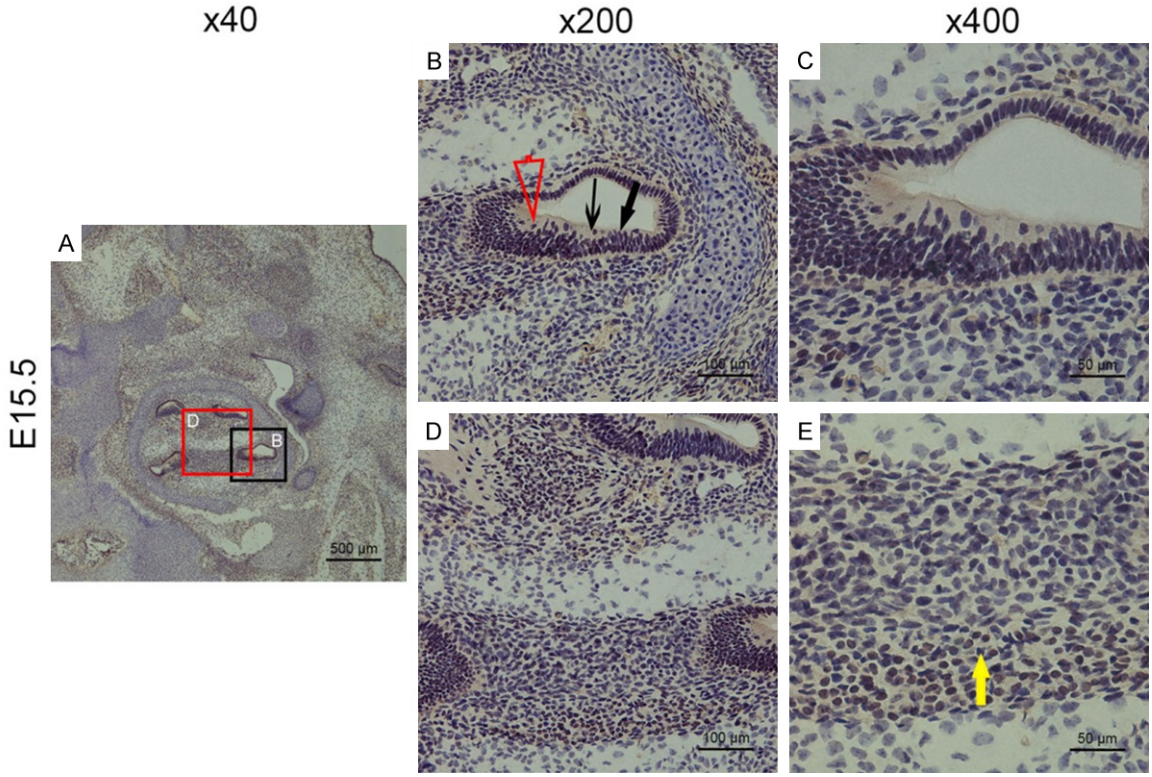

Figure 3. Bmi1 was expressed in the cochlear epithelium and spiral ganglion on E15.5 (A). Images (B) and (C) show that Bmi1 was expressed in the nuclei of the cochlear epithelium in the apical, middle, and basal turns. The red hollow arrowhead, black thin arrow, and black thick arrow indicate Kölliker's organ, inner hair cells, and outer hair cells, respectively. Bmi1 was clearly observed in the auditory hair cells in the basal turn. Images (D) and (E) show that Bmi1 was also expressed in the spiral ganglion. The yellow arrow indicates a spiral ganglion neuron.

the cochlea. Our data showed that Bmi1 was expressed in the dorsal and ventral part of the otocyst (**Figure 1**), indicating that Bmi1 might play important roles in the proliferation and specification of the prosensory domain.

Bmi1 was expressed in the embryonic cochlear duct

Between E13.5 and E14.5, the cells in the primordial organ of Corti stop proliferating due to

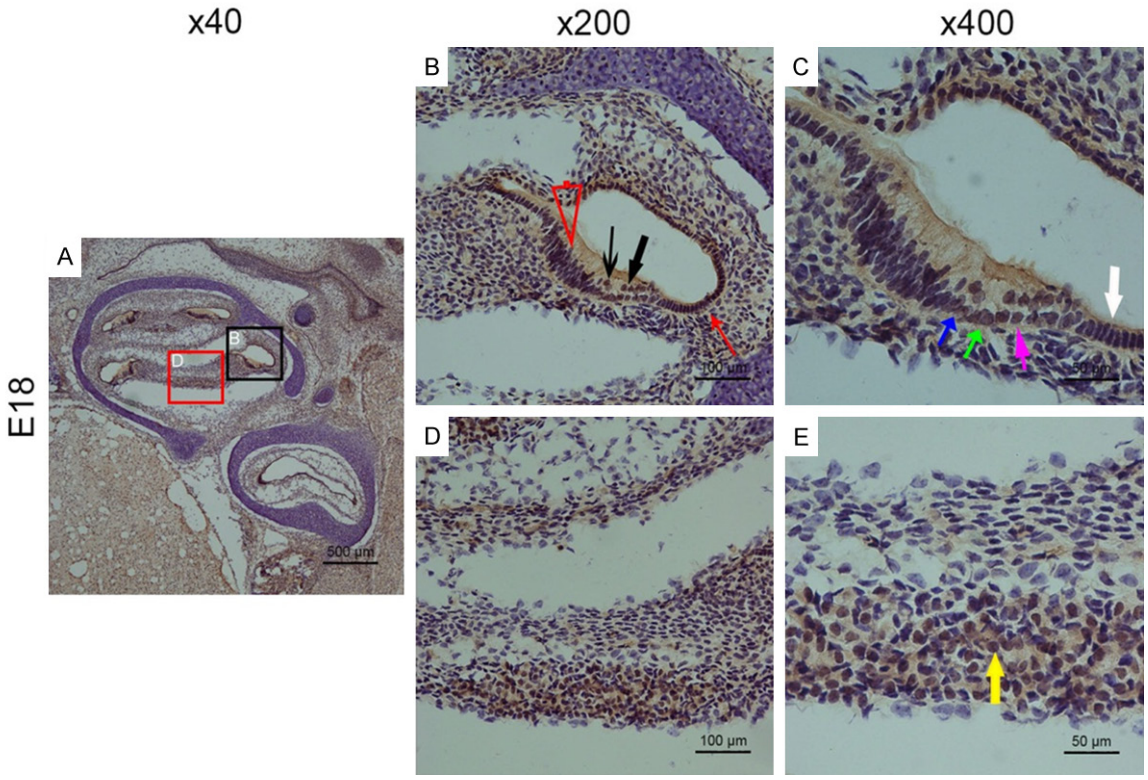

Figure 4. Bmi1 was expressed in the cochlear epithelium and spiral ganglion on E18 (A). Images (B) and (C) show that Bmi1 continued to be expressed in the nuclei of the cochlear epithelium in all three turns and could be distinctly seen in outer hair cells, inner hair cells, and surrounding supporting cells (inner phalangeal cells, Deiters' cells, pillar cells, and Hensen's cells). The red hollow arrowhead, black thin arrow, black thick arrow, and red arrow in (B) indicate Kölliker's organ, inner hair cells, outer hair cells, and the striae vascularis, respectively. The white arrow, pink arrow, green arrow, and blue arrow in (C) indicate Hensen's cells, Deiters' cells, pillar cells, and inner phalangeal cells, respectively. Images (D) and (E) show that Bmi1 was also expressed in the spiral ganglion. The yellow arrow indicates a spiral ganglion neuron.

the expression of the cyclin-dependent kinase inhibitors p27Kip1 and p19Ink4d in the cochlear duct. These nonproliferating cells then differentiate within the sensory primordium to form a precise mosaic of hair cells and supporting cells [1]. Hair cell differentiation starts around E14.5 in the cochlea as indicated by the expression of Math1 [14], and at E14.5 we found that Bmi1 was broadly expressed in the nuclei of the cochlear epithelium (**Figure 2**). Starting at E15.5, myosin 7a, one of the hair cell-specific markers, is expressed at the base of the organ of Corti [15]. As **Figure 3** shows, Bmi1 was clearly observed in auditory hair cells in all three turns at E15.5, indicating that Bmi1 might participate in the differentiation process of inner ear progenitor cells. By E15.5, the spiral ganglion neuron had reached to the cochlear modiolus, and Bmi1 was also expressed in the spiral ganglion (**Figure 3**).

Hair cells complete their differentiation between E17 and E18. We examined the E18 cochlear duct and found that Bmi1 was expressed in the nuclei of the cochlear epithelium in the apical, middle, and basal turns and could be distinctly seen in outer hair cells, inner hair cells, surrounding supporting cells (inner phalangeal cells, Deiters' cells, pillar cells, and Hensen's cell), and Kölliker's organ. Bmi1 was also expressed in the whole cochlear duct, including the striae vascularis and tympanic border cells (**Figure 4**).

Bmi1 was expressed in the postnatal cochlea

Like the E18.5 cochlear duct, Bmi1 was widely expressed in auditory hair cells, supporting cells, Kölliker's organ, the striae vascularis, and tympanic border cells in all three turns of the cochlea at P0 (**Figure 5**). At P0, the spiral gan-

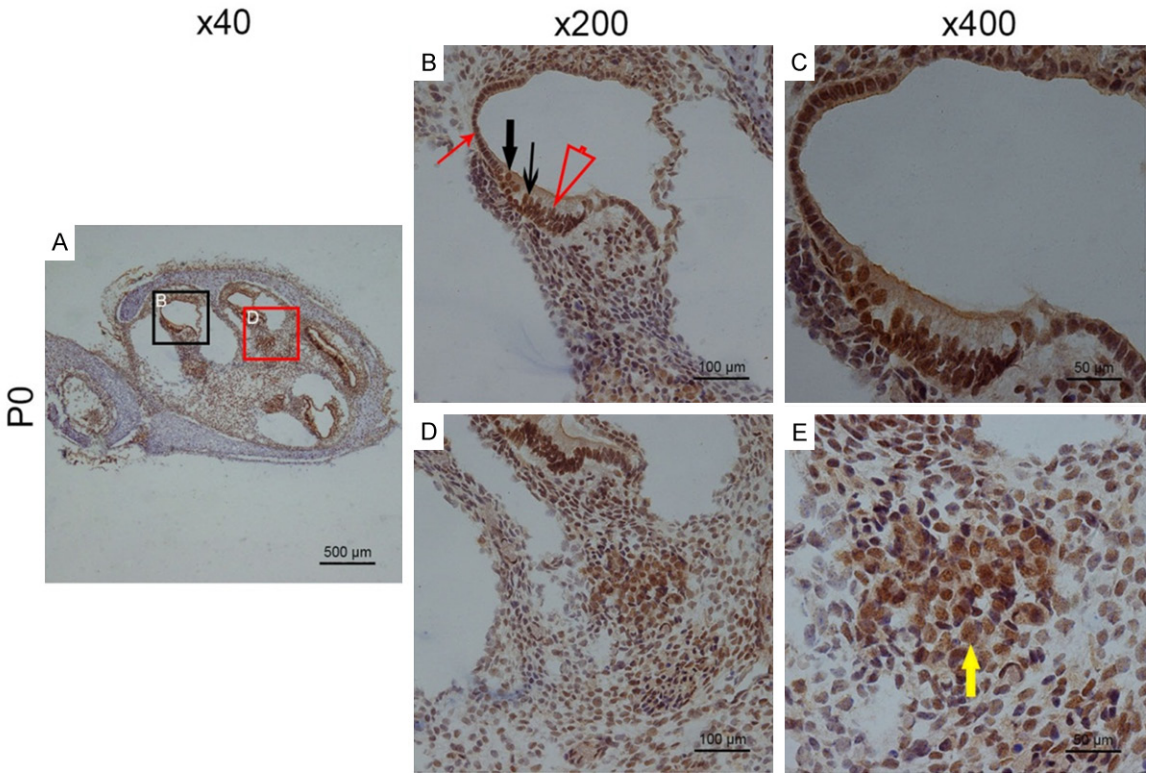

Figure 5. Bmi1 was expressed in the cochlear epithelium and spiral ganglion at P0 (A). Images (B) and (C) show that Bmi1 continued to be expressed throughout the entire cochlear duct. The red hollow arrowhead, black thin arrow, black thick arrow, and red arrow indicate Kölliker's organ, inner hair cells, outer hair cells, and the striae vascularis, respectively. Images (D) and (E) show that Bmi1 was also expressed in the spiral ganglion. The yellow arrow indicates a spiral ganglion neuron.

glion neurons were chiefly within Rosenthal's canal. The spiral ganglion neurons developed from the base to the apex, and Bmi1 continued to be expressed in spiral ganglion neurons similar to its expression in the organ of Corti and the striae vascularis (**Figure 5**).

Discussion

The inner ear is one of the most complex and delicate organs, and it functions to convert sound waves into nerve impulses-which it transmits to the brain-and to maintain body balance. The cochlea is an anatomical structure of the inner ear, and it conducts and senses sound as part of the peripheral auditory system. The organ of Corti is the core part of the cochlea and is the auditory transduction organ that physically transforms signals from the middle ear into nerve impulses. The organ of Corti is located throughout the full length of the basilar membrane and contains two types of hair cells and at least four types of supporting cells, all of which are terminally differentiated cells that are derived from the same precursor cells during embryonic development [16].

The otic placode invaginates to form the otocyst by E11, and at this time the otocyst is already regionalized into the sensory epithelium that will generate hair cells and supporting cells and the non-sensory epithelium that will generate the endolymphatic duct, the endolymphatic sac, and the vestibular membrane [2]. In this study, we showed that Bmi1 protein was expressed in all of the nuclei throughout the entire otocyst, and this indicates that Bmi1 might be involved in the development of the otocyst. From E12.5 to E14.5, the prosensory area differentiates into hair cells and supporting cells. Bmi1 was expressed in the cochlear duct on E14.5, and this implies that Bmi1 might take part in the differentiation of hair cells and supporting cells. From E18 to P0, Bmi1 was located in the hair cells and supporting cells, which suggests that Bmi1 might play important

roles in cell maturation and survival of the organ of Corti.

The striae vascularis is an important structure in the cochlear lateral wall and is composed of three types of cells: marginal cells, intermediate cells, and basal cells. The striae vascularis consists of two layers of epithelium-the epithelial-syncytium layer and the marginal cell endolymph layer-with the intrastria space located between them. The fluid in the intrastria space contains a low concentration of K^+ ions and has a positive potential, which electrically insulates it from the endolymph and perilymph. The K^+ ions of the striae vascularis are transferred to the endolymph through the KCNQ1/KCNE1 K^+ channels of the marginal cells, thus the generation of the endocochlear potential is dependent on both the electrical insulation of the intrastria space and K^+ ion transfer. The striae vascularis of the basal turn of the cochlear duct begins to differentiate on E16.5 and is markedly thickened on P0. The striae vascularis and the cochlea develop gradually from the basal turn to the apical turn, and by P14 the cochlea has fully developed and the striae vascularis is mature [17]. We found that Bmi1 was expressed in the striae vascularis of the basal turn on E18, and by P0 the expression of Bmi1 was located in the striae vascularis of the cochlear duct except in the apical turn because the striae vascularis of the apical turn is not yet developed at that age. These results indicated that Bmi1 might be involved in the development of the striae vascularis and the generation of the endocochlear potential.

The spiral ganglion of the cochlea is located in Rosenthal's tube of the modiolus, and spiral ganglion neurons include both bipolar neurons and afferent nerves. The peripheral processes of the spiral ganglion radiate out and are distributed in the organ of Corti, and its central processes form the cochlear nerve. The ventromedial part of the otocyst gradually develops into the cochleovestibular ganglion on E9. The cochleovestibular ganglion and geniculate ganglion from the first gill plate form the facial-acoustic ganglion primordia. The cochleovestibular ganglion neurons gradually separate and enter into their respective regions on E12.5 and form the cochlear ganglion primordium and vestibular ganglion primordium on E13.5. The cochlear neurons gradually emit projections that reach their peripheral target organs on E15. At P0, the spiral ganglion neurons are not yet mature and there are still some round or polygonal naïve cells that are not wrapped by a compact medullary sheath [1]. We found that Bmi1 was expressed in the cochlear spiral ganglion neurons, which shows that Bmi1 might take part in the development and maturation of the spiral ganglion neurons.

Our data showed that Bmi1 was broadly expressed in various cell types during the development and maturation of the inner ear, which indicates that Bmi1 might cooperate with different genes to play different roles in different cell types during different developmental stages. In support of this, it has been reported that Bmi-1 cooperates with Foxg1 to maintain neural stem cell self-renewal in the forebrain [18]. Our results provide the basic expression pattern of Bmi1 and might be helpful for future investigations of the detailed role of Bmi1 during the development of the cochlea.

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Disclosure of conflict of interest

None.

Authors' contribution

Conceived and designed the experiments: YC and RC. Performed the experiments: LL, YZ, WL, and WN. Wrote the paper: LL, RC, and YC.

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