

Expression Patterns and Implications of LaminB1 in Rat Cochleae*

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Summary: LaminB1, a major component of the nuclear lamina, is a potent regulator of cellular proliferation and senescence and also known to be essential for neuronal migration and brain development. However, the expression patterns of LaminB1 in the rat cochleae are still not fully revealed. Utilizing immunofluorescence, Western blotting, and quantitative real-time PCR, we identified the distribution and expression of LaminB1 in the rat cochleae. Immunofluorescence staining indicated that LaminB1 was mainly localized in the auditory hair cells (HCs), spiral ganglion cells (SGC), stria vascularis (STV, including spiral ligament), Reissner's membrane (RM), and limbus laminae spiralis (LLS). Western blotting analysis illustrated that the distribution of LaminB1 in rat cochleae was characterized by tissue specificity. The LaminB1 protein was expressed more in SGC and basilar membrane (BM) than in STV. Meanwhile, the mRNA expression of LaminB1 displayed difference in cochlear tissues. These observations preliminarily revealed the expression patterns of LaminB1, providing a theoretical basis for further study on the role of LaminB1 in auditory function.

Key words: LaminB1; cochleae; rat; hair cell; spiral ganglion cell; stria vascularis

Sensorineural hearing loss (SNHL) is the most common form of hearing loss at present^[1]. The loss of peripheral tissue or cell death in the cochlea is the typical cause of SNHL^[2]. In the cochlea, especially in the scala media, many interconnected cell types are essential for hearing, such as the hair cells and the supporting cells. The auditory sensory epithelium hair cells transmit the signal of mechanical stimuli to the spiral ganglion neurons (SGNs) through their dendrites. Then the information will be carried to the brain by the axon^[3]. Many studies have focused on the biochemical, molecular, and intracellular mechanisms in normal-state hearing function and in pathological processes that impact the viability of cochlear sensorineural tissues^[2].

The nuclear lamina is an intermediate filament meshwork, located in the inner membrane of the nuclear envelope and involved in the maintenance of the physiological equilibrium of cells^[4]. The major structural proteins of the lamina are divided into A- and B-types^[5]. The two similar but functionally distinct

isoforms of vertebrate A-type lamins, Lamin A and C, are the major products of alternative splicing of *LMNA*^[6]. Lamin B1 and Lamin B2 are the two major B-type lamins in most vertebrates. They are encoded by the *LMNB1* and *LMNB2* genes, respectively^[7]. At least one B-type lamina continues to be expressed in all cells throughout development^[8].

LaminB1 is thought to be essential for chromatin organization, DNA replication and regulation of gene expression^[9, 10]. LaminB1 also has a crucial role in the assembly of the mitotic spindle, and that the spindle complex is disrupted by a dominant negative LaminB1 mutant^[11]. In addition, LaminB1 seems to play a specific role in the proliferation, survival and differentiation of certain tissues and cell types^[12], especially in the nervous system. Specifically, a duplication of the human LaminB1 gene (*LMNB1*) is thought to cause adult-onset autosomal dominant leukodystrophy (ADLD)^[13], a kind of laminopathy affecting myelination of the central nervous system (CNS). The deficiency of *LMNB1* results in perinatal lethality, reduced brain size, disorganized layering and apoptosis of cortical neurons during embryonic corticogenesis^[14]. Meanwhile, *LMNB1* knockout mice exhibit abnormal lung development and bone ossification during embryogenesis and die shortly after

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birth^[15].

These pathological phenotypes associated with LaminB1 above suggested that mammalian cells would be unlikely to thrive in the absence of LaminB1. However, there is currently no publication showing the role of LaminB1 in peripheral auditory nervous system. This study is aimed to explore the distribution of LaminB1 in cochlea tissues and provide a theoretical basis for further study on the physiological functions of LaminB1 in auditory system.

1 MATERIALS AND METHODS

1.1 Animals

Twenty-six 21-day-old Sprague-Dawley rats were purchased from the Model Animal Research Center of Wuhan University (China). All of the Sprague-Dawley rats had no history of noise exposure, otitis media or application of any ototoxic drug. The care and experimental treatment of the animals were approved by the Animal Research Committee, Tongji Medical College, Huazhong University of Science and Technology, China.

1.2 Cochleae Sections Processing

Each cochlea of six rats was isolated after euthanasia under a dissecting microscope. The cochleae were fixed by perfusing the round and oval window with 4% paraformaldehyde in 0.1 mmol/L phosphate-buffered saline (PBS), pH 7.4, and immersed in the solution overnight at 4°C. Then the cochleae were washed with PBS and decalcified in 10% sodium ethylenediamine tetraacetic acid (EDTA; pH 7.3–7.4) for 5 days, followed by an overnight incubation in 30% sucrose. Thereafter, the decalcified and dehydrated specimens were embedded in O.C.T compound (Sakura Finetek USA Inc., USA) for 24 h. Serial mid-modiolar sections of 8 µm thickness were cut and collected onto polylysine-coated glass slides.

1.3 Immunofluorescence

Cochlea sections were washed thrice with 0.01 mol/L PBS for 5 min. Then, the sections were permeated with 0.5% Triton X-100 for 20 min and blocked with blocking solution (10% goat serum in PBS with 0.01% Triton X-100) for 40 min at room temperature. After washing with PBS, the specimens were incubated with primary antibodies against LaminB1 (1:500; Abcam, USA) overnight at 4°C. After rinsing three times with PBS, tissue was incubated with secondary antibody (DyLight™ 488-conjugated goat anti-rabbit IgG; Multi-Sciences, China) diluted 1:600 for 1 h at room temperature. The slides were then washed thrice in PBS, followed by counterstaining with 1 mg/mL 4, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma, USA) for 10 min. After final wash, the sections were coverslipped, and then observed using a fluorescence microscope (Olympus BX 51;

Olympus, Japan). Images were recorded at the same magnification and time of exposure^[16]. All the tests were repeated three times.

1.4 Quantitative Real Time PCR (qRT-PCR)

The tissues of stria vascularis (STV), spiral ganglion cells (SGC), and basilar membrane (BM) from 10 rats were separated respectively in RNase-free D-Hanks' solution under a dissecting microscope. Total RNA was extracted using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol. One microgram total RNA was reversely transcribed to cDNA by using ReverTra Ace (Toyobo, Japan). CDNA samples amplification was performed using SYBR Green premix Ex Tag™ (Tli RNaseH Plus; TaKaRa, Japan) on a Light Cycler 480II (Roche, Switzerland).

The sequences of primers used in this study were as follows: For GAPDH, forward: 5'-GTCGGTGTGAACGGATTTGG-3', and reverse: 5'-GACTGTGCCGTTGAACTTGC-3'; for LaminB1, forward: 5'-GTCCTTCTTCCCGAGTGACC-3', reverse: 5'-CGCCTCTGATTCTTCCACAT-3'.

RT-PCR was performed by preincubation at 95°C for 3 min followed by 40 cycles consisting of denaturation at 95°C for 15 s, annealing at 60°C for 1 min and extension at 72°C for 30 s. Each sample was run in triplicate and the mean values were calculated. The relative expression of genes were calculated using the 2^(-Delta Delta CT) method^[17].

1.5 Western Blotting

The tissues of STV, SGC, and BM were isolated respectively and homogenized ultrasonically in RIPA buffer supplemented with a protease inhibitor cocktail (Sigma, USA). Then the homogenate was centrifuged (Eppendorf model 5417R, Eppendorf, Germany) at 12 000 r/min for 30 min at 4°C, and the protein concentration in the supernatant was measured by BCA protein assay. Equalized amounts of protein (20 µg) were separated by electrophoresis at 80 V on 10% SDS-PAGE gels. Then the proteins were transferred onto polyvinylidene difluoride membranes (Millipore, USA) and blocked for 1 h at room temperature in Tris-buffered saline containing 0.2% Tween-20 and 5% bovine serum albumin. The membranes were probed with primary antibody against LaminB1 (1:1000; Abcam, USA) and β-actin (1:2000; Sigma, USA) overnight at 4°C. Then membranes were washed three times in Tris-buffered saline with Tween-20 and incubated for 1 h at room temperature with secondary antibody dilute (1:2000; Sigma, USA). Protein bands were visualized using enhanced chemiluminescence reagents (Amersham Biosci., USA) with a chemiluminescence system (PTC-200; Bio-Rad Laboratories, USA). All the tests were repeated more than three times.

1.6 Statistical Analyses

All the data were presented as the mean±SEM and statistically analyzed with SPSS (19.0; SPSS Inc.,

USA). One-way analysis of variance (ANOVA) with Student-Newman-Kuels (SNK) correction was used for statistical analysis. $P < 0.05$ was considered to be statistically significant.

2 RESULTS

2.1 Distribution of LaminB1 in Cochleae

Fig. 1 shows that the immunofluorescent staining for LaminB1 was present in nucleus of all cells bordering the scala media (fig. 1A and 1C). Strong

immunoreactivity was observed in SGC and limbus laminae spiralis (LLS) (fig. 1A, 1C, 1J and 1L). Intense staining was also seen in the fibers coursing toward the organ of Corti (OC) as well as the fibers located beneath the hair cells (fig. 1A, 1C, 1G, and 1I). Moderate labeling was detected in hair cells and supporting cells, and the expression of LaminB1 was stronger in outer hair cells (OHCs) than in inner hair cells (IHCs) (fig. 1G and 1I). In the STV, a weak immunoreactivity was also occasionally detectable (fig. 1A, 1C, 1D and 1F).

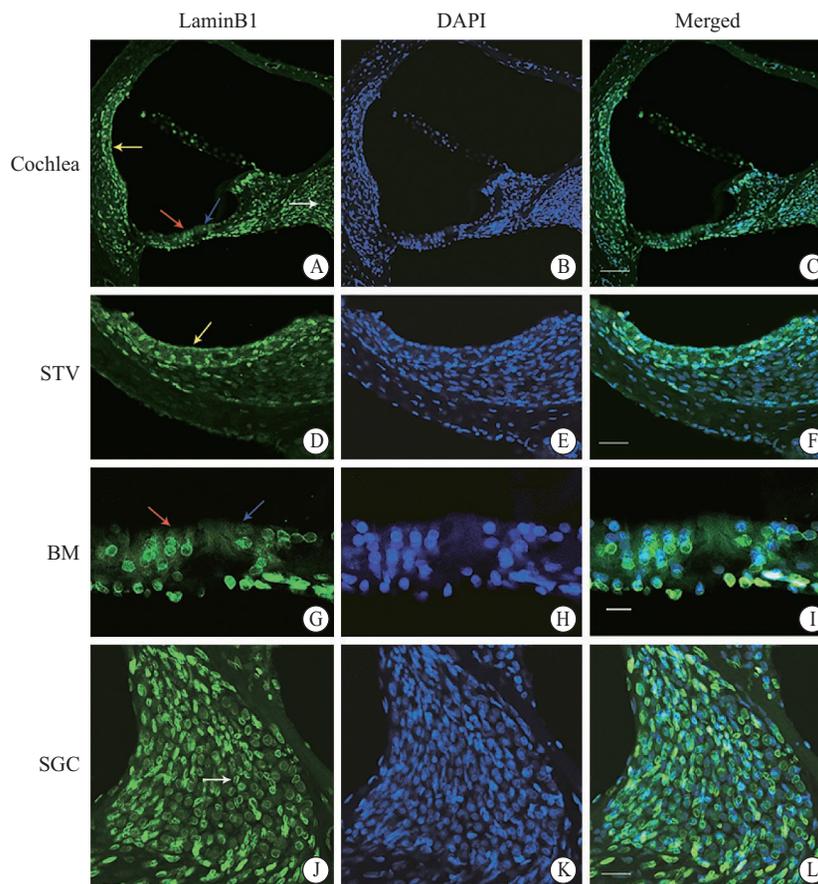


Fig. 1 Expression of LaminB1 in the cochlea

LaminB1 is labeled by green fluorescence (A, D, G, J) and the nuclei are counterstained with DAPI in blue (B, E, H, K). C, F, I, L: showing an overlap of LaminB1 (green) and DAPI (blue). LaminB1 is mainly expressed in spiral ganglion cells (SGC) (J, L), hair cells (both OHCs and IHCs; G, I), Reissner's membrane (RM) (A, C), stria vascularis (STV, including spiral ligament) (D, F), and limbus laminae spiralis (LLS) (A, C). OHCs: red arrow; IHCs: blue arrow; STV: yellow arrow; SGC: white arrow. Scale bar: A–C, 100 μm ; D–F, 50 μm ; G–I, 10 μm ; J–L, 25 μm

2.2 Expression of LaminB1 mRNA in Cochleae

As shown in fig. 2, there was different expression of LaminB1 mRNA in STV, SGC, and BM (One-way ANOVA, $F=129.115$, $d.f.=2$, $P < 0.001$). The mRNA level of LaminB1 obtained was as follows: STV group, 0.764 ± 0.062 ; SGC group, 13.054 ± 1.598 ; BM group, 2.032 ± 0.153 . The expression of LaminB1 was weaker in STV (SNK test, $q=21.514$, $P < 0.001$) and BM (SNK test, $q=20.317$, $P < 0.001$) than in SGC. Meanwhile,

there was also a significant difference between STV and BM (SNK test, $q=2.943$, $P < 0.05$).

2.3 Expression of LaminB1 Protein in Cochleae

Fig. 3 shows the relative protein expression of LaminB1 in different tissues of cochlea. A unique 70-kD band corresponding to LaminB1 and a unique 43-kD band corresponding to β -actin were present in fig. 3A. Normalized to β -actin, the LaminB1 protein levels displayed significant difference in STV, SGC and BM

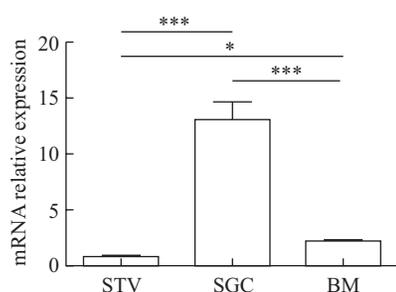


Fig. 2 Expression of LaminB1 mRNA in the cochlea
The relative expression of LaminB1 mRNA in STV, SGC and BM. * $P < 0.05$, *** $P < 0.001$

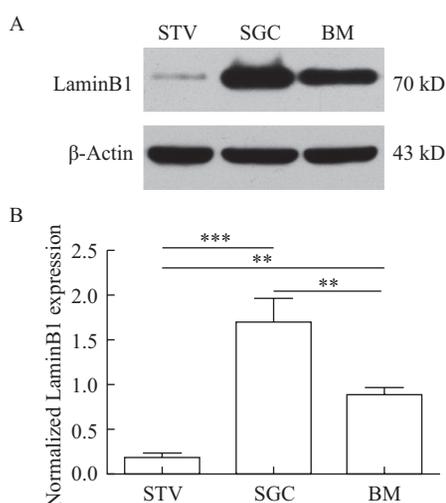


Fig. 3 Expression of LaminB1 protein in the cochlea
A: The molecular weight of LaminB1 is 70 kD detected by Western blotting. B: the relative expression of LaminB1 protein in STV, SGC and BM. ** $P < 0.01$, STV or SGC vs. BM; *** $P < 0.001$, STV vs. SGC

(one-way ANOVA, $F=22.069$, $d.f.=2$, $P < 0.001$) (fig. 3B). LaminB1 was expressed stronger in SGC (SNK test, $q=9.382$, $P < 0.001$) and BM (SNK test, $q=4.256$, $P < 0.01$) than in STV. In addition, the expression of LaminB1 in the BM decreased significantly in comparison with SGC (SNK test, $q=5.126$, $P < 0.01$). These results suggested that the expression of LaminB1 protein in rat cochleae is different.

3 DISCUSSION

LaminB1 plays crucial roles for mammalian health and disease, involved in complex physiological processes. It has been shown that LaminB1 losing can serve as a hallmark for cellular senescence *in vitro* and *in vivo*^[18]. Moreover, fluctuations of LaminB1 have an effect on cell proliferation in WI-38 cells^[19]. LaminB1 is also essential for the differentiation of functional olfactory sensory neurons^[20] and murine neural stem cells^[21]. However, the expression patterns

and the functions of LaminB1 in the cochleae are still unknown.

This study revealed that the immunoreactivity of LaminB1 was present in the nucleus of all cells bordering the scala media (fig. 1), which was consistent with the previous reports that LaminB1 was expressed ubiquitously in various tissues^[13, 22]. Besides this, we identified that the localization of LaminB1 was characterized by tissue specificity in the rat cochlea. Strong immunoreactivity of LaminB1 was found in SGC, LLS, and nerve fibers towards hair cells. What's more, positive labeling was also observed in OC and STV (fig. 1). The results of qRT-PCR (fig. 2) and Western blotting (fig. 3) also illustrated that LaminB1 was localized in cochlear tissues selectively. LaminB1 was expressed more strongly in SGC and BM than in STV. Then we try to explain the differential and specific expression of LaminB1 in cochlea tissues.

Firstly, various forms of posttranslational modifications such as phosphorylation^[23] and farnesylation^[24] perhaps contribute to the differential expression of LaminB1 in individual cochlear tissues. Secondly, LaminB1 modulated nuclear rigidity, and was associated with nuclear ion channel open in ADLD fibroblasts^[25]. We might speculate that differential expression of LaminB1 affects the elastic properties of the nucleus, leading to different nuclear ionic signalings in STV, SGC, and BM. LaminB1 may be involved in the signal transduction between sensory epithelium hair cells and SGNs. Endocochlear potential (EP) maintenance might be also related to the expression patterns of LaminB1. Thirdly, LaminB1 levels could mediate myelin formation of the oligodendrocytes in a mouse model of ADLD^[26]. Thus, we assume that LaminB1 may be a modulator of myelination of the glial cells and essential for the growth and maintenance of the SGC.

In summary, this study revealed that LaminB1 was localized widely in the cochleae and the distribution of the LaminB1 was characterized by tissue specificity. Our findings provide a theoretical basis for further study on the role of LaminB1 in auditory system.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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