International Journal of Pediatric Otorhinolaryngology (2009) 73, 315–319

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International Journal of Pediatric Otorhinolaryngology

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The expression of NOB1 in spiral ganglion cells of guinea pig

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Received 13 August 2008; received in revised form 25 October 2008; accepted 30 October 2008
Available online 19 December 2008

KEYWORDS
NOB1;
Zinc ribbon domain;
Immunohisto-chemistry;
Guinea pigs;
Spiral ganglion cells

Summary

Objective: NOB1 was a transcription-associated protein, consisting of one zinc ribbon domain. This study aimed to investigate the NOB1 expression in spiral ganglion cells of normal guinea pigs and deaf ones.
Methods: Twelve guinea pigs were randomized equally into experimental group and control group. In experimental group, guinea pigs received a single intramuscular injection of gentamicin, while the control group was treated with physiological saline. Auditory brainstem responses (ABR) test was performed before and 10 days after injection, respectively. Guinea pigs of both groups were sacrificed and temporal bones were removed. The expression of NOB1 in the spiral ganglion was evaluated by immunohistochemistry.
Results: NOB1 staining was found expressed in spiral ganglion cells from the basal turn to the apical turn of the cochlea. The expression of NOB1 was found significantly stronger in spiral ganglion cells of deaf guinea pigs as compared with that in normal ones.
Conclusions: The NOB1 was presented in spiral ganglions cells of the guinea pig and might play a role in hearing transduction.

1. Introduction

The human NOB1 gene spaned about 13 kb on genome, consisting of nine exons and eight introns [1]. The assembled cDNA of NOB1 was 1749 bp in length, containing an ORF from 33 to 1271 nt. The ORF

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doi:10.1016/j.ijporl.2008.10.028

encoded a protein of 412 amino acids with a predicted molecular weight of 46 kDa. The results of RT-PCR showed that the expression of NOB1 was mainly detected in adult human lung, liver and spleen. The human NOB1 gene encoded a transcription-associated zinc ribbon protein. Recently, zinc ribbon protein has been found related to multidrug resistance and carcinogenesis of gastric cancer [2—6]. Thus, NOB1 was assumed to be associated with transcription regulation and might play potential roles in mediating some physiologic and pathological functions.

Our laboratory had a long standing interest in the roles of NOB1. We have successfully established one MAb, who could recognize NOB1 protein derived from human cancer cell lines by Western blotting and immunohistochemistry [7]. This MAb would be helpful for understanding the distribution of NOB1 in tissues and its function. So far, it was still unknown whether NOB1 was correlated with the auditory pathways. We have assumed that NOB1 might be involved in the nervous system function as following: firstly, NOB1 was considered as one transcription-associated factor. Secondly, NOB1 was found expressed in the human brain tissues [1]. Thus, we conducted an immunohistochemical analysis of the NOB1 protein expression in the inner ear of normal guinea pigs and deaf ones.

2. Materials and methods

2.1. Experimental animals and treatment groups

Twelve young white guinea pigs weighing 250—350 g with a normal Preyer's reflex were used in this study. Animals were randomized to one of the following two groups. Group 1 received gentamicin at 150 mg/(kg day) (i.m.) dosage for 10 days, while group 2 received the physiological saline at 2.5 ml/(kg day) (i.m.) dosage and was used as control. We verified deafening by measuring ABR thresholds, and excluded animals that did not show a threshold greater than 50 dB SPL (numbers of animals given above do not include excluded animals). All animals were killed upon completion of the study. The procedures concerning animals reported in this study were approved by the Animal Care and Use Committee at the Fourth Military Medical University.

2.2. Auditory brainstem response (ABR) recording

To assess the physiological condition of experimental and control ears, auditory brainstem response (ABR) audiometry was performed. ABRs were measured twice, once before the injection and once before sacrifice. The guinea pigs were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), and needle electrodes were placed subcutaneously. The reference electrode was inserted beneath the pinna of the measured ear, the ground beneath the apex of the nose, and the active electrode beneath the skin on the top of the head. The stimulus signal was generated through Intelligent Hearing Systems (Bio-logic Systems, USA) controlled by computer and delivered by an earphone. Stimuli were presented at a repetition rate of 10/s. Around threshold, responses for 1024 sweeps were averaged at each intensity level, which varied in steps of 5 dB sound pressure level (SPL). Threshold was determined by visual inspection of the responses and defined as the lowest intensity level at which a clear waveform was visible in the evoked trace.

Data was expressed as mean $\pm$ S.D. SPSS13.0 (SPSS Inc.) software was used for statistical analysis. The ABR threshold levels between the experimental and control ears were evaluated using Student' s paired t tests for significance ($p < 0.05$).

2.3. Tissue samples

Guinea pigs of both groups were deeply anaesthetized with sodium pentobarbitone and perfused with 100 ml cold PBS followed by 300 ml cold 4% paraformaldehyde (pH 7.4). Both temporal bones were removed and immersed in the same fixative at 4 °C overnight. Then the samples were decalcified in 10% EDTA for 10—14 days, cryo-protected in 30% sucrose for 24 h and transferred into a cryo-mold filled with OCT embedding compound (Electron Microscopy Sciences). The molds containing the samples were exposed to a gentle vacuum for 10 min, to remove trapped air bubbles [8]. The tissues were kept in OCT at 4 °C for 24 h to allow infiltrations into the inner ear. The tissues were aligned for sectioning, snap-frozen in a dry ice/ethyl alcohol bath, and then stored at −80 °C until sectioning. Sections (10 μm thick) were cut on a CM 3000 cryostat. A series of sections were collected onto electrostatic glass slides, and then stored at −80 °C.

2.4. Antibodies and reagents

Mouse monoclonal antibody (MAb) against NOB1 (clone H11) was raised in our laboratory by immunizing with full-length recombinant NOB1 expressed in E. coli and standard cell fusion techniques [7]. The MAb could recognize NOB1 protein in both native and denatured forms. The SP immunostaining