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GFAP aggregates in the cochlear nerve increase the noise vulnerability of sensory cells in the organ of Corti in the murine model of Alexander disease

Masatsugu Masuda [a,*], Kenji F. Tanaka [b,c], Sho Kanzaki [a], Kenichiro Wakabayashi [a], Naoki Oishi [a], Takafumi Suzuki [a], Kazuhiro Ikenaka [b,c,**], Kaoru Ogawa [a]

[a] *Department of Otolaryngology, Keio University, School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*
[b] *Division of Neurobiology and Bioinformatics, National Institute for Physiological Sciences, 5-1 Higashiyama, Myodaiji, Okazaki, Aichi 444-8787, Japan*
[c] *Department of Physiological Sciences, School of Life Science, The Graduate University for Advanced Studies (Sokendai), Hayama, Miura, Kanagawa 240-0193, Japan*

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Abstract

Outer hair cell (OHC) loss in the auditory sensory epithelium is a primary cause of noise-induced sensory-neural hearing loss (SNHL). To clarify the participation of glial cells in SNHL, we used an Alexander disease (AxD) mouse model. These transgenic mice harbor the AxD causal mutant of the human glial fibrillary acidic protein (GFAP) under the control of the mouse GFAP promoter. It is thought that GFAP aggregates compromise the function of astrocytes. In the auditory pathway, the formation of GFAP aggregates was observed only in GFAP-positive cells of the cochlear nerve. The presence of GFAP aggregates did not change auditory function at the threshold level. To assess the change in vulnerability to auditory excitotoxicity, both transgenic and control mice were treated with intense noise exposure. Auditory threshold shifts were assessed by auditory brainstem responses (ABR) at 1 and 4 weeks after noise exposure, and OHC damage was analyzed by quantitative histology at 4 weeks after exposure. Transgenic mice showed more severe ABR deficits and OHC damage, suggesting that cochlear nerve glial cells with GFAP aggregates play a role in noise susceptibility. Thus, we should focus more on the roles of cochlear nerve glial cells in SNHL.

Keywords: Alexander disease; Astrocyte; Cochlear nerve; GFAP aggregates; Glia; Hair cell; Noise-induced hearing loss

1. Introduction

Intense noise exposure can lead to morphological and functional changes of sensory, neuronal, and non-neuronal cells in the auditory pathway, and leads to hearing loss in a typical model of sensory-neural hearing loss (SNHL). The cochlea is a mammalian auditory organ and contains the sensory epithelium, termed the organ of Corti (OC), which is responsive to sound. The OC is comprised of two different kinds of sensory cells, inner hair cells and outer hair cells (OHCs).

It is widely accepted that the degeneration of these sensory cells is a primary cause of permanent noise-induced SNHL (Wang et al., 2002). In particular, elucidation of the factors associated with OHC degeneration will lead to potent treatment strategies as prevention of OHC degeneration reduces the level of hearing loss in animal models (Minami et al., 2004; Takemura et al., 2004; Yamashita et al., 2005). Although there are reports which suggest that non-neuronal cells in the cochlea, including fibrocytes in the lateral wall and spiral limbus, supporting cells in the OC, and cells in the stria vascularis modulate the severity of noise-induced SNHL (Hakuba et al., 2000; Masuda et al., 2006; Wang et al., 2002), the relationship between non-neuronal cells and noise-induced SNHL has not been elucidated yet. Here we investigated this relationship using model mice that have a compromised function of non-neuronal cells in the auditory pathway.

* Corresponding author. Tel.: +81 3 3353 1211; fax: +81 3 3353 1261.
** Corresponding author. Tel.: +81 564 59 5245; fax: +81 564 59 5247.
E-mail addresses: masoeur13@mac.com (M. Masuda), ikenaka@nips.ac.jp (K. Ikenaka).

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Glial fibrillary acidic protein (GFAP) is well known as a marker protein in astrocytes in the central nervous system (CNS). GFAP is also expressed outside the CNS; i.e. in the auditory pathway, non-myelinating Schwann cells along auditory nerve axons, supporting cells of sensory cells, fibrocytes in the spiral limbus, and cochlear Schwann cells in the OC (Rio et al., 2002). However, GFAP is never expressed in sensory cells and neuronal cells in the auditory pathway.

The coding mutations of GFAP cause a specific astrocytic disease called Alexander disease (AxD), which is characterized by GFAP aggregates in astrocytes and by severe neural dysfunction (Li et al., 2002; Mignot et al., 2004). Recently, we established AxD model mice in which mutant human GFAP (hGFAP) is expressed under the control of mouse GFAP promoter (Tanaka et al., 2007). GFAP aggregates are known as Rosenthal fibers, which primarily consist of GFAP and several heat shock proteins (Head et al., 1993; Iwaki et al., 1989). The presence of mutant GFAP per se is not called a GFAP aggregate, even if its expression is clear. Furthermore, the presence of mutant GFAP per se is insufficient for aggregate formation and for the onset of the disease. The aggregate formation is dependent on GFAP quality and quantity (Tanaka et al., 2007). Our AxD model mice reproduce the presence of GFAP aggregates in the astrocytes and are vulnerable to excitotoxicity in accordance with the degree of GFAP aggregation.

The infantile or juvenile form of AxD is more severe than the adult form and an abnormal auditory brainstem response (ABR), which measures the acoustic stimulus-evoked electrophysiological response of the peripheral auditory pathway and the brainstem (Henry, 1979; Melcher et al., 1996), has been reported (Asahina et al., 2006; Ishigaki et al., 2006). On the other hand, the auditory abnormality has not been reported in the adult form of AxD (Brockmann et al., 2003; Kinoshita et al., 2003). In this study we used our transgenic mouse and examined whether the existence of GFAP aggregates in the auditory pathway affects noise-induced SNHL and normal hearing, although GFAP aggregates in the cochlear nerve have not been reported in SNHL.

Our goal in the present study was to elucidate the effect of malfunction of non-neuronal cells in the auditory pathway in normal and pathological conditions. First, we examined mutant hGFAP expression in the auditory pathway. Second, we tested the auditory function of the transgenic mice at the threshold level by ABR. Third, we examined the difference of noise sensitivity between wildtype and transgenic mice by ABR and the degree of degeneration of OHCs. We demonstrated that mutant hGFAP was expressed in the cochlear nerve and the brainstem in the auditory pathway and formed aggregates only in GFAP-positive cells of the nerve, and its expression affected noise-induced SNHL but not the normal hearing condition. Altogether, our results suggest that GFAP aggregate-positive cells in the cochlear nerve compromise the protection of sensory cells after noise overstimulation, and the present study proposes that this transgenic mouse is a good model for studying the mechanism by which non-neuronal cells contribute to the protection against SNHL in the auditory pathway.

2. Materials and methods

2.1. Animals and anesthesia

Transgenic mice expressing mutant hGFAP were maintained in the C57BL/6J background as hemizygotes as previously described (Tanaka et al., 2007). Mutant hGFAP carrying the R239H mutation was expressed under the control of mouse GFAP promoter (Fig. 1). The 60TM line, which exhibited the most severe phenotype in terms of GFAP aggregates, was used in all experiments. Animals were 4 or 5 weeks of age.

Animals were anesthetized by intraperitoneal application of a mixture of ketamine (80 mg/kg) and xylazine (15 mg/kg). They were more deeply anesthetized when cochlea and brainstems were isolated and dissected.

All experiment protocols were in compliance with the guidelines of the National Institutes of Health and the Declaration of Helsinki, and all procedures were approved and supervised by the Keio University Union on Laboratory Animal Medicine.

2.2. Immunohistochemistry

For analysis of the structure and hGFAP expression of cochleae, transgenic and wildtype mice without noise exposure ($n = 4$ each) and transgenic and wildtype mice 4 weeks after noise exposure ($n = 2$ each) were used. For analysis of the brainstems, transgenic and wildtype mice without noise exposure ($n = 2$ each) were used.

Cochleae were fixed by cardiac perfusion, perilymphatic perfusion, and overnight fixation of 4% paraformaldehyde (PFA) and decalcification as described previously (Masuda et al., 2006). The brainstems were also fixed in 4% PFA overnight at 4 °C. The paraffin sections of 4 μm thickness were made using routine procedures. Specimens were heated at 120 °C for 10 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. Immunolabelling was carried out with an anti-human GFAP-specific antibody (mouse monoclonal IgG, SMI21, 1:1000, Sternberg Monoclonal Incorporation, Lutherville, MD, USA) or anti-GFAP polyclonal antibody (rabbit polyclonal antibody, 1:10,000, DAKO JAPAN, Kyoto, Japan), and then the slides were incubated in biotinylated anti-mouse IgG antibody (Vector Laboratories, CA, USA) or anti-rabbit IgG antibody (Vector Laboratories, CA, USA) each. Finally, the slides were incubated with Vector Elite ABC Reagent or streptavidin-Alexa Fluor 546 conjugate at room temperature. Reaction products of Elite ABC Reagent were developed using 3′,5′-diaminobenzidine as a substrate for peroxidase. Sections were nuclear stained with hematoxylin.

2.3. ABR recording

ABR thresholds were measured as described previously (Masuda et al., 2006). Briefly, electroencephalogram recording was performed using the extracellular amplifier, and waveform storing, stimulus control, and time and frequency domain averaging were performed using the software of ADInstruments (Castle Hill, Australia). Sounds, consisting of either clicks or tone bursts of 4, 12, and 20 kHz, were generated using the system of Tucker-Davis Technologies (FL, USA), and were produced by a coupler type speaker inserted into the external auditory canal of a mouse. For recording, animals were anesthetized. The thresholds of ABR were determined using a 5-dB sound pressure level (SPL) minimum step size down from the maximum amplitude. The hearing threshold was defined as the lowest stimulus intensity that produced a reliable wave of ABR.

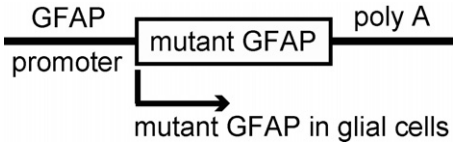

Fig. 1. Construction of the transgene. Mutant human GFAP (R239H) is expressed by glial cells under the control of mouse GFAP promoter. There are an estimated three transgenes.