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Immune defense is the primary function associated with the differentially expressed genes in the cochlea following acoustic trauma

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ABSTRACT

Our previous RNA-sequencing analysis of the rat cochlear genes identified multiple biological processes and molecular pathways in the cochlear response to acoustic overstimulation. However, the biological processes and molecular pathways that are common to other species have not been documented. The identification of these common stress processes is pivotal for a better understanding of the essential response of the cochlea to acoustic injury. Here, we compared the RNA-sequencing data collected from mice and rats that sustained a similar, but not identical, acoustic injury. The transcriptome analysis of cochlear genes identified the differentially expressed genes in the mouse and rat samples. Bioinformatics analysis revealed a marked similarity in the changes in the biological processes between the two species, although the differentially expressed genes did not overlap well. The common processes associated with the differentially expressed genes are primarily associated with immunity and inflammation, which include the immune response, response to wounding, the defense response, chemotaxis and inflammatory responses. Moreover, analysis of the molecular pathways showed considerable overlap between the two species. The common pathways include cytokine–cytokine receptor interactions, the chemokine signaling pathway, the Toll-like receptor signaling pathway, and the NOD-like receptor signaling pathway. Further analysis of the transcriptional regulators revealed common upstream regulators of the differentially expressed genes, and these upstream regulators are also functionally related to the immune and inflammatory responses. These results suggest that the immune and inflammatory responses are the essential responses to acoustic overstimulation in the cochlea.

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1. Introduction

Acoustic overstimulation traumatizes cochlear cells and compromises auditory function. Patients with a history of acoustic trauma often experience various auditory symptoms, including the loss of hearing sensitivity, clarity and dynamic range (Pyykkö et al., 2007; Toppila et al., 2000). Some patients also suffer from tinnitus and hyperacusis (Axelsson and Hamernik, 1987; Mrena et al., 2001). These symptoms worsen later in life, when age-related cochlear degeneration begins to develop. Because the sensory cells in the mammalian cochlea are unable to regenerate once damaged, the understanding of the molecular mechanisms responsible for such damage is essential to prevent noise-induced hearing loss.

Noise-induced cochlear damage is a multifactorial degeneration process. The damage is caused by both direct mechanical stress and

Abbreviations: FPKM, fragments per kilobase of transcript per million mapped reads; DAVID, Database for Annotation, Visualization and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes; IPA, Ingenuity Pathway Analysis; ANOVA, analysis of variance; FDR, false discovery rate; ABR, auditory brainstem response

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subsequent biological and molecular stresses that include oxidative stress, inflammation, energy exhaustion and excitotoxicity. These stress responses cause apoptotic and necrotic cell death (Bohne et al., 2007; Hu et al., 2000; Niu et al., 2003; Shibuya et al., 2003; Wang et al., 2002; Ylikoski et al., 2002). Previous investigations have identified multiple genes that are involved in the cochlear responses to acoustic damage. These genes are functionally associated with various biological processes, such as transcriptional control, oxidative stress and inflammation (Cho et al., 2004; Kirkegaard et al., 2006; Tornabene et al., 2006), and with various molecular pathways, such as the phospho-MEK1/ERK1/2/p90 RSK signaling pathway, the p38/MAPK signaling pathway and the JNK pathway (Jamesdaniel et al., 2011; Maeda et al., 2013; Murai et al., 2008; Pirvola et al., 2000; Wang et al., 2003). Our transcriptional analysis has revealed a time-dependent response of apoptosis-related genes to acoustic overstimulation (Hu et al., 2009). These observations suggest that multiple signaling pathways are involved in the pathogenesis of acoustic trauma in the cochlea.

Recent advances in high-throughput technologies have facilitated the effort to profile the global changes in the expression of cochlear genes. Using RNA-sequencing, we have examined the noise-induced transcriptional changes of the cochlear genes in the rat (Patel et al., 2013). This comprehensive expression analysis identified a group of differentially expressed genes. Additional bioinformatics analysis revealed that these differentially expressed genes are functionally related to multiple biological processes and molecular pathways, including the complement pathway, Toll-like receptor signaling pathway, cytokine–cytokine receptor pathway, chemokine signaling pathway and myosin pathway. This study provides valuable information about the global expression response of the cochlear genes. However, an important question remains as to which processes or pathways that were identified in the rat are common to other rodent species. The determination of common processes and pathways will provide a confirmation for those identified in rats and, more importantly, a better understanding of the essential cochlear response to acoustic overstimulation.

The mouse and the rat are commonly used animal models to investigate the molecular mechanism of noise-induced cochlear damage. In the current study, we performed an RNA-sequencing analysis of the cochlear genes in mice and compared the results with those derived from our previous analysis using the rats that sustained a similar, but not identical, noise exposure. We identified the differentially expressed genes in the mouse and the rat cochlear samples. Noticeably, the major biological processes associated with these differentially expressed genes are remarkably similar between the mouse and rat samples, despite the differences in the differentially expressed genes identified in the two species. The common biological processes are related to immunity and inflammation, which include the immune response, response to wounding, the defense response, chemotaxis and inflammatory responses. Importantly, our study reveals common upstream regulators of the differentially expressed genes and shows that these upstream regulators are functionally related to the immune and inflammatory responses. These results suggest that the immune response is an essential cochlear response to acoustic trauma.

2. Materials and methods

2.1. Animals and experimental procedures

CBA/CaJ mice (male and female, 4–8 weeks old, the Jackson Laboratory, Bar Harbor, ME, USA) and Sprague Dawley rats (male and female, 2–3 months old, Charles River Laboratories, Wilmington, MA) were used. All of the subjects were evaluated for their baseline hearing ability. Only animals with normal hearing sensitivity were included in the study. For the rat RNA-sequencing experiment, the rats were randomly assigned to either a noise group or a control group ($n = 3$ animals for each group). The animals in the noise group were exposed to noise (for details of the noise protocol, see the section on Noise Exposure), and 1 day after noise exposure, the animals' hearing ability was evaluated again. The animals were then sacrificed, and the cochleae were collected for RNA-sequencing analysis. The control animals underwent a protocol identical to that of the subjects in the noise group, except for the noise exposure. For the mouse RNA-sequencing analysis, we performed an intra-subject comparison to reduce the influence of systemic variations on gene expression analysis. Specifically, one ear of each subject was exposed to the noise, while the other ear was protected from the noise exposure and served as the control. At 1 day after noise exposure, the animals were sacrificed, and the cochleae were collected for RNA-sequencing analysis ($n = 4$ cochleae each for the control and noise ears). The procedures involving the use and care of the animals were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

2.2. Noise exposure

A continuous noise (1–7 kHz) at 120 dB (sound pressure level, re 20 μPa) was used. The noise signal was generated using a signal processor (RP2.1, Tucker Davis Technologies, TDT, Alachua, FL, USA). The signal was routed through an attenuator (PA5 TDT, Alachua, FL, USA) and a power amplifier (Crown XLS 202, Harman International Company, Elkhart, IN, USA) to a loudspeaker (NSD2005-8, Eminence, Eminence, KY, USA) that was positioned 30 cm above the animal's head. The noise level at the position of the animal's head in the sound field was calibrated using a sound level meter (LD-PCB, model 800 B, APCB Piezotronics Div., Larson Davis, Depew, NY, USA), a preamplifier (LD-PCB, model 825, Larson Davis, Depew, NY, USA), and a condenser microphone (Larson and Davis, LDL 2559, Depew, NY, USA). The animals were individually exposed to the noise in a holding cage. The duration of the noise exposure was set at 2 h for the rats and 1 h for the mice. Different durations were used to generate a similar level of hearing loss in the rats and the mice.

2.3. Auditory brainstem responses

Auditory brain response (ABR) measurements were performed before and 1 day after noise exposure using a procedure that has been described before (Hu et al., 2012). Briefly, the animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (87 mg/kg) and xylazine (3 mg/kg). The body temperature was maintained at 37.5 °C with a warming blanket. Stainless-steel needle electrodes were placed subdermally over the vertex (non-inverting input) and posterior to the stimulated and non-stimulated ears (inverting input and ground) of the animal. The ABRs were provoked and recorded using an evoked potential measurement system (TDT, Tucker–Davis Technologies, Alachua, FL, USA). The responses were elicited with tone bursts at 5, 10, 30 and 40 kHz for the rats and 4, 8, 16 and 32 for the mice (0.5 ms rise/fall Blackman ramp, 1 ms duration, alternating phase) at the rate of 21/s. In the current study, we used different frequency settings for the rat and mouse assessments because of an update of our ABR testing system during the study. The responses were filtered, amplified and averaged using TDT hardware and software. The ABR threshold was defined as the lowest intensity that reliably elicited a detectable response. To define the change in auditory function after

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