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TRANSCRIPTOME CHARACTERIZATION BY RNA-SEQ REVEALS THE INVOLVEMENT OF THE COMPLEMENT COMPONENTS IN NOISE-TRAUMATIZED RAT COCHLEAE

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Abstract—Acoustic trauma, a leading cause of sensorineural hearing loss in adults, induces a complex degenerative process in the cochlea. Although previous investigations have identified multiple stress pathways, a comprehensive analysis of cochlear responses to acoustic injury is still lacking. In the current study, we used the next-generation RNA-sequencing (RNA-Seq) technique to sequence the whole transcriptome of the normal and noise-traumatized cochlear sensory epithelia (CSE). CSE tissues were collected from rat inner ears 1 d after the rats were exposed to a 120-dB (sound pressure level) noise for 2 h. The RNA-Seq generated over 176 million sequence reads for the normal CSE and over 164 million reads for the noise-traumatized CSE. Alignment of these sequences with the rat Rn4 genome revealed the expression of over 17,000 gene transcripts in the CSE, over 2000 of which were exclusively expressed in either the normal or noise-traumatized CSE. Seventy-eight gene transcripts were differentially expressed (70 upregulated and 8 downregulated) after acoustic trauma. Many of the differentially expressed genes are related to the innate immune system. Further expression analyses using quantitative real time PCR confirmed the constitutive expression of multiple complement genes in the normal organ of Corti and the changes in the expression levels of the complement factor I (Cfi) and complement component 1, s subcomponent (C1s) after acoustic trauma. Moreover, protein expression analysis revealed strong expression of Cfi and C1s proteins in the organ of Corti. Importantly, these proteins exhibited expression changes following acoustic trauma. Collectively, the results of the current investigation suggest the involvement of the complement components in cochlear responses to acoustic trauma.

Key words: RNA-Seq, noise, cochlea, sensory cells, complement components, rat.

INTRODUCTION

One of the leading causes of sensorineural hearing loss in adults is acoustic overstimulation, which can cause mechanical and metabolic stresses to the cochlear structure and consequently induce sensory cell degeneration (Lim, 1986; Bohne and Harding, 2000; Cheng et al., 2005; Henderson et al., 2006). Currently, there are no effective therapies available for preventing sensorineural hearing loss. To minimize cochlear damage, numerous pharmacological therapies have been studied (Abi-Hachem et al., 2010; Bielefeld et al., 2011; Clifford et al., 2011; Guthrie et al., 2011; Oishi and Schacht, 2011). Moreover, the development of gene therapies is also underway (Kesser and Lalwani, 2009; Di Domenico et al., 2011; Zhai et al., 2011). Further progress toward these therapies requires a better understanding of the molecular pathways responsible for controlling the cochlear degenerative process.

Noise-induced cochlear damage involves complex molecular events. To decipher these complex signaling pathways, several high throughput techniques, including microarrays and PCR array, have been employed in large-scale analyses of noise-induced differential expression of cochlear genes. Microarrays can be used to simultaneously quantify a large number of genes, thus producing a wealth of information (Kaminski and Friedman, 2002; Aittokallio et al., 2003; Weeraratna et al., 2004). However, this technique suffers from intrinsic limitations (Shi et al., 2004; Larkin et al., 2005; Kawasaki, 2006; Shi et al., 2008), including the inability to identify novel genes and to define a large dynamic

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Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; C1s, complement component 1, s subcomponent; Cfi, complement factor 1; CT, cycle threshold; CSE, cochlear sensory epithelium; CV, coefficient of variance; DAVID, database for annotation, visualization and integrated discovery; FPKM, fragments per kilobase of transcripts per million fragments mapped; HRP, horseradish peroxidase; RNA-Seq, RNA sequencing; TBS, tris-buffered saline; TBSt, tris-buffered saline with 0.05% TWEEN-20; TDT, Tucker Davis Technologies; qRT-PCR, quantitative real time PCR.

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change in gene expression levels. Furthermore, due to hybridization artifacts and high background noise signals, microarray techniques are often unable to detect low-abundance genes. In contrast, the PCR array technique has an excellent sensitivity and a large dynamic range. However, its throughput is limited.

Recently, the technique of RNA-sequencing (RNA-Seq), which was developed as a deep-sequencing platform, has enabled whole-transcriptome sequencing in a comprehensive way (Mortazavi et al., 2008; Wang et al., 2009; Wilhelm and Landry, 2009; Marguerat and Bahler, 2010). This ultra-high-throughput technology detects both known and unknown gene transcripts. It features a large dynamic range, contributing to its ability to accurately quantify a broader number of differentially expressed gene transcripts (Sengupta et al., 2010; Tang et al., 2010; Tariq et al., 2011). Moreover, it is possible to obtain whole-transcriptome sequencing with high reproducibility. Recently, RNA-Seq has been utilized to compare the transcriptome differences between various normal and pathological conditions in non-cochlear tissues (Beane et al., 2011; Bottomly et al., 2011; Huang et al., 2011; Jager et al., 2011). This technique has the potential to identify novel signaling pathways involved in noise-induced cochlear damage.

In the current study, we applied RNA-Seq to sequence the whole transcriptome of the cochlear sensory epithelium (CSE) and to identify transcriptome changes after acoustic trauma. A bioinformatic pathway analysis revealed the involvement of five signaling pathways in noise-induced cochlear degeneration. The involvement of the complement components was further confirmed through transcriptional analysis of sensory-cell-enriched samples and post-transcriptional analysis of the CSE. This is the first time that complement components have been implicated in noise-induced cochlear degeneration.

EXPERIMENTAL PROCEDURES

Animals and acoustic overstimulation

Sprague Dawley rats, (220–300 g, 2–3 months old, male and female, Charles River Laboratories, Wilmington, MA, USA) were used. For the RNA-Seq analysis, eight rats were divided equally into two groups (normal and noise-traumatized). For the quantitative real time PCR (qRT-PCR) analysis, six rats were divided equally into two groups (normal and noise-traumatized). For an immunohistochemistry analysis, eight rats were divided equally into two groups (normal and noise-traumatized). For a Western blot analysis, three rats from the normal group were used. For the qRT-PCR array analysis, six rats from the normal group were used. In addition, three C57BL/6J (4–6 weeks old, male and female, The Jackson Laboratory, Bar Harbor, Maine, USA) were used to determine the constitutive expression of complement genes under normal conditions. All procedures involving the use and care of animals were reviewed and approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. All efforts were made to minimise the number of animals used and their suffering.

Awake animals from the noise-traumatized group were exposed to a broadband continuous noise (1–7 kHz) at the level of 120 dB (sound pressure level, re 20 μPa) for 2 h. This level of noise was chosen because it is capable of inducing permanent hearing loss and sensory cell apoptosis (Hu et al., 2009; Hu and Cai, 2010). The noise signal was generated with a real-time signal processor (RP2.1, Tucker-Davis technologies (TDT), Alachua, FL, USA), routed through an attenuator (PA5, TDT) and a power amplifier (Crown XLS 202, Harman International Company) connected to a loud speaker (NSD2005-8, Eminence). The speaker was suspended directly above the animal holding cage. The noise level at the position of the animal's head in the sound field was calibrated using a sound level meter (Larson and Davis 800 B, Depew, NY, USA), a preamplifier (Larson and Davis, model 825) and a ½'' condenser microphone (Larson and Davis, LDL 2559). Rats were individually exposed to the noise in the holding cage.

Auditory brainstem response (ABR) test

ABR was measured before and 1 d after the noise exposure to determine the hearing sensitivity of the animals. The animals were lightly anesthetized by an intraperitoneal injection of a mixture of ketamine (87 mg/kg) and xylazine (3 mg/kg). ABRs were recorded using stainless steel needle electrodes subdermally placed over the vertex (noninverting electrode) and posterior to the stimulated and non-stimulated ear (inverting electrode and ground electrode) of the animal. The acoustic stimuli were 5, 10, 20, 30 and 40 kHz tone bursts (0.5 ms rise/fall Blackman ramp, 1 ms duration, alternating phase) at the rate of 21/s, which were generated digitally using a D/A converter (RP2.1, TDT, 100 kHz sampling rate) and fed to a programmable attenuator (PA5, TDT), an amplifier (SA1, TDT) and a closed-field loudspeaker (CF1, TDT). The electrode outputs were delivered to an amplifier (RA4LI and RA4PA; TDT) and then to a medusa base station (RA16BA, TDT). TDT software (BioSig) controlled the auditory-evoked response averaging system. Responses were filtered (100–3000 Hz), amplified and averaged using TDT hardware and software. These responses were then stored and displayed on a computer. Stimulus levels were decreased in 5-dB steps. The lowest intensity that reliably elicited a detectable waveform of the response was defined as the ABR threshold.

ABR thresholds obtained pre- and 1 d post-noise exposure at the five tested frequencies (5, 10, 20, 30 and 40 kHz) were compared using a repeated measures two-way analysis of variance (ANOVA) with post hoc Tukey's test to compare the means.

Euthanasia of animals and harvesting of cochlear tissues

Following the final ABR testing at 1 d after noise exposure, the animals were decapitated under CO_2 gas anesthesia and cochlear tissues were harvested. The