Published in final edited form as:

Hear Res. 2016 March ; 333: 283–294. doi:10.1016/j.heares.2015.10.010.

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

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the immune and inflammatory responses are the essential responses to acoustic overstimulation in the cochlea.

Keywords

RNA-sequencing; inner ear; noise; transcriptome

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 et al., 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 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 to 7 kHz) at 120 dB (sound pressure level, re 20 μ Pa) 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 two hours for the rats and one hour 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 acoustic overstimulation, the threshold shifts were calculated using the pre-noise threshold as the baseline. The ABR measurements were performed by a single observer and the observer was not blinded to the experimental conditions.

2.4. Collection of the sensory epithelia for RNA-sequencing analysis

The mouse and rat samples were collected using an identical procedure of cochlear dissection. Upon completion of the experimental treatments, the animals were decapitated under CO₂ gas anesthesia. The auditory bullae were quickly removed from the skull and placed in ice-cold Dulbecco's PBS (DPBS, GIBCO, Life Technologies, Grand Island, NY, USA) for initial tissue preparation. Then, the cochleae were transferred to an RNA stabilization reagent (RNAlater, Qiagen, Valencia, CA, USA) for the dissection to collect the cochlear sensory epithelium, which contains all of the cells on the basilar membrane between the lateral wall and the modiolus. Specifically, the bony shell of the cochlea was opened and the lateral wall of the cochlea was removed using a fine needle. Then, the sensory epithelium was gently separated from the modiolus. The isolated tissue was transferred to an RNase-free PCR tube and stored at -80°C before the subsequent analysis of gene expression. For the rat RNA-sequencing analysis, the cochlear sensory epithelia from the two cochleae of one animal were collected and pooled to generate one sample. For

the mouse RNA-sequencing analysis, the sensory epithelium from one cochlea was used to generate one sample.

2.5. RNA-sequencing

The RNA-sequencing analyses were performed to define the expression profile of the cochlear genes using a protocol that has been described in our previous publication (Patel et al., 2013). The total RNAs were extracted from the sensory epithelia using a Qiagen RNeasy Micro or Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The quality and quantity of the total RNAs were evaluated using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The RNA integrity number (RIN), an algorithm for assigning integrity values to the RNAs, was measured to determine the quality of the total RNAs extracted from the tissues. The total RNA concentrations were measured for the samples.

The cDNAs were synthesized from the total RNAs for each sample with the Clontech SMARTer™ Ultra Low RNA kit (Clontech Laboratories Inc., Mountain View, CA, USA). A sequencing library was prepared from each sample using an Illumina Paired End Sample Prep kit (Illumina Inc., San Diego, CA, USA) according to the manufacturer's protocol. The average insert size of the libraries was 124 bp. Each cDNA library was sequenced in a 50-cycle single read flow cell lane on an Illumina HiSeq 2000 in the Next-Generation Sequencing and Expression Analysis Core at the University at Buffalo. Four biological repeats (each from one animal) were performed for the mouse samples and three biological repeats (each from one animal) were performed for the rat samples.

For the RNA-sequencing data analysis, the mouse sequence reads were aligned to the mouse reference genome sequence (UCSC Genome Browser, mm10) and the rat sequence reads were aligned to the rat genome sequence (UCSC, rn5) using TopHat version 1.3.2 (Trapnell et al., 2009) and Bowtie (Langmead et al., 2009). The resulting alignments were further assembled and annotated using Cufflinks software (Trapnell et al., 2010). The abundance of gene expression was normalized to the fragments per kilobase of transcript per million mapped reads (FPKM) (Mortazavi et al., 2008). Finally, the program, Cuffdiff, was used to define the genes and transcripts that were differentially expressed after acoustic overstimulation.

2.6. Functional annotation of the differentially expressed genes

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) v.6.7 (Huang da et al., 2009) and Ingenuity Pathway Analysis (IPA) were used to determine the functional relevance of the differentially expressed genes. DAVID is a web-based tool for identifying the enriched gene ontology (GO) terms and molecular functions associated with the genes of interest. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database accessed via DAVID was used to analyze the molecular pathways. To reduce the false positive identification, attention was placed on the captures that had a *P* value or a false discovery rate (FDR) < 0.05.

IPA was used to define the potential upstream transcriptional regulators for the differentially expressed genes. This analysis predicts the genes, molecules, and drugs that could regulate the genes of interest based on the available knowledge of the role of these molecules in downstream regulation. The predictions of the activation and the inhibition states of transcriptional regulators were made based on the direction of the changes in the expression of the downstream genes. The activation Z-score was used to identify the most relevant regulators. This score infers the likely activation states of the upstream regulators based on comparison with a model that assigns random regulation directions. An absolute Z-score of greater than or equal to 2 was used to identify the upstream regulators.

2.7. Protection of one ear from acoustic overstimulation for the mouse RNA-sequencing experiment

For the mouse RNA-sequencing experiment, we used intra-subject comparison to reduce the influence of systemic variation. Specifically, one ear of the subject was exposed to noise, and the other ear was protected from the noise and served as the control. The protection was achieved by disrupting the conductive transmission function of the outer and the middle ear. Specifically, the animal was anesthetized, and silicon grease (Dow Corning Corporation, Midland, MI, USA) was gently pushed into the ear canal to fill the canal and middle ear cavity using a syringe with a fine needle under a surgical microscope. This procedure resulted in a significant threshold elevation, with an average threshold shift of 57.8 ± 10.9 dB. Following recovery from the anesthesia, the animal was exposed to the noise.

2.8. Data analysis

All values are represented as the mean values $\pm$ standard deviation. The significance of the changes or differences between conditions was examined using two statistical programs, SigmaStat for Windows V. 3.5 or Prism 5 for Windows. Based on the experimental designs, a one-way analysis of variance (ANOVA), two-way ANOVA, and Pearson's correlation analysis (see the Results section for details) were used. The identification of significant ANOVA effects and interactions was followed by appropriate pairwise comparisons, using Tukey's or Bonferroni's post hoc tests. The results were considered statistically significant only if the P value was < 0.05 .

3. Results

We first examined the RNA concentrations of the mouse and rat samples. As shown in Table 1, there is no significant difference in the average concentrations between the noise samples and the control samples in both the mouse and rat groups (Student's t test, $t(6) = 1.16$, $P = 0.29$ for the mouse group; $t(4) = -0.24$, $P = 0.82$ for the rat group). However, the average RNA concentration of the rat samples is significantly higher than that of the mouse samples (Student's t test, $t(12) = -15.05$, $P < 0.001$). This difference is likely to be caused by the difference in the amount of the tissues used in the mouse and the rat groups. In the current study, each rat sample contained the tissues pooled from two cochleae, whereas the mouse sample contained the tissues from only one cochlea.

We also examined the numbers of reads generated from the mouse and rat samples. As shown in Table 2, there is no significant difference in the average read number between the noise samples and control samples in both the mouse and rat groups (Student's *t* test, $t(6) = 1.01$, $P = 0.35$ for the mouse samples; $t(4) = 0.40$, $P = 0.71$ for the rat samples). However, the average read number of the rat samples is significantly higher than that of the mouse samples (Student's *t* test, $t(12) = -9.17$, $P < 0.001$). Again, this difference is likely due to the difference in the amount of tissues used for the mouse and rat samples.

Next, we examined the transcriptional profiles of the cochlear genes under normal and noise conditions. The raw sequencing reads were mapped to the reference genome to generate transcriptome assemblies, and those assemblies were merged to calculate the gene expression. To reduce false identification, we used a stringent criterion to define the expressed genes. A gene was considered to be expressed only if its FPKM value was greater than 0.1 in three out of four examined replicates for the mouse samples and in two out of three replicates for the rat samples. Using this criterion, we identified 13,702 constitutively expressed genes in the mouse sensory epithelium. Following acoustic overstimulation, a total of 13,781 genes were detected. These genes accounted for 58.7% (the control samples) and 59.0% (the noise samples) of the 23,361 genes annotated using the mouse mm10 assembly.

We had previously reported the transcriptional profile of the rat samples (Patel et al., 2013). In that study, the sequence reads were aligned to rn4 (Rat Genome Sequencing Consortium). In the current investigation, we used the same RNA-sequence reads, but aligned them to rn5, which is a newer assembly that was released in 2012. A total of 12,026 genes were detected in the control samples, while 12,098 genes were detected in noise overstimulated samples. The detected genes accounted for 70.0 % (the control samples) and 70.5% (the noise samples) of the 17,170 annotated genes. The numbers of detected genes identified with rn5 were similar to those generated using rn4 (12,025 genes for the control samples and 12,096 genes for the noise samples).

3.1. Identification of the differentially expressed genes following acoustic overstimulation

Cuffdiff statistical analyses were performed to identify the genes that were differentially expressed in the rat samples. Using the criteria of a *q* value < 0.05 and a fold change > 1.5 , we identified a total of 555 differentially expressed genes 1 day after noise exposure. Among those genes, 432 (77.8%) were up-regulated, and 123 (22.2%) were down-regulated (Table 3). The total number of identified genes was greater than what had been reported in our previous publication (78 genes) (Patel et al., 2013), which is due to the differences in the reference genome sequence assemblies and the analysis algorithms used in the two analyses. Noticeably, the majority of the 78 genes identified in our previous investigation were identified in the current analysis (Fig. 1A).

The Cuffdiff analysis of the mouse samples revealed a total of 101 genes that displayed significant changes in expression (*q* value < 0.05 and fold change > 1.5) following acoustic overstimulation (Table 4). Among these genes, 76 (75.2%) were up-regulated, and 25 (24.8%) were down-regulated. While the total number of identified genes differed between the mouse and the rat samples (101 for the mouse samples vs. 555 for the rat samples), the

ratios of the up- and down-regulated genes to the total number of differentially expressed genes were similar between the two species (77.8% vs. 75.2% for the upregulated genes and 22.2% vs. 24.8% for the downregulated genes). The predominance of up-regulated genes is consistent with our previous observations (Hu et al., 2012; Patel et al., 2013). The average fold changes of the differentially expressed genes in the mouse cochleae were significantly greater than those in the rat samples (Mann-Whitney rank sum test, $t = 37402$, $P = 0.016$, Fig. 1B). However, the magnitude of the difference was small. Taken together, these analyses reveal the differentially expressed genes in the mouse and rat cochlear sensory epithelium following acoustic trauma. Using these gene lists, we performed subsequent bioinformatics analyses to determine the common functional implications of these differentially expressed genes.

3.2. The common biological processes associated with the differentially expressed genes in the mouse and rat cochleae following acoustic overstimulation

Two bioinformatics tools were used to determine the functional relevance of the differentially expressed genes: the Database for Annotation, Visualization and Integrated Discovery (DAVID v 6.7) and the Ingenuity Pathway Analysis (IPA).

We first performed gene ontology analysis, which is an integrated functional module offered by DAVID. This analysis captured six biological process terms with an FDR < 0.05 in the mouse samples (Table 5). These biological terms included the immune response, response to wounding, the defense response, taxis, chemotaxis and inflammatory response. For the rat samples, the analysis identified 37 biological process terms with an FDR < 0.05 (Table 5). While the numbers of the identified terms differed (6 for the mouse samples vs. 37 for the rat samples), the biological processes that the terms are associated with showed considerable overlap between the two species. All six of the biological terms identified in the mouse samples were also identified in the rat samples. The majority of the terms that were captured in the rat samples were related to the six terms that were identified in the mouse samples. Only six biological terms that were captured in the rat were not related to those identified in the mouse, and those terms were generally non-specific. Together, these analyses suggest that the mouse and rat cochleae have similar biological responses to acoustic trauma and that the common processes are related to the immune defense response.

3.3. The common molecular pathways associated with the differentially expressed genes in the mouse and rat cochleae

KEGG is a web-based database for investigating the functions of biological systems based on information at the genomic and molecular levels. We used KEGG to identify the molecular pathways that were associated with the differentially expressed genes. For the rat samples, the analysis captured 14 pathways, with 9 pathways having a P value < 0.05. Among those pathways, three (viral myocarditis, systemic lupus erythematosus and asthma) were not related to acoustic trauma and were removed from further analysis. The remaining pathways, which are presented in Table 6, were involved in multiple biological processes, and most were related to immune and inflammatory responses (marked by the asterisks in Table 6).

For the mouse samples, the analysis captured six pathways, with five having a P value < 0.05 (Table 7). Among those pathways, four were also identified in the rat: cytokine-cytokine receptor interaction, the chemokine signaling pathway, the Toll-like receptor signaling pathway, and the NOD-like receptor signaling pathway. These analyses again suggest that the immune and inflammatory responses are common cochlear responses to acoustic overstimulation in both species.

3.4. Identification of the common upstream regulators of the differentially expressed genes

IPA was used to determine the upstream transcriptional regulators of the differentially expressed genes. This bioinformatics tool identifies the genes, molecules and chemicals that regulate the transcription of genes of interest. The activation states of transcriptional regulators can be predicted based on the observed differential regulation of the genes in a dataset. To reduce the identification of false positives, we used a stringent bias-corrected z -score > 2 for activation and < -2 for inhibition. We identified 45 upstream regulators in the rat samples and 20 in the mouse samples that were predicted to activate the expression of the differentially expressed genes (Fig. 2). Of those upstream regulators, 10 were identified in both species, and of these 10 common regulators, eight have roles in the immune response and six are cytokines (*Ifng*, *Tnf*, *Il1b*, *Osm*, *Lif*, and *Il6*). This observation suggests that the common regulators of the differentially expressed genes are molecules related to the immune response.

We also identified 18 regulators in the rat and six in the mouse that were predicted to inhibit the expression of the differentially expressed genes (Fig. 3). Among those, three were identified in both species. *Tp73* and *Nkx2-3* are transcription factors, and *MAPK1* is an extracellular signal-regulated kinase that has been implicated in acoustic trauma to the cochlea (Meltser et al., 2010).

3.5. Common cellular components

To analyze the cellular distribution, we identified three terms for the mouse and five terms for the rat that showed an FDR < 0.05 (Table 8). All three terms that were identified in the mouse samples were related to the extracellular space. These terms were also identified in the rat samples, and their FDR values were the lowest among the terms identified in the rat. The two additional terms that were identified in the rat but not in the mouse were related to the plasma membrane and the cell surface, and those two terms were also functionally related to the extracellular space. Together, this analysis suggests that the differentially expressed genes have a strong link to the extracellular space.

3.6. Comparison of the differentially expressed genes between the rat and mouse samples

Given the similarity in the biological functions and the molecular pathways in the mouse and rat cochleae, we sought to determine the common differentially expressed genes that were shared by the two species. A total of 31 genes were identified (Fig. 4A), among which 29 were upregulated and two were downregulated. All of the overlapping genes displayed the same direction of change, i.e., the genes that were upregulated in one species were also upregulated in the other, and vice versa. Moreover, the fold changes in the overlapping

genes correlated between the two species (Pearson's correlation, $r(27) = 0.52$, $P = 0.004$; Fig. 4B).

A large portion of the differentially expressed genes did not overlap between the mouse and rat samples. One reason for this discrepancy is that some genes identified in one species do not have orthologues in the other species. To determine the extent to which this factor contributed, we determined the number of differentially expressed genes identified in one species that did not have an orthologue in the other species. A total of 14 of the 101 differentially expressed genes in the mouse do not have the homologs in the rat, and among the 555 differentially expressed genes identified in the rat, 43 do not have homologs in the mouse, indicating that this factor contributes to a portion of the discrepancy.

3.7. Acoustic overstimulation causes a similar level of auditory dysfunction in mice and rats

To provide the context for comparing the changes in expression of the cochlear genes between the mice and rats, we examined the impact of acoustic overstimulation on auditory function. The ABR thresholds were measured before and 1 day after noise exposure. In our functional assessment of the mouse cochleae ($n = 8$), we found a significant elevation in the ABR threshold at all tested frequencies, with greater loss in the middle frequencies (8 and 16 kHz, Fig. 5). A repeated measures two-way ANOVA (time $\times$ frequency) revealed that the shifts were statistically significant for the time factor ($F(1,28) = 1716$; $P < 0.0001$; Bonferroni's post-test, $P < 0.001$ for all of the tested frequencies). This pattern of middle frequency-dominated hearing loss is different from the flat loss observed in our previous study (Fig. 5)(Patel et al., 2013). However, there were no significant differences in the average threshold shifts between the two species (47.8 ± 13.1 dB for the mice and 44.7 ± 3.2 dB for the rats; Student's t test, $t(46) = 0.599$, $P = 0.55$). These observations indicate that both the mouse and rat ears sustained a similar level of acoustic injury.

4. Discussion

The current study was designed to determine the common biological processes and molecular pathways associated with acoustic trauma in rodents. We analyzed the noise-induced transcriptional changes in the mouse and rat cochleae using RNA-Sequencing data and identified the differentially expressed genes in each species. Our bioinformatics analyses revealed that multiple biological processes and molecular pathways were associated with differentially expressed genes and a significant similarity in these responses between the mouse and the rat. Importantly, we found that a large portion of the common processes and pathways are related to immune and inflammatory responses, suggesting that the immune response plays a key role in the cochlear response to acoustic overstimulation.

Our DAVID gene ontology analysis of the mouse samples reveals six terms that are associated with the differentially expressed genes, including the immune response, response to wounding, the defense response, chemotaxis and inflammatory responses. Our analysis of the rat samples revealed 37 terms, which is a number that is significantly greater than what had been identified in the mouse samples. The difference in the number of terms captured is likely the result of the difference in the numbers of differentially expressed genes that were

identified in the two species, which, in turn, is likely the result of the difference in the read numbers between the two species. Further analyses of the biological terms identified in the rats revealed that many of the rat-specific terms are associated with the same terms that were identified in the mouse. These common terms are associated with the immune response, inflammatory responses, defense response and response to wounding.

Consistent with our functional analysis of the biological processes, our pathway analysis reveals the common pathways shared by the mouse and the rat. These pathways are related to immune and inflammatory responses, which include cytokine-cytokine receptor interactions, the chemokine signaling pathway, the Toll-like receptor signaling pathway, and the NOD-like receptor signaling pathway. Some of the genes that are linked to these pathways have been implicated in the cochlear response to acoustic overstimulation. For example, the exposure to intense noise increases Fos expression in the cochlea (Cho et al., 2004), and this increase appears in the Hensen cells, Claudius cells and Deiters cells of the basal and second turns of the cochlea (Shizuki et al., 2002). Mice lacking Ccr2 function display a greater susceptibility to acoustic overstimulation (Sautter et al., 2006). Our recent study has shown the involvement of Tlr3 and Tlr4 in supporting the cellular response to sensory cell damage following acoustic trauma (Cai et al., 2014). These analyses suggest that the immune and inflammatory responses are the common cochlear responses to acoustic overstimulation in both species. The functional role of the immune and inflammatory responses in cochlear injury and repair has not been well established. Because treatments with steroid hormones are known to have a protective effect (Fakhry et al., 2007; Takahashi et al., 1996; Takemura et al., 2004) and because the protective effect is mediated via NF- κ B (Tahera et al., 2006) and TNF- α (Maeda et al., 2005), which are both immune and inflammatory regulators, the inflammatory response likely contributes to sensory cell damage.

IPA allowed us to predict the transcriptional regulators that would be involved in modulating the differential expression of these genes and whether the regulators activate or inhibit the genes of interest. Using this bioinformatics tool, we determined the common regulators in mice and rats for the genes that showed differential expression following acoustic overstimulation. Lipopolysaccharide is an endotoxin released from Gram-negative bacteria. This molecule is able to provoke a strong immune defense response via a Tlr4-dependent pathway. The identification of this molecule suggests that the Tlr4 pathway is activated, possibly by the endogenous molecules released from the damaged tissues. IL6 and TNF are inflammatory molecules, and their involvement in acoustic injury has been reported (Fujioka et al., 2006; Wakabayashi et al., 2010). Interferon gamma is an important activator of macrophages and induces the expression of major histocompatibility complex II, an antigen presenting protein (Mach et al., 1996; Romieu-Mourez et al., 2007). We have observed an increase in the expression of major histocompatibility complex II in mononuclear phagocytes of the cochlea following acoustic injury (Yang et al., 2015b), suggesting the activation of an antigen-presenting function. Creb1 encodes cAMP-responsive element binding protein 1, and this protein is involved in the transcriptional regulation of cAMP-inducible genes. Together, this bioinformatics analysis of the potential upstream regulators reveals the potential molecular players and pathways involved in the regulation of the cochlear response to acoustic trauma.

Previous investigations have implicated multiple genes in the cochlear responses to acoustic trauma. However, many of the previously reported genes were not identified in the current study. This discrepancy could result from the differences in the experimental paradigms, such as the noise level used, the time of sample collection and the cell populations sampled. In addition, the differences in the expression analysis platforms used in the different studies could contribute to this discrepancy. RNA-sequencing analysis simultaneously compares a large number of genes, yet the sample sizes are relatively small due to high sequencing costs. Small sample sizes compromise the power of the analysis. Moreover, in our recent variation analysis of RNA-sequencing data, we found a marked increase in the variations in cochlear gene expression following acoustic trauma (Yang et al., 2015a). These unstable genes are functionally associated with cell death, apoptosis and macro-molecular metabolisms. Clearly, our RNA-sequencing analysis was unable to identify these genes because of these variations. It would be expected that as the sample size increases, more differently expressed genes could be identified.

Despite the presence of a marked overlap in the functional relevance of the differentially expressed genes, the gene sets identified in the two species differed considerably. This difference could suggest species-specific differences; however, several confounding factors complicate this interpretation. First, mice and rats differ in their susceptibility to stress (Wu et al., 2001). This difference could influence their gene expression responses to stress. Although the current functional assessment revealed a comparable level of functional impact, the likelihood of a difference at the cellular level cannot be ruled out. Second, the total RNA concentrations of the mouse samples and rat sample were different, possibly due to the difference in the amounts of the tissues used for RNA extraction. This difference could affect the detection of low abundance genes. Third, the control conditions used in the mouse and rat RNA-sequencing analyses differed in the current study. For the rat study, the control ears were collected from animals that were not exposed to the noise, while for the mouse study, the control ears were those that were protected from the noise. Even with this protection, the control ears in the mouse experiments might still sustain a low level of noise stimulation, which, in turn, could affect the basal level of gene expression. Moreover, the systemic responses induced by exposure to one ear could affect the baseline expression of the genes in the other ear. Because of these confounding factors, our study focused on the similarity in the biological processes rather than the differences in the individual gene sets. Our study clearly demonstrates that even with the differences in the genes sets, the functional relevance associated with the differentially expressed genes is remarkably similar.

Acknowledgments

The authors thank Dr. Youyi Dong for his assistance in the data analyses. The authors also thank Jonathan Bard and Jennifer Jamison for their assistance in RNA-seq data collection and analysis. This research was supported by NIDCD 1R01DC010154.

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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Highlights

- Determination of the common molecular pathways associated with noise injury in rodents
- The common biological processes are related to the immune defense response
- The common molecular pathways are also related to immune defense
- Mice and rats display a marked similarity in their biological responses to acoustic injury

Figure 1. Identification of the differentially expressed genes

(A) The Venn diagrams illustrate the degree of the overlap of the differentially expressed genes identified in the current study using Rn5 and in our previous study using Rn4. The upper panel shows the upregulated genes and the bottom panel shows the downregulated genes. A majority of the differentially expressed genes identified in our previous study was also identified in the current study. (B) A comparison of the average fold changes of the differentially expressed genes that were identified in the mouse and rat cochleae. The average fold changes in the mouse cochleae was slightly, but statistically greater than that of the rat samples (Mann-Whitney rank sum test, $t = 37402$, $P = 0.016$). The dots represent the fold changes of individual genes, and the blue lines represent the means and standard deviations.

Figure 2. Upstream transcriptional regulators that are predicted to activate the differentially expressed genes

The data were generated using IPA with the bias-corrected z-score > 2 to identify the genes, molecules and chemicals that activate the transcription of differentially expressed genes. The regulators in the left/bottom gray area are those identified in the rat samples, and the regulators in the right green area are those identified in the mouse samples. The regulators in the central area are the common regulators identified for both the rat and mouse samples. A total of 45 upstream regulators were identified in the rat samples and 20 in the mouse samples. Of those upstream regulators, 10 were identified in both species, and of these 10 common regulators, eight have roles in the immune response and six are cytokines.

Figure 3. Upstream transcriptional regulators that are predicted to inhibit the differentially expressed genes

The data were generated using IPA with the bias-corrected z-score < -2 to identify the genes, molecules and chemicals that inhibit the transcription of the differentially expressed genes. The regulators in the left/bottom gray area are those identified in the rat samples, and the regulators in the right green area are those identified in the mouse samples. The regulators in the central area are the common regulators identified in both the rat and mouse samples. A total of 18 regulators were identified in the rat and six in the mouse. Among those, three were identified in both species. Tp73 and Nkx2-3 are transcription factors, and MAPK1 is an extracellular signal-regulated kinase.

Figure 4. Common differentially expressed genes identified in both the mouse and rat cochleae (A) The Venn diagram lists the differentially expressed genes that were detected in both the mouse and rat cochleae. The numbers of genes that were detected exclusively in the mouse cochleae and in the rat cochleae and in both species are also presented. (B) Pearson's correlation analysis of the fold changes of the differentially expressed genes detected in the mouse and rat cochleae following acoustic injury. The expression changes between the two species are moderately correlated.

Figure 5. Comparison of the noise-induced threshold shifts of ABR between the mice and the rats

The tests were performed before and 1 day after noise exposure. While there is a difference in the configuration of the hearing loss, there are no significant differences in the average threshold shifts between the two species (47.8 ± 13.1 dB for the mice and 44.7 ± 3.2 dB for the rats; Student's *t* test, $t(46) = 0.599$, $P = 0.55$).

Table 1

Total RNA concentration (ng/μl).

	Mouse Samples		Rat samples	
	Control	Noise	Control	Noise
Sample 1	1.36	1.97	11.33	11.63
Sample 2	1.62	3.05	8	9.39
Sample 3	2.75	2.02	10.4	9.58
Sample 4	1.37	2.02		
Average	1.78	2.27	9.91	10.20

Table 2

RNA-sequence reads of the mouse and rat sensory epithelium samples.

	Mouse Samples		Rat samples	
	Control	Noise	Control	Noise
Sample 1	55,418,554	54,520,647	201,036,188	113,467,961
Sample 2	76,054,269	56,802,014	167,940,585	208,180,678
Sample 3	52,965,010	33,806,938	159,901,482	170,872,626
Sample 4	40,784,841	43,405,709		
Average	56,305,668.5	47,133,827	176,292,752	164,173,755

Table 3

The differentially expressed genes identified in the rat cochleae.

Upregulated genes (the numbers in parenthesis are fold changes)				
Pole (1.58)	Pxk (1.96)	Fgl1 (2.44)	Samsn1 (3.1)	Mpeg1 (4.85)
Stx4 (1.6)	Trpm2 (1.96)	Mlf1 (2.44)	Myh3 (3.11)	Osmr (4.87)
Mycbpap (1.6)	Cyp3a9 (1.96)	Crabp2 (2.45)	Cxcl13 (4.89)	PCOLCE2 (3.15)
Rab13 (1.6)	Pqlc3 (1.96)	Cmpk2 (2.45)	Aspg (3.16)	Abca4 (4.91)
Dbnidd2 (1.61)	P2ry6 (1.97)	Dio2 (2.46)	Prp3 (3.16)	Cyp11a1 (4.97)
Cend1 (1.61)	Wfdc2 (1.98)	Mki67 (2.46)	Bdkrb2 (3.19)	Mgp (5.06)
Pdlim1 (1.62)	Shmt1 (1.98)	Nusap1 (2.46)	Aebp1 (3.19)	Sele (5.06)
Txn1 (1.62)	Cd68 (1.98)	Tyr (2.46)	Parvg (3.21)	Melk (5.08)
Cyp51 (1.63)	Il13ra1 (1.99)	Kif23 (2.46)	Lilrb3l (3.21)	Dmkn (5.2)
Thbd (1.63)	MGC105567 (2)	Slfn2 (2.47)	Tfap2b (3.22)	Gal (5.21)
Clic4 (1.63)	Tmem176a (2)	Fxyd5 (2.5)	Lgals5 (3.22)	Sct (5.32)
Gpr182 (1.63)	Itgam (2.01)	F2rl2 (2.5)	RT1-Da (3.27)	C1s (5.37)
Gpx1 (1.64)	Rac2 (2.01)	Ptpn7 (2.51)	Tubb6 (3.27)	Akr1b8 (5.37)
Mfsd1 (1.66)	Ampd3 (2.01)	Cited1 (2.54)	Ch25h (3.29)	Atp4a (5.47)
Tnfrsf1a (1.66)	Aox3 (2.01)	Trpv2 (2.54)	RT1-Db1 (3.29)	Pbk (5.54)
Tmem54 (1.67)	Chdh (2.02)	Hcls1 (2.55)	Eml2 (3.3)	Calca (5.58)
Abcc3 (1.67)	Cxadr (2.03)	Apol9a (2.56)	Dsn1 (3.34)	Chaf1b (5.68)
Pcolce (1.67)	Hyal1 (2.03)	Itgb2 (2.56)	Jak3 (3.35)	Ifit3 (5.74)
Il10rb (1.68)	Raly (2.03)	Cep41 (2.56)	A3galt2 (3.35)	Rgs16 (5.76)
Inmt (1.68)	C1qb (2.04)	Serpinb8 (2.56)	Cox6a2 (3.36)	C3 (5.89)
Myd88 (1.68)	Cyth4 (2.04)	Mcm3 (2.56)	Ifi204 (3.38)	Tmem40 (5.91)
S100a11 (1.69)	Agt (2.04)	Pla1a (2.56)	Hsh2d (3.39)	Fam111a (5.91)
Col3a1 (1.69)	S100a6 (2.04)	Thbs4 (2.57)	Ccl7 (3.39)	Acer1 (5.97)
Gja4 (1.7)	Scnn1a (2.04)	Myh1 (2.58)	Hck (3.42)	Tsga10ip (6.21)
Fads1 (1.7)	Igtf (2.05)	Cd74 (2.59)	Ptges (3.43)	Uhrf1 (6.25)
Fdps (1.7)	Plp2 (2.05)	Cxcr5 (2.59)	Dok2 (3.43)	Mybphl (6.32)
Agpat3 (1.7)	Tnfrsf26 (2.05)	Pfkfb3 (2.6)	Ifi27l2b (3.44)	Il22ra1 (6.42)
Ldlr (1.72)	Gadd45g (2.05)	Aadat (2.6)	Ly6c (3.44)	Igfbp4 (6.45)
Pros1 (1.72)	Xirp2 (2.06)	Hk3 (2.6)	Socs3 (3.44)	Rrm2 (6.54)
Tagln2 (1.72)	Cldn11 (2.06)	Col15a1 (2.6)	Asf1b (3.45)	Ifit2 (6.91)
Tyrp1 (6.98)	Trex1 (2.06)	Ras10a (2.6)	Duoxa1 (3.47)	Fam180a (1.72)
Csf1 (1.73)	Rarres2 (2.07)	Scnn1b (2.61)	Mybpc2 (3.48)	Kng1l1 (7.14)
Sdc1 (1.73)	Syt5 (2.07)	Tmprss9 (2.61)	Ncf4 (3.48)	Fabp5 (7.15)
Anxa3 (1.73)	Lrcol1 (2.07)	Clec4a3 (2.63)	Cd274 (3.5)	Vtcn1 (7.18)
Fcer1g (1.74)	Card9 (2.07)	Stat2 (2.64)	Steap1 (3.55)	Cxcr3 (7.37)
Erp29 (1.74)	Cyp11b1 (2.08)	Cdca7 (2.65)	Mmp3 (3.62)	Sfrp4 (7.38)
Lbp (7.72)	Klhl40 (2.09)	Wisp2 (2.66)	Fcgr2b (3.62)	Rnase11 (7.44)
Gem (1.74)	Plek (2.09)	Steap4 (2.67)	Pmch (3.62)	Gadd45b (1.74)
Gatsl2 (1.75)	Fos (2.09)	Trim25 (2.69)	C1rl (3.64)	Hamp (7.75)

Upregulated genes (the numbers in parenthesis are fold changes)				
Dhcr7 (1.75)	Ucma (2.1)	Lsp1 (2.69)	Pla2g2a (3.65)	Ecel1 (7.81)
Emp3 (1.76)	Sbno2 (2.1)	Atf3 (2.7)	Ifi47 (3.65)	Gja5 (7.81)
Aldh1a2 (1.77)	Elf3 (2.11)	Sod2 (2.71)	Pkib (3.66)	Tgm1 (8.25)
Sardh (1.77)	Tmed9 (2.11)	Prg2 (2.73)	Fkbp6 (3.68)	Kcnj15 (8.8)
Plau (1.77)	Slc17a9 (2.11)	Coro1a (2.74)	Extl1 (3.68)	Ccl2 (9.01)
Serpinh1 (1.78)	Endou (2.11)	Enpp3 (2.77)	Spc25 (3.7)	Dapl1 (9.6)
Tsku (1.8)	Pkp1 (2.12)	Phf11 (2.77)	Pmp2 (3.71)	Exo1 (9.64)
Pla2g4a (1.81)	Pglyrp1 (2.13)	Psmb8 (2.78)	Cxcl17 (3.76)	Ube2t (9.64)
Irak3 (1.81)	Kcne3 (2.13)	Myo1g (2.78)	Stmn4 (3.78)	Ccr7 (9.87)
Cfh (1.82)	Rasa3 (2.14)	Tk1 (2.78)	Rbp7 (3.78)	Glycam1 (9.91)
Sdf2l1 (1.83)	Mcm10 (2.15)	S100a4 (2.78)	Sectm1b (3.81)	Fosl1 (10.07)
Sphk1 (1.83)	Cdca8 (2.17)	Mt1a (2.78)	Serping1 (3.82)	Trem1 (10.42)
Nrp1 (1.84)	Clic1 (2.18)	Mx1 (2.78)	Ccl4 (3.83)	Ifitm1 (10.55)
Scnn1g (1.85)	Rab32 (2.19)	Bcl2a1 (2.79)	Iqcf3 (3.83)	Clec4a2 (11.02)
Il17rb (15.37)	Slco2a1 (2.19)	C1r (2.79)	Slfn13 (3.85)	Slc15a1 (11.07)
Nol3 (1.86)	Arhgap30 (2.21)	P2rx1 (2.81)	Cenpt (3.85)	Tnfrsf12a (1.85)
Cdo1 (1.86)	PVR (2.21)	Gcgr (2.82)	Tnnc1 (3.87)	Rhbg (15.73)
C5ar1 (1.86)	Gpr77 (2.22)	Cd86 (2.82)	Mcm5 (3.89)	Wfdc18 (16.69)
Tyrobp (1.86)	Smpd3b (2.22)	Syt13 (2.82)	C1qtnf5 (3.89)	Cxcl9 (16.81)
Hpx (18.82)	Slc28a2 (2.24)	Aldh1l2 (2.82)	Duox1 (3.97)	Tmem173 (1.86)
Fkbp11 (1.86)	Slamf8 (2.24)	Kif22 (2.84)	Cidec (4.02)	Vmo1 (19.08)
Prss36 (1.86)	Rps2 (2.24)	Fgl2 (2.85)	Oc90 (4.03)	Ccl12 (19.81)
Hsd3b7 (1.87)	Il2rg (2.26)	Parp3 (2.86)	Ccr1 (4.04)	Dhrs9 (20.98)
Ptpcr (1.87)	Rgs1 (2.26)	Clec5a (2.86)	Plac8 (4.04)	Crybb3 (25.45)
Mcm6 (1.87)	Slc1a (2.26)	Slc6a18 (2.88)	Oasl (4.07)	Cd177 (26.54)
Mvp (1.88)	Carhsp1 (2.26)	Bag2 (2.9)	Cfb (4.15)	Timp1 (35.35)
Fn1 (1.88)	Gngt2 (2.27)	Myh2 (2.9)	Cdca3 (4.17)	Lcn2 (36.67)
Slc1a5 (1.89)	Nqo1 (2.27)	Gpx2 (2.92)	Rbp4 (4.19)	Slpil2 (51.64)
Epcam (1.89)	Cyr61 (2.27)	Traip (2.93)	Rsad2 (4.2)	Slpi (57.54)
Lamc2 (1.89)	Rundc3a (2.28)	C6 (2.94)	Cfi (4.24)	Calcb (95.22)
Rac1 (1.89)	Emp1 (2.28)	Gfap (2.94)	Chi3l1 (4.25)	Defb52 (10.07)
Mgst1 (1.89)	B4galt5 (2.29)	Csf2rb (2.95)	Gbp2 (4.3)	Reg3b (7.35)
Hspb1 (1.9)	Pstpip1 (2.3)	Tac4 (2.97)	Top2a (4.32)	Sprr1a (4.77)
Adm (1.91)	Mlph (2.3)	Tacstd2 (2.97)	Lgals3 (4.37)	RGD1561157 (1.91)
Slc38a5 (1.91)	Zbp1 (2.33)	Tns4 (2.98)	Il18bp (4.4)	LOC100365008 (1.95)
Mmp19 (1.91)	Cish (2.33)	Slc39a14 (3)	Cym (4.41)	LOC300308 (2.01)
Loxhd1 (1.92)	Lgals3bp (2.33)	Procr (3)	Mmp12 (4.44)	Cd300le-ps1 (2.22)
Ccl11 (1.92)	Cd14 (2.35)	Bcl3 (3)	Mt2A (4.45)	Tmem106a (2.24)
Adamts1 (1.92)	C1qtnf9 (2.35)	Upp1 (3.03)	Scn3b (4.48)	RGD1564854 (2.32)
Slc39a1 (1.92)	Napsa (2.37)	Plk1 (3.03)	Plvap (4.5)	LOC688765 (2.35)
Cd36 (1.92)	Ecm1 (2.37)	Prc1 (3.05)	Cst7 (4.54)	LOC298795 (3.19)
Delc1 (1.93)	Gpr84 (2.38)	Plaur (3.05)	Derl3 (4.64)	LOC681325 (3.38)

Upregulated genes (the numbers in parenthesis are fold changes)				
Lcp2 (1.93)	Clec4a1 (2.38)	Ltbp2 (3.06)	Cd27 (4.64)	RGD1561778 (5.89)
Fam70b (1.93)	Psmb9 (2.38)	Cdca2 (3.06)	Gpnmh (4.66)	RGD1559482 (6.86)
Fgr (1.94)	Ier3 (2.41)	Msr1 (3.06)	Aurkb (4.67)	Serpina3n (15.6)
Psmb10 (1.94)	Mgst2 (2.42)	Ripk3 (3.07)	Clec9a (4.67)	
Rom1 (1.95)	Serpine1 (2.42)	Slc43a3 (3.07)	Isg20 (4.69)	
Folr2 (1.96)	Slc6a19 (2.44)	RT1-Ba (3.07)	Gphb5 (4.7)	
Downregulated genes				
Fgf21 (13.85)	Dnajc5b (2.45)	Slc17a7 (1.95)	H1f0 (1.81)	Fam163a (4.24)
Ifit1 (9.1)	Slc4a10 (2.44)	Thsd7b (1.95)	Ppm1h (1.8)	Epb41l1 (1.65)
Cyp17a1 (7.46)	Egr1 (2.43)	Cers5 (1.94)	Ppargc1a (1.8)	Ppm1k (1.65)
Ckmt2 (4.29)	Sik1 (2.41)	Acsbg1 (1.93)	Gira3 (1.79)	Il12rb2 (1.64)
Gpm6a (1.65)	Lcat (2.38)	Twist1 (1.93)	Lbh (1.64)	Slc25a29 (1.79)
Slc25a18 (4.07)	Nrn11 (2.35)	Gpr157 (1.92)	Etnk1 (1.64)	Trappc10 (1.78)
A4galt (3.7)	Slc41a3 (2.3)	Stk11 (1.92)	Spire1 (1.78)	Lmo7 (1.63)
Cytl1 (3.58)	Zfp385b (2.28)	Camk1g (1.91)	Sertad4 (1.78)	Abcg2 (1.63)
Spon2 (3.54)	Crem (2.28)	Elovl2 (1.91)	Ociad1 (1.78)	Slc44a3 (1.63)
Gpr12 (3.45)	Egln3 (2.27)	Agr3 (1.9)	Zic5 (1.75)	Tagln (1.63)
Mstn (3.21)	Ppp1r1a (2.24)	Mlc1 (1.9)	Tspan2 (1.75)	Slc16a4 (1.63)
Spock2 (3.11)	Car14 (2.24)	Apold1 (1.89)	Rab3a (1.74)	Atp11a (1.61)
Tnnc2 (3.06)	Rgr (2.13)	P2ry14 (1.89)	Cables1 (1.73)	Slc38a1 (1.61)
Spt1 (2.94)	Trpm1 (2.13)	Frem1 (1.87)	Ppp1r1b (1.73)	Slc22a23 (1.66)
Il12a (2.92)	Dusp1 (2.11)	Slc7a1 (1.87)	Pygm (1.59)	Dennd2c (1.73)
Lrrc52 (2.9)	Hunk (2.08)	Corin (1.87)	Atrn (1.73)	LOC171161 (3.84)
Kcnc1 (2.89)	SrpX2 (2.03)	Slc16a6 (1.87)	Rin3 (1.72)	RGD1562673 (2.71)
Ali3 (2.78)	Wfdc1 (2.03)	Prkaa2 (1.86)	Lysmd2 (1.71)	LOC641520 (2.3)
Cmah (2.74)	Foxs1 (2.03)	Amd1 (1.85)	Ppip5k1 (1.69)	Rnase1l1 (2.26)
AcsM5 (2.7)	Cpne7 (2.01)	Ca2 (1.85)	Fcrlb (1.68)	Tmem255a (2.19)
Dcc (2.69)	Chac1 (2.01)	Dnaja4 (1.85)	Fgfr2 (1.67)	Serpina1 (2.04)
Slc25a25 (2.66)	Trim45 (2)	Sdk2 (1.83)	Szrd1 (1.67)	LOC497978 (1.91)
F13a1 (2.63)	Serg1 (1.99)	Gzmm (1.83)	Tspan18 (1.66)	RGD1565616 (1.72)
Npas2 (2.5)	Snx22 (1.96)	Ahcy12 (1.83)	Lhfp12 (1.66)	
Mybpc1 (2.48)	Nt5dc2 (1.96)	Kcnj16 (1.82)	Kank4 (1.6)	

Table 4

The Differentially expressed genes identified in the mouse cochleae.

Upregulated genes (the numbers in parenthesis are fold changes)			
Slc7a11 (2.78)	Ly6a (5.19)	Lilrb4 (3.48)	Tsga14 (2.75)
Gpr84 (11.32)	Ccl12 (5.16)	Cd68 (3.43)	Rnf125 (2.68)
Grem1 (4.05)	Wfdc18 (4.76)	Myom1 (3.34)	Mest (2.6)
Has1 (4.71)	Tubb6 (4.73)	Socs3 (3.33)	Xaf1 (2.58)
Saa2 (55.51)	Prss22 (4.67)	S100a9 (3.28)	Chrna1 (2.52)
Ccl2 (33.02)	Cfb (4.65)	Trpv2 (3.28)	Bst2 (2.5)
Serpina3n (24.9)	Isg15 (4.45)	Fos (3.28)	Ifi2712a (2.46)
Slpi (17.45)	Ccl5 (4.43)	Fstl1 (2)	Gbp2 (2.43)
Ccl7 (16.58)	Il18bp (4.35)	Hbegf (3.22)	Medag (2.35)
Lcn2 (15.67)	Aspg (4.29)	S100a8 (3.18)	Adh1 (2.34)
Saa1 (13.04)	Osmr (4.03)	Dio2 (3.16)	Ngp (2.33)
Fabp5 (9.96)	Msr1 (4.01)	Ifit3 (3.04)	Lgals9 (2.25)
Bcl2l15 (9.46)	Ifi44 (4)	Chi3l1 (3.03)	Fxyd5 (2.25)
Mgp (8.31)	Ccl6 (3.98)	Ifitm3 (2.97)	Irgm1 (2.23)
Zbp1 (8.16)	Oasl1a (3.92)	Runx1 (2.89)	Soat1 (2.15)
Lrg1 (7.19)	Elf3 (3.81)	Irf7 (2.88)	Lmcd1 (2.13)
Bpifa1 (7.17)	Ifitm1 (3.81)	Ctla2a (2.87)	Ube2l6 (2.11)
Glpr2 (6.22)	Usp18 (3.57)	Tnfrsf11b (2.81)	5830403L16Rik (4.93)
Plek (6.02)	Pdk4 (3.49)	Ly6e (2.05)	I830012O16Rik (3.27)
Downregulated genes			
Vcam1 (2.04)	Kazald1 (2.28)	Gm525 (2.77)	Fam129c (4.78)
Ces1d (2.11)	Acan (2.44)	Plbd1 (2.79)	Matk (6.61)
Ibsp (2.16)	Col9a1 (2.51)	Otor (2.84)	1600002H07Rik (3.21)
Cthrc1 (2.18)	Mpo (2.66)	Lcat (2.91)	1500015O10Rik (3.46)
Pcp4 (2.24)	Evc2 (2.69)	Wdr86 (2.98)	
S100b (2.26)	Cpxm1 (2.7)	Fmod (3.86)	
Asgr1 (2.27)	Cyt11 (2.74)	Ndp (3.99)	

Table 5

Comparison of the biological terms that are associated with the differentially expressed genes in the mouse and the rat cochleae.

Mouse		Rat	
Biological terms	Gene count	Biological terms	Gene count
Immune response	14	• Immune response	51
		– Activation of immune response	
		– Positive regulation of immune response	
		– Positive regulation of immune system process	
		– Adaptive immune response	
		– Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	
		– Immune effector process	
		– B cell mediated immunity	
		– lymphocyte mediated immunity	
		– Leukocyte mediated immunity	
		– Humoral immune response	
		– Immunoglobulin mediated immune response	
		– Response to bacterium	
		– Response to molecule of bacterial origin	
		– leukocyte chemotaxis	
		– Response to lipopolysaccharide	
		– Defense response to Gram-positive bacterium	
		– Complement activation	
		– Complement activation, classical pathway	
		– Response to cytokine stimulus	
		– Humoral immune response mediated by circulating immunoglobulin	
		– Cell chemotaxis	
Defense response	12	• Defense response	55
Taxis	7	• Taxis	18
Chemotaxis	7	• Chemotaxis	18
Inflammatory response	9	• Inflammatory response	37
		– Regulation of inflammatory response	
		– Activation of plasma proteins involved in acute inflammatory response	
		– Acute inflammatory response	
Response to wounding	11	• Response to wounding	59

Mouse		Rat	
Biological terms	Gene count	Biological terms	Gene count
		– Wound healing	
		<ul style="list-style-type: none">• Others: Response to organic substance, positive regulation of response to stimulus, response to abiotic stimulus, Cell activation, Cell migration, and locomotory behavior	

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Table 6

Molecular pathways identified in the rat cochleae.

Molecular pathways	Gene count	P values
Complement and coagulation cascades*	18	0.000
Cytokine-cytokine receptor interaction*	21	0.000
Chemokine signaling pathway*	18	0.000
Jak-STAT signaling pathway*	13	0.004
Cell adhesion molecules (CAMs)*	13	0.007
Toll-like receptor signaling pathway*	8	0.043
Glycerophospholipid metabolism	6	0.072
NOD-like receptor signaling pathway*	6	0.072
p53 signaling pathway	6	0.090
Adipocytokine signaling pathway	6	0.094
Fc gamma R-mediated phagocytosis*	7	0.098

Note: the pathways are ranked based on their P values. The asterisks indicate the pathways that are associated with immune and inflammatory responses.

Table 7

Molecular pathways identified in the mouse cochleae.

Molecular pathways	Gene symbols	Gene count	P values
Cytokine-cytokine receptor interaction	Ccl12, Tnfrsf11b, Ccl2, Osmr, Ccl5, Ccl7, Ccl6	7	0.001
NOD-like receptor signaling pathway	Ccl12, Ccl2, Ccl5, Ccl7	4	0.003
Chemokine signaling pathway	Ccl12, Ccl2, Ccl5, Ccl7, Ccl6	5	0.008
Cytosolic DNA-sensing pathway	Irf7, Ccl5, Zbp1	3	0.025
RIG-I-like receptor signaling pathway	Rnf125, Isg15, Irf7	3	0.037
Toll-like receptor signaling pathway	Fos, Irf7, Ccl5	3	0.073

Note: the pathways are ranked based on their P values

Table 8

Comparison of cellular components for DF genes of the mouse and rat samples.

Mouse			Rat		
Location	Gene Count	FDR	Location	Gene Count	FDR
Extracellular region	38	0.000	Extracellular region	100	0.000
Extracellular region part	20	0.000	Extracellular region part	61	0.000
Extracellular space	14	0.004	Extracellular space	44	0.000
			Plasma membrane	120	0.000
			Cell surface	30	0.003

Note: FDR = False discovery rate.