

Altered gene expression profile in a rat model of gentamicin-induced ototoxicity and nephrotoxicity, and the potential role of upregulated *Ifi44* expression

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Abstract. As demonstrated by Alport syndrome, the co-occurrence of auditory and urinary system malformations, and gentamicin-induced ototoxicity and nephrotoxicity, the ears and kidneys potentially share certain molecular pathways. In the present study, microarray chips were used to analyze the changes in the gene expression profile using a rat model of gentamicin-induced ototoxicity and nephrotoxicity, using rat liver tissue as a control. A number of genes were identified to exhibit similar expression changes in the rat ears and kidney tissues, among which microtubule-associated protein 44 (*Ifi44*), was selected for further analysis to validate its expression changes and confirm potential involvement in the inflammation process in the disease model. *Ifi44* is a member of the type I interferon-inducible gene family. Reverse transcription-quantitative polymerase chain reaction, western blotting and immunohistochemistry were performed; the results demonstrated that more inflammatory cells were present in cochlear and renal parenchyma in gentamycin-induced rats, and *Ifi44* expression was increased in these two organs compared with control rats. Taken together, with its role in lupus nephritis and expression in the inner ear, the results

suggested that *Ifi44* is potentially involved in the inflammation associated with gentamicin-induced ototoxicity and nephrotoxicity. The approach of the current study has also provided a strategy for delineating common pathways shared by organs involved in specific diseases.

Introduction

The ear and kidney are likely to share certain molecular pathways, which is demonstrated by the variety of congenital syndromes that involve malformations in both the auditory and urinary system, including Branchio-oto-renal syndrome [Online Mendelian Inheritance in Man (OMIM) entry 113650; characterized by co-occurrence of branchial, ear, and renal anomalies], Alport syndrome (OMIM entry 301050; characterized by hematuria, renal failure and hearing impairment) (1), and aminoglycoside-induced ototoxicity and nephrotoxicity. Our previous study demonstrated that *T-box 1*, a gene implicated in ear development, is also expressed in embryonic kidney tissues and interacts with *homeobox D10* (2).

Gentamicin (GM) is an aminoglycoside antibiotic widely used to treat various types of bacterial infection, particularly those caused by Gram-negative microorganisms. The drug inhibits protein synthesis in the bacteria and alters the permeability of bacterial membrane. Following administration, 90% of GM retains its structure without being metabolized by the liver, and is excreted by the renal tubules, particularly the proximal convoluted tubules. However, GM is highly ototoxic and nephrotoxic, but the mechanism is unclear. Research on the ototoxicity of GM demonstrated that there is massive apoptosis of the vestibular hair cells during the course of disease (3). Notably, rats receiving overdosage of GM also exhibited extensive necrosis of the proximal convoluted tubules, and those receiving a clinical dosage of GM still exhibited significant apoptosis without necrosis of the epithelial cells of the proximal convoluted tubules (4).

Aminoglycoside enters cells by endocytosis or ion channel permeation (5-7). Though all cells take up aminoglycoside,

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the majority of them clear the drug (8). However, the kidney and inner ear also retain aminoglycoside, but are susceptible to aminoglycoside-inducible toxicity. The two organs are anatomically unrelated, but they do share common characteristics, including fluid and ion regulation, and protein expression of various ion channels and transporters (9). We hypothesized that certain molecular mechanisms may be associated with the ototoxicity and nephrotoxicity of GM.

GM induces damage by overproduction of reactive oxygen species and inflammation (10). Interferons (IFNs) are important cytokines involved in inflammation (11). Microtubule-associated protein 44 (*Ifi44*) has been reported to be antiproliferative (12). *Ifi44*, also termed interferon-inducible protein 44 or p44 as it aggregates to form microtubular structures, is part of the type I IFN-inducible gene family. Its promoter region contains an IFN- α stimulation responsive elements, which can mediate type I IFN-inducible gene pathway. *Ifi44* is an inflammatory consensus gene (13). In a glial cell line challenged with neurotoxin candoxin, *Ifi44* appears to have an important role in candoxin-induced glial inflammation (14). Thus, *Ifi44* may be associated with the inflammation involved in GM-induced ototoxicity and nephrotoxicity.

The current study used microarrays to analyze the gene expression profiles of ear and kidney tissues derived from a rat model for GM-induced ototoxicity and nephrotoxicity. To filter non-specific genes, gene expression profiles of liver tissue from the model animal were used for normalization. Based on the microarray results and hypothesis that *Ifi44* may be associated with the inflammation of GM-induced ototoxicity and nephrotoxicity, a series of techniques were performed to investigate the expression of *Ifi44*.

Materials and methods

Animal model, group design and sample collection. Wistar rats (n=30; 4 days old), were obtained from Animal Center of China Medical University (Shenyang, China). The animals were housed in stainless steel wire-mesh cages (5 rats per cage) under standard laboratory condition (25°C, relative humidity 60%, and 12 h dark-and-light cycle). The animals were allowed free access to water and food.

The rats were randomly divided into the control and GM groups. For the GM group, each rat received a dose of 80 mg/kg GM via intramuscular injection. For the control group, each animal received an equal volume of normal saline. The injections were administered once a day for 7 days consecutively.

On the 7th day, 300 μ l blood was collected by cardiac puncture. Blood samples were immediately placed in 1.5 ml centrifuge tubes containing heparin. After centrifugation at 2,000 \times g for 10 min, plasma samples were collected and stored at 4°C. Plasma analysis was conducted within 2 days of collection. A total of 10 rats (randomly 5 per group) were sacrificed by overdose of anesthetic. The kidneys, cochlear tissue and liver were collected. The samples were processed soon after. The study was approved by the ethics committee of Sichuan University (Chengdu, China).

Biochemical analysis. Plasma serum creatinine (SCr) and blood urea nitrogen (BUN) levels were determined with

Serum Creatinine kit (Beijing Leadman Biochemistry Co., Ltd., Beijing, China) and Blood Urea Nitrogen kit (Beijing Leadman Biochemistry Co., Ltd.), respectively, with an AU480 Chemistry system (Beckman Coulter, Inc., Brea, CA, USA) according to manufacturer's instructions.

RNA extraction. Total RNA was isolated from tissue samples with TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the manufacturers' instructions. Total RNAs were quantified with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Samples of total RNA from ears, kidney and liver of rats from the same group were pooled for subsequent GeneChip analysis. Prior to the analysis, pooled total RNA samples were purified using an RNeasy Total RNA Mini Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions.

GeneChip analysis. The GeneChip scan was performed with an Affymetrix GeneChip Rat 230 2.0 array (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Gene expression changes were represented as ratios between the GM and control groups. Gene expression profiles of liver tissue were used for normalization. The difference value of expression ratio between cochlear and liver, or kidney and liver was calculated to determine the change tendency. A ratio >1 indicated that the gene expression was upregulated, and vice versa. Selected genes were classified into four categories for further analysis, as follows: Upregulated in cochlear; downregulated in cochlear; upregulated in kidney; and downregulated in kidney. Genes exhibiting similar tendencies were selected.

Histology and immunohistochemistry. Cochlear, kidney and liver tissues from the rats were preserved in 10% phosphate-buffered formalin. Tissues fixed with neutral formalin were embedded in paraffin and sectioned at 3 μ m. Hematoxylin and eosin (H&E) staining was performed to observe GM-induced ototoxicity, indicated by loss of cochlear hair cells and inflammation.

To identify *Ifi44* protein in the cochlear and kidney, sectioned paraffin-embedded tissue samples were deparaffinized for immunohistochemistry. Slides were incubated with 3% H₂O₂ at room temperature for 10 min to eliminate endogenous peroxidases, and washed with distilled water and PBS. The slides were then incubated with 5% goat serum (ZsBio, Beijing, China) at room temperature for 10 min. Primary antibody (rabbit anti-rat-IFI44 primary antibody; GTX32667; 1:100; GeneTex, Inc., Irvine, CA, USA.) incubation was performed at 37°C for 2 h. PBS was used as blank control for primary antibody incubation. After washing with PBS, biotinylated goat anti-rabbit IgG secondary antibody (ZB-2010; 1:200; ZsBio) incubation was performed at 37°C for 30 min. The slides were washed with PBS and incubated with HRP-streptavidin (ZB-2404; 1:500; ZsBio) working buffer at 37°C for 30 min. The slides were washed with PBS and incubated with diaminobenzidine at room temperature for 10 min, followed by washing with water and H&E staining. The sections were imaged with an Eclipse E600 microscope (Nikon Corporation, Tokyo, Japan).

Table I. Primers for reverse transcript-quantitative polymerase chain reaction.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Ifi44</i>	AGC CGT ATG GAG ACC TGG	TGA GTG ATG CTG CCC TTG
<i>Gapdh</i>	TCA CCA CCA TGG AGA AGG C	GCT AAG CAG TTG GTG GTG CA

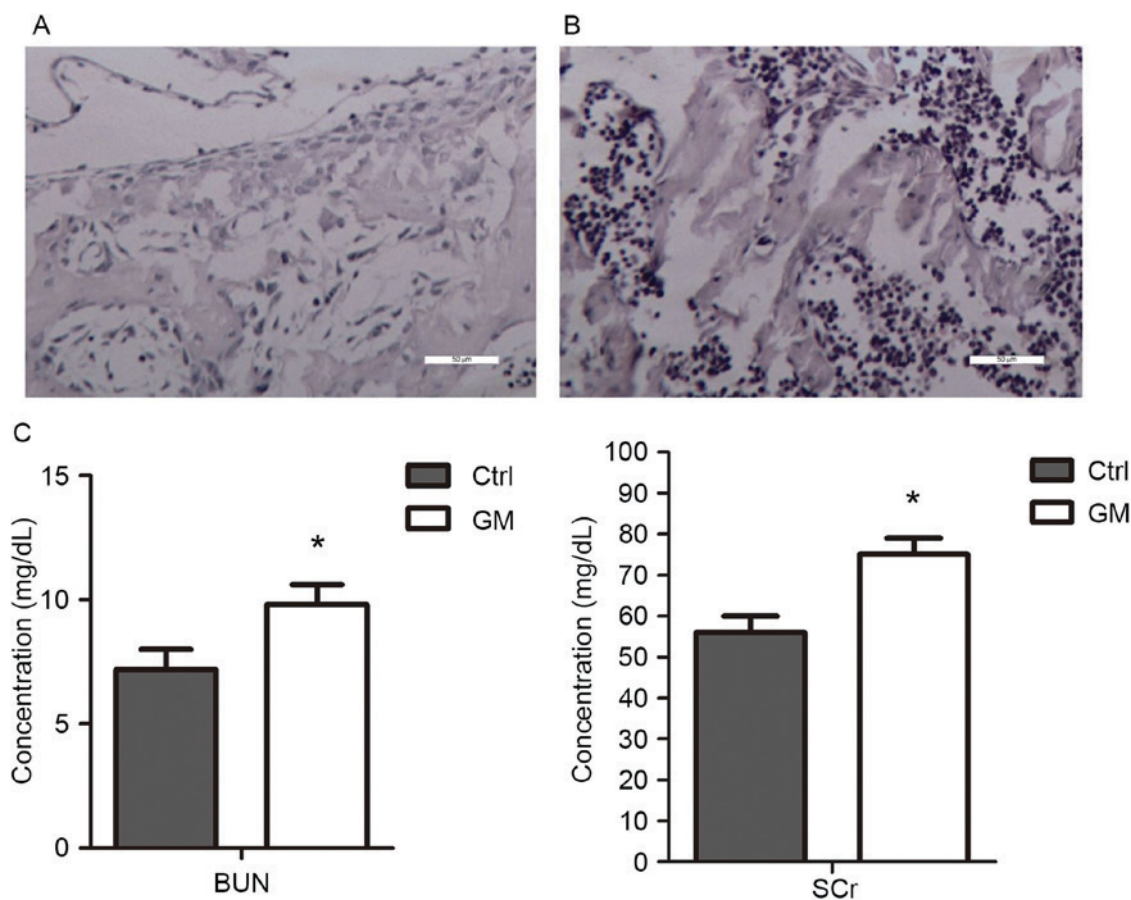


Figure 1. Hematoxylin and eosin staining of cochlear tissue and blood SCr and BUN levels. Compared with (A) the control group, more inflammatory cells can be observed in cochlear tissue from (B) the GM group. (C) Concentration of blood BUN and SCr. In the GM group, both were increased. (* $P < 0.05$). Scale bar, 50 μ m. Ctrl, control; GM, gentamicin; BUN, blood-urea-nitrogen; SCr, serum creatinine.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Extracted RNA was converted to cDNA by reverse transcription of 1 μ g RNA with random primers and AMV reverse transcriptase (Applied Biosystems, Thermo Fisher Scientific, Inc.). The reverse transcription conditions were 42°C for 1 h and 99°C for 5 min. Primers (Table I) were designed using Primer 3 software (<http://primer3.ut.ee>) and synthesized by Genscript Biotech Corporation (Nanjing, China). The reverse transcription and qPCR were performed out on an ABI PRISM 7500 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). qPCR was performed in a total volume of 20 μ l, with each well containing 10 μ l SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), 2 μ l cDNA, and 0.4 μ M *Ifi44* or *Gapdh* primers. The PCR condition consisted of initial denaturation step at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 34 sec. The relative level of gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method (15).

Western blotting. To prepare protein samples for western blotting, prepared tissues (cochlear, kidneys, and livers) were cut into pieces and washed with PBS three times. Tissue pieces were homogenized in RIPA lysis buffer (P0013B; Beyotime Institute of Biotechnology, Shanghai, China) containing PMSF, and centrifuged at 10,000 \times g for 10 min at 4°C. The supernatant was collected and protein concentration was determined using BCA Protein Assay reagents (Pierce, Thermo Fisher Scientific, Inc.). Protein loading buffer (5X; P0015; Beyotime Institute of Biotechnology) was added into the supernatant, and then boiled for 10 min. The protein samples were stored at -70°C until use. Protein samples were loaded, 100 μ g for each well, onto a SDS-PAGE gel and transferred to a PVDF membrane (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The membrane was then incubated with 5% nonfat dry milk for 3 h at room temperature, and then with rabbit anti-rat-IFI44 primary antibody (GTX32667; 1:1,000; GeneTex) and rabbit anti-rat-GAPDH primary

antibody (GTX100118; 1:1,000; GeneTex) for 2 h at room temperature. The membrane was washed with 0.1% TBST for 5 times and incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (ZB-2301; 1:2,500; ZsBio) as the secondary antibody for 1 h at room temperature. After final washing with 0.1% TBST for 5 times, protein bands were detected with an enhance chemiluminescence assay kit (Pierce, Thermo Fisher Scientific, Inc.).

Statistical analysis. All experiments were conducted in triplicate and repeated at least twice. The group mean \pm standard deviations were calculated for each measured parameter. Statistical differences between the groups were evaluated using the Student's *t* test with SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Ototoxicity and nephrotoxicity induced by GM. H&E staining of cochlear tissue from the rats demonstrated distinct inflammatory invasion in the GM group (Fig. 1A and B). Increased blood SCr and BUN levels are indicators of kidney dysfunction. In the GM group, there were significant increases in the levels of both indicators compared with the levels in the control group ($P < 0.05$; Fig. 1C).

Morphological changes in the cochlear tissue, as revealed by H&E staining, along with the biochemical changes in blood, indicated that GM induced ototoxicity and nephrotoxicity in the experimental group rats.

Analysis of GeneChip data. The Affymetrix Rat Genome 230 2.0 microarray contains 31,000 probe sets corresponding to ~24,000 annotated rat genes and 6,693 expressed sequence tags. As GM predominantly causes damage in the kidney and ear, to explore the similarities in gene expression changes between the kidney and ear, genes significantly altered in the liver tissue from both groups were excluded from further analysis. The genes that were unchanged in the liver tissue between the two groups were classified into four categories: Upregulated in the kidneys; downregulated in the kidneys; upregulated in the cochlear; and downregulated in the cochlear (Tables II-V). Compared with the control group, nine genes exhibited similar expression changes in the kidneys and ears in the GM group (Table VI).

Ifi44 was one of the significantly upregulated genes in cochlear and kidney tissue, but not changed in the liver tissue of the GM group with a ratio of 2.45184 (GM cochlear vs. control cochlear) and 3.2915 (GM kidney vs. control kidney), respectively. As *Ifi44* is associated with inflammation processes, further analysis was conducted to verify its expression in the cochlear and kidney tissues.

Changes of *Ifi44* gene expression in the kidneys and ears. To verify the changes of *Ifi44* expression in GM group rats compared with control rats, RT-qPCR, western blotting and immunohistochemistry were performed. As demonstrated in Figs. 2 and 3, the expression of *Ifi44*, at the transcriptional and translational levels, was increased in ear and kidney tissue in the GM group rats compared with control group rats.

Table II. Upregulated genes in the kidney.

Gene	Difference value (kidney ratio-liver ratio)
<i>Abcb1a</i>	2.43477
<i>Abcb1a/Abcb1b</i>	17.722
<i>Adfp</i>	2.20688
<i>Afp</i>	2.188205
<i>Areg</i>	2.44116
<i>Baz1a</i>	1.08197
<i>Bhlhe41</i>	3.47949
<i>Btg2</i>	2.09308
<i>C1qa</i>	0.94038
<i>C2</i>	1.65742
<i>C3</i>	1.77948
<i>Calcb</i>	1.460037
<i>Ccl4</i>	1.140705
<i>Cdkn1a</i>	2.64699
<i>Cebpd</i>	1.867527
<i>Cfi</i>	1.13327
<i>Ch25h</i>	1.50461
<i>Chka</i>	2.850202
<i>Cldn4</i>	1.53799
<i>Clec3f6</i>	1.339374
<i>Clrn3</i>	0.98247
<i>Clu</i>	1.17991
<i>Coro1a</i>	2.17874
<i>Csrnp1</i>	3.07711
<i>Cst7</i>	1.85086
<i>Cxcl11</i>	14.43329
<i>Dhx58</i>	0.80693
<i>Dkk2</i>	1.64244
<i>Dusp8</i>	1.10571
<i>Egfr</i>	1.983407
<i>Emr1</i>	1.25444
<i>F3</i>	1.06992
<i>Fam81a</i>	1.9031
<i>Fcgr2a/LOC498276</i>	1.00979
<i>Fcrla</i>	1.00537
<i>Fga</i>	2.675193
<i>Fgb</i>	2.030199
<i>Fgg</i>	1.409335
<i>Gch1</i>	1.030706
<i>Gnl3</i>	0.92643
<i>Hbegf</i>	1.27193
<i>Hist1h4b</i>	1.20101
<i>Ier2</i>	1.204013
<i>Ifi27</i>	1.85006
<i>Ifi44</i>	0.50785
<i>Ifrd1</i>	2.86349
<i>Il18r1</i>	1.11695
<i>Ino80</i>	1.27326
<i>Irs2</i>	1.04459
<i>Klhd5</i>	1.155802
<i>LOC100134871/LOC689064</i>	1.07074

Table II. Continued.

Gene	Difference value (kidney ratio-liver ratio)
<i>LOC290595</i>	2.58298
<i>LOC679127</i>	1.01209
<i>LOC685277</i>	1.310726
<i>Ly6b</i>	9.520954
<i>Maff</i>	7.34486
<i>Map7</i>	1.05453
<i>MGC105649</i>	2.276752
<i>Mobkl1b</i>	1.7147
<i>Ms4a7</i>	2.84021
<i>Naglt1</i>	1.23055
<i>Nr4a3</i>	1.781463
<i>Nrg1</i>	6.408646
<i>Nupl1</i>	1.326609
<i>Osbp13</i>	1.269767
<i>Parp14</i>	0.89769
<i>Pim3</i>	1.714337
<i>Pla2g15</i>	1.03518
<i>Plcx2</i>	6.208169
<i>PLEK</i>	0.86853
<i>Pltp</i>	1.34507
<i>Ppp1r15a</i>	1.64453
<i>Pspc1</i>	1.44648
<i>PVR</i>	5.07916
<i>Rassf1</i>	1.185572
<i>Rell2</i>	1.575769
<i>RGD1306820</i>	1.081854
<i>RGD1559960/Sult1c2</i>	7.78885
<i>Rnd1</i>	1.91742
<i>Rpp25</i>	0.88792
<i>RT1-EC2</i>	1.44442
<i>Scin</i>	1.574135
<i>Serpinc1</i>	4.471142
<i>Shoc2</i>	0.88887
<i>Slc13a1</i>	1.359457
<i>Slc2a2</i>	1.960617
<i>Slc34a2</i>	1.73034
<i>Spp1</i>	1.538939
<i>Srxn1</i>	1.16717
<i>Stat2</i>	0.87204
<i>Steap1</i>	1.203958
<i>Stra6</i>	1.740229
<i>Thrsp</i>	0.90096
<i>Tinag</i>	5.758012
<i>Tmem140</i>	2.3678
<i>Tubb2c</i>	1.560787
<i>Utx</i>	1.374016
<i>Wdr43</i>	1.370004
<i>Zbtb10</i>	1.09374

Table III. Downregulated genes in the kidney.

Gene	Difference value (kidney ratio-liver ratio)
<i>Aadac</i>	-0.487787
<i>Akr1b7</i>	-0.625335
<i>Alb</i>	-0.744701
<i>Apoc2</i>	-0.52801
<i>Cryab</i>	-0.488116
<i>Dnase1</i>	-0.756643
<i>E030032D13Rik</i>	-0.649601
<i>Egf</i>	-0.382176
<i>Enpp6</i>	-0.76638
<i>Hpgd</i>	-0.629649
<i>Hrg</i>	-0.595822
<i>Inmt</i>	-0.582937
<i>Klk1c10</i>	-0.734721
<i>Mylk3</i>	-1.077123
<i>Ogn</i>	-0.626234
<i>Ppp1r1a</i>	-0.420662
<i>RGD1305645</i>	-0.348252
<i>RGD1305679</i>	-0.491102
<i>Rgn</i>	-0.46558
<i>Slc22a13</i>	-0.657262
<i>Slc34a1</i>	-0.498645
<i>Slc1a6</i>	-0.733911
<i>Sult1c2 /// Sult1c2a</i>	-0.397167

These results were consistent with the results of the GeneChip microarray.

Discussion

By comparing the gene expression profiles, a number of genes were identified that may be specifically involved in GM-induced ototoxicity and nephrotoxicity. Among these, *Ifi44* expression was upregulated in cochlear and kidney tissue from GM treated rats. GM is known to induce damage by overproduction of reactive oxygen species and inflammation (10), and IFNs are important cytokines for inflammation (11). *Ifi44* has been reported to be antiproliferative (12), and its functions include participation in microtubule formation, promotion of apoptosis, inhibition of proliferation and involvement in autoimmune response. The GeneChip analysis indicated that upregulated *Ifi44* expression may be involved in the inflammation associated with GM-induced ototoxicity and nephrotoxicity. Further analysis confirmed that *Ifi44* expression was upregulated at the transcriptional and translational levels.

GM tends to accumulate in renal tubular cells (16), which is in keeping with the expression of protein and cation transporters, namely the giant endocytic complex formed by megalin and cubilin present in the proximal tubule. Intracellular accumulation of GM may be a key factor of GM-induced nephrotoxicity. *Ifi44* was proposed to interact with intracellular GTP (12). Blocking of GTP-associated

Table IV. Upregulated genes in the cochlear.

Gene	Difference value (cochlear ratio-liver ratio)
<i>Acsf6</i>	1.095921
<i>Alb</i>	1.13632
<i>Ankrd34b</i>	1.468724
<i>Apcs</i>	1.163418
<i>C1qa</i>	0.4839
<i>C2</i>	2.07736
<i>Cbln1</i>	1.11505
<i>Chrdl1</i>	0.97534
<i>Cnr1</i>	1.3443
<i>Cxcl11</i>	0.58796
<i>Dhx58</i>	1.48114
<i>Fam19a5</i>	1.051074
<i>Fbp1</i>	1.351329
<i>Gsta3</i>	1.20354
<i>Ifi27</i>	1.8479
<i>Ifi44</i>	0.50785
<i>Krt15</i>	1.338948
<i>Ms4a7</i>	0.76933
<i>Mobp</i>	1.34379
<i>Nefh</i>	1.289564
<i>Neurod1</i>	1.755709
<i>Olig1</i>	0.9484
<i>Parp14</i>	0.18289
<i>Pnlip</i>	1.11708
<i>RGD1306880</i>	0.88192
<i>RGD1560273</i>	1.09038
<i>RT1-EC2</i>	1.20744
<i>Slc6a1</i>	1.03796
<i>Slc7a3</i>	0.90495
<i>Tmem2</i>	0.95757

Table V. Downregulated genes in the cochlear.

Gene	Difference value (cochlear ratio-liver ratio)
<i>Acta1</i>	-1.147199
<i>B3gnt5</i>	-0.605835
<i>Bst1</i>	-0.47024
<i>Car1</i>	-0.625612
<i>Cox8b</i>	-0.537492
<i>Cpox</i>	-0.510466
<i>Ctse</i>	-0.611315
<i>Dhfr</i>	-0.647651
<i>Eraf</i>	-0.465653
<i>Esm1</i>	-0.754375
<i>Hemgn</i>	-0.586487
<i>Igh-6/LOC314509</i>	-0.619679
<i>Klf1</i>	-0.507804
<i>LOC683399</i>	-0.633073
<i>LOC687696</i>	-0.595911
<i>Mb</i>	-0.668166
<i>Mcpt10/8/8l2/9</i>	-0.505066
<i>Myh2/ Myh4</i>	-0.761466
<i>Myl1</i>	-0.696605
<i>Pcsk1</i>	-0.723895
<i>Plek2</i>	-0.549672
<i>Plunc</i>	-0.792194
<i>Rhd</i>	-0.408416
<i>Rrm2</i>	-0.341062
<i>Slc22a4</i>	-0.45902
<i>Spta1</i>	-0.613168
<i>Thbs4</i>	-0.642666
<i>Tnnt3</i>	-0.466837
<i>Tpm1</i>	-0.760636
<i>Trak2</i>	-0.513582

pathways has various effects, including promotion of cell death (12). *Ifi44* potentially participates in GM-induced ototoxicity and nephrotoxicity by depleting intracellular GTP; however, how *Ifi44* is upregulated by GM remains to be explored. Current research on *Ifi44* has focused on its supporting role in the IFN signaling pathway, which is an important part of systemic lupus erythematosus diagnosis. However, the role of *Ifi44* in ear and kidney injury is currently unclear.

GM may also affect the expression of connexin 26 in the cochlear lateral wall (17). Following GM administration, the expression of connexin 26 was increased over time (17). Interaction of connexin proteins with microtubules is essential to allow directed transport of newly synthesized connexin hemichannels to the plasma membrane (18). Considering its function, *Ifi44* may also have a role in the increase of connexin 26 expression induced by GM.

In the present study, GM-induced ototoxicity and nephrotoxicity were confirmed by measurement of blood BUN and SCr levels. H&E staining confirmed that inflammatory

cells aggregated in the cochlear and kidney tissues following GM treatment. RT-qPCR and western blotting also demonstrated that *Ifi44* was upregulated at the transcriptional and translational levels. Immunohistochemistry also demonstrated that *Ifi44* was upregulated in rat cochlear and kidney tissues following the GM treatment. The results suggested that *Ifi44* has a connection to inflammations associated with GM-induced ototoxicity and nephrotoxicity.

Notably, other genes, including *poly(ADP-ribose) polymerase family member 14 (Parp14)*, *DExH-box helicase 58 (Dhx58)*, *interferon α inducible protein 27 (Ifi27)*, *membrane spanning 4-domains A7 (Ms4a7)*, also exhibited similar expression changes in the kidneys and cochlear after GM administration. The role of such genes in the GM-induced ototoxicity and nephrotoxicity requires delineation in further studies.

In summary, the current study identified changes of the expression profiles in ear and kidney tissues following GM administration in rats. Investigation of *Ifi44* gene expression in the cochlear and kidney tissues suggested that *Ifi44*

Table VI. Genes upregulated in cochlear and kidney.

Gene name (symbol)	Cochlear ratio	Kidney ratio	Liver ratio
<i>Complement C1q A chain (C1qa)</i>	2.23839	2.69487	1.75449
<i>Complement C2 (C2)</i>	2.88087	2.46093	0.80351
<i>C-X-C motif chemokine ligand 11 (Cxcl11)</i>	2.37037	16.2157	1.78241
<i>DExH-box helicase 58 (Dhx58)</i>	4.23954	2.75184	1.94491
<i>Interferon alpha inducible protein 27 (Ifi27)</i>	3.71297	2.00469	1.86291
<i>Microtubule-associated protein 44 (Ifi44)</i>	2.45184	3.2915	1.944
<i>Membrane spanning 4-domains A7 (Ms4a7)</i>	2.58608	4.65696	1.81675
<i>Poly(ADP-ribose) polymerase family member 14 (Parp14)</i>	2.10233	2.81713	1.91944
<i>RT1 class Ib, locus EC2 (RT1-EC2)</i>	2.54755	2.78453	1.34011

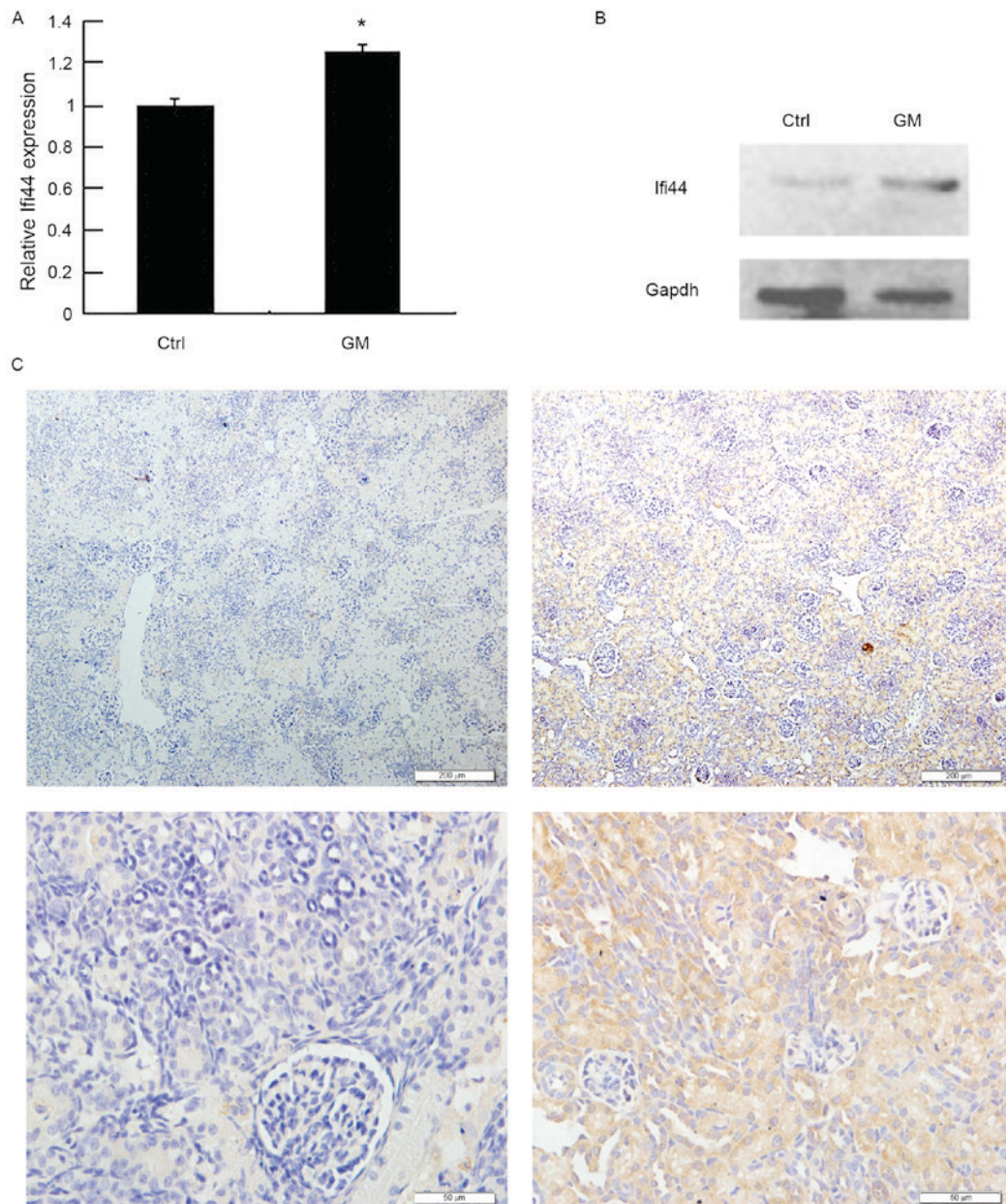


Figure 2. RT-qPCR, western blotting and immunohistochemistry analysis of *Ifi44* expression in the kidney tissue. (A) RT-PCR analysis demonstrated significant upregulation of *Ifi44* transcription in the GM group (* $P < 0.05$). (B) Western blotting confirmed increased *Ifi44* protein in the GM group. (C) Immunohistochemistry analysis also demonstrated upregulation of *Ifi44* in the kidney tissue. Left panels, control group; right panels, GM group; upper panels, 200 μ m scale bar; bottom panels, 50 μ m scale bar. RT-qPCR, reverse transcription-quantitative polymerase reaction; Ctrl, control; GM, gentamicin; *Ifi44*, interferon-inducible protein 44.

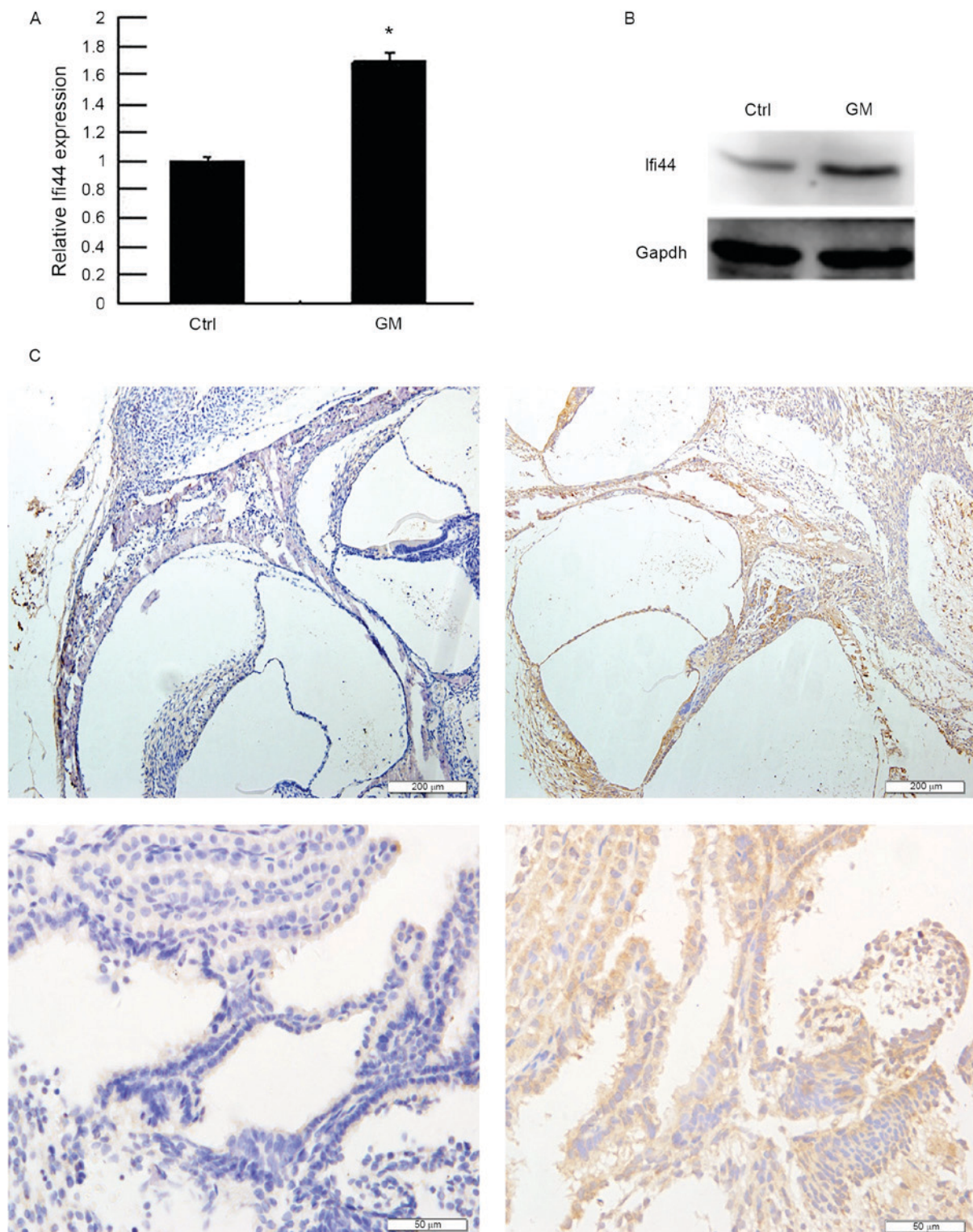


Figure 3. RT-qPCR, western blotting and immunohistochemistry analysis of *Ifi44* expression in the kidney tissue. (A) RT-PCR analysis demonstrated significant upregulation of *Ifi44* transcription in the GM group (* $P < 0.05$). (B) Western blotting confirmed increased *Ifi44* protein in the GM group. (C) Left panels, control group; right panels, GM group; upper panels, 200 μ m scale bar; bottom panels, 50 μ m scale bar. RT-qPCR, reverse transcription-quantitative polymerase reaction; Ctrl, control; GM, gentamicin; *Ifi44*, interferon-inducible protein 44.

may be associated with inflammation during GM-induced ototoxicity and nephrotoxicity. Despite the complex changes in the expression profile, the approach used in the present study may provide a strategy to systematically reveal signaling pathways that are shared by organs involved in specific diseases.

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