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Key genes and potential drugs in age-related hearing loss: transcriptome analysis of cochlear hair cells in old mice

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ABSTRACT

This study aimed to dig new molecular mechanisms and medications for age-related hearing loss (ARHL or presbycusis) by extracting common results of publicly available datasets. Based on five datasets (GSE153882, GSE121856, GSE98070, GSE45026, and GSE98071) in studies of cochlear hair cells, we explored the interrelationships among presbycusis-related genes, including gene interactions, enrichment analysis, miRNA-mRNA matching pairs, and potential new drugs. Together, there were 25 common increased mRNAs. A total of 183 drugs can simultaneously target 11 of these mRNAs. In the interaction network, hub genes included: Cbln1, Prl, Mpp6 and Gh. Meanwhile, there were 74 common decreased mRNAs. The hub genes include Cdkn1a, Egr1, and Ctgf. After de-duplication, the 25 common increased mRNAs had 946 matched miRNAs, with 34 decreased ones; and the 74 decreased mRNAs had 1164 matched miRNAs, with 26 increased ones. Between the inhibitors of increased mRNAs and enhancers of decreased mRNAs, there were 26 common drugs. Besides, we discovered six key genes that may play a crucial role in the onset of presbycusis. In conclusion, by jointly analyzing multiple datasets, we found 25 common increased mRNAs (e.g., Cbln1, Prl, Mpp6 and Gh) and 74 common decreased mRNAs (Cdkn1a, Egr1, and Ctgf), as well as 34 potential therapeutic miRNAs and 26 pathogenic miRNAs, and three candidate drugs (calcitriol, diclofenac, and diethylstilbestrol). They may provide new targets and strategies for mechanistic and therapeutic studies in ARHL.

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Introduction

Age-related hearing loss (ARHL), or presbycusis, is generally caused by disorders of sensory hair cells or auditory neurons (in particular the former)(1-4). It is highly prevalent and affects 25-40% of individuals older than 65 years of age, which even contributes to depression, social isolation, and loss of self-esteem (5, 6). The molecular mechanism of its occurrence and progression is complex and remains to be explored. Important known mechanisms include mitochondrial DNA damage, ROS damage, noise, neuroinflammation, pyroptosis, and so on (7-10). In addition to coding genes, the effects of miRNAs on ARHL have received increasing attention in recent years. Relevant miRNAs that have been reported include miR-183 family and miR-181 family (maybe decreased), miR-29 family and miR-34 family (maybe increased) (11, 12).

However, the current studies on presbycusis have the following problems: (1) there are a large number of conflicting findings while few consistent findings (13-17); (2) there is still a lack of hypotheses for intergenic interactions (e.g., mRNA-mRNA interactions, miRNA-mRNA interactions) based on the various available findings; and (3) potential drugs for presbycusis are still very limited based on the available studies (18-20).

Based on these shortcomings, we aimed to extract logically consistent or common results through the analysis of publicly available datasets, and we explored the interrelationships among presbycusis-related genes, including gene interactions, enrichment analysis, miRNA-mRNA matching pairs, and potential new drugs.

Materials and Methods

Selection of presbycusis-related datasets

We selected the GEO datasets in studies of cochlear hair cells using the term “presbycusis”. The criteria were that the mice were divided into at least two groups: an old group and a young group, where the old group was at least 12 months of age, and the young group was less than or equal to 9 months. Overall, five datasets were collected: GSE153882, GSE121856, GSE98070, GSE45026, and GSE98071. The former three datasets provide the expression profiles of mRNAs, and the last two datasets provide that of miRNAs.

Differentially expressed genes

All the datasets were analyzed with the DESeq2 R package to acquire the differentially expressed genes (DEGs). The fold change (FC) and P-value were obtained for each

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transcript (old vs young). Then, according to the threshold of P-value <0.05 and |log2(FC)|>1 (FC > 2 or < 0.5), the DEGs of each dataset were acquired. The volcano plots of DEGs (including DE-mRNAs and DE-miRNAs) were generated using the ggplot2 R package. In particular, we paid attention to the DE-mRNAs and DE-miRNAs with opposite change directions. The intersection of different sets was presented by a Venn diagram. According to the Venn diagram, DEGs shared by at least two datasets were regarded as common DEGs, including common increased genes and common decreased genes.

Enrichment analysis

Based on common increased and decreased mRNAs, we used the Metascape tool to explore the enriched GO terms, KEGG pathways, Reactome sets, TRRUST sets, and enriched transcription-factor-targets. Accumulative hypergeometric p-values and enrichment factors were calculated and used for filtering. Terms with a p-value < 0.05, a minimum count of 3, and an enrichment factor > 1.5 were collected and grouped into clusters based on their membership similarities. More specifically, p-values are calculated based on the cumulative hypergeometric distribution2, and q-values are calculated using the Benjamini-Hochberg procedure to account for multiple tests. Kappa scores are used as the similarity metric when performing hierarchical analysis.

Interaction analysis

Using the GeneMANIA online tool, we explored the connections among common increased genes and common decreased genes. In particular, the following interactions were probed: (1) Co-expression: Two genes are linked if their expression levels are similar across conditions in a gene expression study. (2) Co-localization: genes expressed in the same tissue, or proteins found in the same location. Two genes are linked if they are both expressed in the same tissue or if their gene products are both identified in the same cellular location. (3) Physical Interaction: Protein-protein interaction data. Two gene products are linked if they were found to interact in a protein-protein interaction study. These data are collected from primary studies found in protein interaction databases, including BioGRID and PathwayCommons. (4) Predicted: Predicted functional relationships between genes, often protein interactions. A major source of predicted data is mapping known functional relationships from another organism via orthology.

The DE mRNA-miRNA pairs

The mRNAs potentially targeted by key miRNAs were predicted using the online tool of miRWalk (http://mirwalk.umm.uni-heidelberg.de/). The following mRNA-miRNA pairs were paid special: increased miRNAs and commonly decreased mRNAs, and decreased miRNAs and commonly increased mRNAs. The threshold was set as follows: binding score = 1.

Potential drugs for presbycusis

Using the CTD database, we collected all the drugs that target the common increased and decreased mRNAs. For increased mRNAs, a drug was screened if it could inhibit the expression/activation/cumulation of the increased genes, and it was called an inhibitor in the potential drug. Conversely, if a drug can increase the expression/activation/cumulation of the decreased genes, it was called an enhancer in the potential drugs. The drugs targeting more than 10 targets of the common increased/decreased genes were selected as preferred novel drugs.

Results

DE mRNAs and miRNAs in presbycusis

First, DE mRNAs were analyzed based on the following datasets: GSE153882, GSE121856, and GSE98070. There were 89, 31, and 2056 increased genes (old vs young) in GSE98070, GSE121856, and GSE153882, respectively (Figure 1 A-D); and not any gene was simultaneously shared by three sets. There were 210, 46, and 3602 decreased genes (old vs young) in GSE98070, GSE121856, and GSE153882, respectively (Figure 1 A-C & E); and the common one was Wisp.

For DE miRNA analysis, the datasets GSE98071 and GSE45026 were used. There were 60 increased and 43 de-

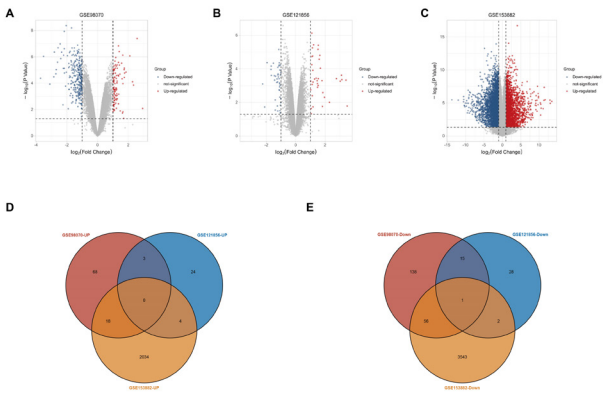

Figure 1. The differential expression of mRNAs in GSE153882, GSE121856, and GSE98070. (A) The volcanic plot of DEGs in GSE98070. (B) The volcanic plot of DEGs in GSE121856. (C) The volcanic plot of mRNAs in GSE153882. (D) The Venn diagram of increased mRNAs in three sets. (E) The Venn diagram of decreased DEGs in three sets (the commonly decreased gene was Wisp1).

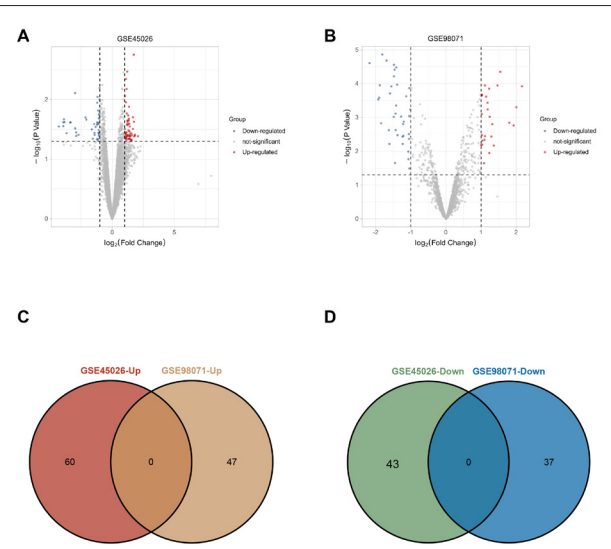

Figure 2. The differential expression of miRNAs in GSE45026, and GSE98071. (A) The volcanic plot of DEGs in GSE45026. (B) The volcanic plot of DEGs in GSE98071. (C) The Venn diagram of increased miRNAs in two sets. (D) The Venn diagram of decreased miRNAs in two sets.

creased miRNAs in GSE45026 (Figures 2A and 2C), and 47 increased and 37 decreased miRNAs in GSE98071 (Figures 2B and 2D). However, no common miRNAs were found in the interaction between the two sets.

Enrichment, interaction, and potential drugs for commonly increased mRNAs

Together, there were 25 common increased mRNAs (3 shared by GSE98070 and GSE121856, 18 shared by GSE98070 and GSE153882, 4 shared by GSE121856 and GSE153882): Cxcl13, Prl, Gh, Lyz2, Cd68, Ms4a7, Clec7a, Hist2h2bb, Fabp5, Snhg11, Gm4349, Kcnk2, BC016579, Gm13648, Dnajc6, Mpp6, Trim30a, Amy1, Otog, Cald1, Zfp418, Cbln1, H2-Aa, Dnase1, and Cndp1. These 25 mRNAs may be important pathogenic genes in the cochlear hair cells in presbycusis.

Next, we analyzed the enrichment, interaction, and potential drugs for these genes. As Figure 3A shows, the following GO terms were significantly enriched: hydrolase activity hydrolyzing O-glycosyl compounds, hydrolase activity acting on glycosyl bonds, cytokine receptor binding, cellular response to biotic stimulus, regulation of system process, response to extracellular stimulus, etc. And a KEGG pathway was enriched: Cytokine-cytokine receptor interaction - Mus musculus. Moreover, a Reactome term was enriched: Neutrophil degranulation. The networks of enriched terms are shown in Figure 3B.

In the interaction network of co-expression, the following increased genes may play important roles: Lyz2, Prl, Cd68, Trim30a, Amy1, H2-Aa, Hist2h2bb, Kcnk2, Otog, Cxcl13, Dnase1, BC016579, Zfp418, Cbln1, and Fabp5 (Figure 4A). In the co-localization network, the hub genes included Cbln1, Dnajc6, Gh, and H2-Aa (Figure 4B). In the physical interaction, Mpp6 and Cbln1 were the hub genes (Figure 4C). In the predicted interaction network, Mpp6, Mpp5, Prl, Gh, and Cbln1 were the key hub genes (Figure 4D).

Based on all increased mRNAs, the inhibitory drugs were collected from the CTD database. The drug numbers of each mRNA were shown in Figure 5A, and the statistical results of drugs with different target numbers were shown in Figure 5B. A total of 183 drugs targeted 11 mRNAs, and these drugs were selected as potential medications for presbycusis (in view of the commonly increased mRNAs).

Enrichment, interaction, and potential drugs for commonly decreased mRNAs

Subsequently, the enrichment, interaction, and potential drugs were analyzed based on the decreased mRNAs. Similarly, there were 74 common decreased mRNAs (56+15+2+1): Col3a1, Agt, Egr3, Hist1h2ao, Hist1h2ai, Hist1h2ab, Hist1h2ap, Hist1h2an, Hist1h2ah, Hist1h2ag, Hist1h2ae, Hist1h2ad, Hist1h2ac, Mepe, Ibsp, Wisp1, Mt2, Tsc22d3, Klf15, Nr4a1, Lzts1, Ddit4, Col11a1, 2810474O19Rik, Slco4a1, Spsb1, Nfkbiz, Fam171b, Cd14, Sbf2, Ednrb, Cdkn1a, 1810011O10Rik, Tnik, Map3k6, Cpm, Mt1, Fam189a1, Poln, Egr1, Crym,

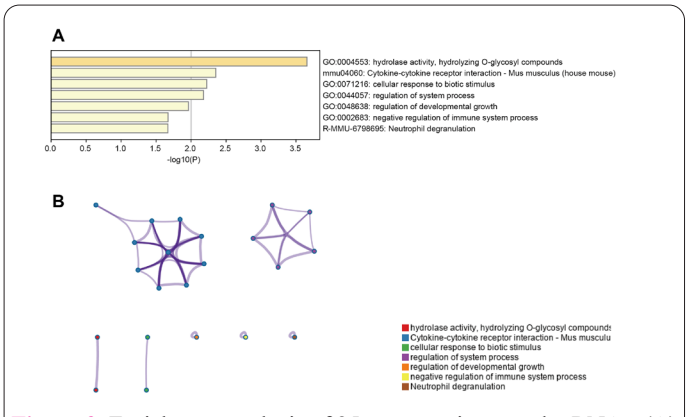

Figure 3. Enrichment analysis of 25 common increased mRNAs. (A) Enriched GO terms. (B) The networks of enriched terms.

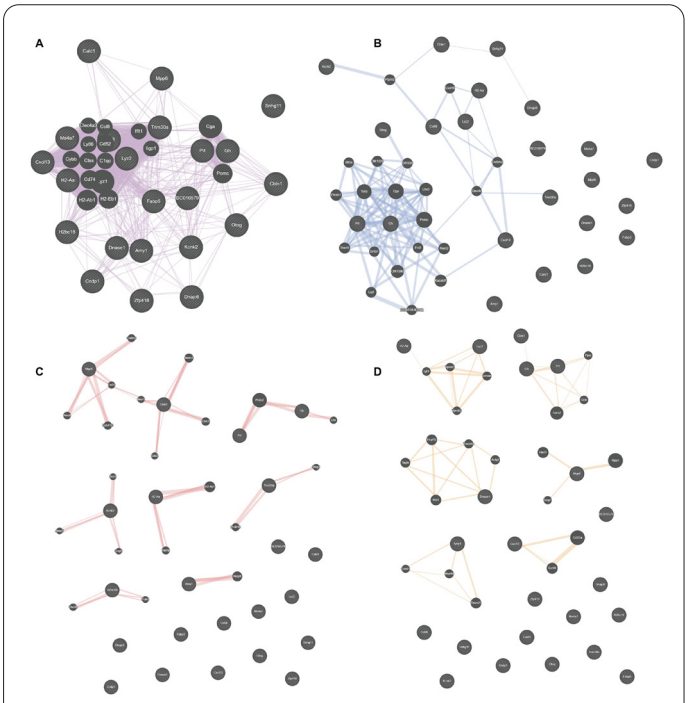

Figure 4. Interaction networks of 25 common increased mRNAs. (A) The interaction network of co-expression. (B) The co-localization networks. (C) The physical interaction networks. (D) The predicted interaction networks.

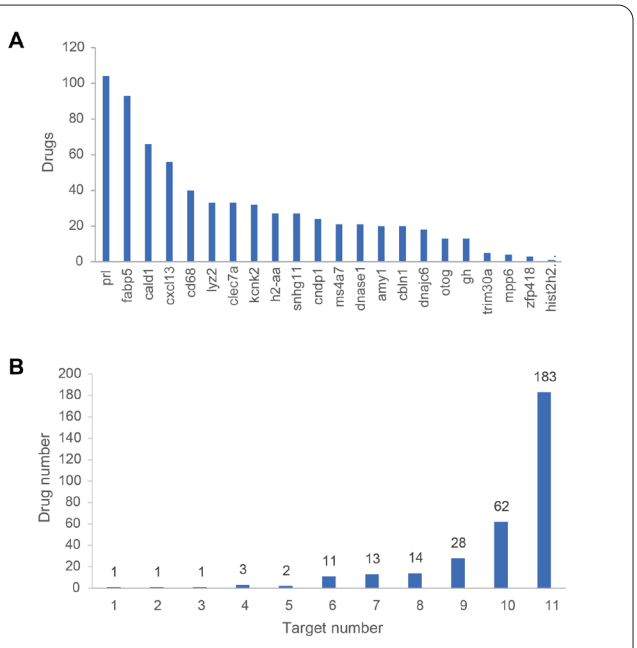

Figure 5. Based on all increased mRNAs, the inhibitive drugs collected from the CTD database. (A) The drug numbers of each mRNA. (B) The statistical results of drugs with different target number. A total of 183 drugs targeted 11 mRNAs, and these drugs were selected as potential medications for presbycusis (in the view of the common increased mRNAs).

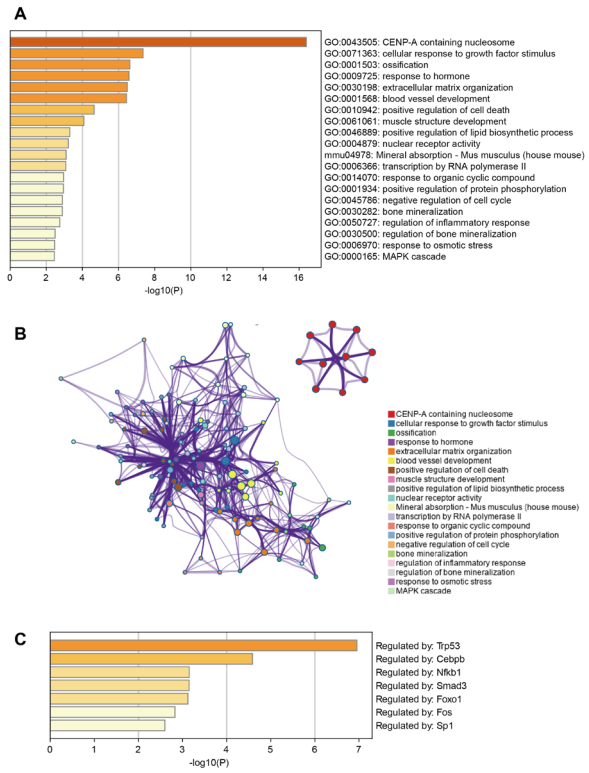

Figure 6. Enrichment analysis of 74 common decreased mRNAs. (A) Enriched GO terms. (B) The networks of enriched terms. (C) The enriched transcription factors based on these decreased mRNAs.

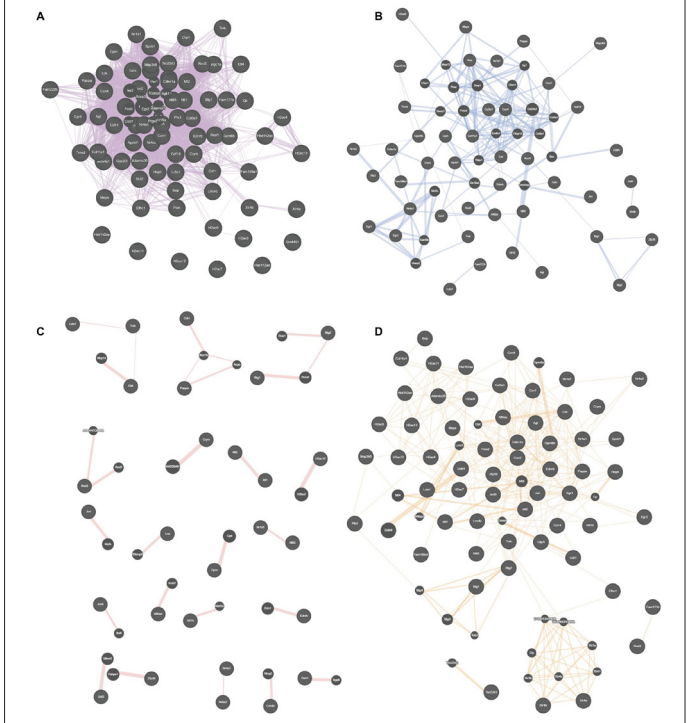

Figure 7. Interaction networks of 74 common decreased mRNAs. (A) The interaction network of co-expression. (B) The co-localization networks. (C) The physical interaction networks. (D) The predicted interaction networks.

Gm11827, Lrrc4c, Btg1, Nfil3, Efhc1, Rnd3, Gm4491, Ctgf, Lox, Heph, Cdt1, Qk, Adamts20, Tnmd, Zfp36, Junb, Fam222b, Ppargc1a, Cyr61, Nr4a2, Btg2, Gas2l3, Gpm6b, Jun, Etl4, Plk3, Apold1, Nr1d1, Clip4, Pappa, Gdf10, Xlr4a, and Xlr4b.These 74 mRNAs may be potential therapeutic genes in the cochlear hair cells in presbycusis.

In enrichment analysis of these 74 genes, enriched terms include (Figure 6A): CENP-A containing nucleosome, cellular response to growth factor stimulus, ossification, response to the hormone, etc.; and the networks of enriched terms were shown in Figure 6B. Based on these decreased mRNAs, the associated transcription factors include Trp53, Cebpb, Nfkb1, Smad3, Foxo1, Fos, and Sp1 (Figure 6C).

In the co-expression interaction, the hub genes include: Dusp1, Fos, Gadd45g, Socs3, Cebpb, Fosb, Errfi1, Klf4, Ptgs2, Junb, Cxcl1, Nr4a1, Cebpd, Mt2, Sik1, Ctgf, Zfp36, Egr1, Nfil3, Plk3, Cd14, Map3k6, Cdkn1a, Ddit4, Btg2, etc. (Figure 7A). In the co-localization network, the hub genes included: Lox, Col3a1, Btg1, Gpm6b, and Egr1 (Figure 7B). In the physical interaction, Tnik was a hub node (Figure 7C). In the predicted interaction network, hub genes include Egr1, Jun, Lox, Cdkn1a, and Ctgf (Figure 7D).

For these commonly decreased mRNAs, the enhancing drugs were collected from the CTD database. The drug numbers of the decreased mRNA were shown in Figure 8A, that cdkn1a has the most drugs for promoting its expression/activation/accumulation, followed by jun and egr1. Besides, the top 30 drugs (with the most targets of decreased mRNAs) were presented in Figure 8B, such as bisphenol A, Tetrachlorodibenzodioxin, Valproic Acid, Benzo(a)pyrene, and Acetaminophen.

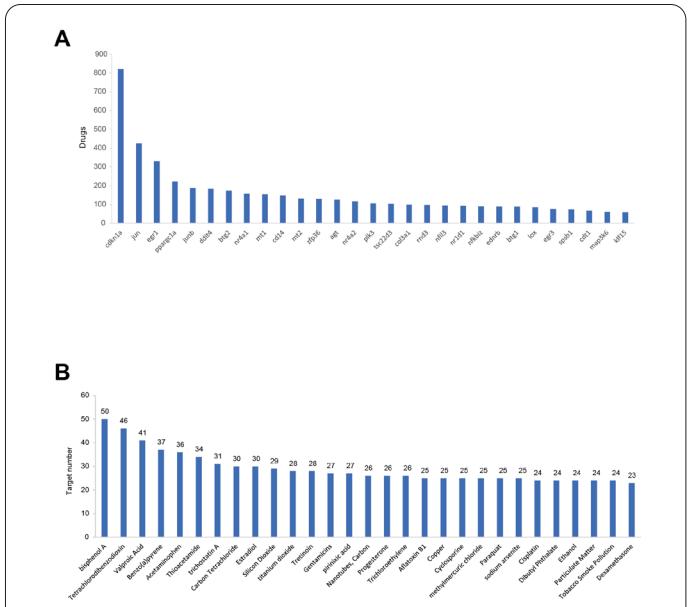

Figure 8. Based on common decreased mRNAs, the enhancing drugs collected from the CTD database. (A) The drug numbers of the decreased mRNA. (B) the top 30 drugs (with the most targets of decreased mRNAs).

The DE mRNA-miRNA pairs

The mRNA-miRNA pairs were predicted using the miRWalk tool. After de-duplication, the 25 common increased mRNAs had 946 matched miRNAs, and in combination with the 80 decreased miRNAs, there were 34 ones in the intersection (Figure 9A), which may be the potential therapeutic miRNAs. These 34 miRNAs were: mmu-miR-211, mmu-miR-504, mmu-miR-92b, mmu-miR-3077, mmu-miR-5107, mmu-miR-5122, mmu-miR-5132, mmu-miR-346, mmu-miR-700, mmu-miR-710,

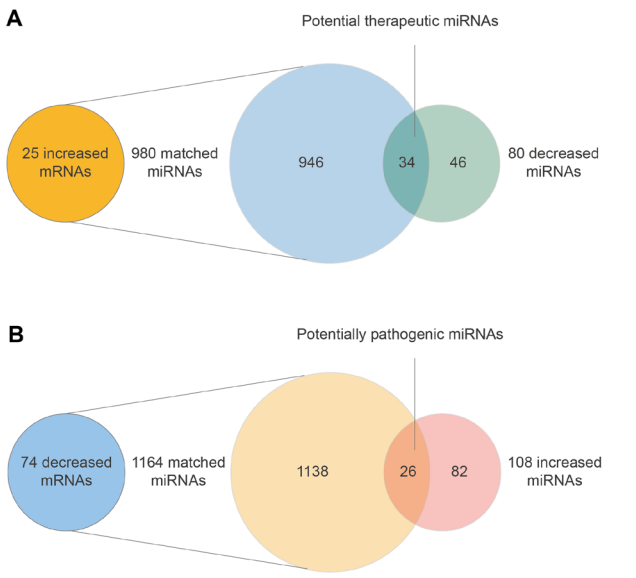

Figure 9. The differentially expressed mRNA-miRNA pairs. The mRNA-miRNA pairs are predicted using the miRWalk tool. (A) After de-duplication, the 25 common increased mRNAs link to 946 matched miRNAs, and in combination with the 80 decreased miRNAs, there are 34 ones in the intersect. (B) The 74 decreased mRNAs link to 1164 matched miRNAs, and 26 common ones shared by the 108 increased miRNAs.

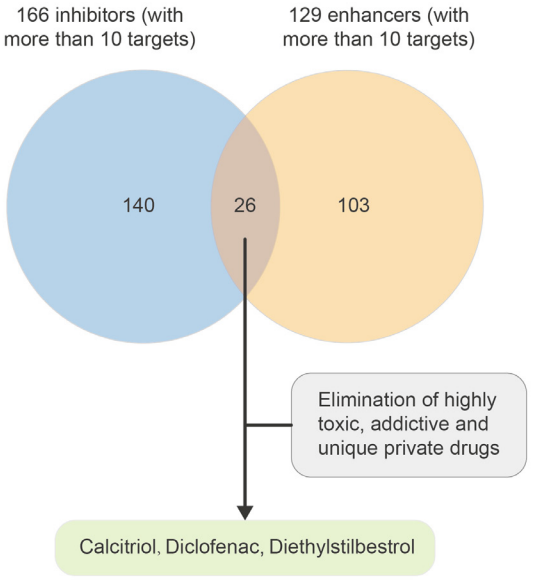

Figure 10. Common candidate drugs for presbycusis. Between the inhibitors of increased mRNAs and enhancers of decreased mRNAs, there are 26 common drugs. After elimination of highly toxic, addictive and unique private drugs, three new candidate drugs are found: calcitriol, diclofenac, and diethylstilbestrol.

mmu-miR-711, mmu-miR-1982, mmu-miR-3090, mmu-miR-149, mmu-miR-705, mmu-miR-1892, mmu-miR-1894, mmu-miR-3102, mmu-miR-1224, mmu-miR-762, mmu-miR-877, mmu-miR-1934, mmu-miR-5126, mmu-miR-1895, mmu-miR-1893, mmu-miR-326, mmu-miR-714, mmu-miR-328, mmu-miR-672, mmu-miR-1907, mmu-miR-2137, mmu-miR-3472, mmu-miR-652, and mmu-miR-3104. Similarly, the 74 decreased mRNAs had 1164 matched miRNAs, and 26 common ones were found when referring to the 108 increased miRNAs (Figure 9B), which merit further investigation as potentially pathogenic miRNAs of presbycusis. These miRNAs were: mmu-miR-140, mmu-miR-141, mmu-miR-15a, mmu-miR-17, mmu-miR-466a, mmu-miR-574, mmu-miR-872, mmu-miR-669o, mmu-miR-466m, mmu-miR-466p, mmu-miR-455, mmu-miR-200b, mmu-miR-148b, mmu-miR-199b, mmu-miR-34a, mmu-miR-130a, mmu-miR-148a, mmu-miR-22, mmu-miR-3074-1, mmu-miR-3074-2, mmu-miR-669c, mmu-miR-221, mmu-miR-34b, mmu-miR-27a, mmu-miR-322, and mmu-miR-362.

The key genes based on the above results

To further explore the key genes, we have selected genes that appear simultaneously in the results of interaction-network hub genes and miRNA-mRNA pairing. To the 34 key miRNAs, 15 (out of 25 increased mRNAs, Figure 9A) were linked: Ms4a7, Kcnk2, Dnajc6, Mpp6, Clec7a, Cald1, Trim30a, Gh, Otog, Lyz2, Cxcl13, Cbln1, BC016579, Zfp418, and Cndp1. And following genes have the most miRNA links (>=5): Kcnk2 (with 14 miRNAs), Dnajc6 (with 11 miRNAs), Cald1 (with 7 miRNAs), and Cbln1 (5 miRNAs). In the interaction network, the following genes link to most nodes (>6): Lyz2, Prl, Cd68, Trim30a, Amy1, H2-Aa, Hist2h2bb, Kcnk2, Otog, and Cxcl13. And two genes (Kcnk2 and Cbln1) were shared by the key members of the gene-interaction network and the mRNA-miRNA network. These two may be the key increased genes in presbycusis development. Similarly, among the decreased genes, 39 genes have more than ten linked nodes in the co-expression network, and among them, there are 13 genes also located in the co-localization network. In the mRNA-miRNA network associated with 26 key miRNAs (Figure 9B) based on decreased mRNAs, 41 mRNAs were linked, and 24 genes have the most miRNA links (>=5): such as Gpm6b, Etl4, Qk, Gas2l3, Tnik, Egr3, Lox, etc. Between the 24 key decreased genes in the mRNA-miRNA network and the 13 key decreased ones in the gene-interaction network, there are four common genes: Cdkn1a, Nfkbiz, Btg1 and Lox. Together, the six key genes (Kcnk2, Cbln1, Cdkn1a, Nfkbiz, Btg1 and Lox) may play a crucial role in the onset of presbycusis.

Common candidate drugs for presbycusis

Finally, we screened the inhibitive/enhancing drugs with more than 10 targets and probed the common candidate drugs for presbycusis (Figure 10). Between the inhibitors of increased mRNAs and enhancers of decreased mRNAs, there were 26 common drugs. After eliminating highly toxic, addictive and unique private drugs, three new drugs were screened: calcitriol, diclofenac, and diethylstilbestrol, which may be new candidate drugs for presbycusis.

Discussion

In this study, we found 25 common increased mRNAs and 74 common decreased mRNAs, as well as 34 potential therapeutic miRNAs and 26 pathogenic miRNAs, and three candidate drugs. The direct relationship between the above key genes/drugs and ARHL is not completely clear, and these results will provide new targets and strategies for mechanistic and therapeutic studies in ARHL.

Among the increased mRNAs, Cbln1, Prl, Mpp6 and Gh were repeatedly noticed to locate at the key positions. No study has reported a direct relationship between Cbln1

and presbycusis. Cerebellin-1 (Cbln1) controls diverse aspects of axon growth and guidance throughout the central nervous system; it has previously been shown to function in early and late neural development to influence synapse organization (21). Cbln1 is a ligand for an orphan GluD2, a bidirectional synapse organizer of the C1q family and is released from lysosomes in axons but not dendrites of cerebellar granule cells (22, 23). Released Cbln1 induces dynamic axonal structural changes by interacting with GluD2 during cerebellar synapse formation (24). Increased Cbln1 may also mediate presbycusis through GluD2-associated signals. Prolactin (PRL) is a helix bundle peptide hormone predominantly expressed by lactotrophic cells located in the anterior pituitary gland; prolactin expression is exclusive to the female mice, which provides a possible mechanism for an age-related hearing loss sub-type that is associated with gender and provides clues as to how this gender bias in hearing loss (25). In a study published in 2013, besides PRL, the closely related hormone Growth Hormone (Gh) was also highly upregulated, and the potent PRL inhibitor calcitonin-a (CalcA) was downregulated (25). However, no published article has revealed a clear correlation between Gh and presbycusis, and only one associated study showed that Gh treatment does not affect incidences of middle ear disease or hearing loss in infants and toddlers with Turner syndrome (26). Again, no link between presbycusis and Mpp6 has been reported, however, a rise in Mpp6 does correlate with postnatal hearing loss (27).

Among the commonly decreased mRNAs, Cdkn1a, Egr1, and Ctgf are notable hub genes. A study about cisplatin-induced ototoxicity in auditory cells indicated that Cdkn1a (p21) may be involved in hearing impairment or ototoxicity (28). Moreover, the histone deacetylase inhibition can protect hearing against acute ototoxicity; meanwhile, after treatment, the pro-survival gene Cdkn1a (p21) was elevated (29). Hence, Cdkn1a is indeed a protective gene against hearing loss, and the decrease of Cdkn1a may be included similarly in the process of ARHL. Egr1 has been found associated with presbycusis, but the present evidence is still weak. Transcriptomic analysis has highlighted the cochlear inflammation associated with ARHL, but they found that Egr1 was increased in the old mice (30). Since the methodology of our grouping is consistent with that study, the reason of the opposed result is to be further explored. Notably, intense permanent threshold shift noise can induce several immediate early genes, including c-Fos and Egr1 (31). Therefore, the change direction and the exact role of Egr1 in ARHL are still not fully determined so far. The role of Ctgf in presbycusis is obscure. However, CTGF is a type IV fibrocyte marker, and damage to type IV fibrocytes has been associated with noise-induced trauma (32). An animal study showed that age-related inflammation and oxidative stress in the cochlea are exacerbated by long-term short-duration noise stimulation (33). In that study, following long-term noise exposure, both young/adult and aged rats displayed a significant decrease in the immunostaining of type IV fibrocytes relative to unexposed rats. Together, Ctgf may be a preferred gene for the treatment of ARHL.

Basal on the DE mRNA-miRNA pairs, we found 34 miRNAs may be the potential therapeutic miRNAs and 26 potentially pathogenic miRNAs of presbycusis. The association of these miRNAs with ARHL has almost been unreported. Therefore, further study should first verify their differential expression by real-time qPCR and subsequently observe their pathogenic or therapeutic effects by expression regulation in animal experiments. In particular, for the therapeutic miRNAs, it may be possible to encapsulate the corresponding mimics for drug delivery through various vectors, which may help to block the development of ARHL.

Finally, we here screened and proposed three drugs (calcitriol, diclofenac, and diethylstilbestrol) that are in urgent need of tapping their therapeutic efficacy. Calcitriol has been widely used in osteoporosis, fractures and falls, and cancer treatment (34-38). There is no evidence showing its efficacy in ARHL. Interestingly, on one hand, a study claimed that a vitamin D-deficient diet can rescue hearing loss in Klotho mice, which normalizes serum calcitriol levels (39). They concluded that hearing loss in Klotho mice is caused by elevated renal 1α-hydroxylase expression and consequent excessive production of calcitriol. On the other hand, sensorineural hearing loss progressively develops at an earlier age in VDR KO mice (40). Given the contradictory findings and the fact that our conclusion is more supportive of the latter, further validation experiments remain to be conducted. Diclofenac is a proven, commonly prescribed nonsteroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory, and antipyretic properties, and has been shown to be effective in treating a variety of acute and chronic pain and inflammatory conditions (41, 42). Again, the link between diclofenac and presbycusis is totally unknown. In 2017, a study reported that diclofenac sodium is ototoxic in chronic use which may lead to loss of hearing especially when used topically in chronic otitis cases with tympanic membrane damage (43). Therefore, its efficacy for ARHL is still highly questionable and needs to be verified in presbycusis animal models. Also, there is no data about the therapeutic role of diethylstilbestrol in presbycusis. In contrast, a report in 2003 showed the risk of deafness in the second generation of intrauterine-exposed fetuses to diethylstilbestrol (44). However, the influence of diethylstilbestrol merits investigation, for that there were clear gender differences in the incidence of presbycusis, as reported by independent studies (45-49). In particular, estrogen may play an essential role in modulating the pathophysiological mechanisms in the cochlea, and the effects of hormone replacement therapy on hearing loss are complex (50, 51).

In conclusion, by jointly analyzing multiple datasets, we found 25 common increased mRNAs (e.g., Cbln1, Prl, Mpp6 and Gh) and 74 common decreased mRNAs (Cdkn1a, Egr1, and Ctgf), as well as 34 potential therapeutic miRNAs and 26 pathogenic miRNAs, and three candidate drugs (calcitriol, diclofenac, and diethylstilbestrol). They may provide new targets and strategies for mechanistic and therapeutic studies in ARHL.

Ethics approval and consent to participate

Not applicable to this type of manuscript.

Competing interests

We declare no competing interests exist.

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Data availability

The original data are available from the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

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