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MicroRNAs in acquired sensorineural hearing loss

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Abstract

Objective. This review summarises the current literature on the role of microRNAs in presbyacusis (age-related hearing loss) and sudden sensorineural hearing loss.

Methods. Medline, PubMed, Web of Science and Embase databases were searched for primary English-language studies, published between 2000 and 2017, which investigated the role of microRNAs in the pathogenesis of presbyacusis or sudden sensorineural hearing loss. Quality of evidence was assessed using the National Institutes of Health quality assessment tool.

Results. Nine of 207 identified articles, 6 of good quality, satisfied the review's inclusion criteria. In presbyacusis, microRNAs in pro-apoptotic and autophagy pathways are upregulated, while microRNAs in proliferative and differentiation pathways are downregulated. Evidence for microRNAs having an aetiological role in sudden hearing loss is limited.

Conclusion. A shift in microRNA expression, leading to reduced cellular activity and impaired inner-ear homeostasis, may contribute to the pathogenesis of presbyacusis.

Review Article

Prof D A Nunez takes responsibility for the integrity of the content of the paper

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Introduction

Presbyacusis, also known as age-related hearing loss, gradually occurs with age. The 2012/2013 Canadian Health Measures Survey reported on the marked age-dependent difference in the prevalence of audiometric confirmed hearing loss; this increased from 10 per cent of the population aged 50 years and younger, to 65 per cent at ages 70–79 years.[1] The global population of people aged 60 years and over was 962 million in 2017, equating to 13 per cent of the total population, and this number is projected to be 1.4 billion in 2030 and 2.1 billion in 2050.[2]

Despite the increasing proportion of the population affected by age-related hearing loss, the underlying pathogenetic mechanism remains poorly understood. Based on post-mortem pathological analysis, Schuknecht and Gacek classified age-related hearing loss into six types: sensory (loss of greater than 10 mm of cochlear sensory hair cells); neuronal (loss of greater than 50 per cent of spiral ganglion neurons); strial (atrophy of more than 30 per cent of the stria vascularis); cochlear conductive (where the pathological changes do not meet any of the previous criteria, but there is a characteristic audiometric hearing loss pattern); mixed (a combination of the first three types); and unclassified.[3] Age-related hearing loss is believed to be a consequence of a combination of genetic, cumulative environmental exposures, and age-related pathophysiological change.[4] Hypertension, diabetes, noise exposure and ototoxicity are amongst the proposed risk factors for its development.[5] No cure has been identified so far.

Sudden sensorineural hearing loss (SNHL) is another acquired SNHL syndrome, of unknown aetiology in 85–90 per cent of the cases. It is defined as a hearing loss of greater than or equal to 30 dB, spanning at least three contiguous audiometric frequencies, which develops over 72 hours.[6] The incidence rate reported in large twenty-first century national studies varies between 27 and 60 per 100 000 population in the USA and Japan respectively.[7,8] The US study identified a slight male predominance and the Japanese study reported a 3:1 female predominance. Alexander and Harris' study findings supported earlier reports that the incidence rate increased with age.[7] In this respect, sudden SNHL mirrors the age-dependant prevalence pattern of age-related hearing loss.

The recovery rate varies, and is proposed to depend on several factors, including: the initial degree of hearing loss, the time to treatment, age, the presence of vertigo and the pattern of hearing loss.[9] The overall spontaneous clinically important hearing recovery rate can be as high as 73 per cent.[10] The recovery rate with current treatment protocols is similar, reported as 78 per cent in a recent study (Lee *et al.*, unpublished data).

Histopathological changes associated with sudden SNHL are similar to those of age-related hearing loss, including the loss of sensory hair cells and cochlear neurons, and atrophy of the stria vascularis.[11] The pathogenesis of sudden SNHL, like age-related hearing loss, is unknown.

MicroRNAs are small (approximately 21–23 nucleotides long), non-coding, single-stranded RNAs. They are found in animals, plants and some viruses that repress

messenger RNA transcription by binding to complementary sequences in the 3′ -untranslated region (3′-UTR) of messenger RNA. MicroRNAs are highly evolutionarily conserved, and have been found to participate in the regulation of gene expression of nearly all cellular processes (e.g. cell proliferation, differentiation, migration and apoptosis). Currently, more than 2500 microRNAs have been discovered in humans. Altered microRNA expression has been identified in ageing, and pathological states such as cancer, and cardiovascular and neurological diseases.

Recent studies have determined that microRNAs play a fundamental role in inner-ear development.[12] There is increasing evidence that microRNA regulation of gene expression in the post-developmental inner ear contributes to the development of acquired hearing loss.[13]

Identifying changes in microRNA expression in acquired SNHL may offer new and exciting insights into disease pathogenesis. Therefore, a review of studies investigating the role of microRNAs in age-related hearing loss and sudden SNHL was undertaken.

Materials and methods

A narrative review of English-language articles published between January 2000 and December 2017 was undertaken. Medline, PubMed, Web of Science and Embase databases were searched, using the Medical Subject Headings (MeSH) term 'microRNA', combined with the MeSH terms 'hearing loss, sensorineural', 'hearing loss, sudden', 'presbycusis', 'organ of Corti', 'labyrinth supporting cells', 'hair cell, auditory', 'ear, inner', 'deafness' and the non-MeSH term 'progressive hearing loss'.

Inclusion criteria were primary studies investigating the role of microRNAs in the pathogenesis of age-related hearing loss or sudden SNHL, in animal and/or human subjects aged over 18 years. Studies were excluded if they: did not use the American Academy of Otolaryngology – Head and Neck Surgery sudden SNHL diagnostic criteria;[6] did not record the authors; or included mostly patients with conditions such as diabetes mellitus or acoustic trauma, which are known hearing loss aetiological factors. Similarly, studies reporting on non-mammals or microRNA biogenesis components only were excluded.

The results of the search were tabulated in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses ('PRISMA') guidelines recommended format.[14] The quality of evidence for individual studies was graded independently by two authors (HHRC and PW) as poor, fair or good, using the National Heart, Lung, and Blood Institute of the National Institutes of Health Study Quality Assessment Tools (Table 1).[15] The grade recorded was that agreed by both evaluators. When there was a discrepancy between the two evaluators that was not resolved through discussion into a consensual grade, then the senior author (DAN) assessed the study and allocated the study grade.

Results and discussion

A total of 207 records were identified with the MeSH terms search after removing duplicates. Eighty-six records were excluded for the following reasons: review articles ($n = 51$), conference abstracts ($n = 22$), letters ($n = 3$), editorial ($n = 1$), survey ($n = 1$), report ($n = 1$), non-English language ($n = 6$) and anonymous authorship ($n = 1$). On further screening, of the remaining 121 primary article abstracts, 112 failed to meet the study inclusion criteria. Figure 1 shows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart.

Evidence of microRNA modulation was reported in eight age-related hearing loss studies and in one sudden SNHL study; these formed the basis of this review. Two were animal studies,[16,17] four included findings from animal cell culture studies,[18–21] one comprised findings from animal and human subjects,[13] and two concerned findings from human subjects alone.[22,23]

The quality of evidence of the studies, based on the National Institutes of Health quality assessment criteria, was judged as good in six, fair in one and poor in two studies (Table 2).

MicroRNAs in programmed cell death

Programmed cell death, which includes apoptosis, autophagy, and programmed necrosis or necroptosis, is defined as regulated cell death executed by an intracellular programme.[24] Accumulated evidence has shown a close interaction between apoptosis, autophagy and necroptosis, and the molecular mechanism underlying the crosstalk is regulated by microRNAs. One microRNA may target more than one component of a programmed death pathway and even more than one death pathway.

Apoptosis is a process by which the body rids itself of up to 70 billion superfluous cells a day without triggering an inflammatory process. It occurs normally during development and ageing, and as a homeostatic mechanism to maintain cell populations in tissues. Inappropriate apoptosis is a factor in many human conditions, including neurodegenerative diseases, ischaemic damage, autoimmune disorders and many types of cancer. Many microRNAs have been reported as regulating the apoptotic signalling pathways, such as executioner caspases, and key regulators transcription factor p53 and death-associated protein kinase (DAPK).[24]

Pro-apoptotic microRNA upregulation in presbyacusis

Pro-apoptotic microRNAs, such as miR-29a/b/c, miR-34a/b/c, let-7a/b/c/e/f/g/i, miR-141, miR-146, miR-203 and miR-429, work through different direct and indirect pathways to induce apoptosis.[16] Upregulation of both pro-apoptotic miR-29a/b/c and miR-34a/b/c families in the organ of Corti likely contribute to hair cell apoptosis and tissue degeneration in age-related hearing loss. MiR-29 regulates genes upstream of p53 pathways and miR-34a regulates genes downstream of p53 pathways, suggesting that microRNAs play a role in p53-mediated apoptosis in the organ of Corti.[25] The protein p53 is a key transcription factor which regulates many genes that initiate anti-proliferative responses, such as cell-cycle arrest, DNA repair, apoptosis and cellular senescence. At an organism level, p53 activity has been implicated in tissue degeneration and ageing.

In age-related hearing loss animal models, signalling pathways miR-34a/B-cell lymphoma2 (Bcl-2)[18] and miR-34a/sirtuin 1 (SIRT1)/p53[21] were demonstrated to be associated with cochlear hair cell apoptosis. Genes *Bcl-2* and *SIRT1* are direct targets of miR-34a.[18,21] The *Bcl-2* family contains both anti-apoptotic and pro-apoptotic members, and their interactions determine the likelihood of the cell undergoing apoptosis.[26] Anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-2l1,

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Table 1. NIH Quality of evidence assessment criteria for observational cohort studies and case–control studies[15]

Assessment criteria for observational cohort studies on animals	Assessment criteria for case–control studies on humans & cell lines
1. Was the research question or objective in this paper clearly stated & appropriate?	1. Was the research question or objective in this paper clearly stated & appropriate?
2. Was the study population clearly specified & defined?	2. Was the study population clearly specified & defined?
3. Was the participation rate of eligible persons at least 50%?	3. Did the authors include a sample size justification?
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion & exclusion criteria for being in the study pre-specified & applied uniformly to all participants?	4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)?
5. Were sample size justification, power description, or variance & effect estimates provided?	5. Were the definitions, inclusion & exclusion criteria, algorithms, or processes used to identify or select cases & controls valid, reliable & implemented consistently across all study participants?
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured? Were the cases clearly defined & differentiated from controls?	6. Were the cases clearly defined & differentiated from controls?
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure & outcome if it existed?	7. If less than 100% of eligible cases &/or controls were selected for the study, were the cases &/or controls randomly selected from those eligible?
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g. categories of exposure, or exposure measured as a continuous variable)?	8. Was there use of concurrent controls?
9. Were the exposure measures (independent variables) clearly defined, valid, reliable & implemented consistently across all study participants?	9. Were the investigators able to confirm that the exposure or risk occurred prior to the development of the condition or event that defined a participant as a case?
10. Was the exposure(s) assessed more than once over time?	10. Were the measures of exposure or risk clearly defined, valid, reliable & implemented consistently (including the same time period) across all study participants?
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable & implemented consistently across all study participants?	11. Were the assessors of exposure or risk blinded to the case or control status of participants?
12. Were the outcome assessors blinded to the exposure status of participants?	12. Were key potential confounding variables measured & adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?
13. Was loss to follow up after baseline 20% or less?	
14. Were key potential confounding variables measured & adjusted statistically for their impact on the relationship between exposure(s) & outcome(s)?	

NIH = National Institutes of Health

Bcl-2l2, Mcl-1 and Bcl-2a1) are thought to exert their effects by stabilising the mitochondrial membrane potential and preventing the release of cytochrome C and apoptosis-inducing factors. The binding of miR-34a to the 3'-untranslated region of the *Bcl-2* gene halts its transcription, thus cancelling the anti-apoptotic effect of *Bcl-2*. In aged mice, higher expression of *Bcl-2* and *Bcl-211* genes was demonstrated to exert a protective effect during cochlear ageing.[25]

Similarly, miR34a overexpression suppressed *SIRT1* gene messenger RNA and induced cochlear hair cell apoptosis via a p53-mediated pathway.[21] The SIRT1 protein is a nicotinamide adenine dinucleotide dependent deacetylase that regulates apoptosis in response to oxidative and genotoxic stress by deacetylating its substrates, including p53.[27] Thus, miR-34a blockage of *SIRT1* transcription induces cochlear hair cell apoptosis via a p53-mediated pathway.

The *E2F3* gene (E2 transcription factor 3), which regulates cell-cycle progression, might be another direct target of miR-34a.[28] Pang and colleagues' (2016) study demonstrated a simultaneous increase of miR-34a expression in the auditory pathways and plasma in aged mice.[13] The messenger RNA levels of genes *SIRT1*, *Bcl-2* and *E2F3* were correspondingly decreased within the auditory pathway and plasma with ageing. However, simultaneous increases of miR-34a expression in non-hearing organs such as the heart and liver suggest that miR-34a exerts a non-tissue-specific ageing effect in mice.

Human studies on the relationship between circulating miR-34a expression level and auditory function are inconclusive. MiR-34 expression level was significantly upregulated in age-related hearing loss patients, compared to normal hearing age-matched and younger controls.[13] However, the messenger RNA levels of miR-34a target genes *SIRT1*, *Bcl-2* and *E2F3* were similar in age-related hearing loss patients and controls, which questions the validity of a miR-34a/Bcl-2 or miR-34a/SIRT1/p53 mediated pathway in the pathogenesis of human age-related hearing loss. In addition, the similar circulating miR-34a expression levels in healthy human controls of different age groups indicates that ageing in humans is not associated with a non-tissue-specific increase in miR-34a level as demonstrated in animal models.[13]

MiR-29b overexpression was shown to induce mitochondrial dysfunction and hair cell loss via downregulation of the SIRT1/PGC-1α (peroxisome proliferator-activated receptor-gamma coactivator 1α) in aged mice.[20] SIRT1 is a direct target of miR-29b. Oxidative stress is believed to cause age-related hearing loss by apoptosis of auditory system cells.[29] SIRT1 regulates intracellular oxidative stress by the deacetylation of its substrates including PGC-1α, a transcriptional co-regulator that binds to

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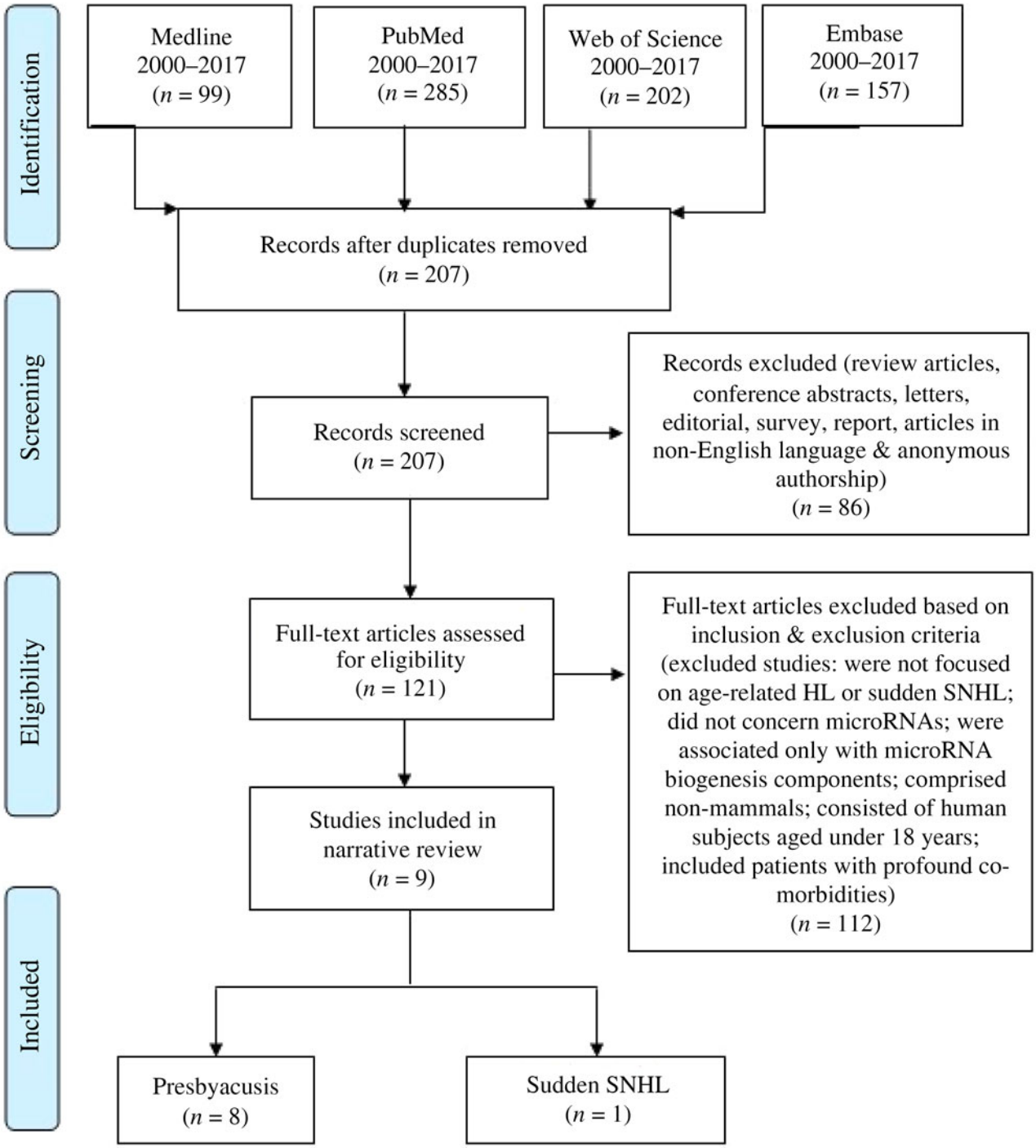

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses ('PRISMA') literature review flow chart. HL = hearing loss; SNHL = sensorineural hearing loss

numerous transcription factors to promote mitochondrial biogenesis and oxidative metabolism.[30] Previous studies, as cited by Xue *et al.*,[20] have shown that the upregulation of miR-29 microRNAs promotes p53-dependent apoptosis by suppressing several key regulators of cell survival, such as genes *p85α* and *CDC42* (cell division cycle 42). There is evidence from animal studies that miR-29 contributes to age-related hearing loss, but supporting human studies are lacking.

Anti-apoptotic microRNA downregulation in presbyacusis

With ageing, more microRNA expression levels are downregulated than upregulated.[16,23] Amongst them are miR-181 and miR-183 family members, which are known mediators of cell proliferation and differentiation pathways. The miR-181 family consists of four members: miR-181a, miR-181b, miR-181c and miR-181d. All of these, with the exception of miR-181c, are associated with organ of Corti degeneration in aged mice.[16] Upregulation of miR-181a correlated with inner-ear sensory epithelium regeneration in chickens, by suppressing protein p27, a cell-cycle inhibitor and differentiator in the auditory sensory epithelium.[31]

The miR-183 family is a triad consisting of miR-96, miR-182 and miR-183; these originate from a common primary transcript that begins to be expressed as early as embryonic day 9.5 within the otic vesicle.[32] Its unique expression

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Table 2. Summary table of reviewed papers' findings

Study (year)	Objectives	Study design	Major findings	Evidence quality (NIH criteria[15])
Pang *et al.* (2017)[19]	(1) To investigate miR-34a mediated cochlear cell death & age-related HL via modulating autophagy. (2) To identify strategies that inhibit miR-34a activity	Prospective cohort of 60 C57BL/6 mice: 20 in each group of different ages (1, 3 & 12 months) – Cochlear tissue	In aged mice: ABRs were elevated; hair cell loss correlated with hearing loss; miR-34a level was upregulated; autophagy marker *p62* mRNA & protein expressions were elevated; & autophagy marker LC3-II protein expression decreased	Good
		HEI-OC1 cells: 2 transfection groups (miR-34a mimic & *ATG9A* siRNA groups)	In HEI-OC1 cells: miR-34a mimic transfection blocked autophagosome–lysosome fusion & reduced cell survival; miR-34a inhibition unobstructed autophagic activity & increased cell survival rate; & ursodeoxycholic acid treatment rescued miR-34a induced cell death by restoring autophagosome activity	
Huang *et al.* (2017)[18]	To demonstrate miR-34a/Bcl-2 signalling pathway in hair cell apoptosis during ageing	Prospective cohort of 57 C57BL/6 mice: 24 aged 3–6 weeks, & 33 aged 12–15 months – Cochlear tissue	In aged mice: hair cell loss correlated with hearing loss; miR-34a level was upregulated; & *Bcl-2* mRNA level decreased	Good
		HEI-OC1 cells: transfected with miR-34a mimic & kmiR-34a inhibitor	In miR-34a mimic transfected cells: miR-34a was overexpressed & promoted apoptosis; & *Bcl-2* mRNA levels were reduced In miR-34a inhibitor transfected cells, the effects were opposite	
Pang *et al.* (2016)[13]	(1) To characterise the profiles of miR-34a, miR-29a & miR-124 in age-related HL. (2) To determine the level of miR-34a targets SIRT1, Bcl-2 & E2F3 in the cochlea, auditory cortex & plasma. (3) To assess the correlation between circulating miR-34a levels & degree of hearing loss	Prospective cohort of 48 C57BL/6 mice: 12 in each group (aged 2, 6, 12 & 20 months) – Peripheral blood, cochlea, auditory cortex, heart & liver	As mice aged: average hearing thresholds increased; miR-34a level was upregulated; miR-29a & miR-124 expression levels were unchanged; & mRNA levels of target genes *SIRT1*, *Bcl-2* & *E2F3* decreased	Fair
		24 age-related HL patients & 58 healthy controls – Peripheral blood	In age-related HL patients: miR-34a level was upregulated; & miR-29a, miR-124 levels & miR-34a target genes *SIRT1*, *Bcl-2* & *E2F3* mRNA levels were unchanged	
Xue *et al.* (2016)[20]	(1) To investigate miR-29b/SIRT1/PGC-1α pathway's role in hair cell death & age-related HL pathogenesis. (2) To explore age-related HL treatment strategies via miR-29b activity inhibition or SIRT1 functional restoration	Prospective cohort of 60 C57BL/6 mice: 30 aged 1–2 months, & 30 aged 12–16 months – Organ of Corti	In aged mice: miR-29b upregulated; mitochondrial dysfunction increased; & cochlear hair cells degenerated	Good
		HEI-OC1 (cell culture): case–control: under 7 conditions (control group, hydrogen peroxide group & 5 transfection groups)	In miR-29b mimic transfected cells: miR-29b overexpression promoted apoptosis, suppressed cell proliferation & induced mitochondrial dysfunction; & SIRT1 & PGC-1α mRNA & protein expressions were reduced	
Xiong *et al.* (2015)[21]	(1) To demonstrate miR-34a/SIRT1/p53 pathway's role in hair cell death & age-related HL pathogenesis. (2) To develop strategies to inhibit miR-34a activity, or restore SIRT1 function	Prospective cohort of: (1) 97 C57BL/6 mice: 47 aged 1–2 months, & 50 aged 12–16 months (2) 28 mice aged 2–3 months: 15 in treatment group & 13 controls – Cochlear tissue	In aged mice: miR-34a was upregulated; p53 expression & acetylation increased; SIRT1 localisation & expression decreased; cochlear hair cell loss & hearing loss increased	Good
		HEI-OC1 cells: case & control under different conditions	In miR-34a mimic transfected cells: *SIRT1* mRNA expression decreased; p53 acetylation increased; apoptosis was promoted, resulting in decreased cell survival; & resveratrol treatment decreased p53 acetylation, with no change in SIRT1 expression	
Zhang *et al.* (2014)[17]	(1) To identify miRNAs that are differentially expressed in the lateral wall of the cochlear duct during ageing. (2) To explore their potential apoptosis-related target genes	Prospective cohort of: (1) 15 C57BL/6J mice: 5 in each group, aged 21 days, 9 & 16 months – Cochlear duct lateral wall (2) 15 CBA/J mice: 5 in each group, aged 21 days, 9 & 16 months – Cochlear duct lateral wall	As the mice aged: a total of 95 & 60 miRNAs were differentially expressed in the cochlear duct lateral wall of C57BL/6J & CBA/J mice, respectively; miR-29 family, miR-203, miR-762 & miR-1224 were upregulated (approx. 2-fold); miR-107 family, miR-127, miR-130a/b, miR-342-3p, miR-351, miR-379, miR-455 & miR-467a were downregulated; & most anti-apoptotic genes were downregulated & pro-apoptotic genes were upregulated	Good

Zhang *et al.* (2013)[16]	To identify miRNAs that were differentially expressed in the organ of Corti	Prospective cohort of: (1) 15 C57BL/6J mice: 5 in each group, aged 21 days, 9 & 16 months – Organ of Corti (2) 15 CBA/J mice: 5 in each group, aged 21 days, 9 & 16 months – Organ of Corti	As the mice aged: 111 & 71 miRNAs were differentially expressed in C57BL & CBA mice, respectively; miR-29a/b, miR-34a & miR-124 were upregulated (>2-fold); & miR-181a/b/d & miR-183 were downregulated (<2-fold)	Good
Sekine *et al.* (2017)[23]	To investigate the expression profile of miRNAs in the inner ear of older adults	8 humans split into 2 groups: 4 young elderly (60–74 years) & 4 old elderly (>85 years) – Autopsy temporal bones	All subjects aged >85 years had hearing loss. 16 miRNAs were significantly upregulated in the young elderly, indicating a reduction in target proteins involved in inner-ear homeostasis. In old elderly, downregulated miRNAs (n = 131) preceded by upregulated miRNAs (n = 9), indicating reduced cellular activities within the inner ear	Poor
Li *et al.* (2017)[22]	To seek miRNAs that might contribute to sudden SNHL pathogenesis	9 sudden SNHL patients & 3 healthy volunteers – Peripheral blood	In sudden SNHL patients: 24 miRNAs were differentially expressed; miRNAs-34a/548n/15a/143/23a/210/1255a/18b/1180 & 99b may play a role in sudden SNHL pathogenesis; & 1038 target genes were predicted	Poor

NIH = National Institutes of Health; HL = hearing loss; ABR = auditory brainstem response; mRNA = messenger RNA; HEI-OC1 = House Ear Institute-organ of Corti 1; siRNA = small interfering RNA; miRNA = microRNA; SNHL = sensorineural hearing loss

pattern within the inner ear correlates with inner-ear development and differentiation.[32]

MiR-182 is expressed during the differentiation of inner-ear stem/progenitor cells into a hair-cell-like fate. Its function may be associated with its putative target protein Tbx1 (T-box transcription factor 1), a transcription factor that has been implicated in inner-ear development and hair cell fate.[33] MiR-182 overexpression induced ectopic hair cells in developing zebra fish, while knockdown of the miR-183 family led to reduced numbers of sensory hair cells and defects in the semi-circular canals.[34]

MiR-96 overexpression demonstrated the same effect as miR-182 in developing zebra fish.[34] Mutation in the miR-96 seed region (2–7 nucleotides in length), which confers binding specificity,[35] results in autosomal dominant, progressive hearing loss in humans[36] and in mice.[37] In addition, a mutation located in the non-seed region of the miR-96 identified in non-syndromic hearing loss in humans was shown to interfere with miR-96 pre-microRNA secondary structure.[38] MiR-96 mutation in mice alters the function and gene expression profile of the organ of Corti.[37] Its downstream effect on *Oncomodulin*, *Pitpnm1*, *Slc26a5* (*prestin*), *Ptprq* and *Gfi1* genes results in impaired hair cell function and hair cell degeneration, and causes deafness.[38] Interestingly, Zhang and colleagues' (2013) microarray analysis detected miR-96 downregulation only in C57BL/6J mice, and not in CBA/J mice, suggesting that the different genetic backgrounds of these two mouse strains may result in differences in the microRNAs involved in the pathogenesis of age-related hearing loss.[16]

Anti-autophagy microRNA upregulation in presbyacusis

In a recent study, miR-34a was shown to induce cochlear hair cell death via the suppression of autophagy.[19] Autophagy is a regulated intracellular programme that seeks to clear accumulated intracellular toxins; if unsuccessful, it results in cell death. It is triggered by growth signal deficiency, nutrient deprivation, genotoxic stress, hypoxic stress, endoplasmic reticulum stress, and/or reactive oxygen species accumulation.

In Pang and colleagues' (2017) study, miR-34a overexpression was associated with a reduction in autophagy in aged mice and HEI-OC1 (House Ear Institute-organ of Corti 1) cells.[19] In HEI-OC1 cells, miR-34a overexpression suppressed protein ATG9A (autophagy-related protein 9A) level and impaired autophagy via p62 elevation.[39] *ATG9A* is one target gene of miR-34a, and its corresponding protein AT9A is necessary for optimal autophagy.[40]

Further research is required to demonstrate the role of miR-34a mediated autophagy in human age-related hearing loss.

MicroRNAs in stria vascularis

A number of differentially expressed microRNAs have been reported in the ageing lateral wall of the cochlear duct or stria vascularis.[17] MiR-203, a pro-apoptotic microRNA, was upregulated in the lateral wall of aged mice. Genotoxic stress agents (e.g. camptothecin) were demonstrated to induce miR-203 expression by p53 acetylation.[41] MiR-203 overexpression downregulates anti-apoptotic genes *Bclw* or *Bcl-2l2* (a member of the Bcl-2 family), and promotes cell death in a p53-dependent manner. Other upregulated microRNAs (miR-762 and miR-1224) and downregulated microRNAs (miR-107, miR-145, miR-342 and miR-455) are also suggested

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to play a crucial role in the function and maintenance of the stria vascularis.

Potential microRNA–messenger RNA networks in age-related hearing loss

Zhang *et al.* (2014) proposed networks of microRNA–messenger RNA interactions in age-related hearing loss based on studies of ageing mice.[17] Differentially expressed microRNAs and their predicted target messenger RNAs associated with apoptosis were used. The networks were complex, with examples of downregulated and upregulated microRNAs targeting upregulated and downregulated pro- and anti-apoptotic genes. For example, *Tnfsf10*, a critical pro-apoptotic gene was predicted to be upregulated by the downregulation of anti-apoptotic miR-181, amongst others.

Lewis *et al.* (2016) also explored the potential networks controlled by miR-96 in miR-96 mutant mice (diminuendo mice).[42] Three methods of network analysis were used: a manually created regulatory network, protein–protein interactome analysis and gene set enrichment analysis. *Myc*, *Gfi1* and *Fos* genes were suggested as important targets of miR-96 mediated regulatory networks involved in hearing and deafness. *Fos* was consistently identified by more than one network analytical method. It regulates genes *Slc26a5* (prestin) and *Ocm* (oncomodulin), both of which are associated with hearing and deafness.[43] *Gfi1* controls *Fos* via *Myc*.[42] However, more work is required in this area.

MicroRNAs in sudden sensorineural hearing loss pathogenesis

Li *et al.* predicted that the microRNAs hsa-miR-34a/548n/15a/143/23a/210/18b regulated target genes which may have a critical role in sudden SNHL, based on a small sample of nine sudden SNHL patients and three controls.[22] Experimental studies not specifically focused on sudden SNHL corroborate a potential role for miR-34a,[13] miR-210,[44] miR18B,[45] miR-23A[46] and miR15a-5p[47] in sudden SNHL. Further studies of larger patient samples are required to validate a role for microRNAs in human sudden SNHL.

Two human-subject studies investigated microRNA biogenesis associated components in sudden SNHL, though neither sought direct evidence of altered microRNA levels.[48,49] The microRNA biogenesis pathway proteins DiGeorge syndrome critical region gene 8 (DGCR8) and argonaute 2 (AGO2) directly influence the biosynthesis of all microRNAs.[48] AGO2 is a component of the RNA-induced silencing complex (RISC) that silences microRNA activity.[50] DGCR8 is a unit of the microprocessor complex, which mediates microRNA biogenesis via the generation of a precursor microRNA (pre-microRNA).[51]

Han and colleagues' study identified elevated *AGO2* gene expression in sudden SNHL patients' peripheral blood samples, and it was positively correlated with *DGCR8* gene expression in both sudden SNHL patients and healthy controls.[48]

Kim and colleagues' study on circulating messenger RNA levels of RNAse III endonucleases *Dicer* and *Drosha* identified dysregulation of *Dicer*, another RISC component that was reduced in sudden SNHL patients compared to controls.[49] *Drosha* expression was not altered, suggesting that the processing of microRNAs at the nuclear level was not affected. Dicer is essential for the processing of mature microRNAs from their pre-microRNA form.[52] Normal development of the functioning inner ear is strongly dependent on normal microRNA maturation, and interrupting the microRNA maturation process results in profound inner-ear malformation.[53]

The expression of individual microRNA biogenesis related components has been found to be changed in various human diseases.[54] Therefore, the impact of alterations in AGO2 and Dicer expressions on the differential expression of microRNAs in sudden SNHL patients is worthy of further study.

Conclusion

MicroRNA regulation of gene expression plays a role in the development, differentiation, proliferation, autophagy and apoptosis of cells. In age-related hearing loss, microRNAs in apoptotic pathways, predominantly miR-34 and miR-29 families, are significantly upregulated, while regenerative and developmental pathway microRNAs, namely miR-181 and miR-183 families, are downregulated. MicroRNA autophagy-mediated effects may also contribute to age-related hearing loss. The observed changes in microRNA expression levels are consistent with the sensory hair cell loss and elevated hearing thresholds associated with age-related hearing loss. There is limited evidence that microRNAs have an aetiological role in sudden SNHL.

Competing interests. None declared

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