Published in final edited form as:

Gene. 2019 February 20; 686: 49–55. doi:10.1016/j.gene.2018.10.075.

Role of microRNAs in Inner Ear Development and Hearing Loss

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Abstract

The etiology of hearing loss tends to be multi-factorial and affect a significant proportion of the global population. Despite the differences in etiology, a common physical pathological change that leads to hearing loss is damage to the mechanosensory hair cells of the inner ear. MicroRNAs (miRNAs) have been shown to play a role in inner ear development and thus, may play a role in the development or prevention of hearing loss. In this paper, we review the mechanism of action of miRNAs in the auditory system. We present an overview about the role of miRNAs in inner ear development, summarize the current research on the role of miRNAs in gene regulation, and discuss the effects of both miRNA mutations as well as overexpression. We discuss the crucial role of miRNAs in ensuring normal physiological development of the inner ear. Any deviation from the proper function of miRNA in the cochlea seems to contribute to deleterious damage to the structure of the auditory system and subsequently results in hearing loss. As interest for miRNA research increases, this paper serves as a platform to review current understandings and postulate future avenues for research. A better knowledge about the role of miRNA in the auditory system will help in developing novel treatment modalities for restoring hearing function based on regeneration of damaged inner ear hair cells.

Introduction

Hearing loss affects a significant proportion of human population. The World Health Organization (WHO) report estimates that 466 million people worldwide, of whom 34 million are children, are affected by disabling hearing loss (WHO, 2018). This number is

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Conflict of Interest

The authors declare no conflict of interest.

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estimated to rise to nearly 630 million by 2030 and to over 900 million people by 2050 (WHO, 2018). Hearing loss can be caused by a number of factors including genetic mutations, age, exposure to therapeutic drugs as well as noise, smoking, adiposity, and chronic diseases, or a combination of these can result in a varying decrease in hearing function (Cunningham and Tucci, 2017). Hearing loss has been classified into three different types, conductive, sensorineural and mixed (Shearer *et al.* 2017). Conductive hearing loss is due to the obstruction in the transmission of sound from outer to inner ear (Edmiston and Mitchell, 2013). Sensorineural hearing loss (SNHL) occurs from damage to the sensory hair cells and/or neural structures in the cochlea and accounts for majority of cases of hearing loss (Walling and Dickson, 2012). When both conductive and sensorineural are present at the same time, this is referred to as 'mixed' hearing loss. Hearing loss can also rarely be caused from damage to the auditory cortex in the brain (Korver *et al.* 2017). Although a number of factors have been implicated in the development of hearing loss, genetics is the most common etiology (Yan *et al.* 2016; Cai *et al.* 2017; Mittal *et al.* 2018; Azadegan-Dehkordiet *et al.* 2018; Liu *et al.* 2018; Li *et al.* 2018; Wang *et al.* 2018). Given the serious impact that hearing loss can have on the cognitive function in patients (Li *et al.* 2014; Roland *et al.* 2016; Contrera *et al.* 2016; Tseng *et al.* 2016), efforts to discover novel causes of, and treatments for, hearing loss has gain an ever-increasing importance in the auditory and neuroscience community.

An avenue for current research efforts in this area pertains to the development and function of the inner ear. Despite the variety in the potential etiologies of hearing loss, the pathological mechanism of action for many of these causes converges at the inner ear where damage to the mechanosensory hair cells and the neurons necessary for auditory signal transmission leads to sensory hearing loss and neural hearing loss, respectively (Zilberstein *et al.* 2012; Cunningham and Tucci, 2017). Damage to the hair cells can result in permanent hearing loss as these cells are not regenerated after their death.

In this manner, the role of microRNAs in the development and function of the auditory system has emerged as a novel area of research. MicroRNAs, or miRNAs, are defined as a group of noncoding small strands of RNA that help to regulate gene expression (Patel and Hu, 2012). Since their discovery in 1993, numerous miRNA that play a role in the proper development of the inner and outer ear have been identified (Lee *et al.* 1993; Lagos-Quintana *et al.* 2001; Appasani 2008; Friedman *et al.* 2009a; Torres *et al.* 2016). Since studies suggest that a majority of proteincoding genes in vertebrates are regulated by miRNAs, the ability of miRNAs to regulate mRNAs in the auditory system merits further investigation (Miranda *et al.* 2006; Friedman *et al.* 2009b). Thus, although hearing loss can be caused by many factors, recently there has been increased interest in the auditory community to decipher the role of miRNAs in inner ear development and hearing loss (Table 1 and Table 2). The aim of this article is to discuss the recent advancements regarding our understanding about the role of miRNA in the inner ear development and hearing loss.

Since studies suggest that a majority of protein-coding genes in vertebrates are regulated by miRNAs, the ability of miRNAs to effect gene expression in the auditory system merits further investigation (Miranda *et al.* 2006; Friedman *et al.* 2009b). Before understanding

their applications in the inner ear, however, it is important to understand the mechanisms of miRNAs with regards to general physiological function.

Mechanism of action of miRNAs

miRNAs have a complex mechanism of function and work to either degrade mammalian mRNA or block protein translation to prevent gene expression. In mammals specifically, miRNA genes are a single gene or clusters of genes that are co-transcribed together as a singular unit (Song and Rossi 2017). miRNAs, containing about 22 nucleotides, are a type of noncoding RNAs (ncRNAs) and help regulate many plant and animal cells, with over 1500 human, mouse, and zebrafish miRNAs identified since March 2009 (www.mirbase.org) (Andreassen and Hoyheim 2017; Cui *et al.* 2017; Papageorgiou and Court 2017; Shi *et al.* 2017; Wang and Cai, 2017; Zhang *et al.* 2017). miRNA genes are generally transcribed with the host protein-coding gene and regulated by a common promoter (Gerlach and Vaidya 2017). The miRNA gene is then transcribed into a long RNA molecule and cut by the Drosha/DGCR8 protein complex to form precursor miRNA (pre-miRNA). This pre-miRNA is later exported from the nucleus to the cytoplasm by the protein Exportin 5. Dicer1 then recognizes the pre-miRNA in the cytoplasm, and excises a fully formed 16-29-nucleotide-long, double-stranded miRNA molecule from the pre-miRNA. Ago2 protein complex then binds to the double-stranded miRNA and incorporates one strand into a riboprotein complex called miRISC (miRNA-induced silencing complex) that aids in gene transcription and translation (Bartel 2004; Song and Rossi 2017). The other strand is released and degraded. The RISC complex can either block protein translation or degrade mRNA strands (Figure 1), both of these mechanisms can result in gene silencing.

miRNAs within the miRISC complex regulate expression of *cis* complementary sequence genes. miRNA translationally suppresses mRNAs with faulty complementary sequences (Bartel *et al.* 2004; Kenny and Ceman 2016; Li *et al.* 2017). Potentially >60% of vertebrate protein-coding mRNAs are directly attributed to miRNAs, which quite possibly influence all organic cellular processes (Friedman *et al.* 2009b; Gerlach and Vaidya 2017). The seed sequence (nucleotides 2-8) of the mature miRNA is a key determinant of which mRNAs will be regulated by each miRNA. Mammals have most of these targeted mRNAs, which contain complementary sequences in their 3'-UTRs (three prime untranslated region: section of mRNA following translation termination codon that influences mRNA efficiency, localization, and stability). The binding of a miRNA to the 3'-UTRs suppresses mRNA translation, with more binding sites in turn leading to greater suppression (Bartel 2004; Feig *et al.* 2015). miRNAs can also reverse downregulation and instead upregulate translation of target mRNAs, depending on the stage of the cell cycle (Vasudevan *et al.* 2008). miRNA binding sites can also occur at other regions, like the 5'UTRs, where the effect of miRNAs may vary with respect to location (Ørom *et al.* 2008). miRNAs are imperative for proper development in organisms, as deleterious changes to miRNA can lead to a lack of viability in the embryonic stage (Crowley *et al.* 2015; Azevedo-Pouly *et al.* 2017). Studies in zebrafish have demonstrated how miRNA influences brain formation, physiological changes, heart processes, and other variations of organogenesis (Giraldez *et al.* 2005; Schier and Giraldez 2006; Takacs and Giraldez 2016; Bhattacharya *et al.* 2017; Liu *et al.* 2017).

Although hearing loss can be caused by many factors, recently there has been increased interest in the auditory community to decipher the role of miRNAs in inner ear development and hearing loss (Table 1), and a number of miRNAs have been demonstrated to play a significant role in the inner ear. Besides genetics, epigenetic modifications that regulate the expression of genes such as those coding for miRNAs have also been implicated in the pathophysiology of hearing loss (Friedman and Avraham 2009). Epigenetic modification through miRNAs play a crucial role in the formation and survival of mammalian organs and tissues as well as in the pathogenesis of human diseases (Bianchi *et al.* 2017; Elia and Quintavalle, 2017; Rastgoo *et al.* 2017; Wang *et al.* 2017).

miRNAs in inner ear development

miRNAs in the inner ear area were first investigated in zebrafish model, where embryos coexpressed three miRNAs (miR-182, miR-96, and miR-183) (Wienholds *et al.* 2005) (Table 2). These three miRNAs are also called the miR-183 family, as they are normally transcribed together as a polycistronic (multiple polypeptides can be coded separately within a RNA molecule) gene. miR-182 and miR-183 share similarities, though miR-96 is not always a part of their coexpression (Mahmoodian-sani *et al.* 2016). The miR-183 family is expressed in hair cells, lateral line neuromasts, sensory cells in the eye and nose, the cranial ganglia, and the epiphysis (Li *et al.* 2010).

Similar to zebrafish development, mice exhibit similar patterns of cochlear differentiation during developmental stages (Patel and Hu, 2012). In the cochlea and vestibule specifically, the miR-183 family proves highly relevant to mouse inner-ear development, beginning from the otic vesicle when invaginated from the otic placode (Sacheli *et al.* 2009; Soukup *et al.* 2009). After mice birth, cochlear miRNA expression becomes restricted to hair cells and the spiral ganglion, where after cochlear maturity, it becomes even more limited in hair cells (Sacheli *et al.* 2009). After cochlear maturity, miRNA expression is found in other cochlear tissues like the inner sulcus and spiral limbus. Such a radical transition of miRNA expression suggests that miRNA is strongly relevant for cell differentiation and eventual maturation. On par with these findings, exclusive deletion of the miR-183 group led to dysfunctional sensory epithelia in mice, including the organ of Corti highlighting the crucial role of miRNAs in the inner ear (Fan *et al.* 2017). However, while knockout of the miR-183 cluster led to deleterious effects in mice, overexpression of the miR-183 family in the cochlea of chicken embryos did not result in ectopic hair cells beyond sensory domains nor any obvious changes in hair cell morphologies within the sensory domain, outside of a mild bias towards hair cell fate within the middle of the sensory basilar papilla (Zhang *et al.* 2015).

Another study in 2006 revealed the function of miR-9 as a potential regulator of *COL9A1*, a gene responsible for producing a component of hyaline cartilage (Sivakumaran *et al.* 2006). From that point forward, studies have been published linking various miRNAs to autosomal dominant hearing loss, cholesteatoma growth, regulation of the number of hair cells in the inner ear, and other pathologies of the auditory system (Friedland *et al.* 2009; Li *et al.* 2009; Mencía *et al.* 2009). Furthermore, certain miRNAs seem to be necessary for normal regulation of inner ear tissue differentiation and maintenance (Friedman *et al.* 2009a). Above

all, from the research published thus far on miRNAs' role in the auditory system, we can conclude that there is strong evidence linking miRNA to the proper development of the inner ear, with improper miRNA function leading to hearing loss in both animal models and humans.

Further research on the exact expression trajectory, however, remains inconclusive. While the miR-183 family is expressed in the inner ear and neuromasts of vertebrates--in particular cases specifically in sensory cell, miR-183 RNAs may also be expressed in nonciliated and supporting cells for nematodes (Pierce *et al.* 2008). They may also be found in additional organs such as the submandibular gland in postnatal mammals (Jin *et al.* 2009). For the miRNAs in the mouse inner ear in particular, microarrays can be used to compare profiles of mature miRNAs in cochleae and vestibules within mice pups (Friedman *et al.* 2009a; Friedman and Avraham 2009). Differences in miRNAs among the epithelia among these systems prove relatively sparse, with no difference in average expression levels regardless of different types of cells. The miRNA microarray does indicate the existence of over 100 forms of miRNA within the inner mouse ear.

miRNA expression in the postnatal and the developed inner ear

When miRNAs in embryonic inner ears are compared to postnatal miRNAs, more significant differences may be observed (Weston *et al.* 2006). A study selected six miRNAs (miR-15a, miR-18a, miR-30b, miR-99a, miR-199a-3p, and miR-182) to follow their expression levels for inner-ear development from embryonic day (E) 16.5 to postnatal day (P) 30 (Friedman *et al.* 2009a). By using the more precise and in-depth qRT-PCR assay, it was found that some miRNAs had highest expression levels in embryonic inner ears while others peaked in postnatal, developed ears thus suggesting that microarrays may fail to emphasize more subtle differences. The study also reported that each miRNA has a unique function in inner ear, depending on where and when miRNA was expressed. For example, MiR-30b is expressed in cochlear hair and supporting cells in the newborn mouse cochlea, while only expressed in vestibular hair cells in the adult animals. Additionally, use of the qRT-PCR and Western Blot techniques led to the discovery of the relationship of miR-194 and neuron morphogenesis in inner ear development via RhoB-mediated F-actin rearrangement (Du *et al.* 2017).

To further explore the role of miRNAs in the cochlea, a study created viable offspring that do not express miRNAs in the inner ear through knocking out *Dicer 1*. The cre-loxP recombination system was used to knock out *Dicer 1* conditionally in the developing mouse inner ear, discarding *Dicer1* in cells expressing Cre enzyme. The inner ear sensory epithelia showed normal morphology at E18 and by P38, signs of degeneration were observed (Friedman *et al.* 2009a; Friedman and Avraham 2009). Recently, a study determined miRNA expression in the cochleovestibular ganglion (CVG), neural crest (NC), and otic vesicle (OV) from paraffin embedded human specimens in the Carnegie developmental stages 13–15 employing HTG miRNA whole transcriptome assays (Chadly et al. 2018). There was distinct miRNA expression profile in the CVG, NC and OV. There was differential expression of miR-183 family (miR-96, miR-182, and miR-183) in the CVG compared to NC and OV at Carnegie developmental stages 13. However, due to very small sample size,

the results of this study should be assessed with great caution. Further studies using large sample size will shed light on miRNA expression and their precise role in the human inner ear.

The role of miRNAs in regulating genes expressed in the inner ear including *Prps1* have also been explored. Expression of *Prps1* in murine vestibular and cochlear hair cells has been demonstrated using *in situ* hybridization (Liu *et al.* 2010). It was observed that continuous expression of *Prps1* occurs in hair cells while only postnatal expression occurs within the spiral ganglion. A study demonstrated that only unedited pri-miR-376 RNAs can be detected in the cochlea (Yan *et al.* 2012), proposing the notion that *Prps1* activity in the inner ear may be insensitive to regulation by miR-376. It has also been observed that levels of miR-24-3p, miR-16-5p, miR-185-5p and miR-451a are upregulated in response to loud noise while both up and de-regulation of pro-cell death miRNAs can induce organ of Corti degeneration (Mahmoudian-sani *et al.* 2017). In great similarity, miR-124 was found to promote neuron differentiation and inner ear stem cell growth, where proteins TrkB and Cdc42 are prominently involved (Jiang *et al.* 2016).

Mutations in a noncoding microRNA (miRNA) gene, MIR96, which is expressed specifically in inner ear hair cells, were linked with progressive autosomal dominant postlingual hearing loss (DFNA50) in humans as well as hearing impairment in Dmdo mice (Mahmoodian-Sani *et al.* 2016; Lewis *et al.*, 2009). The locus DFNA50 segregated in a Spanish family with postlingual, progressive, NSHL at all frequencies (Modamio-Hoybjor *et al.* 2004). Further analysis revealed a mutation in miR-96 (+13 G>A), causing replacement of the fourth nucleotide within the conserved seed region of the mature miR-96 sequence, which segregated with the hearing impairment in the family (Mencia *et al.* 2009). Another mutation in the seed region of miR-96 (+14 C>A) was found in another Spanish family with autosomal dominant, progressive, high-frequency hearing loss. Notably, this is the first report showing a nucleotide substitution in the seed region of a miRNA that is causing a human Mendelian disorder (Mencia *et al.* 2009).

Furthermore, additional miRNAs were found to have essential roles in the development and survival of inner ear hair cells. Over 100 miRNAs can be linked to epigenetic regulation, whereas DNA methylation modifies roughly only 50% (Rudnicki *et al.* 2014a). Indeed, transcriptomic analysis of inner ears presents a platform for understanding epigenetic modifications that regulate particular miRNAs (Smeti *et al.* 2012; Rudnicki *et al.* 2014b). miRNAs were shown to manipulate the enzymatic stimulators of epigenetic alterations (Iorio *et al.* 2010). An additional miRNA, *miR-210*, was found to be involved in auditory hair cell regrowth when overexpressed (Riccardi *et al.* 2016). It is also pivotal for cell reactions involving metabolism, DNA repair, and cell development (Chan and Loscalzo 2010). More recently, the epigenetic modifier DNMT3A was confirmed as upstream to one of the precursor otic genes *Gbx2*, and is subsequently imperative for formulation of early stage otic placodes (Roellig and Bronner 2016).

Moreover, it has been recognized that Cx26, a protein responsible for congenital deafness, reinforces the K⁺-recycling defect hypothesis where inner ear gap junctions are distorted and the pathway to the cochlea deteriorated (Mittal *et al.* 2017; Zhao 2017). As such, the

question of miRNA intercellular transfer influence is raised, highlighting the paucity of data on cochlear developmental disorders. A study supports interest in miRNA-34a overexpression, which potentially limits the occurrence of sirtuin1 (SIRT1) proteins. With this, there possibly exists a deleterious factor in miR-34a regarding hearing loss treatment associated with Type 2 diabetes (Lin *et al.* 2017). Congruently, there exists significant evidence that miR-29b/SIRT1 signaling is directly responsible for the process of ARHL, as it contributes to mitochondrial degradation within HEI-OC1 cells that are pivotal to an inner ear cell line (Xue *et al.* 2016). In summary, miRNAs play a pivotal role in the auditory system, and further studies are warranted to decipher their role in hearing loss.

Conclusions and Future Directions

Hearing loss is a prevalent disability that affects an ever-increasing number of people around the world. Regulation of genes via miRNAs has been shown to be necessary for proper development of various tissues and organ systems in many animal models. Given that the proper development and function of the inner ear is necessary for the ability to hear, miRNAs that play a role in this development are focal points for further study and examination for the role they play in hearing loss. While the miRNA microarray has been used to discover over 100 miRNA forms in the inner ear of the mouse model, the use of the qRT-PCR assay has allowed researchers to measure the expression of different miRNAs at various embryonic and postnatal stages. This has allowed us to discover that miRNAs, such as the miR-183 family, mir15a, miR-30b and many others, each have a unique function in the inner ear depending on when and where the miRNA is expressed. Knocking out the expression of miRNAs in the inner ear of a mouse model showed signs of degeneration in the sensory epithelia of the mouse inner ear, revealing the potentially causal link between inadequate expression of miRNA and hearing loss (Fan *et al.* 2017).

Thus, it naturally follows that mutations in miRNAs have the potential to contribute to improper development of the inner ear and subsequent hearing loss. Study of mutations in miR-96 and expression levels of miR-210, miR-34a, and miR-29b, have contributed to additional understanding of the unique roles each of these miRNAs play in either promoting or preventing hearing loss. miR-34a, miR-29b, miR-299-3p, and miR-431 can be used to improve the sensitivity and specificity of measures trying to identify the damage affecting inner ear cells and central hearing pathways in patients with MELAS (myopathy, encephalopathy, lactic acidosis, and stroke like episode syndrome) (Di Stadio *et al.* 2018). Future research should continue study of the over 100 unique miRNA that play a role in epigenetic regulation of the genes that contribute to inner ear development. The findings of current studies lead us to believe that each of these miRNAs may have their own unique influence on the development of the inner ear, particularly the mechanosensory hair cells of the cochlea. Thus, an understanding of the mechanism of action of miRNA may help us not only to identify the specific miRNA that can be mutated, under-expressed, or over-expressed and contribute to hearing loss, but we may also identify the preceding causal factors that led to this erroneous behavior in the first place. Furthermore, miRNAs such as miR-210 have shown promising ability to regenerate auditory hair cells (Riccardi *et al.* 2016). This is of crucial importance, as many causes of hearing loss, whether they be miRNA related or non-miRNA related, seem to share the damage of these hair cells. Thus, future research aimed at

not only identifying the miRNAs that can lead to hearing loss when damaged or improperly expressed, but also identifying the miRNAs that could potentially be used to restore hearing will gain increasing relevance in the future. A better knowledge about the signaling pathways involved in hair cell regeneration regulated by miRNAs will pave the way for developing novel treatment modalities for hearing loss.

Acknowledgements

This research work in Dr. Liu's Laboratory is supported by grants R01 DC005575, R01 DC01246, and R01 DC012115 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

Abbreviations

miRNAs	MicroRNAs
WHO	World Health Organization
ncRNAs	noncoding RNAs
CVG	cochleovestibular ganglion
NC	neural crest
OV	otic vesicle

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Highlights

- MicroRNAs (miRNAs) plays a crucial role in inner ear development.
- Abnormal function of miRNAs in the auditory system has been associated with hearing loss.
- A better knowledge about the signaling pathways involved in hair cell regeneration regulated by miRNAs will pave the way for developing novel treatment modalities for hearing loss.

Figure 1: Topographical representation of mRNA suppression by miRNAs.

First, DNA polymerase II transcribes the noncoding miRNA sequence (1) and forms the Pri-mRNAs, which are bound to by the Drosha/DGCR8 complex (2). Once cleaved by the Drosha/DGCR8 complex, the individual hairpins are exported from the nucleus into the cytoplasm by the Exportin 5 - RanGTP complex (3). Once in the cytoplasm, Dicer binds to the hairpin pre-MiRNA and cleaves it into individual pieces (4). Half of these pieces (shown in pink) are degraded by intracellular processes, while the other half (shown in orange), binds to a complex of proteins to form the RISC protein complex (5). This complex can then either bind to mRNA strands to prevent translation of proteins or can facilitate degradation of mRNA, both of which facilitate gene silencing.

Table 1:

A summary of miRNAs that play a crucial role in the inner ear.

miRNA	Targeted Gene Expression and Effects	Function and Influences	References
miR-9	COL9A1 (Collagen Type 9 Alpha 1 Chain)	Encodes an alpha chain of type 9 collagen	Sivakumaran et al., 2006
miR-224	Ptx3 (Pentraxin 3)	Encodes member of pentraxin protein family	Rudnicki et al., 2014a Yao et al., 2014
miR-183	Taok1 (TAO Kinase 1)	Transferase, transporting phosphorus-containing groups and protein tyrosine kinase inducement	Li et al., 2010
miR-34a	Sir1 (Saccharomyces cerevisiae 1), E2F trans. factor 3, B-cell lymphoma-2	Heterochromatic silencing of chromatin	Mencia et al., 2009
miR-96	TRK (tyrosine kinases) receptor, epidermal growth factor receptor	Regulates synaptic strength and plasticity in nerves	Chen et al., 2014
miR-182	Tbx1 (T-box protein 1)	Transcription factors that lead to tissue and organ formation	Wang et al., 2012
miR-376	Pps1 (protein phosphatase S 1)--non-syndromic to syndromic hearing deficiency	DNA synthesis phase of cell cycle	Yan et al., 2012
miR-140	NR2F1 (nuclear receptor subfamily 2 group F member 1), Klf9 (Krueppel-like factor 9)	Transcription factoring, sequence-specific DNA binding and coactivator activation	Rudnicki et al., 2014b
miR-96/-182	CLIC5 (chloride intracellular channel 5) 3'-UTR	Hair cell stereocilia formation, myoblast proliferation, cell maintenance	Kuhn et al., 2011
miR-135b	PSIP1-P75	Neuroepithelial cell differentiation and neurogenesis	Elkan-Miller et al., 2011
miR-29b	SIRT1 (NAD-dependent deacetylase sirtuin-1)/PGC-1 α (gamma coactivator-1 α)	Transcription regulation of cellular metabolism, regulation of mitochondrial genesis	Xue et al., 2016
miR-21	Cholesteatoma growth, vestibular schwannomas	Destructive growth in middle ear/mastoid part of temporal bone, intracranial tumor of nerve	Mahmoudian-sani et al., 2017

Table 2:

A summary of expression profile and molecular mechanisms through which miRNA exert effect on the auditory system.

miRNA	Summary of Study	Reference
miR-182, miR-96, and miR-183	One of the first studies of miRNAs in the inner ear, in which zebrafish embryos showed of miR-183 family co-expression of these three miRNAs.	Wienholds et al., 2005
miR-182, miR-96, and miR-183	Cochlear miRNA expression becomes restricted to hair cells and the spiral ganglion after mice birth. After cochlear maturity, miRNA expression becomes even more limited in hair cells.	Sacheli et al., 2009
miR-182, miR-96, and miR-183	Exclusive deletion of the miR-183 group leads to dysfunctional sensory epithelia in mice, including the organ of Corti.	Fan et al., 2017
miR-182, miR-96, and miR-183	Overexpression of the miR-183 family in the cochlea of chicken embryos did not result in ectopic hair cells beyond sensory domains nor any obvious changes in hair cell morphologies within the sensory domain, outside of a mild bias towards hair cell fate within the middle of the sensory basilar papilla.	Zhang et al., 2015
miR-9	Evidence that miR-9 could be a potential regulator of <i>COL9A1</i> , a gene responsible for producing a component of hyaline cartilage.	Sivakumaran et al., 2006.
miR-15a, miR-18a, miR-30b, miR-99a, miR-199a-3p, and miR-182	Study showing some miRNAs had highest expression levels in embryonic inner ears while others peaked in postnatal, developed ears. It also reported that each miRNA has a unique function in inner ear, depending on where and when each was expressed.	Friedman et al., 2009a
miR-194	Discovery of the relationship of miR-194 and neuron morphogenesis in inner ear development via RhoB- mediated F-actin rearrangement.	Du et al., 2017
miR-182, miR-96, and miR-183	Study showing there was distinct miRNA expression profile in the cochleovestibular ganglion (CVG), neural crest (NC), and otic vesicle (OV) in whole human transcriptome assays. There was differential expression of miR-183 family in the CVG compared to NC and OV at Carnegie developmental stages 13.	Chadly et al., 2018
pre-miR-376 RNAs	Showed pre-miR-376 RNAs can be detected in the cochlea proposing the notion that Prps 1 activity in the inner ear may be insensitive to regulation by miR-376.	Yan et al., 2012
miR-24-3p, miR-16-5p, miR-185-5p and miR-451a	Upregulated in response to loud noise while both up and de-regulation of pro-cell death miRNAs can induce organ of Corti degeneration.	Mahmoudian-sani et al., 2017
miR-124	Study that showed miR-124 promotes neuron differentiation and inner ear stem cell growth, with involvement of proteins TrkB and Cdc42.	Jiang et al., 2016
microRNA (miRNA) gene, MTR96	Showed mutations in a noncoding miRNA gene, MIR96, which is expressed specifically in inner ear hair cells, were linked with progressive autosomal dominant post-lingual hearing loss (DFNA50) in humans as well as hearing impairment in mice.	Mahmoudian-Sani et al., 2016
miR-210	Study that showed miR-210 was found to be involved in auditory hair cell regrowth when overexpressed.	Riccardi et al., 2016