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REVIEW ARTICLE

MicroRNAs: effective elements in ear-related diseases and hearing loss

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Abstract miRNAs are important factors for post-transcriptional process that controls gene expression at mRNA level. Various biological processes, including growth and differentiation, are regulated by miRNAs. miRNAs have been demonstrated to play an essential role in development and progression of hearing loss. Nowadays, miRNAs are known as critical factors involved in different physiological, biological, and pathological processes, such as gene expression, progressive sensorineural hearing loss, age-related hearing loss, noise-induced hearing loss, cholesteatoma, schwannomas, and inner ear inflammation. The miR-183 family (miR-183, miR-96 and miR-182) is expressed abundantly in some types of sensory cells in inner ear specially mechanosensory hair cells that exhibit a great expression level of this family. The plasma levels of miR-24-3p, miR-16-5p, miR-185-5p, and miR-451a were upregulated during noise exposures, and increased levels of miR-21 have been found in vestibular schwannomas and human cholesteatoma. In addition, upregulation of pro-apoptotic miRNAs and downregulation of miRNAs which promote differentiation and proliferation in age-related degeneration of the organ of Corti may potentially serve as a helpful biomarker for the early detection of age-related hearing loss. This knowledge represents miRNAs as promising diagnostic and therapeutic tools in the near future.

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Introduction

Hearing loss also known as silent disability affects over 275 million people worldwide most of which live in low- and moderate-income countries. The number of people with debilitating from mild to severe hearing impairment has been estimated to be more than 360 million [1]. Social problems due to hearing impairment include heavy socio-economic costs imposed on families, delayed acquisition of cognitive and verbal skills, and education achievement-related problems in children, as well as difficulties with finding or keeping job in adults. Meanwhile, costs of education of children with special needs can cause a great national economic burden. The main causes of hearing loss include infectious diseases, such as meningitis, measles, mumps, and chronic ear infections, exposure to noise, damage to ear and head, aging, and taking certain drugs with toxic effects. Hearing loss and hearing impairment can be prevented or, through the early diagnosis and appropriate management, treated (by surgery, use of certain instruments, such as hearing aid, and cochlear implant) in half of the cases [2].

The genome of eukaryotes consists of both coding and non-coding DNA. Coding DNA consists of all open reading frames (ORFs) and exons that are transcribed to RNA sequences and even translated to protein sequences. However, the function of non-coding regions, including introns has not fully understood, although some regulatory roles have been observed for introns [3].

Another important factors are miRNAs which were discovered and found to be involved in regulating expression

and function of protein-coding RNAs by Lee and co-workers in 1993 [4]. These small non-coding RNAs [4] are usually 22- to 42-nucleotide long and are highly conserved in plants and animals. These regulatory elements are evolutionarily ancient component for the process of gene regulation [5]. These molecules regulate the expression of over 30% of genes in different biological processes, such as proliferation, differentiation, survival, migration, apoptosis, and death [6].

Cellular reprogramming occurs during the development of eukaryotic organisms via both extracellular [7] and intracellular agents. Among intracellular factors, histone modifications, DNA methylation, and gene expression regulation via microRNAs have been described. microRNAs are found in animals, plants, and some viruses, and they function in silencing and post-transcriptional regulation of gene expression [8]. Most of miRNAs are located within the cell, but some miRNAs are found in extracellular environment, such as serum, biological fluids, and the media of cell culture [9]. microRNAs can silence the gene expression via cleavage of the mRNA strand, shortening of the poly(A) tail, and affecting the mRNA translation into proteins by ribosomes; phenomena that can be predicted using computational biology [10].

To date, over 2500 human miRNAs have been identified [11]. Many pathological processes, such as cardiovascular and neurological diseases, diabetes, and cancers are associated with abnormal expression of miRNAs [12, 13]. Interestingly a number of studies have pointed out the importance of miRNAs in hearing-related diseases. For instance, there are evidences that miRNAs can be used as biomarker to diagnose noise-induced and age-related hearing loss [20, 21]. In this work, we have reviewed the role of microRNAs in ear-related diseases and hearing loss. We have studied the current evidences on the role of miRNAs in inner ear evolution and recent research accomplishments and developments on the role of miRNAs in inner ear biology and pathogenesis.

Biogenesis of MicroRNAs

Biogenesis of miRNAs occurs in nucleus and cytoplasm. In nucleus, first, RNA polymerase II transcribes pri-miRNAs from either coding or non-coding areas in the genome. pri-miRNAs have a loop-stem structure with poly(A) tail at 3′-end and CAP at 5′-end. pri-miRNAs are converted to pre-miRNAs by an RNAse III-containing complex which is specialized to digest a double-stranded RNA (Drosha), and a double-stranded RNA-binding protein, DGCR8. pre-miRNAs are then transported to cytoplasm by exportin-5. In cytoplasm, final processing is conducted on pre-miRNA by TRBP/Dicer complex. Dicer function leads to formation of a 31 to 42-nucleotide, double-stranded RNA with a guide strand and a passenger strand. Passenger strand is destroyed and guide strand forms miRNA via binding to RNA-induced silencing complex (mi-RISC) containing members of Argonaute protein family. This complex is complementary to 3′-UTR of target mRNA. If miRNA sequence is completely complementary to target mRNA, target mRNA is destroyed after binding. However, translation is inhibited, in case the miRNA binding to mRNA is incomplete. Each miRNA can target several mRNAs [14–18] (Fig. 1).

MicroRNAs in hearing loss

Little is known about molecular basis of progressive hearing loss. Recently, the potential role of miRNA as a therapeutic agent in regeneration has been demonstrated in research on regulatory factors involved in ear diseases [19, 20]. miRNAs play a very important role in gene expression, physiological and pathological processes, and progressive sensorineural hearing loss (SNHL) which is mainly due to cochlear hair cells defect or loss. Role of miRNAs in pathogenesis of different ear diseases has been studied via detecting genetic and somatic mutations in miRNAs and their binding sites in target genes [21, 22]. Recently, mutations in miR-96 have been demonstrated to be associated with progressive hearing loss in humans and mice. Similar to histone modifications and DNA methylation, miRNAs have been found to play roles in survival and development of organs and cells, such as mechanosensory hair cells [23]. Moreover, miRNAs have been suggested to be essential and new regulatory factors for the formation of induced pluripotent stem cells (iPSCs) and differentiation of these cells into hair cell [24, 25]

MicroRNAs are involved in formation of the inner ear

miRNAs are considered as important factors that widely affect the development of inner ear. Microarray analysis, quantitative real-time PCR, and in situ hybridization have demonstrated that miRNA-182, miRNA-140, miRNA-200c expressions have distinct temporal and spatial patterns at E13.5 (mouse embryonic day 13.5 of development) and E16.5 [26]. mir-96, mir-182, and mir-183 are the well-investigated miRNAs that were first detected in the otic vesicle, cochlea-vestibule ganglion, and cochlear hair cells at E9.5, E11.5, and E17.5, respectively. According to some studies, the expression continued to at least P30 (Postnatal Day 30). The expression of other miRNAs detected by in situ hybridization is shown in Fig. 2 [27].

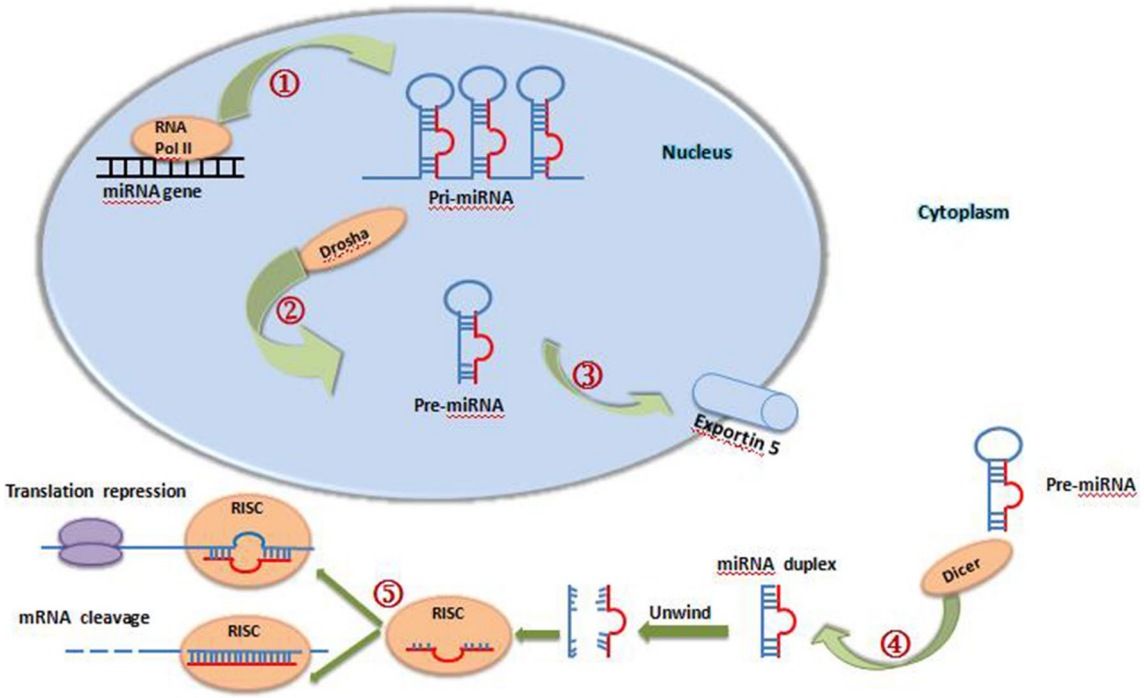

Fig. 1 Biogenesis of miRNA: miRNA biogenesis is a multistep process. First, miRNA genes are transcribed by RNA polymerase II in the nucleus (step 1). The resulting primary transcript is cleaved by Drosha and DGCR8 to produce pre-miRNA (step 2). After exportin-5- and RanGTP-mediated transport to the cytoplasm (step 3), the pre-miRNA undergoes its final processing step, which consists of Dicer-dependent cleavage just below the stem loop to produce a duplex molecule (step 4). The duplex is then separated and usually one strand is selected as the mature miRNA and directed to target-specific mRNAs (step 5)

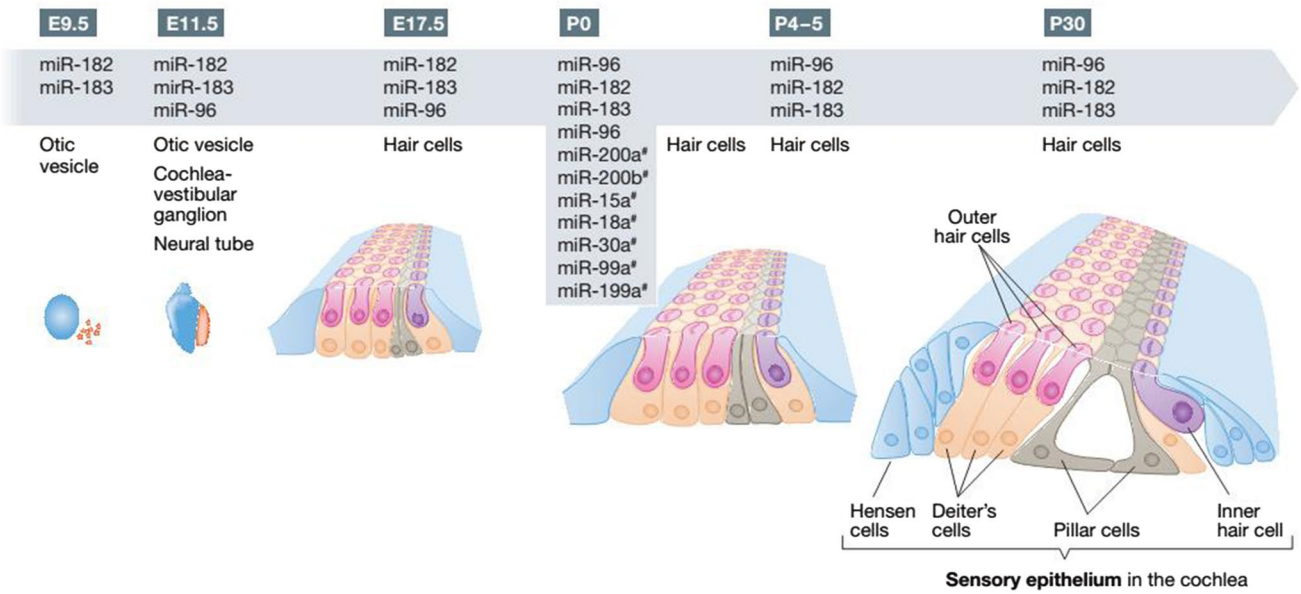

Fig. 2 Timeline for miRNA expression during development and early postnatal stages of the inner ear for a subset of miRNAs (27). Those *marked with numbers* were only examined at the stage indicated

The miRNA-183 family in hair cell development and deafness

miRNA-183 family (96, 182, and 183) is expressed abundantly in specific sensory cell types in the inner ear. They play either distinct or common roles in inner ear cell-fate determination [28]. In vertebrate hair cells, such as zebrafish, the miR-183 family is highly expressed and induces extra and ectopic hair cells. Contrariwise, knockdown causes reduction in numbers of hair cells in the inner ear, smaller spiral ganglion neuron (SGNs), and defects in semicircular canals [29].

Among the members of this family, miR-96 is expressed as a sensory organ-specific miRNA in the mammalian cochlea during development. Indeed, in humans and mice, some types of non-syndromic progressive hearing loss are due to miR-96 mutations [30]. One example is the results of the study conducted by Mencia and co-workers who found that point mutations in the seed region of miR-96 result in progressive hearing loss with autosomal dominant inheritance pattern [31]. Further investigations on the role of a SNP (Single-nucleotide polymorphism) in the seed region of hsa-miR-96 revealed two key biological processes involved in progressive hearing loss. This SNP leads to a significant enrichment of two sets of allele-specific target genes of hsa-miR-96, including neurotrophin in TRK receptor signaling pathway and epidermal growth factor receptor signaling pathway [21]. miRNA profiling, target analysis, and validation suggest that miRNAs (e.g. miR-183/Taok1 target pair) are involved in regulating the degenerative process of the cochlea after acoustic overstimulation [32]. In inner ear hair cells, chloride intracellular channel 5 (CLIC5) expression is regulated by miR-183 family. In other words, CLIC5 3′-UTR contains a highly conserved sequence which is a single predicted miR-96/-182-binding site. This binding site which is located between nucleotides 760–766 is the target where miR-96/-182 can regulated the CLIC5 [33].

MicroRNAs are involved in age-related hearing loss

The main cause of age-related hearing loss (ARHL) is degeneration of the organ of Corti (OC) caused by alteration of miR-29 and miR-34 families that regulate pro-apoptotic pathways. The expression levels of miR-34a exhibit a significant stimulator to increase the cochlea, auditory cortex, and plasma in mice during aging. These increases are accompanied by elevated hearing thresholds and greater hair cells loss. Levels of silent information regulator-1 (SIRT1), B-cell lymphoma-2, and E2F transcription factor 3 as targets of miR-34a may potentially serve as useful biomarkers to detect ARHL early [34]. Moreover, several studies have shown that acetylation of p53 and apoptosis is increased via miR-34a/Sirtuin1 (SIRT1)/p53 signaling pathway in cochlear hair cells during aging. The findings, therefore, suggest a link between age-related apoptosis of cochlear hair cell and miR-34a/SIRT1/p53 signaling, which may provide a potential target for ARHL [35]. Unlike upregulation of this pro-apoptotic miRNA, many miRNA families are important for proliferation and differentiation. Two examples are miR-181 and miR-183 whose downregulation has been reported to be associated with cell death resulting in ARHL [36].

MicroRNA expression profiles in noise-induced hearing loss

There are evidences that noise stimulation or occurrence of noise-induced hearing loss (NIHL) affects miRNA expression. One of the major causes in pathogenesis of NIHL is oxidative stress, because the cochlea is metabolically active [27]. Cellular response to noise exposure can be detected as plasma miRNAs biomarker that may provide new insights in pathogenesis of NIHL. In fact, miRNA target analysis may reveal the pathways involved in NHIL and help to develop new drugs through identification of the cellular stress response components [37]. After noise exposure in animal model with noise-induced deafness, the expression of members of miRNA-183 family exhibits significant increase, which shows that this family members may play significant roles in the pathogenesis and development of NHIL [38]. Moreover, the plasma levels of miR-16-5p, miR-24-3p, miR-185-5p, and miR-451a are also known to be upregulated in noise exposures [39].

MicroRNA in inner ear inflammation

Inflammation of the inner ear can cause several forms of immune-mediated hearing loss. Therefore, understanding the inflammatory pathways and its molecular components is very helpful to develop drugs and treatments for these types of hearing loss [40, 41]. A number of studies have discussed the mechanisms of neuroprotective agents against cytotoxicity [42, 43]. In addition to identification of genetic factors involved in inflammatory hearing loss, miRNAs may contribute to gene expression regulation and affect the outcome of inflammation in ear. The biological effects of miRNAs have been found by ionizing radiation (IR)-induced cell death in auditory cells using miRNA mimics or inhibitors. Studies conducted by Tan and co-workers on house ear institute OC 1 (HEI-OC1) cells revealed that miR-207 is upregulated following IR resulting in IR-induced apoptosis and enhanced DNA damage. Therefore, inhibition or downregulation of miR-207 could be a potential strategy for protecting cochlear hair cells against IR [44]. Another miRNA involved in inner ear inflammation is

miR-224 which is a transcriptional target of the key mediator of innate immunity, nuclear factor kappa B pathway. It is well documented that miR-224 diminishes the innate immune response through downregulating Ptx3 expression that stimulates the innate immune response [40].

MicroRNAs and epigenetics in inner ear

DNA methylation and histone modifications are epigenetic mechanisms that are implicated in human deafness, suggesting that different levels of non-coding genes (such as miRNAs) are required for normal hearing [23]. Sensory hair cells and supporting cells in the auditory organ of the mammalian inner ear arise from a common sensory progenitor. Cell fate in developing of organ of Corti is controlled by miR-124 which is an epigenetic protective agent for two inhibitors of the Wnt pathway, Sfrp4 (secreted frizzled-related protein4), and Sfrp5. These proteins play essential roles in fine-tuning of the expression of genes critical for cell patterning (e.g., Hes1 and Hes5) during cochlear differentiation [23].

microRNA expression in cholesteatoma and schwannoma

The role of miR-21 has been described in some of ear-related diseases, such as human cholesteatoma growth and proliferation, and vestibular schwannomas [45, 46]. Upregulation of miR-21 is implicated in potential harmful growth in the middle ear or in the mastoid bone [45]. Increased levels of miR-21 have been also observed in vestibular schwannomas [46].

Other miRNAs in hearing loss

Experimental analyses of vestibular hair cells have shown that expression of PSIP1-P75 which is a transcriptional co-activator in the inner ear may be regulated by miR-135b. Indeed, some differences between the cochlear and vestibular hair cells are determined by miR-135b which acts as a cellular effector [22]. Furthermore, since protection of SGNs from ongoing degeneration is an essential step to prevent progressive hearing loss, miRNA-base strategies can be designed to prevent or reverse SGN damages. Downregulation of TMPRSS3 (transmembrane protease, serine3) has been reported to be necessary for SGNs development through overexpression of miR-204. Therefore, alteration of miR-204 may serve as a potential therapeutic target in SNHL [47]. Another key regulator in development of hearing organs is miR-6716-3p. This miRNA is involved in actin reorganization, sensory hair cell bundle development, cell adhesion, and inner ear morphogenesis [48]. Since miRNAs can regulate important signaling pathways, it is not surprising that they are also involved in cytotoxicity. Indeed, recent findings have suggested a role of miR34a and miR34c in antibiotic-induced ototoxicity in a dose-dependent manner in cochlear cells [42].

MicroRNA editing and transfer

In addition to all above-mentioned roles for various miRNAs, an important factor affecting the function of miRNAs is their proper trafficking and transfer. An example is a membrane protein Cx26. This protein that is required for cochlear development is responsible for intercellular communication, including miRNA intercellular transfer between native cochlear supporting cells. Deficiency of Cx26 disrupts miRNA intercellular transfer in the cochlea and leads to cochlear developmental disorders and congenital deafness [49]. In addition to proper transit, all miRNAs require a fully functional editing system in the cell; therefore, any failure in editing may result in vigorous outcome in organ development. For instance, a spectrum of non-syndromic to syndromic hearing loss is associated with mutations in phosphoribosyl pyrophosphate synthetase 1 (PRPS1), due to the failure of unedited pri-miR-376 cluster (miR-376a-3p, b-3p, c-3p) in regulating the activity of PRPS1 in the inner ear [50] (Table 1).

Discussion

To develop effective therapeutic strategies to treat hair cell damage, a fundamental and significant step is to identify regulatory mechanisms involved [32]. Recently, a common mechanism has been demonstrated to be involved in evolution and reprogramming of inner ear cells in a number of different species of vertebrate. The regulatory role of miRNAs, particularly miRNA-183 family, and certain genes, such as GFI1, POU4F3, and ATOH1, has been confirmed in differentiation of stem cells into hair cells and protection of hair cells [24]. Moreover, since hundreds of transcripts may be regulated simultaneously by the same miRNA, miRNAs can certainly be considered as potential therapeutic agents to repair or regenerate hair cells at least in animal models [41]. Similarly, since a large proportion of human transcriptome is regulated by miRNAs, they can be used in diagnosis and prognosis as well as development of drugs. Despite advances in the use of cochlear implant to treat SNHL, the rate of hearing improvement is not fully satisfying in people undergoing this therapy. The combination of molecular-based methods and stem cell therapies can be a promising alternative to cochlear implant. Supporting cells differentiation into hair cells that does not occur under normal conditions may theoretically be induced in adult mammals by alteration and modification of miRNAs level in the

Table 1 Summarization of the evidences and validation of miRNA-gene targets found in the inner ear

miRNA	Target genes	References
miR-96	Neurotrophin TRK receptor and epidermal growth factor receptor (two key biological processes for progressive hearing loss)	[17]
	Slc26a5, Ocm, Gfi1, Ptprq and Pitpnm1, Ptprq. Ptprq, a gene which is considered to be important for maturation and maintenance of hair cells	[47]
miR-140	Nuclear receptor subfamily 2 group F member 1 (NR2F1), Klf9	[48]
miR-135b	PSIP1-P75. a transcriptional co-activator in the inner ear	[18]
miR-96/-182	CLIC5 3′-UTR	[30, 49]
miR-124	Secreted frizzled-related protein 4 (Sfrp4) and Sfrp5, two inhibitors of the Wnt pathway	[49]
miR-204	TMPRSS3 (transmembrane protease, serine 3) involved in development of SGNs	[43]
miR-34a targets	Silent information regulator 1, B-cell lymphoma-2, and E2F transcription factor 3	[31]
miR-183	Taok1	[29]
miR-224	Ptx3	[37]
miR-9	COL9A1	[50]
miR-96	Osbpl2	[51]
miR-182	Tbx1, a transcription factor that has been implicated in inner ear development and hair cell fate	[52]
miR-376	Phosphoribosyl pyrophosphate synthetase 1 are associated with a spectrum of non-syndromic to syndromic hearing loss	[46]

cells as well as administration of their transit and cellular trafficking. Molecule-based therapies using anti-miRNA LNA (miravirsen) and mimic miRNA (MRX34) are being investigated in clinical trials, but most of miRNA-based therapies are being studied in preclinical studies [17]. Recently, circulating miRNAs have attracted attention, because they are considered to be frequent and stable biomarkers in serum and plasma. Further studies are required to shed light onto the role of miRNA profile alteration during the hearing loss process before miRNAs can be used as a biomarker to diagnose hearing loss [34, 53–57]. Briefly, in the near future, the patient's serum miRNAs levels might be used, as molecular markers, for diagnosis and prognosis of hearing loss. Moreover, they are potential therapeutic agents for repair or regenerate hair cells, cell reprogramming, and regenerative medicine.

Conclusion

Efforts are being made to offer an appropriate molecular marker with capability to predict hearing loss or even being used as a diagnostic and prognostic agent alongside other pathological or clinical approaches. Among them, miRNAs have been demonstrated to become dysfunctional during hearing loss and play essential roles in the development and progression of this disorder. miRNAs have the potential to be used in treating hearing loss. Considering the nature of miRNAs and the fact that most common approaches to screen hearing loss at the early stages fail to diagnose this disorder, it is a highly promising strategy to study and identify circulating miRNAs as molecular markers for progression of hearing loss due to any causes, such as ARHL, NIHL, inflammation, Schwannomas, and cholesteatoma.

Compliance with ethical standards

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Conflict of interest Authors declare no conflict of interest for the manuscript.

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