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REVIEW

Non-coding RNAs in the development of sensory organs and related diseases

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Abstract Genomes are transcribed well beyond the conventionally annotated protein-encoding genes and produce many thousands of regulatory non-coding RNAs (ncRNAs). In the last few years, ncRNAs, especially microRNAs and long non-coding RNA, have received increasing attention because of their implication in the function of chromatin-modifying complexes and in the regulation of transcriptional and post-transcriptional events. The morphological events and the genetic networks responsible for the development of sensory organs have been well delineated and therefore sensory organs have provided a useful scenario to address the role of ncRNAs. In this review, we summarize the current information on the importance of microRNAs and long non-coding RNAs during the development of the eye, inner ear, and olfactory system in vertebrates. We will also discuss those cases in which alteration of ncRNA expression has been linked to pathological conditions affecting these organs.

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Abbreviations

lncRNA	Long non-coding RNA
miRNAs	microRNAs
NATs	Natural antisense transcripts
ncRNAs	Non-coding RNAs
NFAT	Nuclear factor of the activated T cells
NGS	Next-generation sequencing
OB	Olfactory bulb
OE	Olfactory epithelium
OMP	Olfactory marker proteins
PRC2	Polycomb repressive complex 2
RGC	Retinal ganglion cells
RNCR2	Retinal non-coding RNA 2
RPE	Retinal pigment epithelium
Uchl1	Ubiquitin carboxy-terminal hydrolase L1

Introduction

The continuous improvement of high-throughput technology is providing constantly evolving information on the structure and function of the genome of different species and a high-resolution map of their transcriptional landscape. The resulting picture shows that genomes are at the same time complex and flexible [1], perhaps with the surprise that they are transcribed well beyond the conventionally annotated protein-encoding genes. Indeed, genomes produce many thousands of regulatory non-coding RNAs (ncRNAs). These includes "housekeeping" ncRNAs transcripts (ribosomal RNA, transfer RNA, small nuclear RNA, and small nucleolar RNA), and "regulatory ncRNAs", including long non-coding RNAs, microRNAs,

Table 1 Examples of classes of non-coding RNAs

Class	Description	References
A) Small ncRNAs		
miRNAs	Small ncRNAs involved with RISC complex in post-transcriptional silencing of protein-coding genes	[182, 183]
Piwi-interacting RNA (piRNA)	Small-ncRNA often linked to both post-transcriptional and epigenetic gene silencing of retro-transposons	[184]
tiRNAs	Tiny RNAs associated with transcription start sites	[185]
Enhancer-like long ncRNAs	lncRNAs displaying enhancer activity, mainly for genes regulating development and differentiation	[186]
snoRNAs	Small nucleolar RNAs implicated principally in directing chemical modifications of other RNAs	[187]
PROMPTs	Promoter upstream transcripts located upstream of active transcription start sites	[4]
TSSa-RNAs	Transcription start site-associated ncRNAs probably linked to the maintenance of gene transcription	[188]
B) Long ncRNAs		
T-UCR	Non-coding RNAs transcribed from ultra-conserved regions	[189]
NATs	Natural antisense transcripts located in intergenic regions and organized in antisense with protein-coding genes	[190]
lincRNAs	Long intergenic non-coding RNAs generally associated post-transcriptional silencing by blocking protein production	[191]
ceRNAs	Competing endogenous ncRNAs involved in modulating RNA transcripts by competing for microRNAs	[56]

piRNAs, natural antisense transcripts (NATs), [1–3] and several other poorly characterized ncRNAs that derive from the transcription of expression control sequence elements [4, 5]. Regulatory ncRNAs display dynamic spatial and temporal expression profiles in specific cellular contexts and contribute to tissue patterning and to the control of different cellular programs, such as cell proliferation, differentiation, migration, or apoptosis [6–8]. Table 1 lists a few examples of the main classes of transcripts that have been ascribed to the ever-growing group of regulatory ncRNAs.

ncRNAs have received increasing attention because of their implication in the function of chromatin-modifying complexes and in the regulation of transcriptional and post-transcriptional events [3]. In this review, we will summarize the evidence that ncRNAs contribute to regulate the development and survival of sensory tissues development, paying particular attention to microRNAs and lncRNAs.

Biogenesis and function of non-coding RNAs

MicroRNAs (miRNAs) are a class of small endogenous, single-stranded, non-coding RNAs [9] that were first identified with classical genetic approaches as regulators of *Caenorhabditis elegans* development [10, 11]. Their existence in eukaryotic organisms was thereafter determined on the basis of sequence conservation across species [12]. miRNAs are transcribed as longer primary transcripts (pri-miRNAs) and processed first into ~70-nucleotide (nt)-long precursors (pre-miRNAs) and then into 20–25 nt mature miRNAs by the subsequent activity of two RNAse III-like endoribonucleases, namely Drosha and Dicer [13]. The best-studied function of mature miRNAs is the control of gene dosage at the post-transcriptional level [14–16]. miRNAs bind specific proteins with catalytic activity, called Argonaute, and form a miRNA-induced silencing complex that impairs either translation or mRNA stability by binding, with imperfect base pairing, to specific sites of the 3′-untranslated regions (3′ UTRs) of their target mRNAs [17]. Occasionally, they have being found also binding to the coding regions or the 5′ UTRs of mRNAs [18–20]. In transcriptional repression events, miRNAs guide heterochromatin formation at promoter regions by both imperfectly and perfectly matched double-stranded RNA–DNA interaction [21, 22]. Notably, miRNAs have been also detected as extracellular nuclease-resistant entities, enclosed in small exosomal vesicles or packaged with RNA-binding proteins [23]. These forms of miRNAs have been proposed to act as secreted signaling molecules that bind and activate receptors to influence the phenotype of the recipient cells [24].

Long non-coding RNAs (lncRNAs; Table 1) are molecules longer than 2 kb, with a coding potential of less than

100 amino acids [2, 25–29]. The biological relevance of these transcripts is supported by their developmental regulation [30–32], cell-specific expression pattern [33, 34], sub-cellular distribution [35–38], and possible association with human diseases [39–41]. lncRNAs can have an intragenic or, more often, an intergenic genomic localization. In many instances, they are localized in the vicinity of protein-coding genes, with respect to which they can be organized in sense or antisense orientation. In the latter case, they are referred to as NATs or opposite-strand (OS) transcripts. The majority of lncRNAs are transcribed from the nucleus or, with less frequency, from the mitochondrial genome [42]. As it happens with coding genes, lncRNAs undergo post-transcriptional processing, including 5′ capping, alternative splicing, RNA editing, and polyadenylation [25, 28]. The activity of only a small fraction of the lncRNAs so far identified has been experimentally defined, revealing a wide variety of functions, which, at present, can be summarized as follows. (1) Modulation of chromatin structure at specific genomic sites by recruitment of histone and chromatin modifying complexes. One of the most representative examples occurs during X chromosome inactivation. The lncRNA named "Xist" physically associates with the Polycomb repressive complex 2 (PRC2), resulting in the localization of the PRC2 and H3K27me3 (histone H3 trimethylated at lysine 27) to the inactive X chromosome [43]. Similarly, other lncRNAs (i.e., Air, Kcnq1ot1, HOTAIR etc.) mediate genomic imprinting in *cis* and/or in *trans,* thereby regulating gene expression in a genome-wide scale through the association with chromatin-modifying complexes [44–46]. (2) Recruitment of transcription factors to chromatin, as in the case of Mll1-dependent transcriptional activation of *Hoxa6* and *Hoxa7* by the lncRNA Mistral in differentiating ES cells during mouse germ layer specification [47]. (3) Modulation of nuclear-cytoplasmic trafficking, as in the case of NRON, the non-coding repressor of nuclear factor of the activated T cells (NFAT), which seems to act as a modulator of NFAT nuclear trafficking [48]. (4) Control of intracellular compartmentalization: the lncRNA nuclear-enriched autosomal transcript 1 contributes to the formation of "paraspeckles", dynamic structures of the interchromatin space [35, 49]. (5) Modulation of post-transcriptional RNA processing: lncRNAs have been shown to bind with partial base-pairing to complementary sequences present in the 3′ UTR of specific mRNAs, thus creating a recognition site for Staufen, which is a protein that binds double-stranded mRNA and induces its decay. Therefore, lncRNAs represent an unexpected mean to recruit proteins to mRNAs and thus promote its degradation [50]. By contrast, the mRNA of BACE1, a β-secretase responsible β-amyloid production, is stabilized and protected from RNAse cleavage by base-pairing of its antisense (BACE1-AS) [51]. (6) Modulation of local protein synthesis. The antisense AS-Uchl1, transcribed in the opposite strand of the Ubiquitin carboxy-terminal hydrolase L1 (Uchl1) gene, can specifically modulate the translation of Uchl1 through an embedded a short interspersed nuclear element (SINE) B2 repeat and by binding, with perfect base pairing, to the first 73 nucleotides of the sense mRNA [52]. (7) Interference with miRNA action: this is the case of the emerging class of competing endogenous RNAs (ceRNAs) that, by sequestering microRNAs, can regulate mRNA transcripts containing common microRNA recognition elements [53]. Through the above mechanism, ceRNAs (also known as "target mimicry" in plants) [54] have been found to play a role in the genesis of cancer [55] and in cell differentiation [56]. ceRNAs have been also proposed to orchestrate the crosstalk between RNAs [57], which is a so-far-unexplored large-scale regulatory network across the transcriptome.

By means of one or more of the above-described modalities, lncRNAs can play significant roles in the development and differentiation of several organs and tissues, particularly in the CNS. One of the first reported examples is represented by *Evf2*, which, through an in vivo loss-of-function approach, was found to be essential for the proper development of GABAergic neurons [58]. In non-neural tissues, the muscle-specific ceRNA linc-MD1 acts as an endogenous decoy for miRNAs, thereby controlling the timing of muscle cell differentiation [56], whereas the lncRNA *Braveheart (Bvht)* is required for the commitment of the cardiovascular lineage [59]. Specific examples of lncRNAs with a role in cell differentiation of sensory organs will be reported below.

ncRNAs associated to the development of the inner ear and to deafness and balance disorders

The vertebrate inner ear is an elaborate organ that mediates different aspects of hearing, motion, and space orientation. The mammalian inner ear is actually composed of six distinct sensory organs: the three cristae of the semicircular canals, the two maculae of the saccule and utricle and the organ of corti in the cochlea. The latter structure mediates hearing, whereas the cristae and the maculae are vestibular structures that respond to angular and linear acceleration, respectively. These three types of organs have a similar and relatively simple epithelial conformation, with two basic cell types: the sensory hair cells and the surrounding non-sensory supporting cells. These epithelia are covered at their apical surface with an extracellular structure known as cupula (cristae), otoconial (macula), or tectorial (organ of corti) membrane. The supporting cells are adhered to a basal lamina and surround with their lateral membranes the hair cells, which are thus isolated from one another and from the basal lamina. The apical portion of the supporting cells are

connected one another and with the adjacent hair cells by tight and adherens junctions. A hair bundle, composed of highly specialized microvilli that detect mechanical stimuli and transduce them into electrical signals, occupies the apical surface of the hair cells [60].

All the structures of the inner ear develop from the otic placode, a thickening of the head ectoderm adjacent to the rhombencephalon. The placode invaginates to form the otic pit, which detaches from the ectoderm to form a pear-shaped otocyst. A patch in the ventromedial wall of the otocyst, characterized by the expression of the Notch signaling components *Serrate1* and *Lunatic fringe* [61], acquires pro-sensory characteristics and then differentiate into the six sensory organs described above, under the influence of the transcription factors *Pax2*, *Dlx5*, *Otx1*, and *Hmx3,* among many other regulators (see [60, 62–64], for up-to-date reviews). The otocyst also gives rise to the neurons that innervate the hair cells of the six organs. These neurons form through a process of delamination that takes place before the formation of the sensory organs. A complex series of morphogenetic events also contributes to the final shape of the inner ear [65, 66].

Most of the events implicated in the development of the inner ear as well as in its adult homeostasis require the activity of a large number of miRNAs (Fig. 1) [67]. Indeed, robust expression of hundreds of miRNAs, distributed with precise cellular and temporal patterns, has been detected in the auditory and vestibular systems at embryonic, postnatal, and adult stages [68–74].

Pioneer studies aimed at defining the function of miRNAs in the developing inner ear of the mouse took advantage of the conditional inactivation of Dicer, mediated by Pax2-Cre, which is transiently expressed in the otic placode and thus leads to enzyme depletion in most sensory, supporting, and hair cells [75]. The otic sensory neurons of the resulting embryos formed normally but rapidly underwent apoptosis [76], similarly to what was observed for the hair and sensory cells of the cochlea after Pou4f3- or Atoh1-Cre-mediated inactivation of Dicer [71, 77]. In contrast to this restricted defects, Foxg1-Cre conditional inactivation of Dicer leads to broader defects with an evident disruption of inner ear development marked by extensive cell death [78]. These studies soon led to hypothesize that general miRNA dysfunction could contribute to cochlear dysfunction and disease, such as age-related degeneration and noise- or toxic cochlear damage [79–82].

In a few cases, the role of individual miRNAs in inner ear development/function has also been dissected (Table 2). Among the hundreds of miRNAs expressed in developing and adult ear tissues, the miR-183 cluster is one of the most studied. This cluster is composed of three miRNAs, miRs-183, -182, and -96 that originate from a common primary transcript [68, 74, 77, 83, 84]. Their seed region is identical, suggesting potential common properties in mRNA target recognition. However, the involvement of additional miRNA nucleotides critical for target recognition downstream the seed sequence likely diversifies their cellular functions [85]. The expression of miR-182 and -183 begins at E9.5 in the otic vesicles and then the expression of the entire cluster, including miR-96, becomes localized to the sensory cells of the cochlea, the vestibular end-organs, and the spiral and vestibular ganglia to become finally restricted to the inner sulcus and the spiral limbus of the mature cochlea [77, 83]. After an ENU-induced mutation of the miR-96 seed region, hair cell development begins normally but irregular bundles and persistent clusters of ectopic stereocilia appear at late postnatal stages, followed by progressive cell degeneration and hearing loss [86]. These observations suggest that

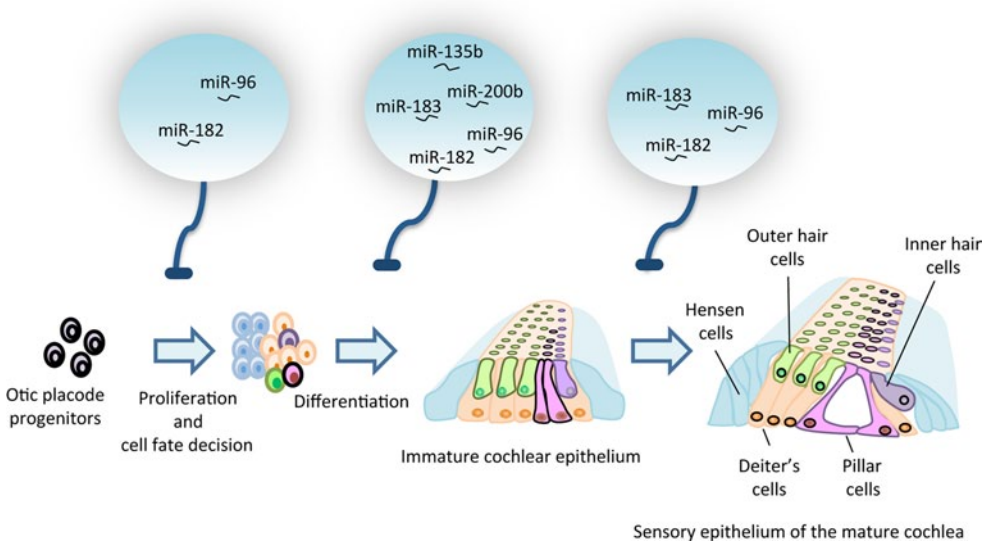

Fig. 1 Role of miRNAs in inner ear development. The drawing depicts the development of the organ of Corti viewed in the transversal plane, showing the relative position of the inner hair cells (*violet*) and the three rows of sensory outer hair cells (*green*). The *pale blue circles* on the top indicate the main miRNAs implicated in the control of the different steps of inner ear development and its homeostatic role in the mature organ (see text for further details)

Table 2 List of the main miRNAs, their proposed target genes, and regulated events during sensory organ development

MicroRNAs	Target genes	Function
miRNAs associated to the development of the inner ear		
miR-182; miR-183; miR-96	T-box1	Regulation of inner ear progenitor cell differentiation [88]
miR-182; miR-183; miR-96	Sox2	Hair cell fate specification and differentiation [77]
miR-135b	PSIP1	Regulation of cochlear and vestibular hair cell differentiation and maintenance [70]
miRNAs associated to eye development		
miR-24a	Casp9 and Apaf1	Repression of apoptosis in the developing neural retina [122]
miR-124	Sema3A	Control of the sensitivity of retinal growth cones to the guidance cue Sema3A [125]
miR-124a	Lhx2	Control of the maturation and survival of retinal cone photoreceptors [133]
miR-204	Meis2	Regulation of lens and retinal development [127]
miR-133b	Pitx3	Control of the maturation and function of dopaminergic amacrine cells [135]
miR-218	Robo1, Robo2, and GLCE	Regulation of normal vascularization of the retina [137]
miRNAs associated to olfactory system development		
miR-7a	Pax6	Regulation of the differentiation of dopaminergic neurons in the olfactory system [178]

the functions of the three members of the miR-183 cluster are not completely redundant, although the precise reasons are unclear [86, 87]. It is possible that the mutated miR-96 recognizes novel target and/or that the remaining members of the miR-183 cluster fail to compensate miR-96 activity, despite their similar seed regions. This would imply that a threshold and balanced level of all members of the cluster is needed for proper inner-ear development. This idea is further supported by single and combined knock-down of the expression of members of this cluster in zebrafish embryos, which led to hair cell loss, the magnitude of which is strictly related to the number of affected miRNAs. By contrast, over-expression of miR-182 or -96 causes the growth of extra or ectopic hair cells, whereas injection of miR-183 has no effect [87].

The genes targeted by this cluster in the ear are poorly characterized, but some information has been obtained using cancer cell lines. Aquaporin5, Myosin-, and Rab-interacting protein, a member of the protein kinase A-anchoring family, the outer dense fiber protein, a critical component of the centrosome, and two components of the non-canonical Wnt signaling pathway, Celsr2 and Ryk, have been biologically validated as miR-96 targets [86]. MiR-182 instead modulates *T-box1* gene expression and this in turn has a significant impact on the genetic control of otocyst-derived cell differentiation [88]. Targets of the entire miR-183 family are two genes expressed in hair cells: the transcription factor *Sox2*, which may repress hair cell differentiation [77], and the chloride intracellular channel 5 involved in hair cell function [89].

Besides the miR-183 cluster, other miRNAs have been shown to contribute to inner ear development and function. The auditory and vestibular defects of the Twirler mice, affected by deafness and balance disorders, have been associated to ZEB1/miR-200b activity [90]. Furthermore, miR-135b inhibits the translation of the transcription factor PSIP1, thereby regulating the identity, differentiation, and homeostasis of cochlear and vestibular hair cells [70], whereas miR-181a diminishes hair cell proliferation, which occurs in response to ototoxic injury in cultured avian cochlea [91].

Probably, the most compelling evidence for the relevance of miRNAs in inner ear homeostasis is the identification of a mutation in the seed region of miR-96, which is responsible for autosomal dominant non-syndromic deafness in humans. Notably, this finding also represented the first example of a hereditary disease caused by a mutation in a miRNA [92]. A subsequent mutational screening that took into account the three members of the miR-183 cluster identified an additional mutation but again in miR-96 [93]. MiRNAs likely contribute to other ear pathological conditions. For example, miR-21 has been associated with the development of human vestibular schwannomas [94] and with the growth and proliferation of cholesteatoma [95], epidermal cysts that can lead to loss of auditory functions.

More than a thousand lncRNAs have been identified in the mouse genome and a good proportion of them is specifically expressed in the nervous system [34]. Unfortunately, the information related to their function during inner ear development is limited to the observation that disruption of *Rubie*, a lncRNA upstream of Bmp4 expression, causes vestibular developmental defects with an undetermined molecular mechanism [96].

Non-coding RNAs in eye development and ocular degenerative diseases

The eye is a bilateral organ that originates from a single field of cells located in the center of the anterior neural plate surrounded rostrally and laterally by telencephalic precursors and caudally and medially by cells that will form the hypothalamus. Cell specification, mostly mediated by Wnt signaling [97] and by the concomitant expression of a few transcription factors, such as *Rx, Pax6*, *Hes1*, *Otx2*, *Lhx2,* and *Six3*, coupled to midline convergence of telencephalic precursors results in the formation of the optic pits. The optic pits begin to evaginate and give rise to the optic vesicles, the first morphologically visible eye structures. Folding and progressive specification of the vesicles further originate functionally specialized eye tissues: the optic stalk, the neural retina, and the retinal pigment epithelium (RPE), which, together with the ectodermal-derived distal components (lens, cornea, and iris), compose a functional eye [98]. The neural retina then undergoes proliferation to finally differentiate in six neuronal and one glial cell types: the retinal ganglion cells, the amacrine, horizontal and bipolar cells, the rod and cone photoreceptors, and the Müller glial cells.

The genetic networks that control eye development, composed by the interplay among different morphogenetic signaling pathways and transcription factors, have been intensively investigated. Most of the fundamental regulators have been identified in different species revealing a robust evolutionary conservation of the basic genetic programs and their properties. This extensive information, summarized in several recent reviews [99–102], has provided an excellent ground to identify the role of ncRNAs in eye development.

Indeed, the spatio-temporal distribution of miRNAs suggests their essential contribution to the control of the genetic networks involved in the development of all eye tissues, leading to the proposal that perhaps in the eye each cell type has its own miRNome [84, 103–112]. However, this information is insufficient to predict possible functions because, for example, ubiquitously expressed microRNA may target gene(s) expressed only in a small subset of cells or vice versa.

As in the case of the ear, the broad picture of miRNA activity in the eye derives from the use of conditional inactivation of Dicer in mice (Fig. 2). Mice lacking Dicer expression in the developing lens placode and in the presumptive corneal epithelium are microphthalmic with dystrophic lens and structural defects of the corneal epithelium, likely caused by an increased apoptosis and a reduced cell proliferation [113]. Dicer inactivation in the neural retina has been pursued with different Cre transgenic lines with either a broad and early or a late and more restricted pattern of expression. According to the Cre expression, the resulting mice are characterized by more or less severe phenotypes. With Rx- and Dkk3-Cre lines, in which Dicer inactivation occurred quite early in the development of the eye, embryos presented a pronounced microphthalmia associated with cell autonomous apoptosis that, in the case of Rx, was also extended to the preoptic region [114, 115]. As a consequence, Rx-Cre-mediated Dicer inactivation also led to significant pathfinding defects of the retinal ganglion cell (RGC) axons at the optic chiasm [114]. The use of αPax6- or Chx10-Cre transgenic lines causes instead variable defects in cell differentiation such as a prolonged production of early generated neuronal types followed by a loss of late-born cells [116] or the formation of rosette-like clusters of photoreceptors, which undergo a progressive degeneration, thereby decreasing the retinal response to light stimuli [117]. Similar, although more severe, phenotypes were observed after Dicer inactivation in *Xenopus* retinal progenitors, which in addition failed to laminate properly [118]. Comparison of Dicer inactivation driven by elements expressed with different spatio-temporal and yet partially overlapping distribution -Tyrp2-Cre, Z/AP, αPax6, and Pou4f3-Cre- demonstrated novel and stage-dependent roles for miRNAs in both the morphogenesis and neurogenesis of the eye [119], whereas forced expression of Cre recombinase into postnatal retinas indicates that miRNAs are required for cell survival [115]. Unexpectedly, generation of a BEST1-Cre conditional Dicer1 knockout, which expresses the recombinase in the RPE, caused the accumulation of dsRNAs of the *Alu* type. This accumulation, in turn, is responsible for RPE degeneration, demonstrating that Dicer has other RNA processing activities besides the generation of miRNAs [120].

In addition to this large body of evidence supporting that miRNAs contribute to modulate eye development, studies in fish, frogs, and mice have also provided at least a partial view on the role of individual miRNAs (Fig. 2; Table 2). Studies in *Xenopus* embryos, for example, have shown that interference with miR-196a function causes microphthalmia [121], whereas miR-24a negatively regulates the pro-apoptotic factors caspase9 and apaf1 in retinal cells [122]. During retinal neurogenesis, miR-124 seems to control cell proliferation and differentiation [123, 124] as well as RGC growth cone sensitivity to Sema3A, an important axon guidance cue [125]. Similarly, differentiation of late-developing retinal cell types seems to require the simultaneous activity of a set of miRNAs composed by miR-129, -155, -214, and -222 [126]. In medaka fish (*Oryzias latipes*) instead, miR-204 affects both lens and retina development via repression of the *Meis2* gene with a consequent alteration of *Pax6* expression in both tissues [127].

In mammals, the miR-204/211 family participates to the differentiation of the RPE [128, 129], whereas the miR-183/96/182 cluster is expressed in photoreceptors and retinal interneurons. The latter complex has been functionally inactivated in photoreceptors by generating a “sponge”

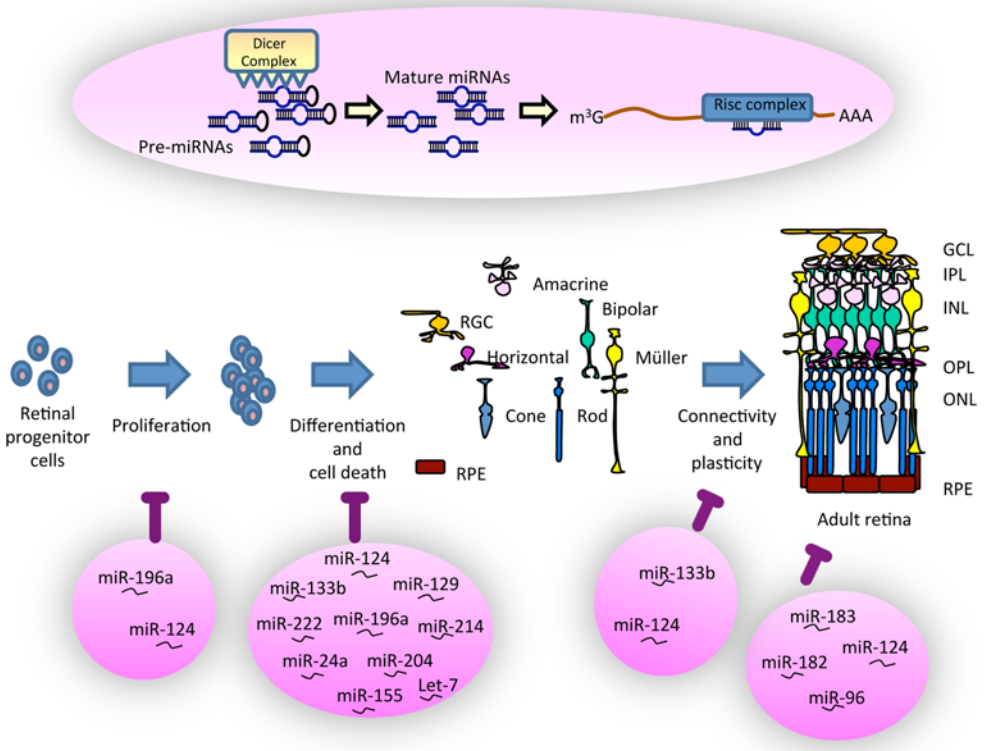

Fig. 2 MicroRNAs are essential for the development and function of the vertebrate retina. A graphical overview of the canonical pathway of miRNA maturation is represented on the *top*. MiRNA biogenesis consists of a series of reactions that convert the primary miRNA transcript (pri-miRNA) first into a precursor miRNA (pre-miRNA) and finally into a biologically mature and active miRNA. The main steps of retinal development are depicted in the *middle*. The six types of neurons of the vertebrate retina originate from a population of neuroepithelial precursor cells in the eye primordium. *Pink circle* at the bottom indicates the main miRNAs that regulate the different steps of retinal development. Their function extends to homeostasis of the mature retina. Details of the interaction of these miRNAs during eye development are discussed in the text. *GCL* Ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *RPE* retinal pigmented epithelium

transgenic mouse carrying ten copies of each of the sequence complementary to the three members of the cluster into the 3′-UTR of the enhanced green fluorescent protein gene under the control of the Opsin promoter. The photoreceptors of the "sponge" mice become extremely sensitive to light-stress, although they showed no alterations under normal light conditions [130]. More recently, the analysis of a mouse deficient in the miR-183 cluster, generated using a gene-trap embryonic stem cell clone, revealed the presence of early onset and progressive synaptic defects of the photoreceptors followed by retinal degeneration [131]. In contrast and despite its abundant expression in the retina, targeted deletion of miR-182 alone in mice has little morphological and molecular consequences [132]. These data together with the work reported above in the inner ear support that the miR-183 cluster has an important protective role for different sensory organs.

Additional studies in mice have shown that miR-124 and the let-7 microRNA signaling pathway are respectively required to control retinal cone survival [133] and Müller glial cell differentiation [134]. In the first case, miR-124 acts by suppressing the expression of *Lhx2* [133]. Similarly, miR-133b controls rat retinal dopaminergic amacrine cells differentiation and synaptogenesis by suppressing the expression of its target *Pitx3* [135].

The development of the ocular vasculature is also under miRNA control. MiR-31, -150, and -184 are involved in retinal and choroidal neovascularization but their target genes have not been identified [136]. Slit–Robo signaling promotes endothelial cell migration and vessel formation. In the eye, miR-218 controls the levels of Robo receptors and of the heparan sulphate biosynthetic enzymes, thus influencing the organization of the blood vessels [137].

Vision depends on elaborate cellular and molecular events, including, among others, a rapid dark- and light-adaptation. This process is also highly dependent on miRNAs at least in mice [138]. Furthermore, miRNA levels are altered in different mouse models of retinitis pigmentosa, ischemic

retina, autoimmune uveoretinitis, and diabetic retinopathy, indicating that a balanced expression of these molecules is required for proper function of the eye [136, 139–143]. This is also supported by the observation that miRNA expression profiles are altered in human pathological conditions such as lens cataract [108, 144]. Notably, mutations in the seed region of miR-184 are responsible for familial keratoconus with cataract [145], a degenerative disease of the cornea that progressively changes its shape. In a mouse model for secondary cataract, this miRNA antagonizes the miR-204-regulated RNA network [146]. This suggests that this network might also be affected in human keratoconus, opening the attractive possibility of treating eye inherited defects with gene transfer mediated by adeno-associated viral vectors facilitated by the relatively easy accessibility of this organ [147–149].

There are other miRNAs that might be useful targets for the treatment of eye-related diseases. Indeed, miR-200b and miR-126 seem to inhibit vascular alteration in diabetic retinopathy and ischemia-induced retinal neovascularization, respectively [150, 151], whereas modulation of miR-328 might be beneficial in Pax6-related high myopia [152].

Similar to miRNAs, the large number of lncRNAs expressed during eye development present cell-specific and spatio-temporal restricted expression profiles [37, 153–157], but their function remains largely unexplored, with limited exceptions. *Tug1*, one of these exceptions, promotes photoreceptor development and survival likely by altering the chromatin structure and thus activating the expression of the photoreceptor-specific transcription factors *Crx* and *Nrl* [158]. The *Retinal Non-coding RNA 2* (*RNCR2*), also known as *Gomafu* or *Miat*, is instead expressed in retinal progenitors and later on in Müller, amacrine and retinal ganglion cells [30, 37] as well in other cells of the nervous system [37]. *Gomafu/RNCR2* has been shown to control amacrine and Müller cell differentiation [159] and the pluripotency of embryonic stem cells [160], with yet undetermined mechanisms. Notably, however, *Gomafu* RNA escapes nuclear export and accumulates in the nucleus, in an uncharacterized compartment [37]. Its sequence contains tandem repeats of UACUAAC that bind to the SF1 splicing factor with high affinity, affecting splicing kinetics. These observations suggest that *Gomafu* could regulates splicing efficiency by changing the local concentration of splicing factors within the nucleus [161], suggesting a possible mechanism for its effect in retinal differentiation.

LncRNAs of the antisense type (NAT or OS) are also relevant to retinal cell differentiation and survival. This is the case of *Six3OS* and *Vax2os1*. Manipulation of *Six3OS* expression interfered with retinal cell specification, possibly because this lncRNA serves as a transcriptional scaffold [155]. *Vax2os1* instead is involved in cell cycle progression of photoreceptor progenitors [156].

MiRNAs function in primary olfactory system development

The olfactory system, one of the most ancient sensory systems, serves to discriminate odors and transmit this information from the nose to the brain [162–164]. The primary olfactory system is composed of the olfactory epithelium (OE) and the olfactory bulb (OB). The development of these two structures begins simultaneously but proceeds, at least initially, with independent programs. The OE differentiates from the olfactory placodes, bilateral epithelial thickening localized in the rostro-lateral regions of the head. Soon after the closure of the neural tube, these placodes invaginate to form the nasal pits, the marginal rims of which then fuse originating the nostrils. The nostrils thereafter become more convoluted and transform into the nasal cavity and the vomero-nasal organ that arises from the medial wall of the epithelium [165]. Upon differentiation, the OE becomes pseudo-stratified and composed of basal and supporting cells, among which mature and immature sensory neurons reside. Olfactory sensory neurons are renewed throughout life and derive from a population of self-renewing stem cells, probably a subtype of basal cells, that sequentially give rise to two types of transit-amplifying cells marked by the expression of different neurogenic basic helix-loop-helix transcription factors. Their daughter cells differentiate into the olfactory sensory neurons, which can be identified by a variety of markers, including the olfactory marker protein (OMP) [166].

The OB instead develops from a predetermined region of the rostral telencephalon, likely under the influence of FGF signaling. In mouse embryos, its development begins around E11.5 although the OB becomes morphologically visible as an evagination of the rostral telencephalon only at around E12.5. There is considerable debate on whether further OB development is influenced by the arrival of the olfactory sensor axons derived from the OE. However, the onset of mitral cell differentiation prior to the arrival of the sensory axons and the evidence that in absence of the OE, the OB maintains its differentiation program supports that the OB is independently formed [167].

The first information on miRNA expression in the olfactory system derived from studies in zebrafish embryos [73, 168] followed by microarray-based analysis in mice [169]. This analysis was thereafter largely validated by real-time RT-PCR studies [170]. Altogether, these data demonstrate that the OB is particularly enriched in miR-200a, miR-200b, miR-200c, miR-141, and miR-429 expression when compared to the rest of the brain. In both mice and zebrafish, miRNA expression remains relatively stable during postnatal stages [171–174], suggesting that these molecules controls the function of the olfactory system.

The miR-200 family, composed of five members divided into two clusters, has been particularly studied (Fig. 3).

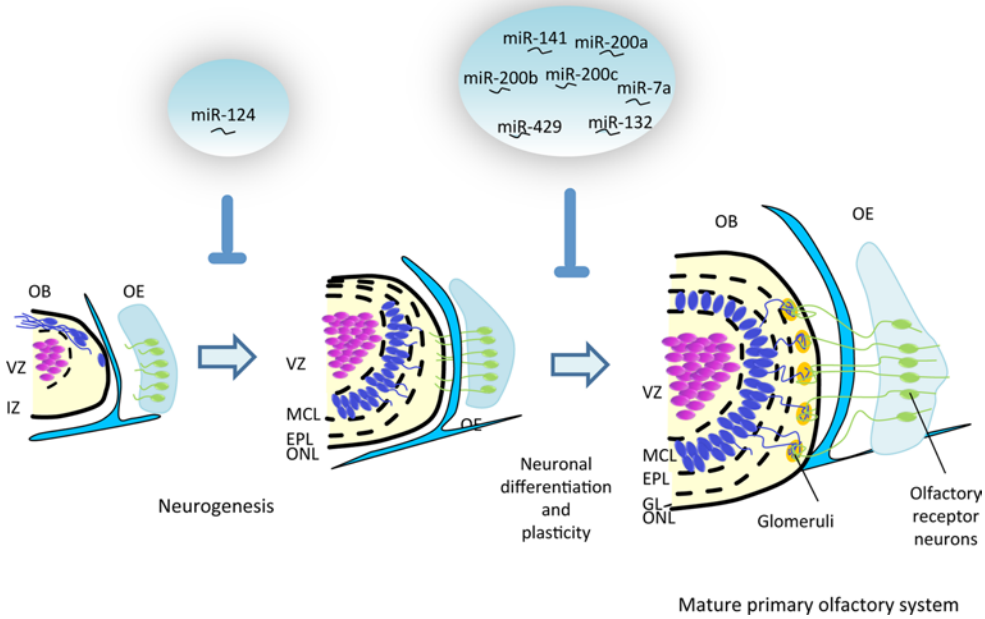

Fig. 3 Emerging roles of miRNAs in olfactory system development. The drawings provide a schematic representation of olfactory system development, staring from the olfactory placode stage to the adult organization. The two *blue circles* on the top indicate the miRNAs that have been so far described as modulators of these events (see text for further details). *VZ* ventricular zone, *IZ* intermediate zone, *MCL mitral* cell layer, *EPL external plexiform, ONL* olfactory nerve layer, *OE* olfactory epithelium, *GL* glomerular layer

In humans, the first cluster, composed of miR-200a, miR-200b, and miR-429, localizes to chromosome 1, whereas the second, formed by miR-200c and miR-141, is found on chromosome 12 [175]. In mice, miR-200 expression starts at E9.5 in the olfactory placodes and then becomes restricted to a subregion of the olfactory pit to finally localize to the immature and mature neuronal cells of the OE [171]. Genetic inactivation of Dicer in mice with a broad (Foxg1-Cre) or more restricted driver (OMP-Cre) showed that miRNAs are required for progression but not initiation of olfactory neurogenesis. However, when the OMP-Cre driver was used to inactivate Dicer at adult stages, both olfactory and vomeronasal neurons appeared normal [171]. Individual or combined inactivation of miR-141/miR-200a or miR-200b/miR-429 using antisense morpholinos had also little effect on the development of the zebrafish olfactory system and only the simultaneous interference with the expression of all the members of the miR-200 family caused abnormal terminal differentiation of olfactory cells and apoptosis. A list of possible target genes, related to neuronal differentiation, was predicted with bioinformatic approaches and two of them, *lfng*, a regulator of the Notch signaling, and *zfhx1*, an enhancer of TGFβ pathway, were further validated in zebrafish. Nevertheless, how miR-200-mediated regulation of these genes fits in the molecular pathway underlying olfactory system development is still unclear [171].

Other miRNAs have important roles in OB development. MiR-124 inactivation impairs neurogenesis and induces the appearance of ectopic cells with astrocyte characteristics [176]. The continuous neurogenesis of OB neurons derived from the subventricular zone involves the function of miR-132 that controls survival of new-born neurons and their dendritic complexity and spine density, likely regulating their plasticity [177]. Furthermore, specific miR-7a-mediated regulation of Pax6 in ventral neural stem cells regulates the molecular network that specify postnatal and adult dopaminergic neurons in the olfactory system [178] (Table 2).

Whether miRNAs are involved in diseases that affect the olfactory system has remained so far unexplored.

Conclusions and perspectives

The recognition of the fundamental and pervasive role of ncRNAs in virtually all biological processes represents one of the most notable outcomes of the Human Genome Project and related efforts [25]. MiRNAs and lncRNAs are now widely recognized as key players in gene regulation processes. As such, their role in the pathogenesis of both monogenic and complex genetic disorders is starting to be unraveled [179]. Nevertheless, there are many unresolved issues concerning both their identity and function. This is particularly evident in the case of lncRNAs, which, despite the remarkable progresses of the past few years [180], still represent a heterogeneous group of transcripts that need to be more finely dissected in the near future.

The extraordinary advances in next-generation sequencing (NGS) technology applied to transcriptome analysis [181] should lead to a more comprehensive definition of the non-coding RNA repertoire of the human and other vertebrates' transcriptomes. Similarly, the use of whole genome or whole-exome NGS-based approaches will likely shed

further light on the pathogenic contribution of ncRNAs to human genetic diseases. Sensory organs are among the structures for which the study of the functional role of ncRNAs has been most rewarding so far. It is remarkable that sensory organs represent the targets of the first two examples of human Mendelian disorders caused by mutations in miRNA sequences [92, 145]. It can be also anticipated that a more precise knowledge of ncRNA function will turn these molecules into therapeutic targets for human disease. Disorders affecting sensory organs, in particular the eye by virtue of its accessibility, may constitute ideal conditions to explore this therapeutic potential in the near future.

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