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INVITED REVIEW

Roles of miR-182 in sensory organ development and cancer

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Abstract

Micro ribonucleic acids (miRNAs) are a cluster of small non-coding RNA molecules predicted to regulate more than 30% of coding messenger (m)RNAs in the human genome and proven to be essential in developmental and pathological processes. The miR-182 gene was first found to be abundantly expressed in sensory organs and regulates the development of the retina and inner ear. Further studies revealed its roles in osteogenesis and T cell differentiation. In addition, the involvement of miR-182 in cancer initiation and progression has recently been uncovered by a growing body of evidence, the majority of which supports its promoting effects in cell proliferation, angiogenesis, and invasion, as well as distant metastasis of various cancer types. Clinical analyses demonstrated the link of miR-182 expression to poor prognosis in cancer patients. Mechanistically, multiple downstream genes including missing-in-metastasis, microphthalm-associated transcription factor, FoxO1, cylindromatiosis, and others, can be targeted by miR-182 and mediate its roles in cancer. miR-182 is also interconnected with prominent cancer-related signaling pathways, such as transforming growth factor beta and nuclear factor kappa beta. Interestingly, it was shown that *in vivo* targeting of miR-182 prevented liver metastasis of melanoma. miR-182 is emerging as an important regulator of malignancies, which warrants further study to establish the application potential of miR-182 in cancer diagnosis and treatment.

Introduction

Micro ribonucleic acids (miRNAs) are a group of non-coding RNA molecules with a length of 19–24 nucleotides, and bind to perfectly or imperfectly complementary regions at the 3′ untranslated region (3′UTR) of target messenger (m)RNAs, leading to either mRNA degradation, translation, inhibition or both.[1] Since the identification of the first worm miRNA in 1993[2] and the first mammalian miRNA in 2003,[3] the functions of miRNAs have been intensely studied, revealing their micro steering, but critical roles in various biological processes.

miR-182, together with miR-183 and miR-96, belongs to a polycistronic miRNA cluster that is located within a 4-kilobase area on murine chromosome 6q (Fig 1A). The human orthologous region is on chromosome 7q32.2, where the three family members are oriented in the same order as in the mouse genome. These miRNA siblings share similar seed sequences (miR-96 and miR-182 are identical, Fig 1B), and are all expressed specifically in sensory organs including the retina, nose, and inner ear.[4] However, these miRNAs seem to have distinct functions by targeting different downstream genes. For example, glypican-3 (GPC3) can be regulated by miR-96, but not miR-182.[5] Moreover, they display distinct expression patterns during inner ear development[6] and, when overexpressed individually, lead to unique phenotypes in hair cell development and prosensory organization of zebrafish.[7] These observations suggest that the difference in the roles of

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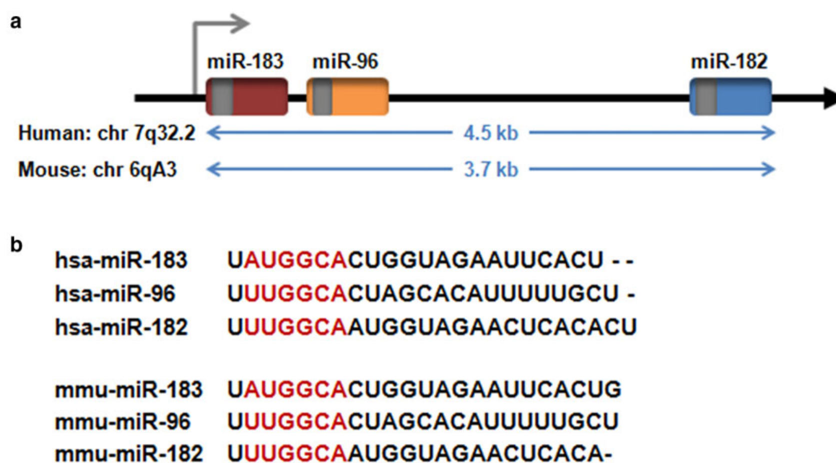

Figure 1 (**a**) The chromosomal locations of human and mouse miR-183/96/182 clusters; (**b**) the seed sequences of these micro ribonucleic acids (miRNAs).

the miRNA family siblings could be expanded to other biological processes. Therefore, this review primarily focuses on miR-182 to discuss its role in development and cancer.

miR-182 and FoxO1

To date, multiple mRNA targets of miR-182 have been identified, among which the forkhead transcription factor FoxO1 was repeatedly reported to be suppressed by miR-182 in different cell types.[8–11] Because of the versatile roles of FoxO1 in various biological processes, miR-182 has been connected to osteogenesis, T cell development, and tumorigenesis. Therefore, we summarize here the studies revealing the interaction of miR-182 and FoxO1 in particular.

FoxOs are a large family of forkhead proteins characterized by the presence of a winged-helix DNA binding domain called the forkhead box, and play crucial roles in redox balance, as well as oxidative stress resistance. FoxO1 is the main FoxO protein in bone and is indispensable for bone homeostasis. The transcription factor positively regulates bone formation by promoting protein synthesis and resistance to oxidative stress in osteoblasts.[12,13] Kim *et al*. reported that miR-182 repressed the 3′UTR luciferase reporter activity of FoxO1 and the endogenous protein level, resulting in reduced osteogenesis.[8] Activation of miR-182 in mesenchymal stem cells and preosteoblasts induced cell apoptosis and prevented osteoblast differentiation. More importantly, *in vivo* delivery of miR-182 into zebrafish embryos impaired skeletogenesis. In addition, forced induction of FoxO1 rescued all of the phenotypes caused by miR-182 activation, confirming the mediating-role of FoxO1 suppression in miR-182-induced osteogenesis inhibition.[8]

Unlike its role in osteoblasts, FoxO1 is a suppressor of T helper (Th) cell proliferation by inducing the cell cycle inhibitor $p27^{Kip1}$.[14] It is expressed in resting Th cells, but inactivated by various mechanisms once the cells are antigen-activated and switch to clonal expansion. At the onset of Th cell activation, FoxO1 is expelled from the nucleus by antigen receptor-mediated phosphorylation.[15] However, in the late phase of clonal expansion, FoxO1 is no longer regulated by phosphorylation-dependent nuclear exclusion; rather it is post-transcriptionally suppressed by miR-182, which is induced by interleukin 2 (IL2), a cytokine activated by antigen stimulation of the T cell receptor.[9] Inhibition of miR-182 resulted in elevated FoxO1 mRNA and protein abundance, as well as reduced Th population expansion, and prevented Th-mediated inflammation and arthritis.[9] Therefore, miR-182 is critical to maintain the prolonged clonal expansion of Th cells.

In cancer cells, FoxO1 is also targeted by miR-182.[10,11] FoxO1 functions as a putative tumor suppressor that can be disregulated by chromosomal deletion.[16] miR-182 was sufficient to reduce the expression of FoxO1 in Ishikawa endometrial cancer cells,[10] while miR-182 inhibition in MCF7 breast cancer cells restored FoxO1 expression and promoted cell apoptosis, a phenotype rescued by small interfering (si)RNA of FoxO1.[11] Together, these studies firmly established FoxO1 as a direct target of miR-182 and a mediator of its functions in development, immune response, and cancer.

miR-182 in sensory organ development

Retina

Murine miR-182 was first identified as a retina-specific miRNA, with its expression abundantly increasing postnatally and reaching the peak in adult retina.[4] In addition, the

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retinal expression of miR-182 displayed a diurnal variation in mice kept in 12-hour light and 12-hour dark cycles, peaking respectively at one hour post light-on and light-off, suggesting a role of this miRNA in retina development and the regulation of mammalian circadian rhythm.[4] Accordingly, 3′UTR luciferase reporter assays indicated that miR-182 directly targets microphthalm associated transcription factor-M (MITF), which is required for the establishment and maintenance of retinal pigmented epithelium, and ADCY6, whose expression responds to circadian fluctuation with a pattern opposite to that of miR-182.[4] However, Jin *et al.* generated miR-182-knockout mice and did not observe any apparent abnormalities in the miR-182-depleted retina, suggesting either that miR-182 is not a major determinant of retinal development, or the existence of functional redundancy of miR-182 with other genes.[17] Therefore, the actual roles of miR-182 in retina development or circadian rhythm regulation must still be characterized.

Inner ear

In mice, the miR-183/96/182 family is expressed specifically in hair cells of the cochlear and vestibular end-organ, as well as in the spiral ganglion.[18] Follow-up studies revealed spatiotemporal and tissue-cell specificity in the expression of the miRNA family in sensory organs of both zebrafish and mice, which correlated with the development of the inner ear.[6,19] In mice, depletion of mature miRNAs in hair cells by Dicer1 conditional knockout resulted in disregulated gene expression profiles and progressive loss of hair cells,[19] corroborating the critical roles of miRNAs for hair cell development, although a direct link to miR-182′s contribution was lacking. Functional characterization of the miR-183/96/182 family for inner ear development was more definitive in zebrafish.[7] Overexpression of miR-182 or miR-96 in zebrafish resulted in excessive otocysts, sensory patches, and hair cells, while silencing of these miRNAs led to the loss of hair cells and neurons in the inner ear.[7] Collectively, these data demonstrated a central role of the miR-183/96/182 cluster for hair cell maintenance and survival.

miR-182 in cancer

Breast cancer

miR-182 is frequently overexpressed in human breast tumor tissues and cell lines,[11,20,21] and is also significantly elevated in the serum of breast cancer patients compared with that of healthy controls, suggesting the potential of miR-182 as a valuable biomarker for breast cancer diagnosis.[22] Previously, we analyzed the correlation of miR-182 to the risk of breast cancer relapse and observed an association of elevated miR-182 expression with poor prognosis.[23] Interestingly, the serum level of miR-182 was found to be linked to the hormone receptor status of breast cancer, with elevated concentrations in estrogen receptor (ER)- or progesterone receptor (PR)-negative patients compared with their receptor-positive counterparts,[22] indicating a positive correlation of miR-182 expression with the triple-negative/basal-like tumors, a breast cancer subtype characterized with unfavorable clinical outcomes. On the other hand, another study reported that, in ER-positive patients, the expression level of miR-182 was positively correlated with the ER mRNA level. In addition, the ER-positive patients with higher levels of miR-182 had longer progression-free survival after tamoxifen treatment than those with lower miR-182 expression, thus indicating a link of miR-182 expression with tamoxifen responses.[24] Therefore, these seemingly contradictory observations argue for a possibility of molecular subtype-dependent correlation of miR-182 with breast cancer prognosis.

In addition to the correlative studies of miR-182 expression, the role of miR-182 in breast cancer has also been intensively studied. Its oncogenic trait was first unveiled by the observation of FoxO1 repression by miR-182, which led to the release of cancer cells from proliferation suppression by the tumor suppressor protein.[11] Another study then connected miR-182 to DNA damage response, showing that miR-182 inhibited the expression of BRCA1 and impaired homologous recombination-mediated DNA repair in HL60 leukemia cells.[25] Furthermore, miR-182 overexpression rendered the cancer cells hypersensitive to irradiation and inhibition of poly adenosine diphosphate ribose polymerase 1 (PARP1), a factor essential for DNA repair following environmental stress.[25] Such a role of miR-182 in DNA repair could be attributed to the targeted inhibition of BRCA1, as evidence showed miR-182 suppression of the wild type, but not the mutant of BRCA1 3′UTR, as well as the selective association of BRCA1 transcript with the miR-182/AGO1 complex. Most importantly, miR-182-induced impairment of DNA repair can be fully rescued by overexpression of miR-182-insensitive BRCA1.[25] miR-182 can also function to promote metastasis, an effect that can be attributed to the regulation of cancer cell cytoskeleton.[23] In multiple cell lines of breast cancer, we proved that miR-182 suppressed the putative metastasis suppressor missing-in-metastasis (MIM, also called MTSS1) by luciferase reporter assays of MIM wild type and mutant 3′UTR, as well as analyses of endogenous MIM protein levels following miR-182 overexpression and inhibition. Functional characterization revealed that MIM significantly inhibited cancer cell motility, invasiveness, and *in vivo* lung metastasis by inactivating the cytoskeleton regulator RhoA. Concordantly, miR-182 expression rescued RhoA from MIM suppression and promoted cytoskeleton rearrangement with concomitant elevation of metastasis capabilities. Furthermore, RhoA inhibitor treatment reversed the metastatic

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phenotypes induced by miR-182 overexpression or MIM knockdown.[23] These data demonstrated the role of miR-182 in breast cancer metastasis, but the underlying mechanism may not be limited to MIM targeting. Indeed, miR-182 was also shown to be transcriptionally activated by the Wnt pathway effector β-catenin, and promote colony formation, as well as invasiveness of MDA-MB-231 breast cancer cells, partially by repressing the matrix metalloproteinase inhibitor reversion-inducing-cysteine-rich protein with kazal motifs (RECK).[20] miR-182 inhibition was able to promote the protein level, but not the mRNA level, of RECK in MDA-MB-231 cells. Luciferase reporter assays also indicated that miR-182 suppressed the activity of RECK 3′UTR, although mutating the target site of miR-182 was not able to completely block the suppression.[20] Nevertheless, these studies demonstrated that miR-182 is involved in cancer cell invasion and metastasis through multiple downstream pathways.

Lung cancer

Compared to the consistent data of expression patterns and functions of miR-182 in breast cancer, the observations of miR-182 in lung cancer turn out to be more controversial. Its expression was found to be elevated in lung cancer cell lines,[26] in lung cancer tissues compared to adjacent normal lung parenchyma of non-smoking patients,[27] and in primary lung tumors compared to lung metastases of other tumor origins.[28] However, functional studies in lung cancer showed that overexpression of miR-182 significantly inhibited lung cancer tumorigenicity both *in vitro* and *in vivo*.[29,30] Two independent molecular mechanisms were proposed. Sun *et al.* found that miR-182 directly targeted RGS17, a negative regulator of G-protein coupled receptors, to inhibit cell proliferation; a phenotype can be blocked by RGS17 induction.[29] Meanwhile Zhu *et al.* demonstrated that an apoptosis-related gene RASA1 could be suppressed by miR-182.[30] Consistent to these functional data, elevated expression of miR-182 was linked to improved disease-free survival of squamous cell carcinoma and stage II non-small cell lung cancer patients. In addition, the prognostic power of miR-182 in these patients was independent of other clinical parameters, including tumor differentiation and nodal status.[31] Therefore, the exact roles of miR-182 in lung cancer initiation and progression are yet to be elucidated with more rigorous approaches.

Melanoma

The chromosomal segment (7q31-34) harboring the miR-182/183/96 cluster is frequently amplified in melanoma.[32] Concordantly, miR-182 is found to be upregulated in melanoma cell lines and clinical samples, partially attributed to the increased gene copy numbers. miR-182 expression is further elevated in metastatic melanomas as compared to primary melanomas, and is inversely correlated to those of MITF and FoxO3. Both *in vitro* and *in vivo* assays showed that miR-182 overexpression promoted survival, migration, invasion, and lung metastasis of melanoma cells. Mechanistically, miR-182 directly targeted the 3′UTR of MITF and FoxO3 to repress their protein levels.[32] The same group demonstrated that *in vivo* silencing of miR-182 by treating the mice with anti-miR-182 oligonucleotides efficiently reduced the liver metastasis of melanoma, thus establishing miR-182 inhibition as a promising therapeutic strategy for metastatic melanoma.[33]

Glioma

miR-182 was also shown to be upregulated in glioma tumors compared with the paired adjacent noncancerous tissues by both quantitative polymerase chain reaction (qPCR) analysis and *in situ* hybridization. Furthermore, higher miR-182 expression is positively linked to higher grading of glioma and shortened survival of patients.[34] Interestingly, the miR-182 level also correlates significantly with the activation of transforming growth factor beta (TGFβ) and nuclear factor kappa beta (NFκB) signaling in human glioma specimens.[35] miR-182 is a transcriptional target of TGFβ/SMAD signaling with a SMAD-binding element (SBE) in its promoter region, and targets the NFκB negative regulator cylindromatiosis (CYLD). Activation of the NFκB pathway is dependent on K63-linked poly-ubiquitination of the IKK complex component NEMO and the upstream regulators, including tumor necrosis factor (TNF)-receptor-associated factors (TRAFs) and receptor interacting protein (RIP). CYLD is a deubiquitinase that removes the ubiquitin chains of these proteins and, thus, interrupts the assembly of IKK complex.[36–38] The wild type miR-182, but not the mutant form with nucleotide substitutions in the seed sequence, was able to suppress the 3′UTR activity and the endogenous expression of CYLD. In addition, a microRNA ribonucleoprotein complex (miRNP) immunoprecipitation (IP) analysis revealed the physical association of miR-182 with CYLD transcripts.[35] Suppression of CYLD by miR-182 promoted ubiquitin conjugation of NFκB signaling components and led to sustained NFκB activation.[35] Thus, miR-182 serves as a link to bridge the two signaling pathways and is responsible for TGFβ-induced NFκB activation, which leads to an aggressive phenotype, including colony formation, angiogenesis, and invasion of glioma.[35]

Ovarian cancer

The upregulation of miR-182 was also observed in clear cell ovarian cancer, rather than in human normal ovarian surface epithelium and endometrium;[39] in high-grade serous ovarian carcinomas rather than in matched normal Fallopian tubes;[40] and in effusions rather than in primary ovarian carcinomas.[41]

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Knockdown of miR-182 in the ES-2 ovarian cancer cell line elicited a widespread effect on gene expression, causing a global shift in the expression pattern toward a more normal state, although the actual functional role of miR-182 in the cells needs to be characterized.[39] Another study reported that miR-182 transfection in primary ovarian granulosa cells resulted in reduced expression of apoptosis markers TdT and caspase-3, indicating a possible oncogenic trait of miR-182.[42] The study by Liu *et al.* was more confirmative for miR-182′s effect in ovarian cancer.[40] They showed that ectopic expression of miR-182 in immortalized and malignant ovarian cells resulted in increased tumor transformation, matrigel invasion, and *in vivo* tumor growth, as well as metastasis. Mechanistically, the oncogenic properties of miR-182 in ovarian cancer were attributed to its suppression of multiple targets, including BRCA1 and MIM, to impair irradiation-induced repair of DNA double-strand breaks, and enhance cell invasion.[23,25,40] Collectively, these results revealed the upregulation of miR-182 in ovarian cancer and proved its oncogenic property.[40]

Colorectal cancer

The upregulation of miR-182 in colon tumor tissues compared to normal counterparts was consistently reported by multiple studies.[43–48] Furthermore, two independent research groups reported similar observations that upregulation of miR-182 was linked to increased risk of disease progression and poor survival in colorectal adenocarcinomas. These studies demonstrated that enhanced miR-182 expression was significantly correlated to higher histological grades, larger tumor sizes, higher depth of tumor invasion, advanced tumor node metastasis (TNM) stages, positive lymph node metastasis, and most importantly, poor overall survival of patients.[44,49] The preliminary functional study revealed that miR-182 overexpression promoted proliferation and survival of colorectal tumor cells.[48] In addition, miR-182 was shown to be able to target the 3′UTR activities of thrombospondin-1 (TSP1), a putative anti-angiogenic factor,[50] and ENTPD5, a gene involved in energy metabolism,[47] in colorectal cancer cells, although the functional consequences of inhibition of these two genes by miR-182 have not yet been elucidated.

Prostate cancer

miR-182 is also overexpressed in prostate cancer samples compared to matched nonmalignant tissues.[46,51–53] In cancerous tissues, the expression of miR-182 correlates with Gleason scores, tumor stages or grades, clinical progression, and patient survival.[51,53–55] Combining miR-182 expression with Gleason scores significantly improves the prediction of risk of disease progression.[55] These results suggested that miR-182 is a promising biomarker for prostate cancer diagnosis and prognosis. However, the data of miR-182 functional studies in prostate cancer have been dichotomous. Some of these studies showed a pro-proliferative role of miR-182, a phenomenon reconciling with the observations of miR-182 upregulation in prostate cancer. For example, Liu *et al.* reported that miR-182 promoted PC3 proliferation and invasion by targeting the N-myc downstream-regulated gene 1 (NDRG1).[56] NDRG1 is a tumor repressor regulating P53-dependent apoptosis, cell cycle, and metastasis[57,58] and is subject to miR-182-mediated suppression via 3′UTR targeting.[56] In addition, Hirata *et al.* found a pro-tumor activity of miR-182 in PC3 and DU145 cell lines, and such a role could be linked to the inhibition of multiple metastasis suppressor proteins including MIM, RECK, and FOXF2, all of which were identified as authentic targets of miR-182 by luciferase reporter assays and Western analyses.[53] miR-182 also induced mesenchymal to epithelial transition (MET) and growth factor-independent growth by repressing the transcription factor SNAI2 in prostate cells.[59] In contrast, another study reported that miR-182 may mediate the antiproliferative activity of atorvastatin in PC3.[60] Atorvastatin is a type of lipophilic statin used as a cholesterol-lowering drug and was recently found to possess proapoptotic and antimetastatic effects in prostate cancer through inconclusive mechanisms.[61] Peng *et al.* showed that miR-182 was induced by atorvastatin treatment while transfection of miR-182 inhibitors attenuated atorvastatin-induced autophage and proliferation suppression. However, miR-182 was not responsive to atorvastatin in LNCaP cells.[60] Instead, miR-182 was shown to inhibit cell invasion by targeting the G protein GNA13 in LNCaP.[62] Overexpression of miR-182, as well as miR-141/200a, which inhibit cancer cell epithelial to mesenchymal transition (EMT),[63] suppressed the 3′UTR activity and protein level of GNA13 and cellular invasiveness.[62] These contradictory data indicated the cell line-specific and context-dependent functional roles of miR-182 in prostate cancer.

In addition, the aberrant expression and possible oncogenic role of miR-182 in other cancers, including bladder,[64–66] gastric[67–69] and pancreatic cancers[70] were also reported. Thus, miR-182 is involved in a wide spectrum of malignancies and represents a promising target for therapeutics of cancer.

Summary

Although miR-182/183/96 cluster miRNAs are transcribed as a single polycistronic gene and share similar or identical seed sequences, each single member seems to play distinct roles in development and cancer, with miR-182 as the most intensively studied molecule in the family. In developmental processes, miR-182 is involved in circadian rhythm regulation of the retina, affects cell fates in the inner ear, negatively regulates osteogenesis, and maintains clonal expansion of helper T lymphocytes upon antigen activation. At the same time,

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miR-182 is proven to be a prominent regulator of cancer-related processes, such as apoptosis, DNA repair, angiogenesis, EMT, cell motility and invasiveness. Overwhelming data have unambiguously demonstrated that this molecule is upregulated in malignant samples compared with normal tissues in a broad range of cancer types, and its expression is linked to increased pathological aggression of tumors and poor survival of patients. The majority of results from miR-182 functional characterization pointed to its oncogenic roles to promote proliferation, inhibit apoptosis, and enhance cancer cell invasion and distant metastasis, although occasionally opposite functions of miR-182 were observed in lung cancer and prostate cancer. Such a multifaceted role of miR-182 is mediated by an already diversified and still expanding set of target genes. Therefore, it is reasonable to speculate that the molecular routes through which miR-182 exerts its regulatory effects are largely context-dependent, and every single miR-182 target is not sufficient to explain the pluripotency of this miRNA in cancer. Nevertheless, several genes, including MIM, MITF, and FoxO1, are repeatedly found to act downstream of miR-182 in various cell types and experimental systems, indicating that they are among the prominent functional mediators of miR-182. Interestingly, miR-182 is also involved in the signal connection of TGFβ and NFκB, and provides an interface for TGFβ input to the NFκB pathway. Moreover, expected phenotypes in osteogenesis and cancer metastasis were observed in pre-clinical models by *in vivo* delivery of miR-182 molecules or inhibitors. Overall, current data have solidly established the potential of miR-182 as a diagnosis biomarker and a therapeutic target in cancer. It is, therefore, important to optimize miR-182 targeting approaches and develop selection criteria to identify responsive tumors.

Disclosure

No authors report any conflict of interest.

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