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MINI REVIEW

Biological functions of microRNAs: a review

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Abstract MicroRNAs (miRNAs) are a recently discovered family of endogenous, noncoding RNA molecules approximately 22 nt in length. miRNAs modulate gene expression post-transcriptionally by binding to complementary sequences in the coding or 3′ untranslated region of target messenger RNAs (mRNAs). It is now clear that the biogenesis and function of miRNAs are related to the molecular mechanisms of various clinical diseases, and that they can potentially regulate every aspect of cellular activity, including differentiation and development, metabolism, proliferation, apoptotic cell death, viral infection and tumorgenesis. Here, we review recent advances in miRNA research, and discuss the diverse roles of miRNAs in disease.

Keywords · MicroRNA · Biogenesis · Function · Expression · Disease

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Introduction

Recent studies have indicated that microRNAs (miRNAs) play a pivotal role in most critical biological events, including development, proliferation, differentiation, cell fate determination, apoptosis, signal transduction, organ development, hematopoietic lineage differentiation, host-viral interactions and tumorgenesis [1, 15]. Improvements in the characterization of miRNAs and in techniques for their functional analysis have not only uncovered their roles in various cellular processes, but also revealed abnormal patterns of miRNA expression in various diseases. In this paper, we summarize the current state of the miRNA field, including the association of miRNAs, the diverse roles played by miRNAs in eukaryotes, and the types of biological processes that miRNAs regulate.

Discovery of miRNAs

In 1993, Lee, Feinbaum and Ambros discovered that lin-4 in *C. elegans* did not code for a protein but instead produced a pair of short RNA transcripts that each regulate the timing of larval development by translational repression of lin-14, which encodes a nuclear protein [25]. They postulated that this regulation was due in part to sequence complementarity between lin-4 and unique repeats within the 3′ untranslated regions (UTR) of the lin-14 mRNA. Down-regulation of lin-14 at the end of the first larval stage initiates developmental progression into the second larval stage [39, 67]. The second known miRNA, let-7 RNA, is expressed later in development and is complementary to the 3′ UTR of the heterochronic genes lin-14, lin-28, lin-41, lin-42, and daf-12, indicating that the expression of these genes may be controlled directly by let-7. Lin-4 and let-7 are nonhomologous and act in a similar manner to trigger the transition to late-larval and adult stages [42]. Since the discovery of let-7, over 10,000 miRNAs have been identified in organisms as diverse as viruses, worms, and primates through random cloning and sequencing or computational prediction [21].

Biogenesis of miRNAs

The biogenesis of miRNA in animals is a complex multi-step process starting in the nucleus, passing through many post-transcriptional modifications, and ending in the cytoplasm. The canonical maturation pathway, similar to protein-coding genes, initiates at transcription (mostly by RNA polymerase II), generating a primary miRNA (pri-miRNA). The pri-miRNA is characterized by a hairpin RNA structure recognized by the nuclear RNAse III enzyme Drosha, and its cofactor DGCR8 [28, 46]. These proteins work within a complex of several proteins known as the Microprocessor. The Microprocessor cleaves the pri-miRNA to generate a shorter hairpin of about 70 nt length—the pre-miRNA [11]. This intermediate miRNA is exported from the nucleus to the cytoplasm via Exportin-5. In the cytoplasm, a second RNase III enzyme, Dicer, makes the pair of cuts that defines the other end of the miRNA, generating an siRNA-like duplex, the miR/miR* duplex [11]. Assembly of the mature, single stranded miRNA from the duplex into the RNA-induced silencing complex (RISC) completes miRNA biogenesis (Fig. 1).

MiRNAs functions

MiRNAs and nervous system regulation

Recent studies have shown that miRNA is not only required for the development of early embryonic stem cell survival and differentiation, but also plays an important role in maintaining the survival of mature neurons and their function. Overgrowth, and obstacles to synaptic plasticity, lead to the occurrence of many nervous system diseases, including Alzheimer's disease (AD), fragile X-syndrome (FXS) and autism. Studies suggest that growth and synaptic plasticity may be regulated by miR-134, which contributes to synaptic development, maturation and plasticity [47]. miR-133b is expressed specifically in midbrain dopaminergic neurons (DNs) and is deficient in midbrain tissue from patients with Parkinson's disease. miR-133b regulates the maturation and function of midbrain DNs within a negative feedback circuit that includes the paired-like homeodomain transcription factor Pitx3. A role for miR-133b in this feedback circuit in the fine-tuning of dopaminergic behaviors such as locomotion has been demonstrated [50].

Atrophin is a direct target of miR-8. miR-8 mutant phenotypes are attributable to elevated atrophin activity, resulting in elevated apoptosis in the brain and in behavioral defects. Reduction of atrophin levels in miR-8-expressing cells to below the level generated by miR-8 regulation is detrimental, providing evidence for a "tuning target" relationship between them. Previous experiments demonstrate that the neuron-specific miRNA miR-124 directly targets PTBP1 (PTB/hnRNP I) mRNA, which encodes a global repressor of alternative pre-mRNA splicing in non-neuronal cells. During neuronal differentiation, miR-124 reduces PTBP1 levels, leading to the accumulation of correctly spliced PTBP2 mRNA and a dramatic increase in PTBP2 protein. Thus, miR-124 promotes NS development, at least in part by regulating an intricate network of NS-specific alternative splicing [32, 51, 65].

miR-124 was also found to affect some regulators of neuron-specific gene expression. Recent studies

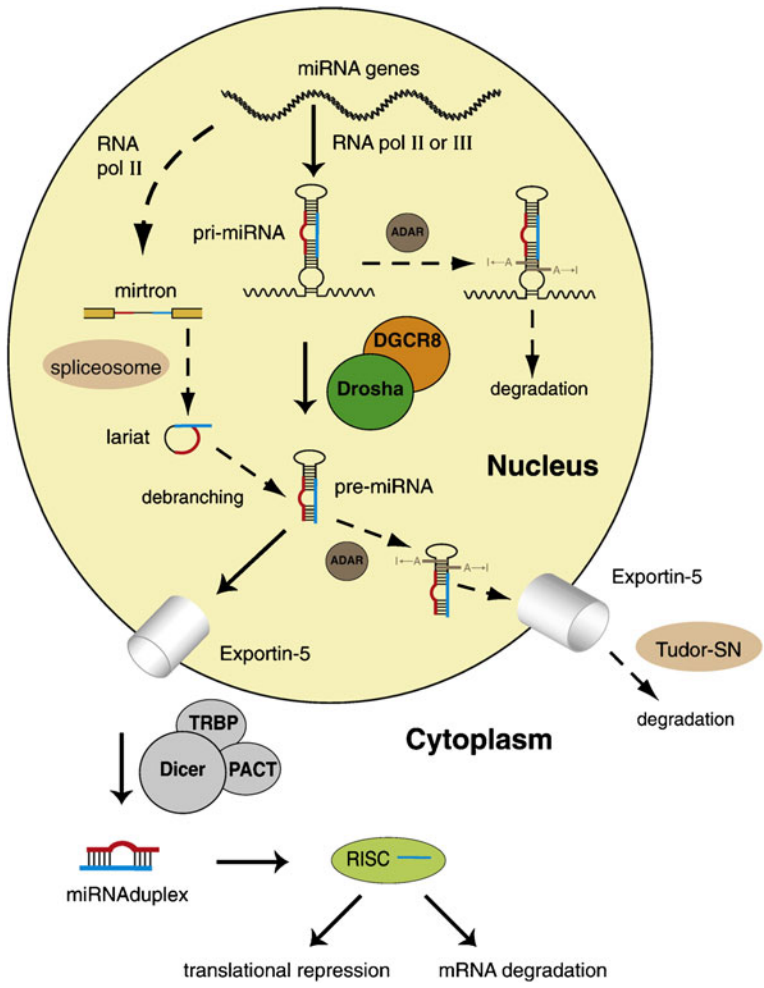

Fig. 1 The microRNA (miRNA) biogenesis pathway

have shown that miR-124 can directly inhibit small C-terminal domain phosphatase-1 (SCP1), which is required for RE1-silencing transcription factor (REST)-mediated repression of neuronal genes [10]. This is a negative feedback loop, in which expression of miR-124 and other neuron-specific genes is inhibited by REST and SCP1 in non-neuronal cells and neuronal progenitors. When cells differentiate to neurons, REST transcription is inhibited and miR-124 promotes the elimination of the biological effects of REST by inhibiting SCP1 [49].

miRNAs and cell differentiation and development

A recent study confirmed that let-7 miRNAs—a family of miRNAs highly expressed in somatic cells—can suppress self-renewal in DGCR8−/−, but not wild-type, embryonic stem cells (ESCs) and stabilize the self-renewing versus differentiated cell fates [33]. The bantam miRNA is physically associated with dFmrp in the ovary. Like dFmr1, bantam is not only required for repressing primordial germ cell differentiation, it also acts as an extrinsic factor for germline stem cell maintenance. Meanwhile, bantam interacts genetically with dFmr1 to regulate the fate of germline stem cells [72]. DGCR8, Dicer, and Ago2 are essential factors for miRNA homeostasis, with critical roles in osteoclast differentiation and function. Gene silencing of GCR8, Dicer, or Ago2 by small interfering RNAs revealed global inhibition of osteoclast transcription factor expression and function, decreased osteoclastogenesis, and decreased bone resorption in vitro. In vivo, CD11b (+) -cre/Dicer-null mice had mild osteopetrosis caused

by decreased osteoclast number and bone resorption. These results suggest that miRNAs play important roles in the differentiation and function of osteoclasts in vitro and in vivo [53]. These results provide hints as to novel molecular mechanisms controlling osteoclast differentiation and function by the miRNA system and specifically by miR-223, which regulates the NFI-A and M-CSFR levels. Zhang et al. [74] demonstrated that over-expression of miR-128 in glioma cells inhibited cell proliferation. A bioinformatics search revealed a conserved target site within the 3′-UTR of E2F3a, a transcription factor that regulates cell cycle progression. The protein levels of E2F3a in gliomas and normal brain tissues were negatively correlated to the expression levels of miR-128 in these tissues.

miRNAs and viral infection

Viruses use miRNAs in their effort to control their host cell; reciprocally, host cells use miRNAs to target essential viral functions. Experimental results have shown that miRNAs are involved in innate immunity and function as gene regulators and as a host cell defense against both RNA and DNA viruses [36, 68]. An anti-viral miRNA , which effectively restricts the accumulation of the retrovirus primate foamy virus type 1 (PFV-1) in human cells, was the first to be reported [24]. The SV40-encoded miRNA miR-S1 helps to keep the infected cell hidden from the immune system. It is expressed late in the viral replication cycle, when it acts to degrade early viral mRNAs encoding T antigen, limiting exposure of the infected cell to cytotoxic T lymphocytes [54]. The miRNA-silencing machinery plays a physiological role in controlling HIV-1 replication, inhibiting virus replication both in peripheral blood mononuclear cells from HIV-1-infected donors and in latently infected cells [61].

The expression of host cell miR-122 can inhibit the replication of hepatitis C virus (HCV) and works through IFN-β [41]. The liver-expressed miR-122 is essential for HCV RNA accumulation in cultured liver cells, but its potential as a target for antiviral intervention has not been assessed [23]. This latter study showed that treatment of chronically infected chimpanzees with a locked nucleic acid (LNA)-modified oligonucleotide (SPC3649) complementary to miR-122 leads to long-lasting suppression of HCV viremia with no evidence of viral resistance or side effects in the treated animals. Furthermore, transcriptome and histological analyses of liver biopsies demonstrated derepression of target mRNAs with miR-122 seed sites, down-regulation of interferon-regulated genes (IRGs), and improvement of HCV-induced liver pathology. The prolonged virological response to SPC3649 treatment without HCV rebound holds promise of a new antiviral therapy with a high barrier to resistance [23].

miRNAs and immunity

miRNA control has emerged as a critical regulatory principle in the mammalian immune system. Genetic ablation of the miRNA machinery, as well as loss or deregulation of certain individual miRNAs, severely compromise immune development and can lead to immune disorders like autoimmunity and cancer. Although individual miRNAs modulate protein output from hundreds of target genes, they may impact physiological processes by regulating the concentrations of just a few key cellular proteins, which may be components of a single or of functionally interrelated pathways in a given cellular context [71]. miR-223 controls the generation and activation of granulocytes; the loss-of miR-223 in mice results in an expanded granulocytic compartment resulting from a cell-autonomous increase in the number of granulocyte progenitors. In addition, granulocytes lacking miR-223 are hypermature, hypersensitive to activating stimuli and display increased fungicidal activity. Thus, miR-223 acts as a fine-tuner of granulocyte production and the inflammatory response [19].

Psoriasis is the most prevalent chronic inflammatory skin disease in adults, with a substantial negative impact on the patients' quality of life. Leukocyte-derived miRNAs and one keratinocyte-derived miRNA-203 were identified as being involved in psoriasis-affected skin. In a panel of 21 different human organs and tissues, miR-203 showed a highly skin-specific expression profile. Among the cellular constituents of the skin, it was expressed exclusively by keratinocytes. The up-regulation of miR-203 in psoriatic plaques was concurrent with the down-regulation of an evolutionary conserved target of miR-203, suppressor of cytokine signaling 3 (SOCS-3), that is involved in inflammatory responses and keratinocyte functions. Results suggest that miRNA deregulation is involved in the pathogenesis of psoriasis and contributes

to the dysfunction of the cross talk between resident and infiltrating cells [52]. The expression of miR-21 in psoriasis was up-regulated and dominantly expressed in T cells [69]. miR-146a is also a negative regulator of the interferon (IFN) pathway. Under-expression of miR-146a contributes to alterations in the type I IFN pathway in lupus patients by targeting key signaling proteins [57]. miR-125a-5p was found to mediate lipid uptake and to decrease the secretion of some inflammatory cytokines (interleukin-2, interleukin-6, tumor necrosis factor-alpha, transforming growth factor-beta) in oxLDL-stimulated monocyte-derived macrophages [7].

miRNAs and angiogenesis

Within the circulatory system, blood flow regulates vascular remodelling1, stimulates blood stem cell formation, and plays a role in the pathology of vascular disease. During vertebrate embryogenesis, vascular patterning is guided initially by conserved genetic pathways that act before circulation. Subsequently, endothelial cells must incorporate the mechanosensory stimulus of blood flow with these early signals to shape the embryonic vascular system. Quite recently, Wang et al. [66] found that an endothelial cell-restricted miR-126 mediates developmental angiogenesis in vivo. Targeted deletion of miR-126 in mice causes leaky vessels, hemorrhaging, and partial embryonic lethality, due to a loss of vascular integrity and defects in endothelial cell proliferation, migration, and angiogenesis. The subset of mutant animals that survives displays defective cardiac neovascularization following myocardial infarction. The vascular abnormalities of miR-126 mutant mice resemble the consequences of diminished signaling by angiogenic growth factors, such as VEGF and FGF. Accordingly, miR-126 enhances the proangiogenic actions of VEGF and FGF and promotes blood vessel formation by repressing the expression of Spred-1, an intracellular inhibitor of angiogenic signaling. Results showed that miR-126 mediated integration of hemodynamics and VEGF signal during angiogenesis. Additionally, knockdown of miR-126 in *zebrafish* resulted in loss of vascular integrity and hemorrhage during embryonic development. miR-126 functions in part by directly repressing negative regulators of the VEGF pathway, including the Sprouty-related protein 1 (SPRED1) and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-beta). Increased expression of SPRED1 or inhibition of VEGF signaling in *zebrafish* resulted in defects similar to miR-126 knockdown [14].

miRNAs and cancer

Abnormal cellular development, as occurs with cancer, has also been associated with miRNAs. In cancer, miRNAs work as regulatory molecules, acting as oncogenes or tumor suppressors [48]. The first study linking miRNA to cancer used patients with chronic lymphocytic leukemia [3]. Calin et al. [3] analyzed the expression of miR-15 and miR-16 in blood samples from patients with CLL. Both miRNAs were absent or down-regulated in the majority (68%) of cases when compared to normal tissue or lymphocytes. This finding suggested that these two miRNAs were causally involved in the pathogenesis of chronic lymphocytic leukemia. Quite recently, O'Connell et al. [37] found increased expression of miR-155 in bone marrow blasts of leukemic patients bearing M4 or M5 subtypes of acute myeloid leukemia (AML). Recent investigations showed that miR-23b was repressed in AML specimens compared to NBM and purified CD34 (+) hematopoietic progenitor cells [16]. The miR-34a, which has been shown to be frequently absent from pancreatic cancer cells, is induced by P53 tumor suppressor protein [6]. The miR-34a responsive genes are involved in regulating cell-cycle progression, apoptosis, DNA repair, and angiogenesis. miR-34a expression is silenced in several types of cancer due to aberrant CpG methylation of its promoter [29].

The let-7 family of miRNAs was the first group of miRNAs shown to regulate expression of a proto-oncogene, the RAS protein [45]. Johnson et al. [20] confirmed experimentally that let-7 can inhibit RAS expression in human cancer cell lines. Loss or reduction of let-7 in lung cancer leads to RAS overexpression, thus promoting cellular growth and contributing to tumorgenesis. Another group independently reported reduced expression of let-7 in lung cancers and found that this correlated with a poor prognosis [55]. Using microchip technology, Liu et al. described a method for profiling human and mouse miRNA gene expression [26]. They analyzed a panel of 20 human RNA samples, finding that each breast cancer tissue has a specific pattern for miRNA

expression. miR-9 is up-regulated in breast cancer cells, directly targeting CDH1—the E-cadherin-encoding messenger RNA—leading to increased cell motility and invasiveness [31]. miR-9 mediated E-cadherin down-regulation results in the activation of β-catenin signalling, which contributes to up-regulated expression of the gene encoding VEGF; this leads, in turn, to increased tumor angiogenesis. Over-expression of miR-9 in otherwise non-metastatic breast tumor cells enables these cells to form pulmonary micrometastases in mice. Significantly, in human cancers, miR-9 levels correlate with MYCN amplification, tumor grade and metastatic status. miR-155 was found to be one of the most potent miRNA suppressing apoptosis in human T cell leukemia Jurkat cells and in MDA-MB-453 breast cancer cells [40]. The mechanism underlying the effect of miR-155 could be ascribed to a blockade of caspase-3 activity [13]. Tumors had reduced levels of miR-26 expression as compared with paired noncancerous tissues, which indicated that the level of miR-26 expression was also associated with hepatocellular carcinoma. Moreover, tumors with reduced miR-26 expression had a distinct transcriptomic pattern, and analyses of gene networks revealed that activation of signaling pathways between nuclear factor B and interleukin-6 might play a role in tumor development. The miR-26 expression status of such patients is associated with survival and response to adjuvant therapy with interferon alfa [17].

miRNAs and cardiac diseases

Dysregulation of miRNAs by several mechanisms has been described in various disease states including cardiac disease. miR-21 regulates the ERK-MAP kinase signalling pathway in cardiac fibroblasts, which has impacts on global cardiac structure and function [22]. miR-21 levels are increased selectively in fibroblasts of the failing heart, augmenting ERK-MAP kinase activity through inhibition of sprouty homologue 1 (SPRY1). In vivo silencing of miR-21 by a specific antagomir in a mouse pressure-overload-induced disease model reduces cardiac ERK-MAP kinase activity, inhibits interstitial fibrosis and attenuates cardiac dysfunction. A recent study has shown that programmed cell death 4 (PDCD4) is regulated by miR-21 and is a direct target of miR-21 in cardiac myocytes [8]. These findings reveal that miRNAs can contribute to myocardial disease by their effects on cardiac fibroblasts [60]. Condorelli et al. [4] found that in vivo inhibition of miR-133 by a single infusion of an antagomir caused marked and sustained cardiac hypertrophy. miR-133a-1 and miR-133a-2 are identical, muscle-specific miRNAs that are regulated during muscle development by the transcription factor SRF. Results showed that mice lacking either miR-133a-1 or miR-133a-2 are normal, whereas deletion of both miRNAs causes lethal ventricular-septal defects in approximately one-half of double-mutant embryos or neonates [27]. Cordes et al. [9] demonstrated that miR-145 can direct smooth muscle fate, and that miR-145 and miR-143 function to regulate the quiescent versus proliferative phenotype of smooth muscle cells.

miRNAs and gastrointestinal diseases

Research has already confirmed that miRNAs can inhibit the expression of oncogenes or anti-oncogenes, and can play a role in the tumorgenesis and progression of gastrointestinal cancers [38, 44]. Studies have shown that some miRNAs are down-regulated in gastrointestinal cancers, which suggests that they may function as tumor suppressors. miR-15b and miR-16, which are down-regulated in human gastric cancer cells, play a role in the development of multidrug resistance (MDR) by modulation of apoptosis via targeting BCL2 [70]. On the other hand, some miRNA genes are over-expressed in gastrointestinal cancers, indicating that they may have roles as oncogenes and accelerate the development of gastrointestinal cancer. Recently, Ueda et al. [62] performed a systematic profile and analysis using 353 gastric samples and identified 22 miRNAs that were up-regulated and 13 that were down-regulated in gastric cancer (GC). This signature has an accuracy of 83% in distinguishing GC samples from benign tumors. Furthermore, the two histological subtypes of GC tissues showed different miRNA signatures: eight miRNAs were up-regulated in diffuse-type and four in intestinal-type cancer [62]. Studies by Nagel et al. [35] found that miR-135a and miR-135b target the 3′-untranslated region of the adenomatous polyposis coli (APC) gene, suppress its expression, and induce downstream Wnt pathway activity. Considerable up-regulation of miR-135a and miR-135b is seen in colorectal adenomas and carcinomas, which correlated significantly with low APC mRNA levels, regardless of the mutational status of the

APC gene. The results uncover a miRNA-mediated mechanism for the control of APC expression and Wnt pathway activity, and suggest its contribution to colorectal cancer pathogenesis [35].

miRNAs and mammalian reproduction

miRNAs regulate physiological processes such as oocyte maturation, luteum development and early embryo development. Yu et al. [73] reported 29 miRNAs from mouse testis that are differentially expressed as the prepubertal testis differentiates to the adult testis, and identify several possible male germ cell target mRNAs. Early in development, primordial germ cells (PGCs) are set aside from somatic cells and acquire a unique gene-expression program. Nanos expression is required during germline development and is post-transcriptionally restricted to PGCs [34]. The miR-430 targets the 3′-UTR of nanos1 during *zebrafish* embryogenesis. A miR-430 target site within the nanos1 3′-UTR reduces poly (A) tail length, mRNA stability, and translation [34]. Repression is disrupted in maternal-zygotic dicer mutants (MZdicer), which lack mature miRNAs, and is restored by injection of processed miR-430. Although miR-430 represses other genes equally in germline and soma, specific regions in the nanos1 3′-UTR compensate for miRNA-mediated repression in PGCs and allow germline-specific expression. Other results support the effects of the loss of maternal inheritance of miRNAs following specific deletion of Dicer from growing oocytes. Mutant mature oocytes were almost entirely depleted of all miRNAs, and failed to progress through the first cell division, probably because of disorganized spindle formation, demonstrating that the maternal miRNAs are essential for the earliest stages of mouse embryonic development [56]. Tesfaye et al. [59] revealed the differential expression of 59 miRNAs, of which 31 and 28 miRNAs were found to be expressed preferentially in immature and mature oocytes, respectively. Expression profiling of selected miRNAs during preimplantation stage embryos showed a distinct temporal expression pattern [59].

miRNAs and diabetes

Several studies to date have demonstrated that miRNAs, which regulate translation of gene transcripts, influence gene expression cascades involved in pancreas development. Some of these miRNAs, e. g., miR-7 and miR-375, are known to be expressed at high levels in pancreas, and are also known to be involved in Zebrafish pancreas development as well as in insulin secretion in mice [12]. This latter study found that miR-375 directly targets 3′-phosphoinositide-dependent protein kinase-1(PDK1) and reduces its protein level, resulting in decreased glucose-stimulatory action on insulin gene expression and DNA synthesis. Furthermore, glucose leads to a decrease in miR-375 precursor level and a concomitant increase in PDK1 protein [12]. miR-375 is differentially expressed in human islet beta as well as non- beta-cells. Though no significant difference in abundance of miR-375 was noted in either cell type, analysis of islet-specific miRNA and mRNA in single cells revealed that non-beta cells contain higher levels of miR-375 [18]. The biological mechanism of a recently discovered association of type 2 diabetes with the ACAA-insertion/deletion polymorphism at the 3′-UTR of the IGF2R gene had remained unclear. The very recently emerging novel polymorphic control layer by miRNAs now sheds light on this issue [30].

Other functions of miRNAs

Signal transmission

Exosomes are small (50–90 nm) membrane vesicles of endocytic origin that are released into the extracellular environment upon fusion of multivesicular bodies (MVB) with the plasma membrane [64]. These vesicles can mediate communication between cells, facilitating processes such as antigen presentation. Studies in animals have confirmed that genetic exchange between cells can occur in exosome-mediated transfer of miRNAs [63]. Studies showed that exosomes from a mouse and a human mast cell line (MC/9 and HMC-1, respectively), contain miRNAs that can be delivered to another cell, and can be functional in this new location [63].

In plants, a recent study has shown that miRNA can move from one cell to another, and affect the signal transmission of gene expression. In roots, radial tissue organization is highly conserved, with a central vascular cylinder in which two water-conducting cell types, protoxylem and metaxylem,

are patterned centripetally. Carlsbecker et al. [5] discovered that a protein called SHORTROOT migrates to the inside of the root skin where it activates another protein SCARECROW by watching the development of cell types through the actions of protein molecules. SHORTROOT, produced in the vascular cylinder, moves into the endodermis to activate SCARECROW. In combination, these molecules trigger the production of miR-165a and miR-166b. These miRNAs are able to cross the cell membrane and penetrate the membranes of neighboring cells. Endodermally produced miRNAs then act to degrade target mRNAs encoding class III homeodomain-leucine zipper transcription factors in the endodermis and stele periphery [5].

Maintain the stability of molecular networks

Researchers have recently claimed to have found RNA fragments that play an important role in functions determining the identity of individuals animals. miR-7 is perfectly conserved from annelids to humans, and yet some of the genes that it regulates in Drosophila are not regulated in mammals. The study found that miR-7 functions in several interlocking feedback and feed-forward loops, and proposes that its role in these networks is to buffer them against perturbation. To directly demonstrate this function for miR-7, the network was subjected to temperature fluctuation; it was found that miR-7 is essential for the maintenance of regulatory stability under conditions of environmental flux, suggesting that some conserved miRNAs like miR-7 may enter into novel genetic relationships to buffer developmental programs against variation and thus impart robustness to diverse regulatory networks [58].

Perspectives

The discovery of miRNAs has led to deeper insights into the regulation mechanism of gene expression and the complexity of this process. Regulatory RNAs could have therapeutic implications, in which disease-related miRNAs could be antagonized or functional miRNAs restored. New knowledge about miRNA function may bring new possibilities and strategies in developing novel disease therapies. It was recently demonstrated that a number of drugs of clinical relevance can modulate miRNA expression in treated cells in vitro, suggesting that miRNAs might be suitable targets for the therapeutic effects of anticancer agents [2, 43]. Although miRNA therapeutic modulation is potentially attractive for treating cancer as well as diagnosis and prognosis, there is still a great need to understand the roles of miRNA in tumorigenesis more deeply. It is also necessary to develop an effective and efficient method for delivering antagomirs in vivo. With further study, we predict that miRNAs will be found to have played a more important role than previously thought in the origin of life, evolution of species, the complexity of gene expression regulation, disease mechanisms and development. Meanwhile, the study of miRNA will also provide a new basis for thought, and provide scope for the application of RNA interference (RNAi) technology. At present, the main challenge is that the miRNAs of a variety of organisms and their target genes, as well as their function, remain to be identified. As miRNA genes are related to growth, development and differentiation, understanding their functions will bring a new revolution of detailed insight into the phenomena of living organism activities.

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