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Novel functions for small RNA molecules

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Abstract

Small RNAs are short (~ 18 to 30 nucleotides), non-coding RNA molecules that can regulate gene expression in both the cytoplasm and the nucleus via post-transcriptional gene silencing (PTGS), chromatin-dependent gene silencing (CDGS) or RNA activation (RNAa). Three classes of small RNAs have been defined, including microRNAs (miRNAs), siRNAs and Piwi-interacting RNAs (piRNAs). Research has indicated that small RNAs play important roles in cellular processes such as cell differentiation, growth/proliferation, migration, apoptosis/death, metabolism and defense. Accordingly, small RNAs are critical regulators of normal development and physiology. More interestingly, increasing evidence indicates that small RNAs are involved in the pathogenesis of diverse diseases including cancer, cardiovascular disease, stroke, neurodegenerative disease, diabetes, liver disease, kidney disease and infectious disease. More than 20 clinical trials are ongoing to evaluate therapies based on small RNA. Additionally, small RNAs may serve as novel biomarkers and therapeutic targets for the majority of diseases.

Keywords

Biomarker; cell biology; disease; microRNA; small RNA; therapy

Introduction

Small RNAs are defined as short (~ 18 to 30 nucleotides [nt]), non-coding RNA molecules that can inhibit the expression of target genes via post-transcriptional gene silencing (PTGS) and chromatin-dependent gene silencing (CDGS), in both the cytoplasm and the nucleus [1–3]. In addition, more recent studies revealed that some small RNAs can increase the expression of target genes via an RNA activation (RNAa) mechanism [4–6]. It is generally agreed that there are three main classes of small RNAs: microRNAs (miRNAs), siRNAs and Piwi-interacting RNAs (piRNAs) [1]. Recently, there has been an increase in the number of studies of small RNAs, reflecting the novel association of these molecules with many critical biological functions [7]. A given small RNA sequence is able to regulate the expression of multiple genes because it can bind to target genes as either an imperfect or perfect complement [8] Thus, small RNAs are functionally as important as transcription factors [9]. As a group, small RNAs may directly regulate more than 30% of the genes in a cell [10]. It is not surprising, therefore, that small RNAs are involved in the regulation of all major cellular functions, including cell differentiation, growth/proliferation, migration, apoptosis/death, metabolism and defense [11, 12]. Given these diverse roles, small RNAs could be pivotal regulators in development, physiology and disease [11]. This review article summarizes the novel functions of these small RNA molecules.

Classification, biogenesis and gene-regulatory mechanism of small RNAs

Although many classes of small RNAs have emerged, various characteristics related to the origins, structures, associated effector proteins and biological roles of small RNAs have resulted in the classification of these molecules into three main categories: miRNAs, siRNAs and piRNAs [1]. miRNAs are ssRNA molecules with a length of approximately 21 or 22 nt. miRNAs are initially transcribed in the nucleus to form large pri-miRNA transcripts, which are subsequently processed in the nucleus into approximately 70-nt pre-miRNAs. These pre-miRNAs are then transported into the cytoplasm to become mature miRNAs [11]. The inhibition of the expression of target genes by miRNAs then occurs via PTGS and CDGS in both the cytoplasm and the nucleus [1, 13]. Currently more than 850 mature human miRNA sequences have been identified [14], many of which are highly conserved among many species. siRNAs were discovered from the phenomenon known as RNAi, in which administrated dsRNAs selectively inhibit the expression of target genes with a homologous nt sequence [1]. Because these products of processed dsRNAs were able to efficiently reconstitute silencing complexes, they were named siRNAs [15]. It is well established that siRNAs can be produced from RNA transcribed in the nucleus (endogenous siRNAs), can be virally derived or can be introduced experimentally as chemically synthesized dsRNAs (exogenous siRNAs) [1]. siRNAs have a distinct size distribution that is able to inhibit the expression of genes by PTGS and CDGS [1]. piRNAs are the most recently discovered class of siRNAs [16] and, as their name suggests, are germ-cell-specific small RNAs that bind to the Piwi clade of Argonaute proteins [17]. Piwi is a critical protein for their target gene regulation (17). In addition to the inhibitory effects of piRNAs on their target genes, recent studies indicate that some piRNAs also are able to increase their target gene expression via an RNAa mechanism [4–6].

Cellular functions of small RNAs

Small RNAs in cell differentiation

During development, the expression of small RNAs occurs in spatiotemporal, tissue- and cell-specific manners, suggesting the involvement of small RNAs in cell differentiation. The first evidence that miRNAs might play a role in the differentiation of embryonic stem (ES) cells came from the identification of changes in the expression of miRNAs during the differentiation of ES cells, in which multiple miRNAs were either downregulated or upregulated [17,18]. The critical role of miRNAs in the differentiation of ES cells was further verified in mice with the genes encoding Dicer (a key enzyme in small RNA biogenesis) inactivated by gene knockout (KO) [18]. In these mice, the inhibition of the effects of miRNA resulted in abnormal differentiation of ES cells and the premature death of embryos [18,19]. More recently, Xu and colleagues reported that the expression of miRNA-145 in EC cells was high during differentiation but low other times [20]. Studies have been conducted to elucidate the roles of miRNAs in adult cell differentiation [21–30]. One example is the study of differentiation in cancer cells. Dedifferentiation is an important feature of cancer cells [21], and miRNAs are dysregulated in many cancers [22,23]. The modulation of miRNAs has strong effects on the differentiation of cancer cells [23]. The role of the miRNA-1 (miR-1) in the differentiation of cardiomyocytes was discovered in 2005 [24]. The miR-1 gene was identified as a direct transcriptional target of several regulators of muscle differentiation [24]. The role of miRNAs in the differentiation of adult vascular smooth muscle cells (VSMCs) was investigated in a study by Zhang and colleagues [25]. miR-145 was identified as a critical controller of VSMC differentiation both in rat VSMCs *in vitro* and in rat carotid arteries *in vivo*, via its target genes such as Kruppel-like factor 5 (*KLF5*) [25]. Further studies revealed that miRNAs also play important roles in the differentiation of other cell types [26–30], for example: miR-143 in adipocyte differentiation [26]; miR-181 in myoblast differentiation [27]; miR-142s, miR-181 and miR-223 in

hematopoietic cell differentiation [28]; miR-25 in airway smooth muscle cell differentiation [29]; and multiple miRNAs in neural cell differentiation [30].

Small RNAs in cell growth/proliferation

During the past 5 years, the biological roles of miRNAs in the growth and proliferation of cancer have been studied. miRNAs were identified as critical regulators of the proliferation of many cancer cells [22,23].

The role of miRNAs in the growth of cardiomyocytes has been documented in recent studies [31–33]. The overexpression of miR-21, miR-23a, miR-23b, miR-24, miR-195 or miR-214 via adenovirus-mediated gene transfer induced the hypertrophic growth of cultured cardiomyocytes; whereas the overexpression of miR-150, miR-181b and miR-1 caused a reduction in the size of cardiomyocytes [31–33]. These effects of miRNAs on cardiomyocyte growth were confirmed by further studies both *in vitro* and *in vivo* [34–36].

In recent studies by Zhang and colleagues, it was demonstrated that miR-221, miR-222 and miR-145 play important roles in the proliferation of VSMCs [31,37]. The overexpression of miR-221 and miR-222 increased the proliferation of VSMCs, whereas knockdown of the two miRNAs decreased proliferation [37]. In contrast, VSMC proliferation was significantly inhibited by the overexpression of miR-145 [31]. The effects of miR-221, miR-222 and miR-145 on VSMC proliferation were also demonstrated by two other independent research groups [38,39]. In addition, miR-143 was identified as a regulator of the proliferation of VSMCs by Cordes *et al* [39].

Small RNAs in cell migration

miRNAs are critical regulators of the migration of cancer cells; for example, miR-23b [40], miR-146b [41] and miR-34a [42,43] are reported to be involved in this process. The effects of miR-221 and miR-222 on the migration of vascular endothelial cells were initially determined by assays of tube formation and wound healing [44]. The results suggest that the influence of miR-221 and miR-222 on the migration of endothelial cells occurs, at least in part, through c-kit [44]. The effects of other miRNAs, such as let-7 [45,46], miR-27b [45,46], miR-126 [47] and miR-210 [48] on human endothelial cell migration have also been demonstrated. For example, Davis *et al* reported that miR-221 had a pro-migratory effect on VSMCs [38].

Small RNAs in cell death and apoptosis

The role of miRNAs in the apoptosis of cancer cells was described in a review article by Wang and Lee [49]. Multiple miRNAs that are deregulated in cancer cells were found to regulate apoptotic pathways. For example, miR-29b [50], miR-15-16[49], let-7[49], miR-98[49], miR-21[51] and miR-17-92 [52] were involved in regulating the apoptosis of cancer cells [49]. The biological roles of miRNAs in cell apoptosis/death were also demonstrated in other types of cells. [53–55]. In studies by Zhang and colleagues, miR-21 was demonstrated to be an important anti-apoptotic miRNA in cardiac cells and in VSMCs both *in vitro* and *in vivo* via the regulation of the target genes of miR-21, such as *PDCD4* [53–55].

Small RNAs in cell metabolism and cell defense

Small RNAs were reported to have roles in cell metabolism, including lipid metabolism [56] and glucose homeostasis [57]. In addition, miRNAs and siRNAs are also involved in cell defensive responses to diverse injuries such as oxidative stress [58], bacterial infections [59] and viral infections [60].

Small RNAs in developmental biology

The roles of small RNAs in development were first demonstrated in Dicer KO mice. These mice did not survive for more than 7.5 days after gastrulation, suggesting a vital role of miRNAs in early development [61,62].

Small RNAs in early embryonic development

miR-15 and miR-16 inhibited Nodal signaling and dorsal mesoderm patterning in the early embryo [63]. Spemann's organizer and head structures were reduced in size by the overexpression of miR-15 and miR-16, but were increased by the inhibition of these miRNAs. miR-430, a highly abundant miRNA that is required for the clearance of maternal mRNAs, was demonstrated to directly decrease the expression of squint mRNA, a member of the Nodal family [64]. Interestingly, lefty mRNA, an antagonist of Nodal, was also downregulated by miR-430. When miR-430 complementary sites of squint were mutated, early embryonic development was disrupted [64].

Small RNAs in cardiac development

The role of miRNAs in cardiac development was demonstrated by investigating the role of miR-1 in this process [24,65]. Cardiomyocytes in *miR-1-2* KO mutant mice failed to exit the cell cycle properly, resulting in hyperplasia [65]. These defects resulted in the prenatal or early postnatal death of approximately half of the mutant mice. In addition to miR-21, miR-133a and miR-206 are also involved in cardiac development [66].

Small RNAs in neuronal development

The role of small RNAs in neuronal development was first demonstrated by the requirement for miR-273 in the establishment of left-right asymmetry in ASE neurons in *Caenorhabditis elegans* [67]. miR-124a, which is specific to neuronal tissue, helps to acquire and maintain the neuronal cellular identity by directly silencing a large number of target mRNAs, including the mRNAs for polypyrimidine tract-binding protein and the RE1-silencing transcription factor [68].

Small RNAs in germline development

Mutations in piRNA-pathway genes including *piwi*, *zucchini* and *squash* resulted in severe defects in oogenesis, including loss of germline stem cells in *Drosophila*. The mouse genome encodes three Piwi homologs: Miwi, Miwi2 and Mili [69]. Mutations in each of the three genes led to the degeneration of the male germline, suggesting piRNAs might play an important role in the mouse female germline [69].

Small RNAs in disease

Small RNAs in cancer

Cancer is a complex disease that involves a variety of changes in gene expression that result in abnormal cell growth, migration and apoptosis [69]. As these genes and cellular functions are regulated by miRNAs, cancer became the first field of miRNA research [22].

miRNA expression profiles have been generated by microarray analysis in multiple cancer types, including bladder cancer [70], breast cancer [71], chronic lymphocytic leukemia (CLL) [72], colorectal cancer [73,74], gastric cancer [75], glioblastoma [76], hepatocellular carcinoma [77], lung cancer [78], nasopharyngeal carcinoma [79], oral cancer [80], ovarian cancer [81], pancreatic tumor [82], pituitary tumor [83] and prostate cancer [84]. These studies demonstrated that many miRNAs are aberrantly expressed in diverse cancers. The uniqueness of miRNA profiling in particular cancer types may provide important

information for cancer diagnosis; different cancers may have different miRNA signatures [7,12]. Further studies demonstrated that many of these aberrantly expressed miRNAs were either tumor suppressors (TS-miRs) or oncogenes (onco-miRs) [22,85,86]. For example, let-7 was reported to be a TS-miR for lung cancer via its target gene *RAS* [87], and miR-34a was reported to be a TS-miR for many cancers via its multiple target genes such as *BCL2* [88]. In contrast, miR-155 [89] and miR-372 [90] were reported to be onco-miRs.

Studies revealed that the some miRNAs are related to tumor invasion and metastasis [91,92]. For example, miR-31 [93], miR-200 [94], miR-193b [95] and miR-23b [40] were reported to be inhibitors of tumor invasion and metastasis. However, tumor invasion and metastasis were promoted by some miRNAs such as miR-373 [40], miR-520c [96] and miR-10b [97]. In addition, studies demonstrated that expression patterns of miRNAs are able to aid in cancer prognosis [98–101]. Thus, small RNAs may play important roles in cancer development and progression, as well as prognosis, diagnosis and the evaluation of treatment response [22,23].

Small RNAs in cardiovascular disease

Cardiac hypertrophy and heart failure are the most common pathological responses to several cardiovascular diseases. Cardiac hypertrophy often leads to heart failure, and is a major determinant of mortality and morbidity in cardiovascular diseases. miRNAs are important regulators for the differentiation and growth of cardiac cells, and it is therefore reasonable to hypothesize that miRNAs play important roles in cardiac hypertrophy and heart failure. Four independent research groups almost simultaneously reported results profiling the miRNA expression signature of mouse hearts in which hypertrophy was induced [31–34]. The modulation of some of these dysregulated miRNAs had strong effects on cardiac myocyte hypertrophy *in vitro* [31–33]. *In vivo*, the overexpression of miR-195, an miRNA that was upregulated in hypertrophic hearts, was sufficient to induce cardiac hypertrophy [33], while a gene mutation or 'decoy' approach confirmed the roles of miR-133 [34] and miR-208 [36] in cardiomyocyte hypertrophy. The roles of miR-23a [102] and miR-1 [103] in cardiac hypertrophy were identified in two studies. Furthermore, the role of miRNAs in human cardiac hypertrophy and heart failure was investigated in several clinical studies [33,104,105]. A critical link between miRNAs and heart failure has also been reported [106–108].

Cardiovascular diseases are the leading cause of death in developed countries. Several recent studies have suggested that miRNAs might play critical roles in the pathophysiology of acute myocardial infarction (AMI) [109–112]. The potential involvement of miRNAs in AMI was suggested in a study in miR-126-null mice that demonstrated the survival rate of miR-126-deficient mice following AMI was significantly lower than of miR-126-expressing mice [110]. van Rooij *et al* studies the expression signature in late phase AMI and found that miR-29 was critical for cardiac fibrosis during the repair process after AMI [112]. In addition, a study in an *in vitro* ischemia/reperfusion (I/R) injury model revealed that myocardial infarct size was reduced in mouse hearts pre-injected with heat-shock-induced miRNAs including miR-21 [112]. Moreover, the miRNA expression signature and the role of miR-21 in the early phase of AMI were identified by Zhang and colleagues [113]. In a mouse model, Ren *et al* found that miR-320 was involved in the regulation of cardiac I/R injury by targeting Hsp20 [114].

The formation of neointimal lesions is a common pathological feature of diverse cardiovascular diseases [53]. Using microarray analysis and a model of neointimal lesion formation, the miRNA expression profile in the vascular wall during neointimal lesion formation was determined [53]. Compared with healthy, uninjured arteries, microarray analysis demonstrated that aberrant miRNA expression was a characteristic of vascular walls

after angioplasty [53]. Modulating an aberrantly overexpressed miRNA, miR-21, via antisense-mediated knockdown had a significantly negative effect on neointimal lesion formation in rat arteries after angioplasty [53]. These results indicated that miRNAs might be important regulators in the development of proliferative vascular diseases.

More recently, a series of studies were conducted by different research groups to determine the roles of miR-221 [37,38], miR-222 [37], miR-143 and miR-145 [25,39,113] in proliferative vascular disease. Zhang and colleagues demonstrated that knocking-down miR-221 and miR-222 inhibited neointimal growth in rat carotid arteries after angioplasty by inhibiting the proliferation of VSMCs [37]. The cellular effects of miR-221 were further demonstrated by another study, in which Davis *et al* demonstrated that miR-221 increased the proliferation of VSMCs and migration through its target gene, *p27Kip1* [38]. miR-145 was also identified as the most abundant miRNA in normal arteries, and the expression of this miRNA was significantly downregulated in injured or atherosclerotic vascular walls with neointimal lesion formation [25,39]. Interestingly, both studies identified miR-145 as a critical regulator for the differentiation and proliferation of VSMCs. The restoration of miR-145 in balloon-injured rat carotid arteries significantly inhibited neointimal growth [25]. Moreover, KO of miR-145 and miR-143 was sufficient to elicit neointimal lesion formation in mouse vessels [113].

Small RNAs in stroke and neurodegenerative disease

In a model of middle cerebral artery occlusion (MCAO), two studies revealed the role of miRNAs in the pathogenesis of stroke [115,116]. In the first study, the expression profiles of miRNAs were first identified by Jeyaseelan *et al*, in which multiple miRNAs were found to be involved in ischemic injury responses in the brain [115]. In the second study, of the 238 miRNAs evaluated, 8 had increased expression and 12 had decreased expression in at least 4 out of 5 reperfusion time points studied between 3 h and 3 days, compared with controls [116]. Bioinformatic analysis indicated a correlation in expression levels between altered miRNA and several mRNAs known to mediate inflammation, transcription, neuroprotection, receptor function and ionic homeostasis [116].

The roles of miRNAs in neurodegenerative disease were described in a review article by Barbato *et al* [117]. Alzheimer's disease (AD) is the best studied degenerative disease affecting the CNS. Several studies demonstrated that multiple dysregulated miRNAs could be associated with aging and could contribute to the development of AD, including miR-9, miR-128, miR-29, miR-107, let-7, miR-101, miR-15a and miR-106b [117–121]. miR-133b was identified as an miRNA expressed in midbrain dopaminergic neurons, but the expression of this miRNA was deficient in midbrain tissue from patients with Parkinson's disease [122]. miR-133b was suggested to regulate the maturation and function of dopaminergic neurons within a negative feedback circuit through suppression of paired-like homeodomain 3 (Pitx3) [123]. In addition, levels of miR-19, miR-101 and miR-130 were related to the severity of neurodegenerative disease because these miRNAs target ataxin-1, which is increased in diseases such as spinocerebellar ataxia type 1 [117].

Small RNAs in diabetes

miRNAs have emerged as novel regulators in both type I and type II diabetes [122]. The first miRNA that was linked to type I diabetes was miR-375 [124–126], which was found to be selectively expressed in pancreatic endocrine cell lines [124]. Overexpression of miR-375 resulted in suppressed glucose-stimulated insulin secretion, whereas inhibition of miR-375 enhanced insulin secretion, both via miR-375-mediated suppression of myotrophin and pyruvate dehydrogenase kinase [125,126]. Mice lacking miR-375 were hyperglycemic [126], and exhibited increased total pancreatic α-cell counts, raised levels of fasting and fed

plasma glucagon, and increased gluconeogenesis and hepatic glucose output [126]. Pancreatic β-cell mass was reduced as a result of impaired proliferation [126]. More recently, the role of miRNAs in type II diabetes was also reported. For example, the overexpression of miR-125a in insulin target tissues was related to type II diabetes in rats [127].

Small RNAs in liver disease

The expression of miRNAs is dysregulated in many liver diseases such as viral hepatitis, hepatocellular cancer and polycystic liver diseases (for a review, see reference [128]). For example, the expression of miR-1, miR-30, miR-122, miR-128, miR-196, miR-296, miR-351, miR-431 and miR-448 was demonstrated to be related to viral and virus-infected cell functions during viral hepatitis [129–131]. The expression of miR-21, miR-224, miR-34a, miR-221, miR-222, miR-106a, miR-203, miR-122, miR-422b, miR-145 and miR-199a was related to hepatocellular cancer [40]. In addition, decreased expression of miR-15a was associated with polycystic liver disease in model cell lines [132].

Small RNAs in kidney diseases

A role for miRNAs in kidney diseases was recently discovered [133–135]. Studies of conditional Dicer KO mice revealed critical roles for miRNAs in kidney development, and the maintenance of the structural and functional integrity of the renal collecting system and glomerular barrier [133]. Kidney podocyte-specific deletion of Dicer resulted in proteinuria and severe renal dysfunction in mice [134]. miRNA expression profiles were characterized in normal and diseased kidneys [133], and miR-15a was implicated in pathways linked to cystic kidney disease [133]. In addition, miR-192 and miR-377 promoted matrix deposition [133], while miR-200 and miR-205 were related to epithelial-to-mesenchymal transition [133]. In contrast, miR-21 might have protective roles in kidney disease [135].

Small RNAs in infectious diseases

Viruses encode miRNAs that target their own mRNA or the mRNA of the cells that they infect [136,137]. For example, miRBART2 is encoded by Epstein-Barr virus (EBV) and inhibits EBV DNA polymerase BALF5 [136]. miR-H2-3p, miR-H6, miR-H3 and miR-H4 provided novel candidate mechanisms for the regulation of viral latency and productive replication [36, 38]. simian virus 40 (SV40) -encoded microRNAs were able to regulate viral gene expression and reduce susceptibility to cytotoxic T cells in cultured TC-7/Db cells [139]. miR-UL112, expressed by human CMV, targeted the MHC class I polypeptide-related sequence B and inhibited NK cell killing of virus-infected cells [140]. In an alternative mechanism, viral miRNA targeted the apoptosis pathway to promote the survival of infected cells, which was supported by miR-BART5, expressed by EBV to inhibit the pro-apoptotic protein PUMA [141]. The detailed roles of individual miRNAs in infective diseases should be investigated in future studies. The role of siRNAs in infective diseases is well characterized and is described in a review article by Ge *et al* [142].

The use of small RNAs in diagnosis

The expression of miRNAs is tissue-specific. Many studies in diseased tissues revealed that different diseases had different miRNA expression signatures. These findings provided an opportunity to use these signatures in the diagnosis of diverse diseases (for reviews of the diagnostic roles of tissue miRNAs, see references [143,144]). Interestingly, studies revealed that miRNAs exist in circulating blood. In contrast to the original hypothesis that miRNAs are only stable within the cells, miRNAs are relatively stable in the blood because of their ability to bind to other materials such as exosomes [145, 146] . Increasing evidence suggests that miRNAs in diseased tissues could be released into circulating blood, which can then be

measured and used as a diagnostic biomarker [145–146]. Circulating miRNAs have been proven to be biomarkers in cancer [145, 146], stroke [145] and heart disease [147]. For example, serum levels of miR-141 (an miRNA expressed in prostate cancer) were able to distinguish patients with prostate cancer from healthy controls [146], and plasma miR-208 has been demonstrated as a biomarker for myocardial injury [147].

Small RNAs in therapy

miRNAs are aberrantly expressed in many diseases, some being upregulated and others downregulated. Thus, two major miRNA-based therapeutic strategies are restoring the expression of miRNAs reduced in diseases and, conversely, inhibiting overexpressed miRNAs (for a review of the therapeutic use of siRNAs, see reference [148]).

Artificial antisense oligonucleotides that downregulate complementary miRNA sequences are designated as anti-miRs, also known as miRNA inhibitors [148,149]. Many chemical modifications of miRNA inhibitors have been designed to enhance miRNA inhibition, increase stability and tissue uptake. miR-122 expressed in the liver was implicated in cholesterol and lipid metabolism. Locked nucleic acid (LNA)-anti-miR-122 was used to efficiently silence miR-122 in monkeys, and resulted in a long-lasting and reversible decrease in total plasma cholesterol, without evidence of LNA-associated toxicities or histopathological changes [150].

The upregulation of a specific miRNA *in vivo* in the clinic is another strategy for the treatment of diverse diseases. Although pre-miRNAs and miRNA mimics are successful in upregulating the expression of miRNAs *in vitro*, the application of these mimics *in vivo* remains questionable due to their weak tissue uptake and short period of effect. Virus expressing miRNAs such as adenovirus was successfully used to upregulate the expression of miRNAs in animal studies [25,37,113]. Additional approaches to upregulate miRNAs in patients should be investigated.

Although small RNA-based modalities have not been accepted as formal therapeutic medicines, the promising results from animal studies prompted an increasing number of clinical trials of small RNA-based therapeutics for the treatment of many human diseases. There are more than 20 ongoing clinical trials of miRNAs [151–153]. Some of the small RNA-based drugs that are currently undergoing clinical trials are listed in Table 1, together with their therapeutic target(s), possible indications and developing companies [151,154–164].

The first siRNA-based clinical trial was initiated in 2004 by Acuity Pharmaceuticals Inc [151,154]. The company tested an siRNA, bevasiranib (now being developed by OPKO Health Inc following its merger with Acuity), for the treatment of wet age-related macular degeneration (Table 1). Bevasiranib targets the expression of *VEGF*. In this clinical trial, an inhibitory effect on the overgrowth of blood vessels behind the retina (the cause of severe and irreversible loss of vision) occurred in patients receiving the siRNA. Bevasiranib, which is in phase III trials for wet age-related macular degeneration, is also undergoing a phase II clinical trial for the treatment of diabetic macular edema [151,154].

The first clinical trial of an anti-infective miRNA is the miR-122 inhibitor (anti-miR-122) SPC-3649 for the treatment of HCV infection, initiated by Santaris Pharma A/S in 2009 [155]. miR-122 is the therapeutic target of this inhibitor because this molecule facilitates the replication of HCV in the liver.

Recently, Genta Inc completed a clinical trial of the RNA-based therapy oblimersen sodium (Genasense), which targets *BCL2*, in the treatment of relapsed or refractory CLL [156,157]

(Table 1). The company had filed an NDA with the FDA after positive clinical findings. The FDA has recommended conducting a confirmatory clinical trial for this potential novel drug [157].

Some disadvantages were identified in the initial clinical trials of small RNA-based therapies, such as off-target effects. However, the interest in these new therapeutic approaches from researchers, clinical doctors and the drug development industry suggests that rapid advances and new applications for small RNA-based therapies should be expected.

Conclusion

In summary, as shown in Figure 1, recent research progress has identified many novel functions of small RNAs in cell biology. Small RNAs have important roles in development and disease via regulation of cell differentiation, growth/proliferation, migration, apoptosis/death, metabolism and defense. Increasing evidence reveals that small RNAs are involved in the pathogenesis of diverse diseases such as cancer, cardiovascular disease, stroke, neurodegenerative disease, diabetes, liver diseases, kidney diseases and infective diseases. Small RNAs may serve as novel biomarkers and therapeutic targets for the majority of diseases. Detailed understanding of target genes of small RNAs and of potential side effects are needed to improve the safety, efficacy and reliability of small RNA-based therapy. Further advanced therapeutic strategies to characterize and modulate the aberrantly expressed miRNAs under different disease conditions should be developed to realize the potential of miRNA-based technology in clinical diagnosis and therapy.

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Table 1

Small RNA-based therapeutics in clinical development.

Small RNAs	Molecular target	Target diseases	Highest development status	Reference
bevasiranib (OPKO Health Inc)	*VEGF*	Wet AMD and diabetic macular edema	Phase III	[151,154]
oblimersen sodium (Genasense) (Genta Inc)	*BCL2*	B-cell lymphoma, breast tumor, chronic lymphocytic leukemia, colorectal tumor, leukemia, macroglobulinemia, melanoma, multiple myeloma, myeloid leukemia, nasopharyngeal carcinoma, non-Hodgkin's lymphoma, NSCLC and prostate tumor	Phase III	[156,157]
AGN-211745 (Sirna-027) (Allergan Inc)	VEGFR-1 mRNA	Wet AMD	Phase II	[158]
PF-4523655 (RTP801i-14) (Quark Pharmaceuticals Inc/ Pfizer Inc)	Hypoxia-inducible gene and RTP801	Wet AMD	Phase II	[159]
ALN-RSV01 (Alnylam Pharmaceuticals Inc/Cubist Pharmaceuticals Inc/ Kyowa Hakko Kirin Co Ltd)	Respiratory syncytial virus N gene	Respiratory syncytial virus infection	Phase II	[160]
imetelstat (GRN-163L) (Geron Corp)	Telomerase	Breast tumor, chronic lymphocytic leukemia, multiple myeloma, ocular disease, NSCLC and solid tumor	Phase II	[161]
shRNA therapeutic (BLT-HIV) with a recombinant virus vector (rHIV7-shl-TAR-CCR5RZ) (Benitec Ltd)	HIV-1 tat and rev shared exons	HIV infection and associated lymphoma	Phase I	[162]
NUCB-1000 (Nucleonics Inc)	HBV genome	HBV infection	Phase I	[164]
CALAA-01 (Calando Pharmaceuticals Inc)	Ribonucleotide reductase M2 subunit	Solid tumor	Phase I	[164]
TD-101 (TransDerm Inc)	Keratins (*K6A*)	Pachyonychia congenital	Phase I	(ClinicalTrials.gov identifier: NCT00716014)
SPC-3649 (Santaris Pharma A/S)	miR-122	HCV infection and hyperlipidemia	Phase I	[155]

AMD age-related macular degeneration, **shRNA** short hairpin RNA