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Therapeutic potential of plant-derived amiRNAs: challenges and strategies

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Abstract: Small RNAs (sRNAs) are potent and accurate genetic regulators capable of targeting a diverse range of genes in eukaryotes. In recent years, the sRNA-based RNA interference (RNAi) technique has emerged as a promising strategy for modifying pathological processes by targeting specific genes or gene families, thereby promoting RNAi-based precise medical therapy. Notably, plant-derived miRNAs have shown promise in the treatment of various disorders, including cancer, neurodegenerative diseases, and infectious diseases. This review examines the therapeutic potential of plant-derived natural miRNAs and artificial miRNAs (amiRNAs), especially sourced from edible plants like crops and herbs. In addition, we discuss the challenges and strategies of utilizing amiRNAs in the therapeutic application and highlight the essential factors for effectively producing desired miRNA mimics in plants.

Keywords: artificial miRNAs; RNAi therapy; sRNA drugs; plant; oral administration; cross-kingdom regulation

1. Introduction

In 1998, Andrew Fire and his colleagues made a milestone discovery that the injection of exogenous double-stranded RNA (dsRNA) caused gene silencing in *Caenorhabditis elegans*, opening the doors to the discovery of RNA interference (RNAi) phenomenon and RNA-mediated regulation [1]. Since then, various types of endogenous small RNAs (sRNAs), typically 20–40 nucleotides (nt) in length, have been characterized, including small interfering RNAs (siRNAs), microRNAs (miRNAs), and PIWI-interacting RNAs (piRNAs) which are classified based on their distinct biogenesis pathways and modes of regulation [2,3]. These sRNAs, along with their associated proteins, specifically silence the expression of one or more target genes in the transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS) pathway [4,5]. According to reports, up to 60% of all protein-encoding

genes are subject to sRNA-based regulation, suggesting sRNAs as central regulators in cellular signaling with a wide range impact on almost every biological process [6,7].

In addition to the cell-autonomous regulatory function within local areas, growing evidence suggests that sRNAs, particularly miRNAs, can be transferred between cells and even organisms to exert a non-cell-autonomous regulatory role [3,8]. The discovery that the MIR168a from rice inhibits low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) mRNAs and reduces the removal of LDL from mice plasma is the first demonstration of plant miRNAs with regulatory function in mammals [9]. Another canonical example involving species-to-species RNAi mediated by miRNAs is that parasitic plant *Cuscuta campestris* derived 22-nt miRNAs ccm-miR12497a, ccm-miR12480 and ccm-miR12495 manipulate the expression of target genes *TIR1*, *AFB2*, *AFB3*, *SEOR1* and *BIK1* in host plants [10,11]. Moreover, a complex regulatory interaction between plants and honeybees has been established, demonstrating that plant-derived miR162a is more abundant in beebread than in royal jelly. This miRNA directly targets *amTOR*, a stimulatory gene in caste development, thereby inducing larval differentiation into worker bees rather than queens [12]. Recent research has also verified that miR399 and miR156 can serve as signaling molecules involving communication between adjacent plants [13]. Bidirectional signaling of sRNAs, such as immune TAS1c-siR483 and TAS2-siR453 from *Arabidopsis thaliana* and virulent Bc-sRNAs from *Botrytis cinerea*, reveals the evolutionary arms race between host and fungal pathogens [14,15].

Over the past decade, research on interspecies regulation by miRNAs has revealed a prevalent and intricate connection between interacting organisms [16–19], and presented a novel therapeutic strategy utilizing miRNAs as pharmaceuticals [20–22]. The advent of sequencing technologies has further confirmed that plant-derived miRNAs, particularly those from edible plants like cabbage [23] and corn [24], can bypass the traditional enzymatic digestion and traverse the gastrointestinal (GI) tract while maintaining their integral structure and regulatory function in mammalian recipient tissues or cells [9]. In 2015, a groundbreaking discovery revealed that honeysuckle, a traditional Chinese herb known for its anti-inflammatory and antibacterial properties [25], contains a specific miRNA named MIR2911, which directly targets multiple subtypes of influenza A viruses (IVAs), including H1N1, H5N1, and H7N9 in mice when administrated through decoction feeding [26]. Five years later, another breakthrough demonstrated that MIR2911 from honeysuckle exhibits a remarkable suppression of SARS-CoV-2 replication and contributes to the conversion of infected patients to a negative status [27]. Moreover, several inspiring discoveries have shown the therapeutic potential of specific plant-derived miRNAs. Ath-miR159a from *Arabidopsis thaliana*, gma-miR159a-3p, and gma-miR159e-3p from *Glycine max*, sharing the identical sequence of 5'-UUUGGAUUGAAGGGAGCUCUA-3', are found to inhibit the growth of xenograft breast tumors in mice when administrated orally [28]. In addition, miR156a, commonly found in dietary green vegetables like spinach and lettuce, has exhibited promise as atheroprotective pharmaceuticals [29]. Two lettuce-derived amiRNAs, specifically amiR471 and amiR519, targeting the HBV surface antigen gene (*HBsAg*) in mice, present an opportunity for the treatment of chronic diseases through plant biogenesis [30].

A systematic review by He *et al.* summarized the therapeutic functions of plant-derived miRNAs, encompassing anti-tumor, anti-inflammatory, immune modulation, anti-apoptosis, and intestinal modulation [31]. Here, we provide supplementary evidence in Table A1.

A recently reported mammalian transmembrane protein SIDT1, which is homologous to nematode systemic RNA interference defective protein 1 (SID-1) and localized in gastric pit cells, has been studied and confirmed as a vital sRNA transporter involved in the dynamic uptake of dietary and orally administrated miRNAs [32]. The Cryo-EM structures further implicate the low-pH-dependent RNA transport activity of human SIDT1 and SIDT2 [33]. Above mentioned findings provide valuable evidence to support the notion that plant-derived miRNAs have the potential to be utilized as therapeutics through dietary uptake. In this review, we highlight the advantage of using plants as bioreactors for producing therapeutic sRNAs and propose a feasible solution to address the challenges encountered with sRNA therapy, specifically through using artificial microRNAs (amiRNAs) in plant bioreactors.

2. Advancement of amiRNAs in gene regulation and therapeutics

The amiRNAs, also known as miRNA mimics, are designed to replace the original miRNA duplex loci and are produced by endogenous biogenesis systems. These engineered amiRNAs possess a complete or partial complementary sequence to the target mRNA of interest, acting as a guide for RNA-induced gene silencing complex (RISC) to regulate gene expression. By resembling endogenous miRNA biogenesis, amiRNAs offer a more "natural" and safer approach with prolonged silencing effects compared to synthetic siRNA or antisense oligonucleotides (ASOs) [34]. Consequently, amiRNAs hold significant promise as effective and versatile tools for RNAi application.

In the field of plants, amiRNAs are predominantly used for crop improvement, gene function studies in biological processes, and control of pests and viruses [35–41]. An early report described the modification of an *Arabidopsis thaliana* miR159 backbone to express two amiRNAs that target mRNA sequences encoding gene silencing suppressors, P69 of *turnip yellow mosaic virus* (TYMV) and HC-Pro of *turnip mosaic virus* (TuMV) [42]. Based on a transportome-scale amiRNA-based screen, the redundant roles in auxin transport and response of *ATP Binding Cassette Subfamily B Member 6 and 20* (*ABCB6 and 20*) are revealed [43]. In tobacco, amiRNA constructs are designed to target ORFs of *Jatropha leaf curl Gujarat virus* (JLCuGV). The inverse relationship between amiRNA levels and viral load, coupled with an analysis of photosynthetic parameters and sugar metabolism, demonstrates that amiRNAs enhance tobacco resistance against JLCuGV [44]. Gu *et al.* developed the cotton leaf crumple virus vector system, which can be applied in gene function analysis by overexpressing miRNAs, small tandem target mimic (STTM), and amiRNAs in cotton. This system provides a persistent gene-silencing approach and a powerful tool for gene discovery in cotton [45].

In the field of mammals, designed amiRNAs have emerged as novel and reliable therapeutic tools against a range of intractable diseases, including neurodegenerative diseases [46,47], cancer [48,49], hepatic fibrosis [50], virus infection [51,52] and others. A recent study has

genetically engineered PR8 viruses to express amiR-30CLK1 (PR8-amiR-30CLK1) and amiR-93SON (PR8-amiR-93SON) as attenuated vaccines. By targeting two essential host genes for viral replication, *Cdc2-like kinase 1* (*CLK1*) and *SON DNA binding protein* (*SON*), mice infected with these engineered influenza PR8 viruses show amelioration of weight loss and lethality caused by lethal-dose PR8 infection. This suggests the potential use of amiRNAs as a new therapeutic influenza vaccine [52]. In addition, a novel lentiviral vector expressing anti-sickling βAS3-globin and amiR7m has been developed, combining gene addition and gene silencing strategies for gene therapy of sickle cell disease (SCD) [53]. A single intravenous injection of the recombinant vector *AAV8* expressing three tandem amiRNAs (AAV8-amiRNA135) effectively suppresses the level of hepatitis B virus (HBV) antigens and inhibits HBV replication for up to 15 months in transgenic mice [54]. Inhibition of *vascular endothelial growth factor receptors* (*VEGFRs*) expression via amiRNAs triple combination significantly reduces tumor growth and represents a novel targeted therapy in pancreatic ductal adenocarcinoma (PDAC) through regulating epithelial-mesenchymal transition (EMT) [55]. The amiRNA/target model has proven to be a more efficient strategy. By differentiating the location of the seed interaction within the binding site, designed amiR-KS3 can specifically target point-mutated genes, such as *Kirsten rat sarcoma viral oncogene homologue (KRAS)*, without affecting their wild-type counterparts [56].

With the understanding of the underlying mechanism, a wide range of advanced techniques utilizing amiRNAs are rapidly emerging, incorporating more sophisticated promoters, backbones, and vectors. However, the ultimate goal of curing human diseases necessitates the direct manipulation of human cells or tissues, which still presents challenges in terms of controllability, safety, and ethics. A less emphasized but potentially valuable alternative approach involves using heterologous amiRNAs, in which plants serve as donors and mammals as recipients through dietary intake. This strategy could contribute to RNAi therapy at present and in the future.

3. Advantages of plant bioreactor in amiRNAs production

Plants play a crucial role in human survival by providing essential primary and secondary metabolites. Apart from serving as a source of food and nutrients, plants have been utilized for medicinal purposes for thousands of years. Advances in the field of life science have given rise to plant bioreactors, also known as plant biological factories. These bioreactors take advantage of genetic manipulation to enhance the production of therapeutic biomolecules through the endogenous biogenesis machinery of plants. The advantages of employing plants as bioreactors include cost-efficiency, absence of animal pathogens, and environmentally friendly production [57,58]. Currently, plant bioreactors are primarily used for the production of recombinant proteins or peptides such as vaccines, antibodies, and other industrial enzymes [59–61]. However, the complexity of biosynthesis and modification processes, coupled with the requirement for intact conformation and suitable physicochemical conditions, constrains the further development of artificial proteins or peptides. In contrast, miRNAs comprise fewer biomolecules (21–24 nt), and the bioactivity

of mature miRNAs relies on their primary structure or specific nucleotide sequence, making them a more promising product through plant bioreactor.

The attention towards plant-derived amiRNAs has been growing due to their exceptional characteristics. Extensive research in recent years has demonstrated the remarkable ability of plant-derived miRNAs to endure harsh conditions, including high temperature, ultrasonic treatment, nuclease digestion, and the acidic gastrointestinal environment when taken orally [62,63]. Plausible explanations have been proposed, particularly focusing on the stabilizing effect of their unique sequence and high GC content. For example, it was experimentally confirmed that MIR2911 (5'-GGCCGGGGGACGGACUGGGA-3'), derived from honeysuckle and possessing a high GC content, exhibits remarkable stability. Mutations in the sequence, specifically changing 5'-GG to 5'-AA and 3'-GGA to 3'-AAA, were found to eliminate resistance to RNase treatment [26]. However, it should be noted that MIR2911, an atypical miRNA derived from ribosomal RNAs (rRNAs), may not be representative of all plant-derived miRNAs in terms of its high GC composition. On the other hand, the 2'-O-methylation modification at the 3' end is recognized as a crucial protective mechanism in the endogenous biogenesis of plant-derived miRNAs, and the 3' end methylation has been found to confer partial resistance to RNase digestion and oxidation [64,65]. Notably, a study comparing two synthetic miRNAs (hsa-let-7a and 2-OMe hsa-let-7a) with mistletoe-derived val-miR218 under the same herb processing condition consistently demonstrated that synthetic miRNA with 2'-O-methylation modification appeared to be more stable. However, the stability of both naked miRNAs was lower than that of mistletoe-derived miRNA, suggesting the involvement of other plant-derived protective molecules such as lipids, proteins, polysaccharides, and vesicle encapsulation in stabilization [66].

Understanding the packaging and delivery process of miRNAs has revealed the presence of certain biomolecules associated with the uptake and stability, which may exist in the crude extracts of amiRNA expression plants. RNA binding proteins (RBPs) such as AGO1, DEAD-box ATP-dependent RNA helicase11 (RH11), RH37, Annexin1 (ANN1), and ANN2 have been implicated in the packaging and stabilization of sRNAs in exosomes for transport [67]. Furthermore, accumulating evidence supports that plants encapsulate functional miRNAs into exosomes, providing additional lipid bilayer protection [68]. In an exosome-dependent manner and facilitated by high-density lipoproteins in plasma, miRNAs can be delivered into the mammalian circulation system. [69,70].

4. Factors influencing amiRNAs production in plant bioreactor

The production of amiRNAs in plant bioreactors is a complex process influenced by several factors. With the fundamental understanding of plant endogenous miRNA biogenesis including structural and molecular dynamics of core miRNA machinery, function process of how miRNAs select substrates from transcriptome, and regulation of miRNAs turnover, insights for modified amiRNAs with application purposes are proposed. In the current section, we will discuss the essential factors and empirical designs that may affect amiRNA expression and functionality throughout the production process.

4.1. Sequence design for amiRNAs

Theoretically, mature miRNAs can guide the RISC to down-regulate target mRNAs as long as their sequences are complementary to any portion of mRNAs of interest. However, different sequences of guide strands designed for the same target show considerable variations in efficacy. Previous research has identified four characteristics associated with higher efficacy: GC content, low internal stability at the sense strand 3'-terminus, lack of inverted repeats, and specific position preference [71,72].

Through a comprehensive analysis of conserved and abundant miRNAs across plants, supported by experimental evidence, an optimal GC content of ~52% has been deduced. Furthermore, the specific positional preference within the miRNA sequences is also important for accurate processing and optimal functionality. In contrast to animal miRNAs, which often exhibit a preference for U at position 1, C or G at position 19 and, A or U at position 10, plant miRNAs show a distinct pattern. Specifically, they prefer G or C at positions 8–9 and 18–19, while favoring A or U at positions 5, 7, 10 and 15 [72,73]. These features could be considered to improve the production of amiRNAs. In plants, Dicer-like proteins (DCL) recognize the secondary structure formed by the backbone of primary miRNAs and conduct two-step cleavages with a 2-nt overhang. Some studies have indicated that AGO loading specificity depends on the intrinsic structural features of miRNA/miRNA* duplex [74]. The thermodynamic stability and 5' nucleotide (such as 5' uridine preference for AGO1) determine the sorting preference for the specific AGO loading of miRNAs [75–77]. In plants, the recognition of miRNAs with target sites typically relies on perfect base pairing [78], while animal endogenous miRNAs usually form partial pairing with target sites through nucleotides 2–8 of the miRNAs, known as the seed sequence, which is considered the minimal element [7,79]. Furthermore, studies have demonstrated that once paired with targets, AGOs undergo a conformational change to expose part of the miRNAs 3' region for sequential recognition [80,81]. The evidence of biased pairing between miRNAs and targets is attributed to the sequence design of amiRNAs. In addition, a recent study replaced two asymmetrical tandem amiRNA duplexes into miR168a precursor backbone of *Arabidopsis thaliana* to silence GFP and endogenous PDS reporter genes, demonstrating a higher efficiency for "two-hit" amiRNA compared to traditional "one-hit" amiRNA [82]. It is worth considering simultaneously deploying multiple amiRNAs to silence one or more targets involved in the disease.

4.2. Selection of amiRNAs expression backbone

In the canonical biogenesis pathways, miRNA genes are transcribed as the 5' capped and 3' polyadenylated primary miRNAs (pri-miRNAs), which sequentially cleaved into a stem-loop structured precursor miRNAs (pre-miRNAs) and then a duplex consisting of the guide strand (mature miRNA) and the passenger strand (miRNA*) [83]. In plants, Dicer-like 1 proteins (DCL) recognize the secondary structure formed by the backbone of primary miRNAs and conduct two-step cleavages with a 2-nt overhang. It also has been hypothesized that HYL1 exhibits affinity for specific structures within the precursors [84–86]. For the accurate

recognition and processing by microprocessors in the nucleus, amiRNAs duplex is embedded into a stem-loop structure called backbones that offers flank nucleotides sequence and secondary structure [87]. However, the lack of unified standards and the abundance of unclear backbone information present challenges in this regard. Generally, the backbone can be divided into three portions: a single-stranded basal segment, a double-stranded stem, and a terminal loop. All of these portions are essential for the integrated processing and transport of miRNAs [88–91]. The single-stranded basal segment is pivotal for the initial recognition of DCL and pri-miRNAs, with the CNNC (N represents any ribonucleotide) nucleotide sequence in the 3' basal segment potentially contributing to DCL recruitment [85,92]. The miRNA duplexes are flanked by two double-stranded stem fragments: a lowed stem adjacent to the proximal base and an upper stem adjacent to the distal loop. This portion, containing the majority of the backbone sequence and both cleavage sites, is crucial for proper miRNA processing. The nucleotide sequence on the double-stranded stem forms a secondary structure with symmetric mismatches, internal loops, and asymmetric bulges [93]. Notably, the GU wobble base pair, which frequently appears in RNA secondary structure, is also present on the stem. Studies have indicated that the arrays of secondary structures, length, and specific motifs of the double-stranded stem are involved in miRNA recognition and cleavage process [34]. The terminal loop primarily functions in the recognition, location, and specific cleavage of pri-miRNAs [86,94]. In addition, a novel approach by high-throughput reveals generic features that define human pri-miRNAs. This approach can be modified to explore features required for regulated processing of the enigmatic features defining pri-miRNAs of plants and optimizes the design of amiRNAs backbones [95]. Due to the complexity and incomplete understanding of the backbone structure, the current strategy is to utilize conserved, accurate, and highly expressive natural backbones, such as pre-miR319a, pre-miR171, pre-miR164/5b, pre-miR159 and pre-miR390 from *Arabidopsis thaliana*, as well as pre-miR390 and pre-miR528 from *Oryza sativa*. Subsequently, the original miRNA duplex is replaced with artificially designed miRNAs via overlapping PCR or other techniques [96–101].

4.3. Vector construction and plant transformation

This step involves the use of two types of vectors: a clone vector for the amplification of the backbone, and an expression vector for the integration of amiRNAs expression cassette into the plant bioreactor genome for expression [102]. The WMD3 design platform (http://wmd.weigelworld.org) facilitates the automatic design of plant amiRNA sequences for vector construction. This platform also provides several vectors containing plant endogenous miRNA precursor sequences, such as *pRS300, pNW55, pChamiRNA 2, and 3*. Three pairs of primers can be automatically designed based on the selected amiRNA sequence and its flanking sequence on the selected clone vector. The pre-amiRNA is then constructed using these primer pairs and selected vectors through four rounds of PCR reactions, and then cloned into a suitable plant expression vector through restriction enzyme ligation. This approach optimizes the steps involved in the construction of traditional RNAi vectors, thereby

reducing the experimental period and improving the experimental accuracy [103]. In addition, the researcher has recently developed two median vectors, pAMIR395a and pAMIR319a, which contain two restriction sites. An artificial miRNA construct can be obtained by inserting an artificial stem-loop, produced from one PCR reaction into the corresponding median vector [104].

Successful integration of suitable vectors carrying amiRNAs into the genomes of transformed plants and efficient expression is also indispensable. Thus far, successful expression of amiRNAs in various plants, including Arabidopsis [103,105], wheat [106], petunia [107], lettuce [30], tomato [39], tobacco [108], and jute [109] have been reported. The potential for the expression of amiRNAs in other types of horticultural or edible plants remains to be explored. Furthermore, to meet pharmaceutical application requirements, the production cost and yield of amiRNAs also need to be improved.

4.4. Administration of amiRNA therapeutics

After the successful production of amiRNAs in transgenic plants, it is essential to prepare appropriate methods of therapeutic administration. Currently, clinically tested miRNA candidates are mainly administrated via skin or intravenous injection, with inhalation administration being tested for certain respiratory diseases. Advanced administration techniques, such as implanting miRNA therapeutics into a biodegradable 3D matrix and encapsulating them into a lipid-like layer to enhance stability and permeability, have been extensively reviewed in the context of miRNA clinical trials [110]. Here, we specifically emphasize the potential of edible administration based on the therapeutic effect of plant-derived miRNAs. A plethora of evidence demonstrates the cross-kingdom regulation of mammalian systems by plant-derived miRNAs present in edible plants such as rice, honeysuckle, cabbage, fruit, and others. The consumed dosage is also considered [111]. In many cases, subjects are administrated with fresh food or miRNAs-contained decoction to achieve a therapeutic effect. Natural plant-derived miRNAs have significant value due to their low toxicity, low dosage, and greater acceptability, unlike chemically synthesized siRNAs. The stability and durability of plant-derived miRNAs are preferred characteristics. Notably, relevant research in Chinese traditional medical herbs has revealed that millions of plant-derived single-stranded RNAs and hundreds of lipids remain stable in decoctions after boiling for thirty to sixty minutes [112]. Growing evidence suggests that certain herbal sRNAs can be orally administrated with herb decoction or medical decoctosome mimic (comprised of lipids, chemical compounds, proteins, and sRNAs), effectively ameliorating various diseases [113–116].

5. Challenges and strategies of deploying amiRNAs

As mentioned earlier, the application of amiRNAs still faces several key challenges, including design, delivery, tissue specificity, target gene effectiveness, safety, production cost, and off-target prediction. Achieving high specificity and optimal expression levels is crucial through design optimization. Overcoming delivery barriers, ensuring stability and

cellular uptake, and achieving tissue-specific delivery are necessary for successful application. The differences in the effectiveness of different target genes and potential adverse effects should also be considered. Improving the scalability and cost-effectiveness of production methods, simplifying synthesis and delivery processes, and enhancing the accuracy of off-target prediction tools are important challenges currently faced.

Nevertheless, amiRNAs have emerged as promising regulators in the treatment of various human diseases. The strength of amiRNAs lies in their ability to precisely regulate the expression of disease-associated genes, thereby improving efficacy and reducing side effects. It offers opportunities for personalized medicine, disease treatment, regenerative medicine, antiviral therapy, and cancer treatment. Customized amiRNAs have the potential for highly targeted and individualized therapeutic interventions, enabling precision medicine tailored to specific genetic profiles. Furthermore, amiRNAs show promise in regenerative medicine by guiding cellular differentiation and tissue regeneration. It also offers the potential to combat viral infections by targeting specific viral genes. In cancer treatment, targeted intervention becomes possible, enabling more effective and personalized treatments with fewer side effects. In addition, amiRNAs hold potential in cardiovascular medicine for the development of more targeted and effective therapies for heart-related conditions. The application of amiRNAs for genetic disorders offers a new avenue for gene therapies to address inherited diseases.

In summary, amiRNAs demonstrate tremendous potential and application prospects in multiple medical fields. With ongoing research and technological advancements, amiRNAs are expected to play an increasingly important role in the future medical field, benefiting human health and quality of life.

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Conflicts of interests

The authors declare no conflict of interest.

Authors' contribution

Conceptualization and supervision, Z.H.; investigation, X.L. and Y.W.; project administration, Z.H.; original draft preparation, X.L.; editing and data curation, Y.W. funding acquisition, Z.H. All authors have read and agreed to the published version of the manuscript.

Appendix

Table A1. Plant-derived miRNAs with therapeutic efficacy.

Plant miRNAs	Derived from	Targets or pathways of regulation	Disease	Years
miR2911	Honeysuckle	H1N1;H5N1;H7N9	Influenza viruses	2015 [26]
oeu-sR34	Olea europaea	SIRT1 and BCL2 protein	Tumor	2018 [117]
miR2911	Honeysuckle	VP1 gene	Enterovirus 71	2018 [118]
mdo-miR7267-3p	Ginger	LGG monooxygenase ycnE	Colitis	2018 [119]
HJT-sRNA-m7	Hong Jing Tian	α-SMA, fibronectin, and COL3A	Pulmonary fibrosis	2019 [120]
miR2911	Honeysuckle	IE62 gene	Varicella-zoster virus	2019 [121]
miR156c; miR159a	*Juglans regia, Juglans californica, Corylus avellana*	TNF receptor superfamily member 1a (Tnfrsf1a)	Inflammation	2019 [122]
miR167e-5p	*Moringa oleifera*	β -catenin	Enterocyte proliferation	2019 [123]
mtr-miR-5754; gma-miR-4995	*Medicago truncatula* Soybean	LncRNA MALAT1 and NEAT1	Cancer cell proliferation	2020 [124]
miR2911	Honeysuckle	SARS-CoV-2 genome	SARS-CoV-2	2020 [125]
gma-miR159a	Soybean	TCF7 gene	Colon cancer and colitis	2021 [126]
rgl-exomiR-7972	*Rehmanniae radix*	GPR161-mediated Hedgehog pathway	Lipopolysaccharide-induced acute lung injury and gut dysbiosis	2023 [127]
nanovesicles contained miRNA	Yam	BMP-2/p-p38-dependent Runx2 pathway	Osteoporosis	2023 [128]
XKC-sRNA-h3	Xia Ku Cao	Angiotensin-converting enzyme inhibitors (ACE)	Angiotensin II-induced hypertension	2023 [115]
BZL-sRNA-20	Ban Zhi Lian	TLR4	Injury induced by bacterial lipopolysaccharide and SARS-CoV-2	2023 [114]

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