Review | Received 1 December 2023; Accepted 28 February 2024; Published 19 March 2024
https://doi.org/10.55092/exrna20240004

A decade later: future perspectives on dietary microRNAs

Chenyu Tao[1], Hongyuan Guo[1,2,*]

[1] Research Unit of Extracellular RNA, Chinese Academy of Medical Sciences, Nanjing, Jiangsu 210023, China

[2] Nanjing Drum Tower Hospital Center of Molecular Diagnostic and Therapy, State Key Laboratory of Pharmaceutical Biotechnology, Jiangsu Engineering Research Center for MicroRNA Biology and Biotechnology, NJU Advanced Institute of Life Sciences (NAILS), Institute of Artificial Intelligence Biomedicine, School of Life Sciences, Nanjing University, Nanjing, Jiangsu 210023, China

* Correspondence author; E-mail: hongyuanguo@nju.edu.cn.

Abstract: MicroRNA (miRNA) is a class of single-stranded non-coding RNAs of approximately 22 nt in length, which are widely distributed in animals and plants. MiRNAs are involved in post-transcriptional regulation and thus influence many physiological processes. The function of dietary miRNAs has long been overlooked because it was thought that they could not survive the gastrointestinal environment. However, there is increasing evidence that these dietary miRNAs are not only capable of being absorbed by consumers such as humans, but also appear to be extensively involved in various physiological activities. In this review, we look back at studies on the stability, absorption, redistribution and function of dietary miRNAs in the decade since their discovery, as well as possible future applications. Although current research on dietary miRNAs is not well developed, we expect that this review will provide new directions for future interpretation or study of the concept of dietary miRNAs in cross-species regulation.

Keywords: microRNA; functional food component; exosome; gastrointestinal tract; plant; mammal; cross-species regulation

1. Introduction

Our daily diet provides essential nutrients required for a wide range of biological functions. Among these, carbohydrates, proteins, fats and nucleic acids make up the biological macromolecules present in food. These macromolecules are broken down into smaller molecules by a cascade of enzymes, culminating in their absorption in the gastrointestinal tract (GI). It is well known that pancreatic ribonuclease breaks down nucleic acids such as RNA into oligonucleotides or mononucleotides in the small intestine. This is followed by the

hydrolysis of oligonucleotides into mononucleotides by diesterases secreted by the small intestinal mucosa. These mononucleotides are further broken down into nucleosides and phosphate by nucleotidase before being absorbed by passive diffusion. Once internalized, they are distributed throughout the body where they are recycled by various cells [1]. Consequently, existing research on the effects of diet has predominantly focused on nutrients and organic compounds in food, often overlooking bioactive RNAs such as miRNAs.

Notwithstanding, a groundbreaking study from our laboratory in 2012 identified a significant presence of plant-derived miRNAs in the serum of Chinese adult volunteers [2]. MiRNAs are single-stranded non-coding RNAs of approximately 22 nucleotides in length, omnipresent in both animals and plants. They play a pivotal role in post-transcriptional regulation, affecting numerous physiological processes [3]. Subsequent research indicated that these serum miRNAs originate from dietary plants, with the majority being encapsulated in exosomes [2]. It is widely accepted that there is a difference in the functional mechanism between animal and plant miRNAs. Unlike animal miRNAs, which typically exhibit imperfect complementarity with their target mRNAs and primarily act by inhibiting translation, plant miRNAs possess near-perfect complementarity with their targets, leading to the cleavage of the target mRNA [4].

We conducted further functional studies on selected plant miRNAs identified in serum, particularly osa-miR168a, which showed the highest levels. The results were incredibly exciting, as we discovered that osa-miR168a can directly target LDL receptor bridging protein 1 (LDLRAP1) in the liver, leading to a reduction in LDL clearance in the bloodstream. This finding is particularly intriguing given the significant consumption of plant-based foods, including grains, vegetables, and fruits, by humans on a daily basis. It raises the intriguing possibility that exogenous plant miRNAs from various sources may survive the gastrointestinal environment, enter the circulatory system, accumulate in specific tissues, and exert significant biological effects [2].

Nevertheless, it has long been believed that RNAs are not orally bioavailable due to the acidic environment in the stomach, which leads to substantial degradation of the RNAs. Additionally, the presence of various digestive enzymes in the gastrointestinal tract can degrade the RNAs, and the negative charge on the RNA backbone can hinder permeation through the intestinal mucosal barrier. Despite the discovery of miRNAs almost 30 years ago, the transfer of miRNAs between distantly related organisms, such as plants and mammals, has only recently been reported.

However, subsequent experiments conducted by other research groups have yielded disappointing results. For instance, Dickinson *et al.* reported no detection of osa-miR168a in the serum of mice after rice consumption, nor did they observe alternations in LDLRAP1 expression levels in the liver [5]. Whereas Dickinson *et al.* did not detect sufficient osa-miR168a signal even in rice chow, the positive control for deep sequencing. Additionally, the experimental methods and manipulations employed may have also affected the reliability of the results [6]. Despite these concerns, various follow-up studies have further explored the field of miRNA-mediated cross-species regulation [7–10]. These studies have provided direct evidence for the absorption and active function of cross-species miRNAs. Notably, Chin *et al.*

identified the first plant miRNA with therapeutic properties, demonstrating that gma-miR159, predominantly found in extracellular vesicles in human serum, was inversely correlated with breast cancer progression and its oral administration significantly reduced xenograft breast tumor growth in mice by targeting TCF7 [11]. Furthermore, our research team conducted a comparative analysis of plant-derived miRNA content across various human body fluids. By evaluating the levels of exosomal miRNAs in a collection of 70 human samples, encompassing serum, saliva, urine, and breast milk, we identified 334 plant-origin miRNAs. Our findings reveal that 82% of the plant miRNAs present in the bloodstream were also detectable in saliva, urine, or breast milk, constituting 21%, 24%, and 18% of the total miRNAs in these fluids respectively [12]. Several other studies have also illustrated the detection of plant miRNAs in tissues of different consumers, including pigs, pandas, and rodents [10,13–15]. Furthermore, the molecular functions and biological significance of plant miRNAs in the prevention and treatment of human diseases, including viral infections, tumors, chronic inflammation, and pulmonary fibrosis, have been extensively documented [11,16–18]. In conclusion, there is increasing evidence that the uptake and utilization of exogenous dietary miRNAs is indeed widespread in nature and involves complex biological processes.

The discovery of dietary miRNAs has sparked increasing interest in this emerging field. One of the most crucial areas of study is unraveling the precise mechanism of miRNA transmission between distantly related species. It is essential to understand why cross-species miRNAs remain stable and how plant miRNAs can traverse the gastrointestinal tract and be absorbed by mammalian. Is there a specific channel protein, carrier, or transporter responsible for the uptake of plant miRNAs? Once absorbed, how do plant miRNAs travel within the body? Gaining a comprehensive understanding of the mechanism behind miRNA-mediated cross-species regulation will shed light on the biological significance of miRNA signaling transmission and provide valuable insights into the nature of miRNAs. This rapidly evolving field promises to uncover further cross-kingdom miRNAs and their effects. In this review, we revisit research from the past decade on dietary miRNAs, with a focus on digestion and function as depicted in Figure 1. Our aim is to lay a theoretical groundwork for future explorations of dietary miRNAs in human health and disease.

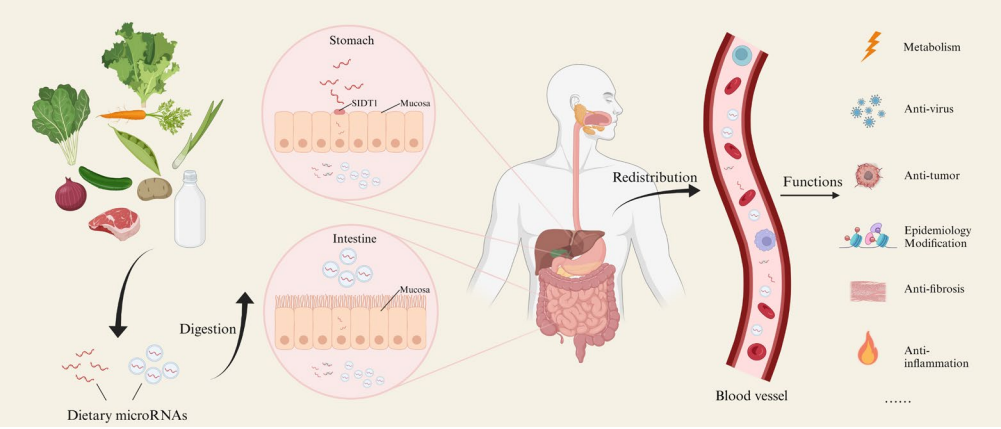

Figure 1. Digestion, redistribution and functions of dietary miRNAs. Created with BioRender.com.

2. The concept of dietary miRNA

Dietary miRNAs include not only those found in edible plants, but also miRNAs derived from meat, dairy products, herbs, *et al.* [16,19–21]. Numerous studies have reported their presence in consumers' serum or tissues, suggesting that this form of cross-species regulation may extend beyond just plant-to-animal interactions, encompassing a complex and multidimensional network of communication in nature. While research on meat miRNAs remains limited, the high conservation of mammalian miRNAs poses a challenge in distinguishing them from human miRNAs [19]. In contrast, plant miRNAs exhibit greater resistance to periodate oxidation due to a 2'-O-methylation modification at their 3'-terminus, a trait retained in consumer serum [2]. Experimental evidence has shown that plant miRNAs in consumers' serum exhibit significant variability and are closely related to their dietary patterns [12]. Yet, the presence of various plant miRNAs in human serum, typically at lower concentrations than endogenous miRNAs, raises questions about their *in vivo* functionality [22].

3. The functions of dietary miRNA

Despite their low bioavailability, dietary miRNAs have been shown to potentially regulate consumer health and their roles have been extensively documented. Tables 1 and 2 list several key dietary miRNAs involved in metabolism, anti-oxidation, immune regulation, anti-tumor, antiviral, *etc.* And the wide range of functions of dietary miRNAs suggests that the composition of one's diet can profoundly impact individual well-being.

Table 1. Studies of the regulatory functions of dietary microRNAs in human.

miRNA	Source species	Target species	Target gene	Function	Reference
osa-miR168a	Rice	Human	LDLRAP1	Decreases LDL removal in liver	[2]
ath-miR156a	Cabbage/Spinach/ Lettuce	Human	JAM-A	Reduce monocyte adhesion induced by inflammatory factors	[23]
bta-miR-148a	Cow	Human	DNMT1	Influence epigenetic modifications, Maintains Th17/Treg homeostasis	[24]
miR2911	Honeysuckle	Human	28 binding sites in the SARS-CoV-2 genome	Hinder SARS-CoV-2 replication	[25]
miR168	Strawberry	Human	TLR3	Suppress the inflammatory response	[18]
miR156	Strawberry	Human	TLR3	Suppress the inflammatory response	[18]
bol-miR874	Cabbage	Human	TLR3	Suppress the inflammatory response	[18]
osa-miR168	Rice	Human	TLR3	Suppress the inflammatory response	[18]
gma-miR159	Soybean	Human	TCF7	Suppress breast cancer growth	[11]
gma-miR4995	Soybean	Human	MALAT1/NEAT1	Suppress tumor growth	[26]

Table 2. Studies of the regulatory functions of dietary microRNAs in animal model or cell lines.

miRNA	Source species	Target species/ cell line	Target gene	Function	Reference
miR159a miR156c	Nuts	Mouse	Tnfrsf1a	Anti-inflammation, Enhanced glucose uptake	[27]
gga-miR-451	Chicken/Pig	Mouse	Foxo3	Protects erythroid cells from oxidative stress	[28]
miR2911	Honeysuckle	Mouse	TGF-β 1,	Promoted T lymphocytes infiltration, Impede colon tumor development.	[29]
miR2911	Honeysuckle	Mouse	BP2, NS1	Hinder influenza A virus replication	[16]
gma-miR159a	Soybean	Mouse	GSK-3 β	Reduce liver fibrosis	[30]
miR2911	Honeysuckle	MRC-5 cell line	IE62	Hinder Varicella-zoster virus replication	[31]
miR2911	Honeysuckle	RD, HEK293T cell line	VP1	Hinder Enterovirus 71 virus replication	[32]
mtr-miR5754	Medicago truncatula	LAN-1, T98G, HepG2, HCT116 cell line	MALAT1/NEAT1	Suppress tumor growth	[26]
miR162a	-	Honeybee/ Fruit Fly	amTOR	Control cast differentiation	[33]

Beyond everyday fruits and vegetables, the miRNAs in herbs, particularly in Chinese medicinal herbs, merit attention. Traditionally, the benefits of herbs have been attributed to small molecules, but the role of larger molecules like RNAs has been underestimated [34–36]. An analysis of herbal miRNAs in tissues showed that thousands of miRNAs were identified in blood cells and tissues from mice that had ingested herbs. Many of these miRNAs were mapped to the herb genome, highlighting the therapeutic potential of herb miRNAs [21]. Studies on the functions of miRNAs in Chinese medicinal herbs have bridged a long-standing gap in understanding the functions of active biomolecules in these herbs, and have played an important role in explaining the pharmacology of Chinese medicinal herbs that could not be explained otherwise. These findings lay a theoretical foundation for incorporating Chinese medicinal herbs as primary or adjunct therapies in the future.

Furthermore, we have also explored the potential of dietary miRNAs for cross-kingdom regulation and synergistic evolution between species. From an evolutionary standpoint, miRNAs have existed long before the emergence of humans on Earth [37]. The presence of miRNAs is vital for the evolution of complex organisms, and the miRNA machinery plays a pivotal role in all evolutionary steps of eukaryotes [38]. Over the past decade, numerous studies have discovered conserved miRNAs across various species, highlighting a direct correlation between the number of miRNAs and the morphological complexity of organisms. Particularly, the number of miRNAs has been hypothesized to increase when some organisms specialized the process of mastication and grinding of food, thus starting a precise process of

morphological evolution [39]. Importantly, in addition to vertical transmission, miRNAs can undergo horizontal transmission between different species, especially between plants and animals through food [40]. Furthermore, the transmission of miRNAs from plants to herbivorous animals makes the study of miRNAs fundamental to shed light on the biological relevance of these molecules at an ecological and evolutionary scale.

3.1. Metabolism

osa-miR168a is considered as one of the earliest plant-derived miRNAs identified to be ingested into the body, and it is widely present in various grains, fruits and vegetables. Overall aggregation of osa-miR168a in the liver was observed in mice after rice feeding, whose abundance is comparable to that of endogenous mammalian miRNAs, suggesting its potential to function in the liver. Further investigation revealed that osa-miR168a in the liver was able to bind to Argonaute 2 protein (AGO2) and thus inhibiting LDLRAP1 in hepatocytes, which is essential for hepatocytes to endocytose circulating LDL. The mice after rice feeding exhibited an accumulation of osa-miR168a in the liver and higher levels of circulating LDL-cholesterol compared to control mice. In addition, a study focusing on osa-miR168a found that ectopic expression of osa-miR168a resulted in the upregulation of glucose transporter protein GLUT1 expression *in vitro* [2]. Further studies revealed that the upregulation of GLUT1 expression might be caused by the inhibition of the key mitochondrial electron transport chain complex I in the oxidative phosphorylation pathway by osa-miR168a. Indeed, mice injected with miR168a did exhibit a decrease in blood glucose concentration, laterally demonstrating the practical possibility of such regulation [41].

In a separate study, miR159a and miR156c, present in nuts, namely *J. californica*, *J. regia* and *C. avellana*, were found to complementarily bind to the mRNA of mammalian TNF receptor superfamily member 1a (Tnfrsf1a), indicating a potential regulatory relationship. Subsequent experiments pointed out that both natural and synthetic miR159a and miR156c were able to downregulate Tnfrsf1a in mouse adipocytes, thus inhibiting the TNF-α pathway. The inhibition led to enhanced glucose uptake, suggesting a possible contribution of nut-derived miR159a and miR156c to insulin resistance, and that these two miRNAs may have anti-inflammatory effects [27].

3.2. Anti-oxidation

miR-144/451 knockout mice are defective in the expression of miR-451, which accounts for nearly half of the total miRNA expression profile in mouse erythrocytes. While miR-144/451 is highly conserved in human and mouse, so do chicken and pig, the common species in human diet. The study found that feeding with wild-type chicken or pig blood was able to revert serum miR-451 to normal levels in miR-144/451 knockout mice. Despite the relatively low abundance, these dietary miR-451 in serum were able to improve the antioxidant capacity of erythrocytes by targeting the 14-3-3ζ/Foxo3 pathway, whereas erythrocytes from mice fed a miR-451-deficient diet exhibited a deficit in antioxidant capacity [28].

3.3. Anti-atherosclerosis

In addition to the previously mentioned osa-miR168a, we also identified another plant miRNA, ath-miR156a, in serum. ath-miR156a is commonly found in green leafy vegetables, like cabbage, spinach and lettuce. Interestingly, we observed significantly decreased levels of ath-miR156a in the serum of patients suffering from cardiovascular disease, suggesting a possible role of this dietary ath-miRNA in the circulatory system. By *In silico* screening and *in vitro* experiments, we found that ath-miR168a can directly target the junction adhesion molecule-A (JAM-A). JAM-A plays a crucial role in promoting monocyte migration to the intima and their adhesion to endothelial cells, thereby facilitating the development and progression of atherosclerosis. Further experiments revealed that ectopically expressed ath-miR168a can inhibit JAM-A in HAEC cells, leading to a reduction in monocyte adhesion induced by inflammatory factors, thus theoretically enabling control of atherosclerosis [23].

3.4. Epidemiology modification

Epigenetic modifications, such as DNA methylation, play a pivotal role in gene expression regulation, and miRNAs have the ability to indirectly modulate the extent of these modifications by targeting key genes involved in epigenetics. Previous studies have revealed that certain miRNAs, like bta-miR-148a in cows, directly inhibit DNA methyltransferase 1 (DNMT1) in mammary epithelial cells. DNMT1 is responsible for CpG cytosine methylation in mammalian DNA, contributing to gene silencing, and its decreased levels alongside elevated bta-miR-148a levels serve as crucial indicators of efficient milk production in dairy cows. Remarkably, it has been discovered that bta-miR-148a present in milk can be absorbed by consumers through cow-derived exosomes, subsequently targeting and inhibiting DNMT1 in consumers and thereby influencing the levels of epigenetic modifications. This phenomenon may be attributed to the high conservation of the DNMT1 sequence across mammals [24].

3.5. Anti-fibrosis

The Chinese medicinal herb *Rhodiola rosea*, which grows at high altitudes and in cold regions such as Tibet, is traditionally used in Chinese medicine as an anti-aging agent and shows potential in treating pulmonary fibrosis. While screening for compounds within *Rhodiola rosea* failed to yield an active ingredient capable of treating pulmonary fibrosis. Fortunately, subsequent experiments revealed that a small RNA in *Rhodiola rosea*, HJT-sRNA-m7, was able to directly inhibit α-SMA, the ECM component fibronectin and COL3A1, key components involved in the pulmonary fibrosis process [42].

In addition to *Rhodiola rosea*, *Lonicera japonica* (also known as honeysuckle) has also been found to have anti-fibrotic properties. The honeysuckle-encoded atypical miRNA, miR2911, targets TGF-β1, a key factor in fibrosis. In a mouse model of CCl4-induced liver fibrosis, oral administration of miR2911 significantly reduced liver fibrosis. The uptake of

miR2911 appeared to be mediated by the SIDT1 protein, as SIDT1 mice had low miR2911 uptake capacity and high levels of liver fibrosis [17].

Another study discovered that gma-miR159a effectively inhibited the activation and proliferation of hepatic stellate cells by downregulating TGF-β1 and PDGF *in vitro*. By iTRAQ-based quantitative proteomic analysis, Yu *et al.* identified GSK-3β as the target of gma-miR159a. Subsequent investigations revealed that gma-miR159a inhibits liver fibrosis by targeting the key protein GSK-3β and the downstream NF-κB and TGF-β1 pathways in human LX-2 cells (a commercial human hepatic stellate cells). Mice treated with intraperitoneal injections of gma-miR159a agomir showed reduced levels of serum markers for liver function and inflammatory cytokines, demonstrating the feasibility of using gma-miR159a as a treatment for liver fibrosis [30].

3.6. Anti-inflammation

MiRNAs play a crucial role in the mammalian immune system, and it appears that plant miRNAs can also exert some influence on the mammalian immune system. According to Cavalieri *et al.*, strawberry (*Fragaria vesca*) miR168 reduces inflammatory response of human dendritic cells (DCs) when challenged with LPS or polyI:C. Further investigation revealed that miR168 suppresses TRIF transcription by binding to the upstream TLR3 in DCs. This, in turn, limits the proliferation of T-cells triggered by DCs in the presence of pro-inflammatory factors. The expanded experimental results found that not only miR168 but also miR156 derived from strawberry, bol-miR874 derived from cabbage, and miR168 derived from rice can attenuate the response of DC cells to proinflammatory factors and thus suppress the inflammatory response in the same manner [18].

Furthermore, as mentioned above, bta-miR-148a from milk, is capable of reducing the methylation level of the FOXP3 promoter in T cells by inhibiting DNMT1. This demethylation promotes the expression of FOXP3, which facilitates the differentiation towards regulatory T cells and ultimately maintains Th17/Treg homeostasis *in vivo* [24].

3.7. Antivirus

Honeysuckle, a well-known Chinese herb used for treating influenza virus infections, has shown promising results in a groundbreaking study. Our team discovered that miR2911, derived from honeysuckle, remains stable and intact in the boiled decoction. *In vitro* studies have implied that miR2911 can effectively target and counteract multiple viral genes of influenza A viruses (IAVs). *In vivo* experiments revealed that administration of the honeysuckle decoction by gavage feeding significantly increased miR2911 levels in the blood and lungs of mice. Consequently, miR2911 accumulated in the lungs and directly inhibited the replication of various IAVs, including H1N1, H5N1, and H7N9, in mouse models. This led to reduced weight loss and mortality caused by viral infections [16]. Subsequent research extended the scope to examine miR2911's efficacy against enterovirus and herpes-zoster virus infections, revealing its ability to target the VP1 coat protein of enterovirus and the IE62 transcriptional regulatory protein of herpes-zoster virus, effectively

halting their replication [31,32]. Following the outbreak of SARS-CoV-2, our investigations into miR2911's role in combating this virus have shown significant inhibition of viral replication and accelerated patient recovery [25]. The cumulative results from these studies underscore miR2911's potent and broad-spectrum antiviral capabilities, suggesting its potential as a novel strategy for broad-spectrum antiviral treatment.

3.8. Antitumor

Chin *et al.* demonstrated that the presence of plant gma-miR159 in the serum of breast cancer patients was inversely correlated with the incidence and progression of the disease, indicating a potential anti-cancer effect of gma-miR159. Further investigations revealed that gma-miR159 could hinder proliferation of human breast cancer cell lines MDA-MB-231 and MCFDCIS *in vitro*, by targeting the transcription factor TCF7, resulting in the down-regulation of MYC protein levels. Subsequent experiments validated that orally administrated gma-miR159 is able to significantly reduce tumor growth in MDA-MB-231 xenograft tumor model [11].

Furthermore, studies on honeysuckle in the field of anti-tumor research discovered that the survival rate of colorectal cancer mice with a dietary intake of honeysuckle was significantly higher than that of the control group, suggesting the potential of honeysuckle in the field of anti-tumor treatment. Additional research revealed that miR2911 derived from honeysuckle could be absorbed by mice and, with the assistance of SIDT1, a transporter protein located in the stomach, downregulate the expression of TGF-β1. *In vivo* experiments further manifested the ability of miR2911 to inhibit the progression of colorectal cancer through dietary intake in CT26 tumor-bearing mice [29].

In addition to the suppression of protein-coding mRNAs, dietary miRNAs can also hinder tumor proliferation by suppressing oncogenic lncRNAs. One of the initial oncogenic lncRNAs studied was MALAT1, which is often upregulated in cancer development and progression. NEAT1 is known to regulate the response to DNA damage, and high expression of NEAT1 is closely linked to poor prognosis in patients. It was observed that plant-derived mtr-miR5754 and gma-miR4995 were able to selectively suppress these lncRNAs and inhibit the proliferation of HCT116 cells *in vitro* [26].

3.9. Co-evolution

The honeybee is a eusocial creature with a strict social structure. While queens and worker bees have the same genetic makeup, queens have the ability to reproduce and have a larger body size, faster development, and longer lifespan compared to workers. On the other hand, worker bees are typically sterile and dedicated to tasks such as housekeeping and food collection. These differences are not predetermined at birth but are a result of variations in larval diet. Larvae that consume royal jelly develop into queens, while those that consume beebread and pollen become worker bees [43]. Mechanistic studies have shown that the gene *Apis mellifera* TOR (amTOR), which plays a role in caste differentiation, is directly inhibited by plant miR162a. This inhibition prevents larvae from developing into queens and instead

promotes the development of worker bee characteristics. Although residual miRNAs in royal jelly do not reach a functional level, queen-destined larvae are able to evade this regulation and develop into more queen-like individuals [33,44,45]. Maintaining a single reproductive queen within a colony is crucial for the stability of honeybee populations. Therefore, the use of plant miRNAs to achieve stability within the population aligns with evolutionary advantages. Plant miRNAs are not randomly included in the honeybee's diet, but are collected purposefully, likely for the survival of the entire colony. In an evolutionary context, the role of plant miRNAs in regulating honeybee development provides valuable insights into co-evolution. We propose that the cross-kingdom regulatory function of plant miRNAs may have evolved through the selection of food sources by honeybees in nature. When honeybees gather pollen for food, they inadvertently pollinate plants, and in return, plants donate miRNAs to regulate caste differentiation. This suggests that flowering plants have developed attractive characteristics for honeybees to facilitate pollination, and the significant regulatory effects of plant miRNAs on honeybees subsequently influence the characteristics of these pollinators. The selection of food sources by honeybees demonstrates an extraordinary evolutionary adaptation for the success of the colony through a partnership between two interacting organisms. As a result, honeybees and plants exert selective pressures on each other in a co-evolutionary relationship, ultimately affecting each other's fate in the interconnected ecosystem. From this perspective, honeybees and flowering plants have long benefited from their interdependence. Further research in this emerging field may provide insights into the impact of food consumption on the evolution of eusociality.

4. How dietary miRNA cross the biological barrier?

4.1. Stability

In general, dietary miRNAs must undergo high temperature processing (such as cooking or simmering) and absorption in the gastrointestinal tract before being transported to relevant tissues through the circulatory system to exert their functions [46]. This process actually involves overcoming at least three biological barriers: high temperature, extreme pH, and various nucleases present in the gastrointestinal tract and serum. Previous study has shown that these dietary miRNAs, like gma-MIR6300 from soybean, can be found distinguishably in both serum and stool of vegetarians or vegans, indicating that these dietary miRNAs may well be able to survive these biological barriers and undergo absorption and redistribution [47]. It is crucial to consider how these dietary miRNAs remain stable, and understanding the underlying mechanisms is fundamental to comprehending their absorption and functionality.

Experimental data strongly supports that a considerable portion of miRNAs, originating from both animal and plant sources, can withstand high temperature treatments [8,19,46]. This suggests the existence of specific mechanisms that protect these miRNAs from external environmental interference and potentially facilitate their uptake.

Exosomes are extracellular vesicles with a phospholipid bilayer structure similar to that of cells, ranging from 30 to 200 nm in diameter. These exosomes carry various cargoes, including proteins, lipids, and nucleic acids, and miRNAs are no exception. Encapsulated in

exosomes, miRNAs can be present in body fluids such as blood, urine, saliva, and milk, remaining stable without being degraded by RNase [48,49]. MiRNAs within exosomes facilitate communication between different tissues or cells, possibly through paracrine or endocrine means, and regulate essential functions of proximal or distal cells [50]. Dysregulation of miRNAs can lead to imbalances in the nervous system, metabolism, immune responses, or tumorigenesis due to their involvement in numerous physiological or pathological processes [51–54]. In milk, numerous miRNAs loaded in exosomes have been identified, and experimental results have demonstrated that these miRNAs are protected from degradation by exosomes under conditions such as RNase degradation, repeated freeze-thaw cycles, or low pH. Synthetic miRNAs, on the other hand, lack this self-protection [20,55–57]. These findings suggest that exosomes present in milk have the ability to protect cargo miRNAs and maintain their stability in various environments or within the gastrointestinal tract.

A similar mechanism exists in plants, where plant-derived extracellular vesicles (EVs), known as exosome-like nanoparticles (ELNs), share a similar structure with animal-derived exosomes and can also selectively load miRNAs [58–60]. A subsequent study in *Arabidopsis* found that key proteins such as Argonaute 1, RNA helicase 11 and RNA helicase 37 contribute to the loading of plant small RNAs (sRNAs) into EVs. While Annexins 1 and Annexins 2 bind non-specifically to sRNAs and play a role in stabilizing sRNAs in EVs [61]. These findings suggest the possibility of an animal-like mechanism in ELNs that protects dietary miRNAs from degradation under extreme conditions.

Apart from nature protective vesicle structure, Qin *et al.* have demonstrated that oral mastication also plays a role in stabilization of dietary miRNAs. *In vitro* mastication simulation homogenized plant miRNAs altogether with other food components, including water, carbohydrate, proteins, lipids, *etc.* Using transmission electron microscope, the researchers found that the emulsion of the food matrix contains spherical microstructures with diameters mostly range from 142 to 615 nm. This surprising discovery indicates dietary microRNAs can self-assemble with other food components to form stable submicron particles which in turn protect them from RNase degradation [62].

In addition to the stabilization of dietary miRNAs by biological membranes like exosomes or ELNs, modifications of miRNAs, intrinsic high GC sequences, or secondary structures also appear to provide protective effects for dietary miRNA stabilization in the gastrointestinal tract. For instance, the stem-loop structure at the 3' end of miR159 was found to protect it from recognition and digestion by RNase. Similarly, miR168a seems to be shielded from RNase by a bulge structure formed by an internal GC pairing [63,64]. In contrast, our stability study of miR2911 revealed that it has a high GC content in its sequence. Furthermore, the two mutants we generated (one with a mutation from 5'-GG to 5'-AA and the other with a mutation from 3'-GGA to 3'-AAA) showed a lack of resistance to RNase, suggesting that the stability of miR2911 may be related to its high GC content [16].

4.2. Absorption

Over the past decade, since the identification of dietary miRNAs in serum, considerable effort has been directed towards understanding their uptake mechanisms. Given the natural encapsulation of dietary miRNAs within animal-derived exosomes or plant-derived extracellular vesicle-like nanoparticles (ELNs), it is hypothesized that these vesicles play a critical role in facilitating miRNA absorption in the body.

An analysis of dietary miRNA uptake has shown that PKH26-stained ELNs were found co-localized with macrophages in the intestinal lamina propria and intestinal stem cells after intragastric administration, demonstrating the capacity of plant-derived ELNs to be internalized by macrophages and stem cells within the mouse intestine [58]. Another independent experiment denoted that ELNs from fruit and vegetable juices are rich in miRNAs such as miR156a-5p, miR166a-3p, and miR168a-5p, and can be absorbed by rat intestinal epithelial cells IEC6 [65]. Similarly, for animal-derived miRNAs in the diet, especially those found in milk, it has been observed that these miRNAs can be taken up by exosome-mediated endocytosis or transcytosis of epithelial cells, thereby regulating infant immunity and promoting intestinal maturation. Additionally, macrophages can phagocytose miRNA-loaded exosomes in milk [66–70]. Although current experimental evidence suggests that the intestine is the main site of dietary miRNA uptake, studies on extracellular vesicle-mediated uptake of these miRNAs are limited to *in vitro* experiments. There is a lack of sufficient *in vivo* evidence to confirm the specific sites and mechanisms of dietary miRNA uptake in mammals, such as humans, under physiological conditions.

In spite of that, considering that not all dietary miRNAs are loaded in exosomes or ELN, it is crucial to understand the fate of these free miRNAs, whether they are degraded or taken up by other means. Our recent research identified a protein called SIDT1, located in the stomach, which mediates the uptake of free miRNAs in an acidic environment. Knockout mice lacking SIDT1 showed a significantly reduced ability to ingest dietary miRNAs, suggesting that the stomach may be the primary site of dietary miRNA absorption. Initially, SIDT1 was thought to only transport mammalian double-stranded RNA, but we discovered that SIDT1 in the stomach can also transport mature single-stranded dietary miRNAs. Nonetheless, the efficiency of SIDT1 in transporting single-stranded miRNAs is not as high as that of double-stranded ones, and we observed variations in uptake efficiency for different types of plant-derived miRNAs, such as miR156a, miR168a, and miR2911, indicating that SIDT1-mediated dietary miRNA uptake is somewhat selective [17].

In addition, the processing of food or herbs may also enhance the absorption of miRNAs. It is widely recognized that Chinese medicinal herbs are often boiled for extended periods of time during preparation, known as decoctions. On the other hand, the impact of this herbal treatment on the pharmacological properties and pharmacokinetics of their internal miRNAs has not been thoroughly investigated. A recent study focused on the herb *Rhodiola crenulata* and identified a small RNA, called HJT-sRNA-m7, which exhibited the ability to alleviate lung fibrosis in both cultured cells and a mouse model by simultaneously downregulating three fibrotic genes [42]. Moreover, the researchers explored the mechanisms by which small

RNAs survive the heat treatment during the decoction process and enter mammalian cells. They discovered that the heating process promotes the co-assembly of small RNAs and lipids in the decoctions, resulting in the formation of heat-stable exosome-like nanoparticles, referred to as decoctosomes. These decoctosomes are composed of lipids, chemical compounds, proteins, and small RNAs, and can effectively enter human cells, thereby displaying significant therapeutic effects in mouse models of pulmonary fibrosis and pulmonary inflammation. Consequently, HJT-sRNA-m7 and other small RNAs present in the decoctosomes exhibit potent anti-fibrosis and anti-inflammatory effects. Overall, these findings suggest an unprecedented pathway in which lipids form liposomes with small RNAs in boiling decoctions, facilitating the uptake of small RNAs into human cells [71]. This groundbreaking study provides valuable insights that factors which might typically be considered detrimental to the stability of dietary miRNAs, such as high-temperature treatment, may actually enhance their stability and uptake.

4.3. Distribution

After absorption in the gastrointestinal tract, dietary miRNAs are released into the systemic circulation, a process that bears resemblance to the release of endogenous miRNAs. In mammals, miRNAs are mainly secreted into the systemic circulation via two pathways: encapsulation in extracellular vesicles (EVs), including exosomes and microvesicles (MVs), or stabilization through binding to RNA-binding proteins such as AGO2 [49]. We tested the levels of the dietary miR168a and miR156a in C57BL/6J MV and MV-free serum by RT-qPCR, and the portion of circulating plant miRNAs in MVs compared to MV-free plasma is similar to most endogenous miRNAs, such as miR-16, miR-21, and miR-150. Our findings indicate that plant-derived miR168a is predominantly found within MVs rather than in MV-free serum, suggesting that MVs may have a protective effect on plant miRNAs in serum. Furthermore, our co-immunoprecipitation experiments revealed that miR168a binds to Ago2, which confers protection against degradation by nucleases in serum [2]. However, contrary to this theory, we observed a divergent outcome with miR2911. We discovered that miR2911 remains stable in serum and does not interact with exosomes or Ago2, indicating that its redistribution in the endosomal environment after absorption does not rely on these established pathways. Subsequent studies demonstrated that high levels of GC content may be crucial for the sustained presence of miR2911 in serum. This suggests the existence of novel pathways or mechanisms for the redistribution of dietary miRNAs *in vivo* [16].

Similar to endogenous miRNAs, the redistribution of dietary miRNAs *in vivo* is also specific to particular organs or tissues, although the exact mechanism underlying this phenomenon remains unclear. Notably, Manca *et al.* stained exosomes derived from milk and unexpectedly discovered their presence in the circulatory system. Intriguingly, these milk-derived exosomes exhibited differential distribution patterns compared to their loaded miRNAs, accumulating in the liver, spleen, and brain. Whether this experiment implies that milk-derived exosomes facilitate the distribution of their cargo miRNAs *in vivo* remains uncertain, as the tissue distribution characteristics of these exosomes differ from those of

their loaded miRNAs. Further investigation is necessary to elucidate the specific biological processes associated with milk-derived exosomes [72].

5. Dietary miRNA inspires novel RNAi delivery strategy

Despite significant advancements in injectable, transdermal, and nasal drug delivery routes, the oral route remains the optimal and preferred method to achieve therapeutic benefits due to its convenience and patient compliance, especially for chronic therapies [73–75]. Meanwhile, the oral administration of miRNAs or siRNAs for RNAi-based therapeutics has long been considered impossible due to the physiological barriers in the gastrointestinal tract. Despite efforts to reduce the impact of digestive enzymes and to enhance the absorption of miRNAs and siRNAs using microparticle carriers, these methods are still limited by their instability, low delivery efficiency, high cost, and significant toxicity [76–78].

Recent advances in plant miRNA-mediated cross-kingdom gene regulation offer hope for overcoming the major challenges associated with oral administration of miRNAs and siRNAs in clinical settings. Plants appear to be ideal carriers for therapeutic RNAs, as some plant miRNAs are resilient and resistant to the harsh conditions of digestion, allowing them to be absorbed by consumers [63,79,80]. Utilizing dietary plants as an oral route presents an intriguing opportunity to develop an innovative, convenient, and potentially effective treatment strategy for oral delivery of siRNAs as therapeutic medications. In fact, pioneering studies have successfully engineered transgenic plants to express therapeutic siRNAs designed to target disease-related genes in animals. These medicinal plants can biosynthesize a significant amount of therapeutic plant miRNAs and facilitate their delivery to consumers for oral gene therapy.

For example, we have engineered an edible lettuce to biosynthesize artificial miRNAs specifically targeting the hepatitis B virus surface antigen (HBsAg) gene using the endogenous miRNA biogenesis machinery of lettuce. After oral administration of lettuce decoction, the artificial miRNAs can be absorbed and delivered to the liver, inhibiting HBsAg expression in p21-HBsAg knock-in transgenic mice at a relatively low concentration compared to treatment with high doses of synthetic siRNAs. Remarkably, after a 15 month of long-term treatment, the expression of HBsAg was dramatically reduced, and liver injury was significantly alleviated in transgenic mice, with no observed toxic effects [81]. This study developed a medicinal lettuce that utilizes the plant's endogenous miRNA biogenesis machinery to produce methylated miRNAs, increasing stability while reducing production costs. Considering that chronic hepatitis B virus (HBV) infection remains a serious public health issue, particularly for patients in the immune-tolerant phase or resistant to conventional antiviral treatment, this study not only provides an effective, convenient, and financially viable treatment strategy for these patients but also minimizes potential side effects by reducing the required dose of miRNA medication and allowing long-term administration. Plant miRNA-based oral therapy has the potential to offer an effective, non-toxic, and affordable treatment option for various diseases, especially chronic ones, and

could initiate a "green revolution" in the bioengineering of edible plants to express miRNA medications.

6. Future perspective

The consumption of miRNAs from dietary sources across species is increasingly acknowledged as a significant form of cross-species regulation. This novel mode of information exchange challenges the traditional belief that exogenous RNAs are simply digested and absorbed without retaining their functional integrity or conveying genetic information. It has been a decade since our groundbreaking discovery of cross-species regulation mediated by dietary miR168a. During this time, numerous subsequent studies have demonstrated that dietary miRNAs can unexpectedly be absorbed in the gastrointestinal tract and perform various functions in the mammalian body. In addition to the previously speculated mechanism of miRNA absorption facilitated by exosomes or ELNs in the small intestine, recent findings have revealed that the protein SIDT1, present in the stomach, also plays a role in mediating the absorption of dietary miRNAs, suggesting that the stomach may be an unanticipated site for dietary miRNA absorption. Intriguingly, SIDT1 exhibits varying efficiency in transporting different miRNAs, indicating a selective uptake of dietary information. Furthermore, investigations into SIDT1 polymorphisms in humans have revealed that 22 out of 135 volunteers possess amino acid substitutions (Val78Met) in SIDT1. Subsequent experiments have implied that individuals with the SIDT1 polymorphism exhibit decreased uptake of dietary miR2911 [82], which inspires us that there may be other alternative mechanisms for the absorption of dietary miRNAs.

Further exploration into the mechanisms of dietary miRNA absorption promises to deepen our understanding of cross-species regulation and optimize its application. Additionally, studies on the diverse functions of dietary miRNAs not only validate the feasibility of cross-species regulation but also expand our knowledge of the functional spectrum of these miRNAs. To some extent, this supports the principles of dietary therapy advocated by traditional Chinese medicine, which advocates for a diet structured to promote health. Research into the functions of dietary miRNAs provides new insights into the potential benefits of the foods we consume every day and lays the groundwork for their use as dietary supplements. Simultaneously, research on dietary miRNAs has inspired novel directions for investigating active substances in food and Chinese medicinal herbs. Going beyond the previous focus on molecular compounds, these miRNAs in Chinese medicinal herbs offer a new insight in understanding their pharmacological aspects. While research on dietary miRNAs is still in its early stages, it is hopefully that more mechanisms and functions will be uncovered in the future. This may pave the way for utilizing oral nucleic acid drugs as a new approach to drug delivery, overcoming the limitations of traditional approaches.

Acknowledgments

This work was supported by grants from the Excellent Postdoctoral Program of Jiangsu Province (2023ZB697) and Natural Science Foundation of Jiangsu Province (BK20230786).

Conflicts of interests

The authors declare no conflicts of interest.

Authors' contribution

Conceptualization, H.G.; writing—original draft preparation, C.T.; writing—review and editing, H.G.; visualization, C.T.; supervision, H.G.; project administration, H.G.; funding acquisition, H.G. All authors have read and agreed to the published version of the manuscript.

References

[1] Zhu WJ, Liu Y, Cao YN, Peng LX, Yan ZY, *et al.* Insights into Health-Promoting Effects of Plant MicroRNAs: A Review. *J. Agric. Food Chem.* 2021, 69(48): 14372–14386.
[2] Zhang L, Hou D, Chen X, Li D, Zhu L, *et al.* Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res.* 2012, 22(1): 107–126.
[3] Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. *Annu. Rev. Biochem.* 2010, 79: 351–379.
[4] Li Z, Xu R, Li N. MicroRNAs from plants to animals, do they define a new messenger for communication? *Nutr. Metab. (Lond)* 2018, 15: 68.
[5] Dickinson B, Zhang Y, Petrick JS, Heck G, Ivashuta S, *et al.* Lack of detectable oral bioavailability of plant microRNAs after feeding in mice. *Nat. Biotechnol.* 2013, 31(11): 965–967.
[6] Chen X, Zen K, Zhang CY. Reply to Lack of detectable oral bioavailability of plant microRNAs after feeding in mice. *Nat. Biotechnol.* 2013, 31(11): 967–969.
[7] Liang G, Zhu Y, Sun B, Shao Y, Jing A, *et al.* Assessing the survival of exogenous plant microRNA in mice. *Food Sci. Nutr.* 2014, 2(4): 380–388.
[8] Philip A, Ferro VA, Tate RJ. Determination of the potential bioavailability of plant microRNAs using a simulated human digestion process. *Mol. Nutr. Food Res.* 2015, 59(10): 1962–1972.
[9] Yang J, Farmer LM, Agyekum AA, Elbaz-Younes I, Hirschi KD. Detection of an Abundant Plant-Based Small RNA in Healthy Consumers. *PLoS One* 2015, 10(9): e0137516.
[10] Yang J, Farmer LM, Agyekum AA, Hirschi KD. Detection of dietary plant-based small RNAs in animals. *Cell Res.* 2015, 25(4): 517–520.
[11] Chin AR, Fong MY, Somlo G, Wu J, Swiderski P, *et al.* Cross-kingdom inhibition of breast cancer growth by plant miR159. *Cell Res.* 2016, 26(2): 217–228.
[12] Chen X, Liu L, Chu Q, Sun S, Wu Y, *et al.* Large-scale identification of extracellular plant miRNAs in mammals implicates their dietary intake. *PLoS One* 2021, 16(9): e0257878.
[13] Ma J, Wang C, Long K, Zhang H, Zhang J, *et al.* Exosomal microRNAs in giant panda (Ailuropoda melanoleuca) breast milk: potential maternal regulators for the development of newborn cubs. *Sci Rep.* 2017, 7(1): 3507.
[14] Luo Y, Wang P, Wang X, Wang Y, Mu Z, *et al.* Detection of dietetically absorbed maize-derived microRNAs in pigs. *Sci. Rep.* 2017, 7(1): 645.
[15] Chen X, Dai GH, Ren ZM, Tong YL, Yang F, *et al.* Identification of Dietetically Absorbed Rapeseed (Brassica campestris L.) Bee Pollen MicroRNAs in Serum of Mice. *Biomed. Res. Int.* 2016, 2016: 5413849.
[16] Zhou Z, Li X, Liu J, Dong L, Chen Q, *et al.* Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. *Cell Res.* 2015, 25(1): 39–49.

[17] Chen Q, Zhang F, Dong L, Wu H, Xu J, *et al.* SIDT1-dependent absorption in the stomach mediates host uptake of dietary and orally administered microRNAs. *Cell Res.* 2021, 31(3): 247–258.
[18] Cavalieri D, Rizzetto L, Tocci N, Rivero D, Asquini E, *et al.* Plant microRNAs as novel immunomodulatory agents. *Sci. Rep.* 2016, 6: 25761.
[19] Dever JT, Kemp MQ, Thompson AL, Keller HG, Waksmonski JC, *et al.* Survival and Diversity of Human Homologous Dietary MicroRNAs in Conventionally Cooked Top Sirloin and Dried Bovine Tissue Extracts. *PLoS One* 2015, 10(9): e0138275.
[20] Zempleni J, Aguilar-Lozano A, Sadri M, Sukreet S, Manca S, *et al.* Biological Activities of Extracellular Vesicles and Their Cargos from Bovine and Human Milk in Humans and Implications for Infants. *J. Nutr.* 2017, 147(1): 3–10.
[21] Huang F, Du J, Liang Z, Xu Z, Xu J, *et al.* Large-scale analysis of small RNAs derived from traditional Chinese herbs in human tissues. *Sci. China Life Sci.* 2019, 62(3): 321–332.
[22] Yang J, Primo C, Elbaz-Younes I, Hirschi KD. Bioavailability of transgenic microRNAs in genetically modified plants. *Genes Nutr.* 2017, 12: 17.
[23] Hou D, He F, Ma L, Cao M, Zhou Z, *et al.* The potential atheroprotective role of plant MIR156a as a repressor of monocyte recruitment on inflamed human endothelial cells. *J. Nutr. Biochem.* 2018, 57: 197–205.
[24] Melnik BC, Schmitz G. MicroRNAs: Milk's epigenetic regulators. *Best Pract. Res. Clin. Endocrinol. Metab.* 2017, 31(4): 427–442.
[25] Zhou LK, Zhou Z, Jiang XM, Zheng Y, Chen X, *et al.* Absorbed plant MIR2911 in honeysuckle decoction inhibits SARS-CoV-2 replication and accelerates the negative conversion of infected patients. *Cell Discov.* 2020, 6(1): 54.
[26] Marzano F, Caratozzolo MF, Consiglio A, Licciulli F, Sbisà E, *et al.* Plant miRNAs Reduce Cancer Cell Proliferation by Targeting MALAT1 and NEAT1: A Beneficial Cross-Kingdom Interaction. *Front Genet.* 2020. 11: 552490.
[27] Aquilano K, Ceci V, Gismondi A, De Stefano S, Iacovelli F, *et al.* Adipocyte metabolism is improved by TNF receptor-targeting small RNAs identified from dried nuts. *Commun Biol.* 2019, 2: 317.
[28] Wang W, Hang C, Zhang Y, Chen M, Meng X, *et al.* Dietary miR-451 protects erythroid cells from oxidative stress via increasing the activity of Foxo3 pathway. *Oncotarget* 2017, 8(63): 107109–107124.
[29] Liu C, Xu M, Yan L, Wang Y, Zhou Z, *et al.* Honeysuckle-derived microRNA2911 inhibits tumor growth by targeting TGF-β1. *Chin. Med.* 2021, 16(1): 49.
[30] Yu WY, Cai W, Ying HZ, Zhang WY, Zhang HH, *et al.* Exogenous Plant gma-miR-159a, Identified by miRNA Library Functional Screening, Ameliorated Hepatic Stellate Cell Activation and Inflammation via Inhibiting GSK-3β-Mediated Pathways. *J. Inflamm. Res.* 2021, 14: 2157–2172.
[31] Huang Y, Liu H, Sun X, Ding M, Tao G, *et al.* Honeysuckle-derived microRNA2911 directly inhibits varicella-zoster virus replication by targeting IE62 gene. *J. Neurovirol.* 2019, 25(4): 457–463.
[32] Li X, Huang Y, Sun M, Ji H, Dou H, *et al.* Honeysuckle-encoded microRNA2911 inhibits Enterovirus 71 replication via targeting VP1 gene. *Antiviral Res.* 2018, 152: 117–123.
[33] Mutti NS, Dolezal AG, Wolschin F, Mutti JS, Gill KS, *et al.* IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate. *J. Exp. Biol.* 2011, 214(23): 3977–3984.
[34] Zeng P, Li J, Chen Y, Zhang L. The structures and biological functions of polysaccharides from traditional Chinese herbs. *Prog. Mol. Biol. Transl. Sci.* 2019, 163: 423–444.

[35] Fresco P, Borges F, Marques MP, Diniz C. The anticancer properties of dietary polyphenols and its relation with apoptosis. *Curr. Pharm. Des.* 2010, 16(1): 114–134.
[36] Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J. Nutr. Biochem.* 2007, 18(7): 427–442.
[37] Higgs PG, Lehman N. The RNA World: molecular cooperation at the origins of life. *Nat. Rev. Genet.* 2015, 16(1): 7–17.
[38] Bartel DP. Metazoan MicroRNAs. *Cell* 2018, 173(1): 20–51.
[39] Berezikov E. Evolution of microRNA diversity and regulation in animals. *Nat. Rev. Genet.* 2011, 12(12): 846–860.
[40] Samad AFA, Kamaroddin MF, Sajad M. Cross-Kingdom Regulation by Plant microRNAs Provides Novel Insight into Gene Regulation. *Adv. Nutr.* 2021, 12(1): 197–211.
[41] Akao Y, Kuranaga Y, Heishima K, Sugito N, Morikawa K, *et al.* Plant hvu-MIR168-3p enhances expression of glucose transporter 1 (SLC2A1) in human cells by silencing genes related to mitochondrial electron transport chain complex I. *J. Nutr. Biochem.* 2022, 101: 108922.
[42] Du J, Liang Z, Xu J, Zhao Y, Li X, *et al.* Plant-derived phosphocholine facilitates cellular uptake of anti-pulmonary fibrotic HJT-sRNA-m7. *Sci. China Life Sci.* 2019, 62(3): 309–320.
[43] Evans JD, Wheeler DE. Differential gene expression between developing queens and workers in the honey bee, Apis mellifera. *Proc. Natl. Acad. Sci. U. S. A.* 1999, 96(10): 5575–5580.
[44] Patel A, Fondrk MK, Kaftanoglu O, Emore C, Hunt G, *et al.* The making of a queen: TOR pathway is a key player in diphenic caste development. *PLoS One* 2007, 2(6): e509.
[45] Zhu K, Liu M, Fu Z, Zhou Z, Kong Y, *et al.* Plant microRNAs in larval food regulate honeybee caste development. *PLoS Genet.* 2017, 13(8): e1006946.
[46] Link J, Thon C, Schanze D, Steponaitiene R, Kupcinskas J, *et al.* Food-Derived Xeno-microRNAs: Influence of Diet and Detectability in Gastrointestinal Tract-Proof-of-Principle Study. *Mol. Nutr. Food Res.* 2019, 63(2): e1800076.
[47] Tarallo S, Ferrero G, De Filippis F, Francavilla A, Pasolli E, *et al.* Stool microRNA profiles reflect different dietary and gut microbiome patterns in healthy individuals. *Gut* 2022, 71(7): 1302–1314.
[48] Pegtel DM, Gould SJ. Exosomes. *Annu. Rev. Biochem.* 2019, 88: 487–514.
[49] Mori MA, Ludwig RG, Garcia-Martin R, Brandão BB, Kahn CR. Extracellular miRNAs: From Biomarkers to Mediators of Physiology and Disease. *Cell Metab.* 2019, 30(4): 656–673.
[50] Wortzel I, Dror S, Kenific CM, Lyden D. Exosome-Mediated Metastasis: Communication from a Distance. *Dev Cell.* 2019, 49(3): p. 347-360.
[51] He X, Kuang G, Wu Y, Ou C. Emerging roles of exosomal miRNAs in diabetes mellitus. *Clin. Transl. Med.* 2021, 11(6): e468.
[52] Duan W, Zhang W, Jia J, Lu Q, Eric Gershwin M. Exosomal microRNA in autoimmunity. *Cell. Mol. Immunol.* 2019, 16(12): 932–934.
[53] Noonin C, Thongboonkerd V. Exosome-inflammasome crosstalk and their roles in inflammatory responses. *Theranostics* 2021, 11(9): 4436–4451.
[54] Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW, *et al.* Extracellular vesicles in cancer - implications for future improvements in cancer care. *Nat. Rev. Clin. Oncol.* 2018, 15(10): 617–638.
[55] Benmoussa A, Ly S, Shan ST, Laugier J, Boilard E, *et al.* A subset of extracellular vesicles carries the bulk of microRNAs in commercial dairy cow's milk. *J. Extracell. Vesicles* 2017, 6(1): 1401897.
[56] Kosaka N, Izumi H, Sekine K, Ochiya T. microRNA as a new immune-regulatory agent in breast milk. *Silence* 2010, 1(1): 7.

[57] Howard KM, Jati Kusuma R, Baier SR, Friemel T, Markham L, *et al.* Loss of miRNAs during processing and storage of cow's (Bos taurus) milk. *J. Agric. Food Chem.* 2015, 63(2): 588–592.
[58] Mu J, Zhuang X, Wang Q, Jiang H, Deng ZB, *et al.* Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. *Mol. Nutr. Food Res.* 2014, 58(7): 1561–1573.
[59] Kameli N, Dragojlovic-Kerkache A, Savelkoul P, Stassen FR. Plant-Derived Extracellular Vesicles: Current Findings, Challenges, and Future Applications. *Membranes (Basel)* 2021, 11(6): 411.
[60] Urzì O, Raimondo S, Alessandro R. Extracellular Vesicles from Plants: Current Knowledge and Open Questions. *Int. J. Mol. Sci.* 2021, 22(10): 5366.
[61] He B, Cai Q, Qiao L, Huang CY, Wang S, *et al.*, RNA-binding proteins contribute to small RNA loading in plant extracellular vesicles. *Nat. Plants* 2021, 7(3): 342–352.
[62] Qin X, Wang X, Xu K, Zhang Y, Ren X, *et al.* Digestion of Plant Dietary miRNAs Starts in the Mouth under the Protection of Coingested Food Components and Plant-Derived Exosome-like Nanoparticles. *J. Agric. Food Chem.* 2022, 70(14): 4316–4327.
[63] Wang X, Ren X, Ning L, Wang P, Xu K. Stability and absorption mechanism of typical plant miRNAs in an in vitro gastrointestinal environment: basis for their cross-kingdom nutritional effects. *J. Nutr. Biochem.* 2020, 81: 108376.
[64] Yang J, Elbaz-Younes I, Primo C, Murungi D, Hirschi KD. Intestinal permeability, digestive stability and oral bioavailability of dietary small RNAs. *Sci. Rep.* 2018, 8(1): 10253.
[65] Ito Y, Taniguchi K, Kuranaga Y, Eid N, Inomata Y, *et al.* Uptake of MicroRNAs from Exosome-Like Nanovesicles of Edible Plant Juice by Rat Enterocytes. *Int. J. Mol. Sci.* 2021, 22(7): 3749.
[66] Izumi H, Tsuda M, Sato Y, Kosaka N, Ochiya T, *et al.* Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. *J. Dairy Sci.* 2015, 98(5): 2920–2933.
[67] Alsaweed M, Lai CT, Hartmann PE, Geddes DT, Kakulas F. Human Milk Cells Contain Numerous miRNAs that May Change with Milk Removal and Regulate Multiple Physiological Processes. *Int. J. Mol. Sci.* 2016, 17(6): 956.
[68] Carney MC, Tarasiuk A, DiAngelo SL, Silveyra P, Podany A, *et al.* Metabolism-related microRNAs in maternal breast milk are influenced by premature delivery. *Pediatr. Res.* 2017, 82(2): 226–236.
[69] Kusuma RJ, Manca S, Friemel T, Sukreet S, Nguyen C, *et al.* Human vascular endothelial cells transport foreign exosomes from cow's milk by endocytosis. *Am. J. Physiol. Cell Physiol.* 2016, 310(10): C800–C807.
[70] Wolf T, Baier SR, Zempleni J. The Intestinal Transport of Bovine Milk Exosomes Is Mediated by Endocytosis in Human Colon Carcinoma Caco-2 Cells and Rat Small Intestinal IEC-6 Cells. *J. Nutr.* 2015, 145(10): 2201–2206.
[71] Li X, Liang Z, Du J, Wang Z, Mei S, *et al.* Herbal decoctosome is a novel form of medicine. *Sci. China Life Sci.* 2019, 62(3): 333–348.
[72] Manca S, Upadhyaya B, Mutai E, Desaulniers AT, Cederberg RA, *et al.* Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. *Sci. Rep.* 2018, 8(1): 11321.
[73] Usach I, Martinez R, Festini T, Peris JE. Subcutaneous Injection of Drugs: Literature Review of Factors Influencing Pain Sensation at the Injection Site. *Adv. Ther.* 2019, 36(11): 2986–2996.
[74] Pafili K, Papanas N. The importance of patient compliance with insulin pens: how can a new user-friendly pen help? *Expert Opin. Drug Deliv.* 2014, 11(5): 629–632.
[75] Hubbard GP, Elia M, Holdoway A, Stratton RJ. A systematic review of compliance to oral nutritional supplements. *Clin. Nutr.* 2012, 31(3): 293–312.

[76] Kang SH, Revuri V, Lee SJ, Cho S, Park IK, *et al.* Oral siRNA Delivery to Treat Colorectal Liver Metastases. *ACS Nano* 2017, 11(10): 10417–10429.
[77] He C, Yin L, Tang C, Yin C. Multifunctional polymeric nanoparticles for oral delivery of TNF-α siRNA to macrophages. *Biomaterials* 2013, 34(11): 2843–2854.
[78] Forbes DC, Peppas NA. Oral delivery of small RNA and DNA. *J. Control. Release* 2012, 162(2): 438–445.
[79] Jia M, He J, Bai W, Lin Q, Deng J, *et al.* Cross-kingdom regulation by dietary plant miRNAs: an evidence-based review with recent updates. *Food Funct.* 2021, 12(20): 9549–9562.
[80] Zhang M, Wang X, Han MK, Collins JF, Merlin D. Oral administration of ginger-derived nanolipids loaded with siRNA as a novel approach for efficient siRNA drug delivery to treat ulcerative colitis. *Nanomedicine (Lond)* 2017, 12(16): 1927–1943.
[81] Zhang S, Sang X, Hou D, Chen J, Gu H, *et al.* Plant-derived RNAi therapeutics: A strategic inhibitor of HBsAg. *Biomaterials* 2019, 210: 83–93.
[82] Zhou Z, Zhou Y, Jiang XM, Wang Y, Chen X, *et al.* Decreased HD-MIR2911 absorption in human subjects with the SIDT1 polymorphism fails to inhibit SARS-CoV-2 replication. *Cell Discov.* 2020, 6: 63.