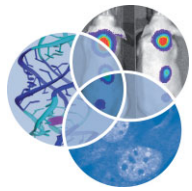
Focus Article



Regulation of mammalian gene expression by exogenous microRNAs

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Communication between cells ensures coordination of behavior. In prokaryotes, this signaling is usually referred to as quorum sensing, while eukaryotic cells communicate through hormones. In recent years, a growing number of reports have shown that small signaling molecules produced by organisms from different kingdoms of nature can facilitate cross-talk, communication, or signal interference. This *trans*-kingdom communication (also termed as *trans*-kingdom signaling or inter-kingdom signaling) mediates symbiotic and pathogenic relationships between various organisms (e.g., microorganisms and their hosts). Strikingly, it has been discovered that microRNAs (miRNAs)—single-stranded noncoding RNAs with an average length of 22 nt—can be transmitted from one species to another, inducing posttranscriptional gene silencing in distant species, even in a cross-kingdom fashion. Here, we discuss several recent studies concerning miRNA-mediated cross-kingdom gene regulation.

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INTRODUCTION

Organisms do not exist in isolation, but instead they associate with each other in interconnected ecosystems. The research field of *trans*-kingdom cell-to-cell signaling has received a great amount of attention in recent years, and it is defined as the concept that organisms from different kingdoms of nature can communicate with each other through an array of hormones or hormone-like chemicals.[1–3] This field evolved from the initial observation that bacteria can communicate with each other through hormone-like signals, a process that was later known as quorum sensing, and expanded with the realization that these bacterial signals can modulate mammalian and plant cell signal transduction and that host hormones can also cross-signal with quorum sensing molecules to modulate bacterial gene expression.[1–3]

Recently, scientists have discovered an entirely new level of gene regulation mediated by small molecules called microRNAs (miRNAs), which are a class of single-stranded noncoding RNAs with an average length of 22 nt that play an important role in posttranscriptional gene regulation (Figure 1).[4,5] Since the first miRNA, lin-4, was identified in 1993,[6] over 20,000 miRNAs have been identified in more than 150 species of animals, plants, viruses, and fungi. It has been estimated that miRNAs account for 1–5% of all expressed human genes and that they regulate the expression of more than 30% of the protein-coding genes.[7] It is now accepted that miRNAs play crucial regulatory roles in numerous biological processes, including cellular proliferation, differentiation, development, apoptosis and metabolism, as well as associate with various diseases such as cancer.[8]

Strikingly, recent findings have suggested that miRNAs not only execute function within original cells, but can also be transmitted from one species to another, inducing posttranscriptional gene silencing in distant species, even in a cross-kingdom fashion. The discovery of this novel mechanism has greatly

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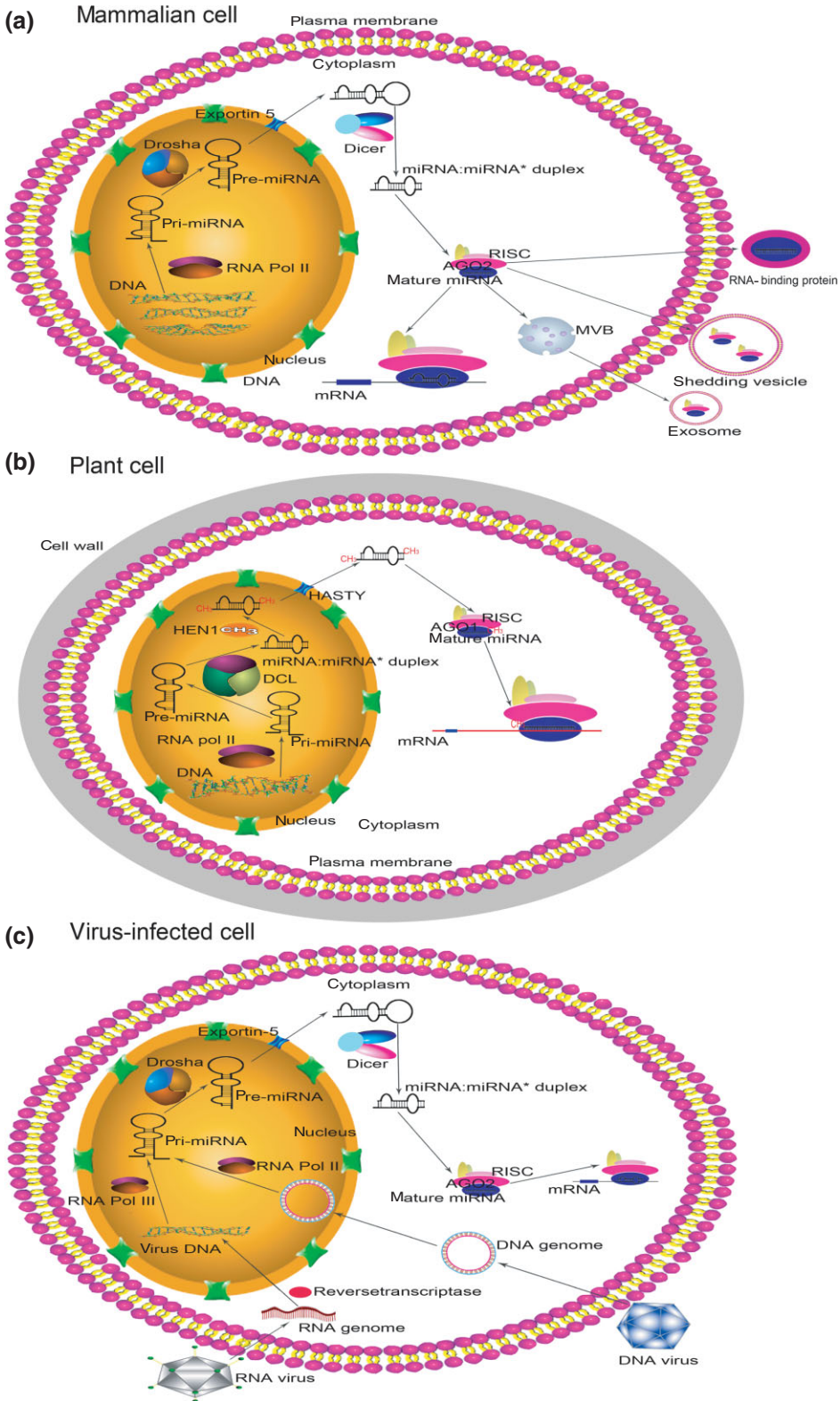

FIGURE 1 | Legend on next page

enhance our understanding of molecular signaling between species, which will likely have had important effects on the evolution of many extant species. Here, we discuss several recent studies concerning miRNA-mediated cross-kingdom gene regulation.

REGULATION OF MAMMALIAN GENE EXPRESSION BY VIRUS-ENCODED miRNAs

The classical example of mammalian gene regulation by exogenous miRNAs comes from virus–host interactions. Numerous studies have demonstrated that viruses can hijack the host cell miRNA machinery to produce their own sets of miRNAs. The existence of viral miRNA was first reported in 2004 by Pfeffer et al.[27] who described five miRNAs produced by Epstein–Barr virus (EBV) infected B cells. Since then, more than 400 viral miRNAs from approximately 23 viruses have been identified. Most of the virus-encoded miRNAs that have been identified come from viruses with DNA genomes, such as Herpesvirus, Adenovirus, and Polyomavirus. These findings have led to a common conception that naturally occurring RNA viruses will not encode miRNAs. However, recent study demonstrated that the bovine leukemia virus (BLV), a retrovirus with an RNA genome, encodes a conserved cluster of miRNAs that are transcribed by RNA polymerase III.[28]

The functions of most viral miRNAs remain to be deciphered. Current evidence indicates that viruses use miRNAs to manipulate host cell gene expression, suggesting an intensive miRNA-mediated *trans*-kingdom communication. Generally, viral miRNAs with *trans*-kingdom regulatory functions can be divided into five groups (Figure 2 and Table 1): (1) they can maintain viral latency through the repression of cellular factors involved in viral lytic

FIGURE 1 | Biogenesis of animal, plant, and virus microRNAs (miRNAs). (a) In general, genes encoding animal miRNAs are transcribed by RNA polymerase II.[9] The hairpins of the primary transcripts (pri-miRNAs) are then recognized by a nuclear protein known as DiGeorge Syndrome Critical Region 8 (DGCR8).[10] DGCR8 associates with the enzyme Drosha to form an RNase III type endonuclease that cleaves off the 5′ and 3′ ends of the pri-miRNAs to leave a 2 nt 3′ overhang.[11] Next, the ~70 nt hairpins, referred to as pre-miRNAs, are rapidly shuttled from the nucleus to the cytoplasm via the Exportin5/RAN–GTPase pathway.[12] Once in the cytoplasm, the pre-miRNAs are recognized by a cytoplasmic RNase III type endonuclease, Dicer, which cleaves off the bulged ends of the hairpins to generate imperfect miRNA:miRNA* duplexes around 20–25 bp in length, with each end having a 2 nt 3′ overhang.[13] Dicer activity is aided by transactivating (TAR) RNA-binding protein (TRBP) and PACT [interferon-inducible dsRNA-dependent protein kinase (PKR) activator], which are both cofactors for strand selection.[14,15] The final step in miRNA biogenesis is the assembly of mature miRNAs into the RNA-induced silencing complex (RISC).[16] The strand with the lower thermodynamic stability at the 5′ end of the miRNA is named 'guide strand' and is incorporated into RISC, while the other 'passenger' strand is released and degraded.[17] The composition of RISC is not fully defined, but it is known that Argonaute proteins are crucial to its function.[18] Once a mature miRNA is incorporated into RISC, it targets the 3′-UTR of mRNAs that show perfect complementarity to the seed sequence (positions 2–8 with respect to the 5′ end of miRNA) but imperfect complementarity to the remainder of the miRNA.[19] The precise mechanisms of miRNA-mediated repression are not fully defined, and both translational repression and degradation of miRNA-bound mRNAs have been observed.[20,21] Recent studies show that mammalian miRNAs can be actively secreted to outer cellular environment through enclosed in small membranous vesicles (e.g., exosomes and shedding vesicles) or packaged with RNA-binding proteins (e.g., high-density lipoprotein), and may function as secreted signaling molecules to influence the recipient cell phenotypes.[22] (b) Instead of being cleaved by two different enzymes inside and outside of the nucleus as in animals, plant miRNAs undergo both cleavages inside the nucleus by the Dicer homolog Dicer-like 1 (DCL1).[23] Before the plant miRNA:miRNA* duplex is transported outside the nucleus by the protein Hasty (HST; a homolog of Exportin 5),[24] its 3′ overhang is methylated by a RNA methyltransferase named Hua Enhancer1 (HEN1).[25] Unlike animal miRNAs, plant miRNAs show perfect or near-perfect complementarity to their targets and regulate target mRNA expression by directing mRNA cleavage at special sites in the coding regions.[26] (c) Biogenesis of DNA virus-encoded miRNAs appears to be mediated solely by cellular factors, as no viral protein involved in miRNA processing have been described so far. Thus, viral miRNA biogenesis and functional targeting are fully dependent on the host molecular miRNA processing and silencing machinery.[27] Up to now, efforts to identify miRNAs expressed by RNA viruses have been failed, except recent study reported the identification of miRNAs encoded by the retrovirus BLV that are expressed in BLV-transformed B cells.[28]

reactivation; (2) regulate viral latency and lytic replication by manipulating the host survival pathway; (3) block cell-cycle progression in host cells; and (4) prevent host cell apoptosis by repressing pro-apoptotic proteins; and (5) facilitate immune evasion.

Besides the way that viral miRNAs regulate host gene expression, there are other mechanisms by which viruses influence the RNA interference (RNAi) pathway of host cells. For example, Cazalla et al.[39] demonstrated that a viral noncoding RNA encoded by Herpesvirus saimiri modulated the expression of a host miRNA in virally transformed T cells. In addition, Lakatos et al.[40] showed that the P19 protein expressed by Cymbidium ringspot virus diminished the amount of free small interfering RNA (siRNA) in cells by forming p19–siRNA complexes, thus making siRNAs inaccessible for effector complexes of RNA-silencing machinery. These findings, combined with the above mentioned, support that miRNAs and other small molecules play an important role in the intricate virus–host interaction network, which offer new insights into diagnosis and control of viral diseases.

On the other hand, viruses can also use miRNAs to control their own gene expression. Interestingly, a recent study by Riley et al.[41] gives new insights into the cotargeting of viral and human genes during latency by viral and human miRNAs. By using high-throughput sequencing and cross-linking immunoprecipitation, they identified mRNA targets of EBV and human miRNAs in EBV-transformed B cells. While majority of the human mRNAs are targeted by EBV and human miRNAs via distinct binding sites, only three viral mRNAs are targeted, suggesting that miRNAs do not control the latent/lytic switch by targeting EBV lytic genes. However, regulation of viral mRNAs by viral miRNAs is not the main topic of this paper, because it does not represent a classical example of *trans*-kingdom communication.

REGULATION OF INSECT AND MAMMALIAN GENE EXPRESSION BY SYSTEMIC RNAi

RNAi is a posttranscriptional pathway in which double-stranded RNA (dsRNA) triggers the degradation of complementary mRNA in the cytoplasm of eukaryotic cells. In plants and in some animals, including *Caenorhabditis elegans*, RNAi can spread intercellularly, a phenomenon referred to as systemic RNAi.[42] This mechanism can mediate passive cellular uptake and cell-to-cell distribution of dsRNA across tissues and cellular boundaries, leading to sequence-specific mRNA silencing in distant cells. Systemic RNAi defective protein 1 (SID-1) is a transmembrane protein that enables systemic RNAi in the nematode *C. elegans*,[43] and it functions as a pore or channel that transports dsRNA into and out of cells. SID-2 is another small transmembrane protein that appears

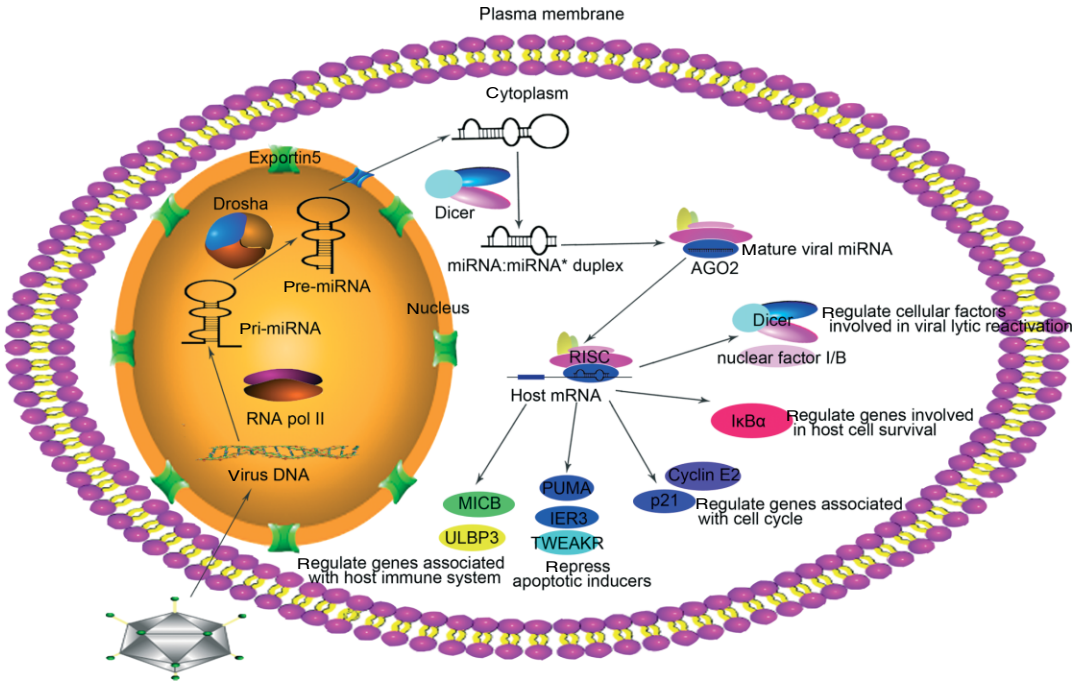

FIGURE 2 | Regulation of mammalian gene expression by virus-encoded miRNAs.

TABLE 1 | Examples of Viral miRNAs with *Trans*-kingdom Regulatory Functions

Group	Viral miRNA	Virus	Target in host cell	Function	References
1	miR-BART6-5p	EBV	Dicer	Regulate viral latency	29
	miR-K3	KSHV	Nuclear factor I/B		30
2	miR-K1	KSHV	IκBα	Rescue NF-κB activity and inhibit viral lytic replication	31
3	miR-US25-1	HCMV	Cyclin E2 and histone proteins	Block cell-cycle progression	32
	miR-K1	KSHV	Cyclin-dependent kinase inhibitor p21		33
4	miR-BART-5	EBV	PUMA	Repress pro-apoptotic proteins	34
	HIV-1-encoded miRNA	HIV-1	IER3		35
	miR-K10a	KSHV	TWEAK receptor		36
5	miR-UL112	HCMV	MICB	Facilitate immune evasion	37
	miR-J1-3p	BKV JCV	ULBP3		38

EBV, Epstein-Barr virus; KSHV, Kaposi's sarcoma-associated herpesvirus; HCMV, human cytomegalovirus; IκBα, nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α; NF-κB, nuclear factor-κB; PUMA, p53 upregulated modulator of apoptosis; TWEAK, tumor necrosis factor-like weak inducer of apoptosis; MICB, major histocompatibility complex class I-related chain B.

to act as a receptor for the uptake of dsRNA from environment. Characterization of SID-2 activity in a variety of nematodes indicates that *C. elegans* SID-2 is required for environmental RNAi.[44] In sum, SID channels are present in a wide variety of invertebrate and vertebrate animals and have relative specificity for small RNA molecules.

It is interesting to note that feeding *C. elegans* with dsRNA-expressing bacteria can lead to the systemic depletion of targeted mRNAs (Figure 3A).[45] Although this phenomenon is not a classical example of *trans*-kingdom regulation of gene expression by exogenous small RNAs (bacterial dsRNA is artificial), it does indicate that bacteria and nematodes from different kingdoms of nature can communicate with each other through a systemic RNAi mechanism. Considering that bacteria express many small RNAs (sRNA),[46] it is intriguing to speculate that bacteria noncoding sRNA may also play a role in the life process of *C. elegans*.

Although RNAi spreading phenomenon is apparent in nematodes, systemic RNAi does not appear to be a common feature in mammals. However, recent reports have shown that the human SID-1 homologue, SIDT1, enhances siRNA uptake in human systems, resulting in increased siRNA-mediated gene-silencing efficacy.[47–49] More strikingly, Elhassan et al.[50] currently show that SIDT1 facilitates rapid, contact-dependent, bidirectional siRNA, and miRNA transfer between human cells, resulting in target-specific non-cell-autonomous RNAi. Furthermore, they showed that SIDT1-mediated intercellular transfer of miR-21, the most famous oncogenic miRNA, contributes to the acquisition of drug resistance. This observation raises the possibility that SID channels may play a fundamental role in the complex intercellular communication, probably via exogenous miRNAs.

REGULATION OF INSECT GENE EXPRESSION BY siRNAs PRODUCED BY TRANSGENIC PLANT-MEDIATED RNAi

Although plants are often considered 'free lunch' by herbivores, their consumption often comes at a price. To promote reproductive success, plants have evolved multilayered defensive tactics to avoid or discourage herbivores. On the other hand, herbivores have themselves evolved intricate strategies to find, eat, and successfully digest plant tissues. Interestingly, recent findings indicate that small RNAs produced by transgenic plants may have an effect on insects. In 2007, Mao et al.[51] identified a cytochrome P450 gene, CYP6AE14, from the cotton bollworm *Helicoverpa*

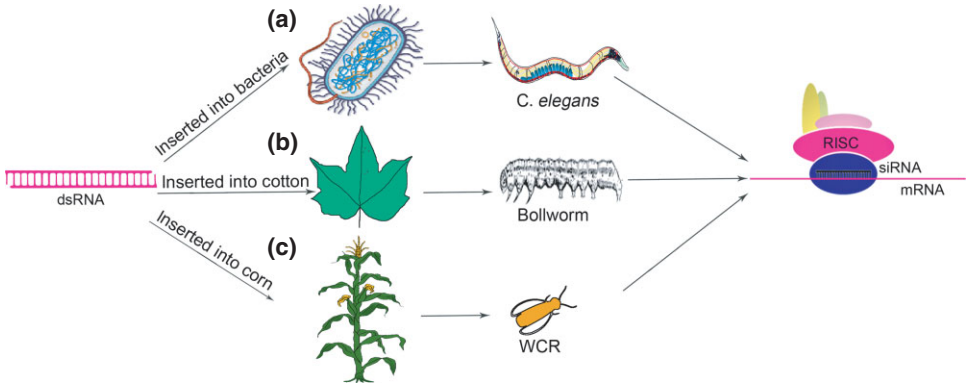

FIGURE 3 | Regulation of insect gene expression by bacteria or plant-mediated RNA interference (RNAi). (a) *E. coli* bacteria expressing double-stranded RNA (dsRNAs) can confer specific interference effects on the nematode larvae that feed on them. (b) The growth of cotton bollworm larvae is retarded when larvae were fed plant material expressing dsRNA specific to CYP6AE14. (c) Transgenic corn engineered to express siRNAs against the V-ATPase of the western corn rootworm can protect itself from the damage caused by rootworm feeding.

armigera that permitted this herbivore to tolerate what would otherwise be inhibitory concentrations of the cotton metabolite gossypol. They showed that when larvae were fed plant material expressing dsRNA specific to CYP6AE14, levels of this protein decreased in the midgut, and larval growth was retarded (Figure 3(b)).[51] These results demonstrate that siRNA can be transmitted from plants to insects, which suggests that plant material expressing siRNA may be used to protect crop plants against insect damage. Baum et al.[52] also showed that transgenic corn plants engineered to express siRNAs against the V-ATPase of the western corn rootworm *Diabrotica virgifera virgifera Leconte* were more resistant to damage caused by rootworm feeding (Figure 3(c)). Furthermore, the study by Ulvila et al.[53] provided insight into the mechanism of dsRNA internalization by insect cells. They identified two Drosophila scavenger receptors, SR-CI and Eater, which together accounted for more than 90% of the dsRNA uptake into S2 cells. Thus, dsRNA fragments might be internalized by receptor-mediated endocytosis. Taken together, these results suggest that it may be possible to exploit the RNAi pathway to control insect pests by expressing siRNA in transgenic plants and crops. However, optimization needs to be carried out before RNAi-mediated insect control will be efficient. Important factors that influence the success of RNAi in specific organisms and with respect to specific targets include the following: siRNA concentration, nucleotide sequences, siRNA fragment length, persistence of silencing, and the life stage of the targeted insect during feeding. The following questions will also need to be addressed: How can we take advantage of the specificity of RNAi to develop crops that are resistant to pest insects but that are not harmful to humans? What can be done to slow or eliminate the development of resistance by insects to these methods? Clearly, there remain many challenges to overcome in this new exciting field. In summary, although expression of siRNAs against insect genes in transgenic plants is an indirect evidence of trans-kingdom communication (plant siRNA is artificial), it does indicate that organisms from different kingdoms of nature can communicate with each other through exogenous small RNAs.

REGULATION OF MAMMALIAN GENE EXPRESSION BY PLANT miRNAs

Recently, we provide direct evidence that food-derived exogenous plant miRNAs can pass through the mouse gastrointestinal track and enter into the circulation and various organs especially the liver where it cross-kingdomly regulates target gene expression and physiological condition (Figure 4). By using deep sequencing and quantitative RT-PCR assay, we first detected putative plant miRNAs in the sera and tissues of humans and herbivorous animals at concentrations similar to some endogenous mammalian miRNAs.[54] Then, we utilized oxidized deep sequencing to assess whether the miRNAs identified are 2′-O-methyl modified on their terminal nucleotide. In oxidized deep sequencing, animal miRNAs with free 2′ and 3′ hydroxyls will be oxidized on their terminal nucleotide, preventing them from being ligated to the adapter and sequenced; in contrast, plant miRNAs bearing 2′-O-methylated 3′ ends are resistant to oxidizing agent sodium periodate. We showed that the putative plant miRNAs were successfully sequenced after oxidation,[54] suggesting that they bear 2′-O-methylated 3′ ends and are genuine plant miRNAs.

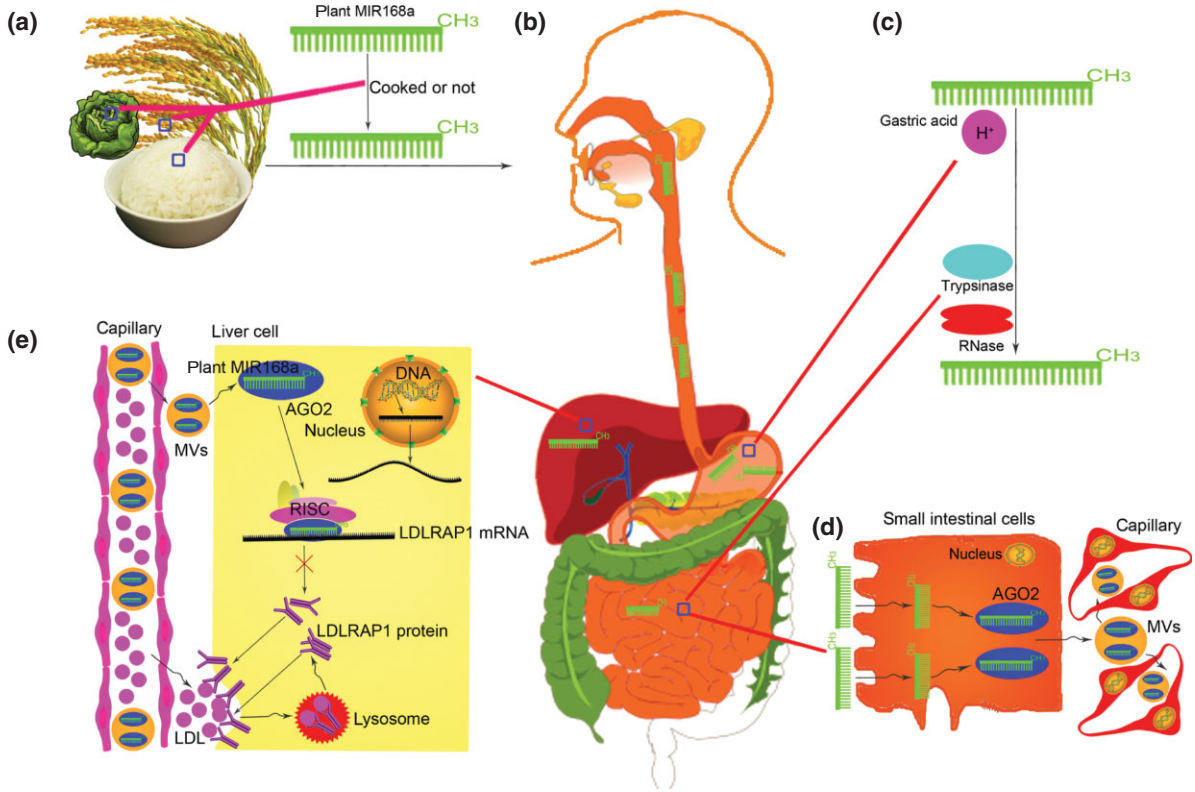

FIGURE 4 | Regulation of mammalian gene expression by plant miRNAs in food. (a) Plant MIR168a is enriched in various plants such as rice (*Oryza sativa*) and crucifers (*Brassicaceae*), and it can survive the cooking process. (b) Plant MIR168a can enter into human body through food intake. (c) Plant MIR168a can survive digestion by the strong acid and RNase in the stomach and gut. (d) Plant MIR168a can be absorbed by intestinal epithelial cells via a currently unknown mechanism. Intestinal epithelial cells package MIR168a and Argonaute2 (AGO2) into microvesicles (MVs), and these MVs deliver MIR168a to various organs through the circulatory system. (e) In a target organ such as the liver, for example, MIR168a binds to its target, the LDLRAP1 mRNA, to block production of the LDLRAP1 protein, which in turn influences the uptake of LDL from the blood.

Furthermore, we demonstrated in a murine model that plant miRNAs accumulated in the serum and tissues as a result of food intake and that exogenous mature plant miRNAs in food could pass through mouse gastrointestinal tract.[54] Moreover, we identified that, in mammals, the low-density lipoprotein receptor adapter protein 1 (LDLRAP1) is a target of MIR168a, which is one of the plant miRNAs that we found to be present at a relatively high levels in human serum and we also showed that MIR168a could directly bind to the coding sequence of LDLRAP1 in human liver cells and influence the uptake of LDL from the blood.[54] We believe this finding is very interesting for a number of reasons: First, it indicates that plant miRNAs may be a previously unknown but essential bioactive compound in food. Whether plant miRNA is equally important as the six types of nutrients in food (H_2O, protein, free fatty acid, carbohydrate, vitamins, and mineral elements) requires further studies. Second, it sheds new light on our understanding of cross-kingdom (e.g., plant–animal) interactions and coevolution. Third, this finding expands the known types of miRNA functions, giving insight into functional miRNA-transmission between higher multicellular eukaryotes. Fourth, the ability of plant miRNAs to affect human cellular behavior might be a way to partially explain the effectiveness of Chinese herbal medicines. Beyond the components traditionally thought to be active in herbs, might it be possible that miRNAs in the herbs comes preprogrammed to tell certain cells how to initiate healing. Further studies are required to test whether some herbs have a unique miRNA signature that gives health promoting properties.

However, mechanism underlying the absorption and processing of exogenous plant miRNAs are insufficiently understood. There exist many challenges for plant-derived miRNAs to be taken up by the body through food sources and to eventually reach their target organs. First, once inside the mammalian gastrointestinal tract, exogenous miRNAs face a number of extreme factors, including RNase, phagocytosis, and a low-pH environment.

These unfavorable conditions require miRNAs to adopt stable structures to protect themselves from degradation prior to reaching recipient cells. It is known that plant miRNAs are methylated on the 2′-hydroxyl group of 3′-terminal nucleotide, which inhibits 3′-end uridylation and subsequent 3′–5′ exonuclease digestion.[25] Consistent with this, we have detected that mature plant miRNAs have slower degradation rates compared with synthetic unmethylated forms,[54] which suggests that methylation contributes to the stability of plant miRNAs *in vivo*. Second, the means that intestinal epithelial cells use to absorb exogenous plant miRNAs is unclear. We hypothesized that SID-1/2 may be the transporter responsible for the uptake of exogenous plant miRNAs. So far, SID-1/2 proteins and their orthologues have been shown to mediate the uptake of mant types of small RNAs, including double-stranded siRNAs, single-stranded hairpin structure pre-miRNAs and single-stranded mature miRNAs.[43,44,47–50] Whether SID-1/2 proteins are capable of transporting natural mature plant miRNAs requires further investigation. Mice with mutant SID proteins are ideal model to study the dietary delivery of the plant-based miRNAs. Third, it is unknown how plant miRNAs are shuttled from the original cells to the target organs, such as the liver in mammals. We proposed that intestinal epithelial cells could take up plant miRNAs and package them into microvesicles (MVs), which could then be released into the circulatory system and delivered to recipient organs and cells. Consistent with this hypothesis, more than half of the MIR168a found in the serum was present inside MVs.[54] Even more intriguingly, MVs may also contain components of the RNA-induced silencing complex (RISC) to ensure that the packaged miRNAs are active. Indeed, MIR168a was found to be associated with Argonaute2 (AGO2), both in the MVs from human intestinal epithelial cells and in the target liver cells. These data suggest that plant miRNAs can utilize mammalian AGO2 to execute their functions. Fourth, how plant-derived miRNAs regulate their target mRNAs in mammalian cells is an interesting question. Plant miRNAs tend to induce mRNA cleavage through perfect or near-perfect complementarity to their target sequences, which are often in mRNA coding regions.[55] On the other hand, mammalian miRNAs generally cause translational repression through partial complementarity to their target sequences, which are often in mRNA 3′-untranslated regions (3′-UTRs).[4,5] The fact that MIR168a decreased LDLRAP1 protein levels in liver cells but did not affect mRNA levels indicates that it behaves, at least in some ways, similar to a mammalian miRNA. However, MIR168a shows near-perfect complementarity to target sequence in the coding region of LDLRAP1, suggesting that, in some respects, it behaves similar to a plant miRNA. It would therefore be interesting to analyze the regulatory mechanisms of other plant miRNAs in mammalian cells. Finally, the following questions should also be addressed: to learn the behavior of plant miRNA absorption, can we track the movement of fluorescently tagged plant miRNAs in the animals eating them? Do carnivores have little or no such *trans*-kingdom signaling or do they get the plant miRNAs from the tissues of the animals they eat? If eating rice inhibits the removal of LDL, do populations whose diets include large amounts of rice suffer from high levels of LDL?

CONCLUDING REMARKS

Once thought to be unstable molecules, miRNAs have recently been demonstrated to transfer horizontally across species and kingdoms, suggesting a novel role for these molecules in interspecies/inter-kingdom communication and coevolution. Utilizing a currently unclear trafficking system, exogenous miRNAs (especially those from plants) can be delivered into recipient mammalian cells, where they can function similarly to endogenous miRNAs, simultaneously regulating multiple target genes and biological processes. These findings indicate that miRNAs endogenous to one species may often exert an influence on the biology of other distantly related species. While the study of interspecies/inter-kingdom interactions is a new and exciting field, much remains to be learned in respect to mechanisms and roles of endogenous miRNAs in both normal physiological and pathological contexts before we can fully understand exogenous miRNA-mediated gene regulation.

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REFERENCES

1. Hughes DT, Sperandio V. Inter-kingdom signalling: communication between bacteria and their hosts. *Nat Rev Microbiol* 2008, 6:111–120.
2. Sperandio V. Striking a balance: inter-kingdom cell-to-cell signaling, friendship or war? *Trends Immunol* 2004, 25:505–507.
3. Pacheco AR, Sperandio V. Inter-kingdom signaling: chemical language between bacteria and host. *Curr Opin Microbiol* 2009, 12:192–198.
4. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004, 116:281–297.
5. Ambros V. The functions of animal microRNAs. *Nature* 2004, 431:350–355.
6. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993, 75:843–854.
7. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009, 136:215–233.
8. Bushati N, Cohen SM. microRNA functions. *Annu Rev Cell Dev Biol* 2007, 23:175–205.
9. Lee Y, Kim M, Han JJ, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004, 23:4051–4060.
10. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 2004, 432:235–240.
11. Lee Y, Ahn C, Han JJ, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003, 425:415–419.
12. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science* 2004, 303:95–98.
13. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001, 293:834–838.
14. Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 2005, 436:740–744.
15. Lee Y, Hur I, Park SY, Kim YK, Suh MR, Kim VN. The role of PACT in the RNA silencing pathway. *EMBO J* 2006, 25:522–532.
16. Schwarz DS, Hutvagner G, Du T, Xu ZS, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. *Cell* 2003, 115:199–208.
17. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell* 2003, 115:505–505.
18. Liu JD, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ. Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 2004, 305:1437–1441.
19. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005, 120:15–20.
20. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene Lin-14 by Lin-4 mediates temporal pattern-formation in C-elegans. *Cell* 1993, 75:855–862.
21. Yekta S, Shih IH, Bartel DP. MicroRNA-directed cleavage of HOXB8 mRNA. *Science* 2004, 304:594–596.
22. Chen X, Liang HW, Zhang JF, Zen K, Zhang CY. Secreted microRNAs: a new form of intercellular communication. *Trends Cell Biol* 2012, 22:125–132.
23. Tang GL, Reinhart BJ, Bartel DP, Zamore PD. A biochemical framework for RNA silencing in plants. *Gene Dev* 2003, 17:49–63.
24. Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS. Nuclear processing and export of microRNAs in Arabidopsis. *Proc Natl Acad Sci USA* 2005, 102:3691–3696.
25. Yu B, Yang ZY, Li JJ, Minakhina S, Yang MC, Padgett RW, Steward R, Chen XM. Methylation as a crucial step in plant microRNA biogenesis. *Science* 2005, 307:932–935.
26. Llave C, Xie ZX, Kasschau KD, Carrington JC. Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science* 2002, 297:2053–2056.
27. Pfeffer S, Zavolan M, Grasser FA, Chien MC, Russo JJ, Ju JY, John B, Enright AJ, Marks D, Sander C, et al. Identification of virus-encoded microRNAs. *Science* 2004, 304:734–736.
28. Kincaid RP, Burke JM, Sullivan CS. RNA virus microRNA that mimics a B-cell oncomiR. *Proc Natl Acad Sci USA* 2012, 109:3077–3082.
29. Lizasa H, Wulff BE, Alla NR, Maragkakis M, Megraw M, Hatzigeorgiou A, Iwakiri D, Takada K, Wiedmer A, Showe L, et al. Editing of epstein-barr virus-encoded BART6 microRNAs controls their dicer targeting and consequently affects viral latency. *J Biol Chem* 2010, 285:33358–33370.
30. Lu CC, Li ZH, Chu CY, Feng JY, Feng J, Sun R, Rana TM. MicroRNAs encoded by Kaposi's sarcoma-associated herpesvirus regulate viral life cycle. *EMBO Rep* 2010, 11:784–790.
31. Lei X, Bai Z, Ye F, Xie J, Kim CG, Huang Y, Gao SJ. Regulation of NF-κB inhibitor IκBα and viral replication by a KSHV microRNA. *Nat Cell Biol* 2010, 12:193–199.
32. Grey F, Tirabassi R, Meyers H, Wu GM, McWeeney S, Hook L, Nelson JA. A viral microRNA down-regulates

multiple cell cycle genes through mRNA 5' UTRs. *Plos Pathog* 2010, 6.

33. Gottwein E, Cullen BR. A human herpesvirus microRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest. *J Virol* 2010, 84:5229–5237.
34. Choy EYW, Siu KL, Kok KH, Lung RWM, Tsang CM, To KF, Kwong DLW, Tsao SW, Jin DY. An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. *J Exp Med* 2008, 205:2551–2560.
35. Klase Z, Winograd R, Davis J, Carpio L, Hildreth R, Heydarian M, Fu S, McCaffrey T, Meiri E, Ayash-Rashkovsky M, et al. HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. *Retrovirology* 2009, 6.
36. Abend JR, Uldrick T, Ziegelbauer JM. Regulation of tumor necrosis factor-like weak inducer of apoptosis receptor protein (TWEAKR) expression by Kaposi's sarcoma-associated herpesvirus microRNA prevents TWEAK-induced apoptosis and inflammatory cytokine expression. *J Virol* 2010, 84:12139–12151.
37. Stern-Ginossar N, Elefant N, Zimmermann A, Wolf DG, Saleh N, Biton M, Horwitz E, Prokocimer Z, Prichard M, Hahn G, et al. Host immune system gene targeting by a viral miRNA. *Science* 2007, 317:376–381.
38. Bauman Y, Mandelboim O. MicroRNA based immunoevasion mechanism of human polyomaviruses. *RNA Biol* 2011, 8:591–594.
39. Cazalla D, Yario T, Steitz JA. Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. *Science* 2010, 328:1563–1566.
40. Lakatos L, Szittya G, Silhavy D, Burgyan J. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. *EMBO J* 2004, 23:876–884.
41. Riley KJ, Rabinowitz GS, Yario TA, Luna JM, Darnell RB, Steitz JA. EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. *EMBO J* 2012, 31:2207–2221.
42. Cohen HC, Xiong MP. Non-cell-autonomous RNA interference in mammalian cells: Implications for in vivo cell-based RNAi delivery. *J RNAi Gene Silencing* 2011, 7:456–463.
43. Feinberg EH, Hunter CP. Transport of dsRNA into cells by the transmembrane protein SID-1. *Science* 2003, 301:1545–1547.
44. Winston WM, Sutherlin M, Wright AJ, Feinberg EH, Hunter CP. *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. *Proc Natl Acad Sci USA* 2007, 104:10565–10570.
45. Timmons L, Fire A. Specific interference by ingested dsRNA. *Nature* 1998, 395:854–854.
46. Storz G, Vogel J, Wassarman KM. Regulation by small RNAs in bacteria: expanding frontiers. *Mol Cell* 2011, 43:880–891.
47. Duxbury MS, Ashley SW, Whang EE. RNA interference: a mammalian SID-1 homologue enhances siRNA uptake and gene silencing efficacy in human cells. *Biochem Biophys Res Commun* 2005, 331:459–463.
48. Wolfrum C, Shi S, Jayaprakash KN, Jayaraman M, Wang G, Pandey RK, Rajeev KG, Nakayama T, Charrise K, Ndungo EM, et al. Mechanisms and optimization of in vivo delivery of lipophilic siRNAs. *Nat Biotechnol* 2007, 25:1149–1157.
49. Tsang SY, Moore JC, Huizen RV, Chan CW, Li RA. Ectopic expression of systemic RNA interference defective protein in embryonic stem cells. *Biochem Biophys Res Commun* 2007, 357:480–486.
50. Elhassan MO, Christie J, Duxbury MS. Homo sapiens systemic RNA interference-defective-1 transmembrane family member 1 (SIDT1) protein mediates contact-dependent small RNA transfer and microRNA-21-driven chemoresistance. *J Biol Chem* 2012, 287:5267–5277.
51. Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen XY. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nat Biotechnol* 2007, 25:1307–1313.
52. Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, et al. Control of coleopteran insect pests through RNA interference. *Nat Biotechnol* 2007, 25:1322–1326.
53. Ulvila J, Parikka M, Kleino A, Sormunen R, Ezekowitz RA, Kocks C, Ramet M. Double-stranded RNA is internalized by scavenger receptor-mediated endocytosis in *Drosophila* S2 cells. *J Biol Chem* 2006, 281:14370–14375.
54. Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res* 2012, 22:107–126.
55. Voinnet O. Origin, biogenesis, and activity of plant microRNAs. *Cell* 2009, 136:669–687.

