Beyond nutrients: Food-derived microRNAs provide cross-kingdom regulation

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Food turns out to be not only the nutrient supplier for our body but also a carrier of regulatory information. Interestingly, a recent study made the discovery that some plant/food-derived microRNAs (miRNAs) accumulate in the serum of humans or plant-feeding animals, and regulate mammalian gene expression in a sequence-specific manner. The authors provided striking evidence that miRNAs could function as active signaling molecules to transport information across distinct species or even kingdoms. Although the mechanism of how miRNAs are shuttled between different organisms is still not well characterized, initial results point to the involvement of microvesicles and specific RNA-transporter-like proteins. These findings raise both speculation about the potential impact that plants may have on animal physiology at the molecular level, and an appealing possibility that food-derived miRNAs may offer us another means to deliver necessary nutrients or therapeutics to our bodies.

Keywords:
- cross-kingdom regulation; microRNA; microvesicle; RNA transporter

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Abbreviations:
Ago2, argonaute-2; **GI,** gastrointestinal; **LDL,** low density lipoprotein; **LDLRAP1,** low-density lipoprotein receptor adapter protein 1; **MV,** microvesicle; **RISC,** RNA-induced silencing complex.

Introduction

The ecosystem in which we are living is inter-connected: individual species are not isolated, but are associated with each other. There is constant communication between all living organisms, as well as between the organisms and their habitat. Cells communicate with each other using common signaling molecules such as hormones, cytokines, growth factors, and second messengers. Recently, secreted microRNAs (miRNAs) have emerged as novel signaling molecules to mediate intercellular communication [1–3]. miRNAs are a class of 19–24-base-long nucleotides derived from the primary miRNA hairpin portion of transcripts. They were first characterized as non-coding RNAs from the nematode *Caenorhabditis elegans* in 1993 [4, 5]. They are known to function in a sequence-specific manner to silence specific protein-coding genes at the post-transcriptional level by targeting the 3′ untranslated region (3′ UTR) of mRNA [6]. Where there is extensive complementary base pairing, miRNAs can direct mRNA cleavage; often, where there is only partial homology, miRNAs can lead to translational repression and mRNA destabilization [6]. Using the recently developed deep-sequencing technology, miRNAs have been found in a wide spectrum of eukaryotes from plants to mammals, including humans, algae, and viruses [7]. So far, over 15,000 miRNA gene loci distributed over 140 species have been annotated in the miRBase database [8]. With a rapid increase in knowledge accumulated over the last decade, miRNAs are now recognized as a crucial regulatory element that can manipulate various biological processes including cell growth and differentiation, development, apoptosis, and metabolism [9].

In mammals, miRNAs have recently been found in serum, plasma, urine, saliva, and other body fluids [10–14]. Surprisingly, contrary to traditional beliefs on extracellular RNA stability, biochemical analyses indicate that, in mammals, these circulating miRNAs are highly stable and resistant to RNase activity, as well as extreme pH and temperature [15, 16]. More importantly, it has been reported that the profiles of circulating miRNAs, particularly serum miRNAs, are tightly correlated with a variety of diseases, including cancer and

diabetes, and with tissue injury. This suggests that miRNAs could be used as biomarkers to diagnose and monitor human diseases [15–23]. In this review, we focus on the findings of Zhang et al. [24] that secreted miRNAs can function as signaling molecules in a cross-kingdom manner.

miRNAs are selectively packaged into microvesicles to transport information from cell to cell

Despite numerous reports of the detection of secreted miRNAs, the exact mechanisms of how these miRNAs are transported and act as signaling molecules are not clear. They have been implicated in stem cell function, hematopoiesis, and immune regulation [3, 25–28]. Recently, several lines of evidence have suggested that miRNAs are selectively packaged into microvesicle (MV) compartments to function efficiently in mammalian cells. MVs are membrane-covered vesicles and can be released by various kinds of cells. Based on the mechanism of formation and the intracellular origin, MVs may be divided into shedding vesicles (SVs), which directly bud from the cell surface, exosomes, which are derived from endosomal membranes [29, 30], and apoptotic bodies in response to apoptotic stimuli [31]. It is believed that being packed in MVs can help miRNAs escape RNase digestion since treatment with detergents, which destroy the lipid structure of vesicles, can lead to the miRNA degradation [3]. This indicates that the vesicle structure is critical for miRNA stability. Some miRNAs are present at a relatively higher level in MVs than in the parental cells [28, 32]. Furthermore, MVs have been shown to possess specific types and levels of miRNAs compared to those found overall in cells under various conditions, supporting the hypothesis that the miRNA packaging process is a selective rather than a random event. Recently, it was demonstrated that miRNAs contained in exosomes are released through a ceramide-dependent secretory machinery, and that these secreted miRNAs can function in recipient cells. For example, a tumor-suppressive miRNA can be transported between cells via this secretory pathway and can produce cell growth inhibition in recipient cells [1].

In addition to MVs, argonaute-2 (Ago2), a protein involved in the RNA-induced silencing complex (RISC) [33], and high-density lipoprotein (HDL) [34] have recently been proposed to act as carriers of miRNAs. These proteins may provide alternative, non-vesicular means of transporting miRNAs between cells.

miRNA/double-stranded RNA regulates gene expression in a cross-kingdom manner

Cross-kingdom regulation through miRNA/double-stranded RNA (dsRNA) has been observed in many organisms and engineered systems. The uptake of exogenous miRNA/dsRNA often leads to an alteration of gene expression in the recipient cells. For example, feeding nematode larvae with *Escherichia coli* expressing target gene dsRNA can effectively initiate RNA interference (RNAi) on the target mRNA, indicating that bacteria-derived dsRNA can regulate gene expression in a higher organism [35]. In addition to *C. elegans*, many other species, such as planaria or certain parasitic nematodes, have the ability to take up environmental dsRNA into their cells [36, 37]. Similarly, feeding insects plants that express dsRNA may also trigger RNAi [38]. For example, when cotton bollworm larvae are fed on plant material expressing dsRNA targeting *CYP6AE14*, whose gene product helps the insect to counteract the deleterious cotton metabolite gossypol, the transcript level of this gene is decreased and causes larval growth retardation [39]. In mammals, miRNAs have been identified in both cow milk and human breast milk [13, 14], suggesting a potential route for inter-individual communication via miRNAs. Another interesting example of miRNA cross-kingdom regulation comes from the discovery that engineered *E. coli* expressing short hairpin RNA (shRNA) against a mammalian gene can induce cross-kingdom RNAi both in vivo and in vitro [40]. These findings may provide the groundwork for future design of RNAi-based therapies.

Cross-kingdom regulation via miRNA/dsRNA also comes from the virus-host interplay. In plants and insects, host cells can process viral dsRNA into small interfering RNAs (siRNAs), which can be maintained and amplified later to target parasitic RNA [41]. Certain animal cells can encode their own miRNAs as a defense mechanism to fight off foreign viral genomes [42]. On the other hand, many viruses are known to encode suppressor proteins that can counter this defense with RNA silencing [41]. For example, Lecellier et al. showed that a cellular miRNA, miR-32, is able to effectively inhibit the accumulation of retrovirus primate foamy virus type 1 (PFV-1) in human cells. Tas, a protein encoded by PFV-1, however, can suppress miR-32-mediated translation inhibition [43].

Plant-derived miRNA may regulate mammalian gene expression

In a recent publication by Zhang et al. [24], the authors explored the function of plant-derived miRNAs as active signaling molecules to mediate intercellular communication. They demonstrated that mature single-stranded plant miRNAs are present in both serum and tissues of mammals that use plants such as rice as their food sources. Using state-of-the-art Solexa sequencing and traditional quantitative RT-PCR, with or without oxidation of small RNAs with periodate, they were able to verify that the miRNAs detected are bona fide plant miRNAs [24]. In addition, in a murine model, the authors tested the hypothesis that the plant miRNAs accumulate in the sera and tissue as a result of food intake. They showed that the exogenous mature plant miRNAs in food can pass through mouse gastrointestinal (GI) tract to reach the sera and organs. Moreover, the authors identified the low-density lipoprotein receptor adapter protein 1 (LDLRAP1) as a target for MIR168a, a plant miRNA that was present at a relatively high level in human sera. The presence of exogenous pre-MIR168a or mature MIR168a miRNA can significantly reduce LDLRAP1 protein level in culture. Furthermore, feeding mice with rice

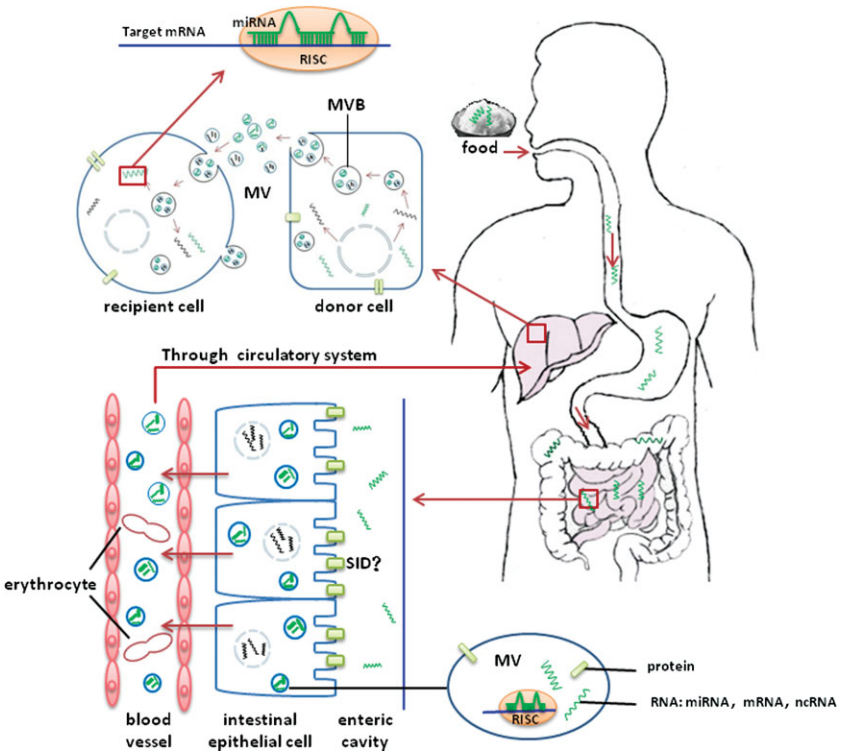

Figure 1. A schematic view for the uptake of food-derived miRNAs into human cells. Food enters the gastrointestinal (GI) tract, where it is digested, releasing small pieces of debris and molecules that human cells can absorb, including amino acids, fatty acids, and miRNAs. Exogenous miRNAs may be taken up into the epithelial cells lining intestine by SID-like transporters and selectively packaged into MVs. miRNAs are secreted into the circulatory system enclosed in MVs, together with components of the RISC. On reaching the final recipient cells in organs such as liver, miRNAs are released and regulate the target gene in a sequence-specific manner. MV, microvesicle; MVB, multivesicular body; SID, systemic RNAi deficient; ncRNA, non-coding RNA; RISC, RNA-induced silencing complex.

that produces MIR168a reduced the amount of LDLRAP1 protein in liver, which in turn resulted in an elevation of the LDL level in mouse plasma. Both the decrease of LDLRAP1 and the increase of LDL in plasma, however, could be blocked by the addition of an anti-MIR168a antisense oligonucleotide [24]. These elegantly executed experiments not only confirm the role of circulating miRNAs in intercellular communication, but also suggest that miRNAs can transport and function in a cross-kingdom manner. A schematic summary of how food-derived miRNAs can regulate mammalian gene expression is shown in Fig. 1.

For the plant-derived miRNAs to be taken up by the animal body from food sources and eventually reach the end organs, they must overcome numerous challenges. First, once in the mammalian GI tract, the exogenous miRNAs face many extreme conditions such as various enzymes, phagocytosis, and a low pH environment. These unfavorable surroundings require miRNAs to have stable structures to protect themselves from degradation prior to being taken up into the recipient cells. Plant miRNAs are methylated at the 2′-hydroxyl group of the 3′-terminal nucleotide, which inhibits 3′-end uridylation and subsequent 3′-to-5′ exonuclease digestion [44]. Consistent with this, plant miRNAs have a slower degradation rate compared to their synthetic forms [24], suggesting that methylation indeed may contribute to the stability of plant miRNAs in vivo.

Second, how does the plant miRNA pass through the GI tract? One possible mechanism may be that the intestinal epithelial cells can somehow take up plant miRNAs from food, albeit the exact trafficking pathway of the plants miRNA from the gut to these cells is not known. In *C. elegans*, a multispan transmembrane protein, SID-1 (systemic RNAi deficient-1), is believed to form a channel that could allow passive diffusion of dsRNA [45]. The mammalian SID-1 homolog is able to enhance siRNA uptake [45, 46]. SID-2 is another recently identified transmembrane protein in *C. elegans* that is important for environmental RNAi [47]. In contrast to the ubiquitous expression of SID-1, SID-2 is expressed in the intestine and is localized to the luminal membrane; it is thought to mediate the endocytosis of dsRNA from the lumen [47]. The presence of an RNA transporter protein on the cell surface may also provide a means for the plant miRNAs to be transported across the intestinal lumen in mammals. However, there are several gaps that need to be filled when trying to identify the potential transporter proteins in mammalian cells for plant miRNAs. Although SID-1/SID-2 proteins are capable of transporting pre-miRNAs and single-strand RNA with hairpin structures [47, 48], whether SID-like proteins are capable of delivering natural plant pre-miRNAs or mature miRNAs still needs to be evaluated. It is worth noting that there is no direct evidence that mammalian Dicer and Ago2, the two key enzymes for mature miRNA biogenesis, can recognize natural plant pre-miRNAs and liberate single-stranded mature miRNAs. However, at least some of the plant miRNAs are known to be taken up by mammalian cells in their mature single-stranded form [24], raising the possibility that a receptor or transporter gene specific for single-stranded RNA is present on mammalian cell surface.

The final challenge is how the plant miRNAs get shuttled from the initial cells that take up these molecules to the end organs such as liver in mammals. Using fluorescently labeled MVs and miRNAs, it was demonstrated that miRNAs packaged

in MVs can be delivered to downstream recipient cells and regulate target genes in these cells [3, 24]. Zhang et al. [24] also hypothesized that plant miRNAs in food could be transported through a similar mechanism: intestinal epithelial cells take up miRNAs, which are then packaged into MVs, to be released into the circulatory system (Fig. 1). Consistent with this, more than half of the MIR168a in the serum was in a MV-packaged form [24].

On reaching the mammalian target cells, the plant miRNAs also develop several strategies to guarantee their functions within the cells. It turns out that MVs package not only miRNAs, but possibly also protein components of the RISC to ensure that packaged miRNAs are active [3, 24]. MIR168a was found to be associated with Ago2 in the MVs from human intestinal epithelial cells [24]. In addition, after being delivered to the target liver cells, MIR168a is still found to be associated with Ago2 along with its target LDLRAP1 mRNA [24]. These data also suggest that plant miRNAs are able to utilize mammalian Ago2 to execute their function.

How do plant-derived miRNAs regulate their target mRNAs in mammalian cells? Whereas plant miRNAs tend to be perfectly or nearly perfectly complementary to their target sequence and induce mRNA cleavage, mammalian miRNAs only possess partial homology to targets and often cause translational repression [49]. The fact that MIR168a decreases the LDLRAP1 protein level, but does not affect the mRNA level, indicates that it behaves like a mammalian miRNA in liver cells. It would be interesting to analyze other plant miRNA regulation patterns in mammalian cells.

Speculations raised by miRNA-mediated cross-kingdom regulation

With the discovery that the exogenous miRNAs or shRNAs derived from plants or bacteria can regulate gene expression of mammalian cells [24, 40], many issues (some related to our daily lives) are raised. First, what is the scope and degree of food-derived miRNAs on regulation of mammalian genes? Solexa sequencing has revealed that there are approximately 30 plant miRNAs in sera from Chinese individuals [24]. What are the mammalian targets (other than LDLRAP1) of these miRNAs? Also, what are the potential effects of these food-derived miRNAs on our metabolism and health? Initially, due to the complexity of the physiology involved, a panel of organs and potential targets need to be further examined in animal models. It would also be interesting to extract the contribution of individual miRNAs in regulating mammalian genes, for example, using engineered bacteria expressing individual plant miRNAs.

Second, this study implies that while we are consuming food, we not only absorb its nutrients, but also inherit signaling and regulatory information from these food sources. This may have an impact on the future design of genetically engineered plants or identification of active ingredients in certain plants that have therapeutic effects. In fact, the success of engineered bacteria expressing an shRNA specifically silencing a mammalian target gene in mice confirms the potential of RNAi-based gene therapy [40]. In China, traditional herbs have been used to cure various diseases for thousands of years. Some researchers are dedicated to isolating the active/functional components from herbs; however, these components continue to mystify and elude us. The miRNAs that are now being discovered may provide additional clues for evaluating the active therapeutic components of herbs.

The fact that oral ingestion of plant-derived miRNA/sRNA can regulate human metabolism and gene expression also raises concerns on transgenic crops. For decades there have been debates on the safety of transgenic food with regards to human health and the environment. This profound discovery by Zhang et al. [24] should make decision takers more cautious when considering the issues that may arise from the consumption of transgenic crops.

Lastly, if plant miRNAs could act in animal cells, is the reverse scenario possible? That is, could mammalian miRNA regulate gene expression in a plant cell? Preliminary studies have shown animal-specific miRNAs can be detected in plant cells cultured on the medium supplemented with dairy milk (Prof. Jianqun Chen, personal communication). These pioneering studies may initiate a new level of understanding of the co-evolutionary processes existing between the kingdoms of life.

Conclusions

By studying circulating miRNAs we are realising that these molecules represent a novel class of signaling molecules for intercellular communication. The discovery that plant-specific miRNAs accumulate in the sera and regulate the gene expression of human or plant-feeding animals extends the function of miRNAs. These could represent a cross-kingdom regulatory signal. Food intake, possibly via the intestinal epithelia of the GI tract, may represent a general pathway for ingestion of food-derived or food-associated miRNAs. Food-derived exogenous miRNAs may also be qualified as a novel nutrient component, like vitamins and minerals, and may provide us with a novel system to deliver therapeutic small RNAs into animals. However, the exact processes of miRNA transport through organisms and the mechanism of cross-kingdom gene regulation are still unclear. Future studies examining the trafficking pathway, the miRNA cross-kingdom recognition and regulatory mechanism, and the extent of cross-kingdom regulation are warranted.

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References

1. **Kosaka N**, **Iguchi H**, **Yoshioka Y**, **Takeshita F**, et al. 2010. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem* **285**: 17442–52.

Recently in press

2. **Pegtel DM**, **Cosmopoulos K**, **Thorley-Lawson DA**, **van Eijndhoven MA**, et al. 2010. Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci USA* **107**: 6328–33.
3. **Zhang Y**, **Liu D**, **Chen X**, **Li J**, et al. 2010. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell* **39**: 133–44.
4. **Lee RC**, **Feinbaum RL**, **Ambros V.** 1993. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**: 843–54.
5. **Wightman B**, **Ha I**, **Ruvkun G.** 1993. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* **75**: 855–62.
6. **Bartel DP.** 2009. MicroRNAs: target recognition and regulatory functions. *Cell* **136**: 215–33.
7. **Janga SC**, **Vallabhaneni S.** 2011. MicroRNAs as post-transcriptional machines and their interplay with cellular networks. *Adv Exp Med Biol* **722**: 59–74.
8. **Kozomara A**, **Griffiths-Jones S.** 2011. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res* **39**: D152–7.
9. **Bartel DP.** 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**: 281–97.
10. **Lawrie CH**, **Gal S**, **Dunlop HM**, **Pushkaran B**, et al. 2008. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol* **141**: 672–5.
11. **Park NJ**, **Zhou H**, **Elashoff D**, **Henson BS**, et al. 2009. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. *Clin Cancer Res* **15**: 5473–7.
12. **Hanke M**, **Hoefig K**, **Merz H**, **Feller AC**, et al. 2010. A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. *Urol Oncol* **28**: 655–61.
13. **Kosaka N**, **Izumi H**, **Sekine K**, **Ochiya T.** 2010. microRNA as a new immune-regulatory agent in breast milk. *Silence* **1**: 7.
14. **Chen X**, **Gao C**, **Li H**, **Huang L**, et al. 2010. Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products. *Cell Res* **20**: 1128–37.
15. **Mitchell PS**, **Parkin RK**, **Kroh EM**, **Fritz BR**, et al. 2008. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* **105**: 10513–8.
16. **Chen X**, **Ba Y**, **Ma L**, **Cai X**, et al. 2008. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* **18**: 997–1006.
17. **Zampetaki A**, **Kiechl S**, **Drozdov I**, **Willeit P**, et al. 2010. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* **107**: 810–7.
18. **Wang LG**, **Gu J.** 2012. Serum microRNA-29a is a promising novel marker for early detection of colorectal liver metastasis. *Cancer Epidemiol* **36**: e61-7.
19. **Zheng D**, **Haddadin S**, **Wang Y**, **Gu LQ**, et al. 2011. Plasma microRNAs as novel biomarkers for early detection of lung cancer. *Int J Clin Exp Pathol* **4**: 575–86.
20. **Wang R**, **Li N**, **Zhang Y**, **Ran Y**, et al. 2011. Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. *Intern Med* **50**: 1789–95.
21. **Moussay E**, **Wang K**, **Cho JH**, **van Moer K**, et al. 2011. MicroRNA as biomarkers and regulators in B-cell chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* **108**: 6573–8.
22. **Etheridge A**, **Lee I**, **Hood L**, **Galas D**, et al. 2011. Extracellular microRNA: a new source of biomarkers. *Mutat Res* **717**: 85–90.
23. **Gilad S**, **Meiri E**, **Yogev Y**, **Benjamin S**, et al. 2008. Serum microRNAs are promising novel biomarkers. *PLoS One* **3**: e3148.
24. **Zhang L**, **Hou D**, **Chen X**, **Li D**, et al. 2012. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res* **22**: 107–26.
25. **Collino F**, **Deregibus MC**, **Bruno S**, **Sterpone L**, et al. 2010. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. *PLoS One* **5**: e11803.
26. **Yuan A**, **Farber EL**, **Rapoport AL**, **Tejada D**, et al. 2009. Transfer of microRNAs by embryonic stem cell microvesicles. *PLoS One* **4**: e4722.
27. **Hunter MP**, **Ismail N**, **Zhang X**, **Aguda BD**, et al. 2008. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* **3**: e3694.
28. **Mittelbrunn M**, **Gutierrez-Vazquez C**, **Villarroya-Beltri C**, **Gonzalez S**, et al. 2011. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat Commun* **2**: 282.
29. **Heijnen HF**, **Schiel AE**, **Fijnheer R**, **Geuze HJ**, et al. 1999. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood* **94**: 3791–9.
30. **Camussi G**, **Deregibus MC**, **Bruno S**, **Grange C**, et al. 2011. Exosome/microvesicle-mediated epigenetic reprogramming of cells. *Am J Cancer Res* **1**: 98–110.
31. **Zampetaki A**, **Willeit P**, **Drozdov I**, **Kiechl S**, et al. 2012. Profiling of circulating microRNAs: from single biomarkers to re-wired networks. *Cardiovasc Res*, in press, DOI: 10.1093/cvr/cvr266.
32. **Valadi H**, **Ekstrom K**, **Bossios A**, **Sjostrand M**, et al. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* **9**: 654–9.
33. **Turchinovich A**, **Weiz L**, **Langheinz A**, **Burwinkel B.** 2011. Characterization of extracellular circulating microRNA. *Nucleic Acids Res* **39**: 7223–33.
34. **Vickers KC**, **Palmisano BT**, **Shoucri BM**, **Shamburek RD**, et al. 2011. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* **13**: 423–33.
35. **Timmons L**, **Fire A.** 1998. Specific interference by ingested dsRNA. *Nature* **395**: 854.
36. **Issa Z**, **Grant WN**, **Stasiuk S**, **Shoemaker CB.** 2005. Development of methods for RNA interference in the sheep gastrointestinal parasite, *Trichostrongylus colubriformis*. *Int J Parasitol* **35**: 935–40.
37. **Newmark PA**, **Reddien PW**, **Cebria F**, **Sanchez Alvarado A.** 2003. Ingestion of bacterially expressed double-stranded RNA inhibits gene expression in planarians. *Proc Natl Acad Sci USA* **100**: 11861–5.
38. **Terenius O**, **Papanicolaou A**, **Garbutt JS**, **Eleftherianos I**, et al. 2011. RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. *J Insect Physiol* **57**: 231–45.
39. **Mao YB**, **Cai WJ**, **Wang JW**, **Hong GJ**, et al. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nat Biotechnol* **25**: 1307–13.
40. **Xiang S**, **Fruehauf J**, **Li CJ.** 2006. Short hairpin RNA-expressing bacteria elicit RNA interference in mammals. *Nat Biotechnol* **24**: 697–702.
41. **Qu F**, **Morris TJ.** 2005. Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. *FEBS Lett* **579**: 5958–64.
42. **Russo A**, **Potenza N.** 2011. Antiviral effects of human microRNAs and conservation of their target sites. *FEBS Lett* **585**: 2551–5.
43. **Lecellier CH**, **Dunoyer P**, **Arar K**, **Lehmann-Che J**, et al. 2005. A cellular microRNA mediates antiviral defense in human cells. *Science* **308**: 557–60.
44. **Yu B**, **Yang Z**, **Li J**, **Minakhina S**, et al. 2005. Methylation as a crucial step in plant microRNA biogenesis. *Science* **307**: 932–5.
45. **Feinberg EH**, **Hunter CP.** 2003. Transport of dsRNA into cells by the transmembrane protein SID-1. *Science* **301**: 1545–7.
46. **Duxbury MS**, **Ashley SW**, **Whang EE.** 2005. RNA interference: a mammalian SID-1 homologue enhances siRNA uptake and gene silencing efficacy in human cells. *Biochem Biophys Res Commun* **331**: 459–63.
47. **Winston WM**, **Sutherlin M**, **Wright AJ**, **Feinberg EH**, et al. 2007. *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. *Proc Natl Acad Sci USA* **104**: 10565–70.
48. **Shih JD**, **Hunter CP.** 2011. SID-1 is a dsRNA-selective dsRNA-gated channel. *RNA* **17**: 1057–65.
49. **Saumet A**, **Lecellier CH.** 2006. Anti-viral RNA silencing: do we look like plants? *Retrovirology* **3**: 3.