Maintaining good miRNAs in the body keeps the doctor away?: Perspectives on the relationship between food-derived natural products and microRNAs in relation to exosomes/extracellular vesicles.

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**Graphical abstract - text**

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Recently, various studies have shown that some natural compounds can control tumor-suppressive and oncogenic miRNAs in a positive manner. In addition, some studies have raised the possibility that food-derived miRNA/EVs might function after oral intake. We will have discussions about their contributions to the prevention of diseases and the maintenance of good health.
**Abbreviations**: DIM, 3,3'-diindolylmethane; EGCG, epicallocatechin-3-gallate; EV, extracellular vesicle; HCV, chronic hepatitis C virus; LDL, low-density lipoprotein; LDLRAP1, low-density lipoprotein receptor adapter protein 1; LNA, locked nucleic acid; mRNA, messenger RNA; miRNA, microRNA; NS1, nonstructural protein 1; PB2, polymerase basic protein 2; siRNA, small-interfering RNA; 1,25(OH)2D, 1,25-dihydroxyvitamin D; 3'-UTR, 3'-untranslated region

**Keywords**: miRNA, natural compound, cancer, exosome, extracellular vesicle, food-derived miRNA, health benefit

**Abstract**

During the last decade, it has been uncovered that microRNAs (miRNAs), a class of small non-coding RNAs, are related to many diseases including cancers. With an increase in reports describing the dysregulation of miRNAs in various tumor types, it has become abundantly clear that miRNAs play significant roles in the formation and progression of cancers. Intriguingly, miRNAs are present in body fluids because they are packed in exosomes/extracellular vesicles and released from all types of cells. The miRNAs in the fluids are measured in a relatively simple way and the profile of miRNAs is likely to be an indicator of health condition. In recent years, various studies have demonstrated that some...
naturally occurring compounds can control tumor-suppressive and oncogenic miRNAs in a positive manner, suggesting that food-derived compounds could maintain the expression levels of miRNAs and help maintain good health. Therefore, our daily food and compounds in food are of great interest. In addition, exogenous diet-derived miRNAs have been indicated to function in the regulation of target mammalian transcripts in the body. These findings highlight the possibility of diet for good health through the regulation of miRNAs, and we also discuss the perspective of food application and health promotion.

1. Introduction

Since their discovery in the early 1990s, small non-coding RNAs, termed microRNAs (miRNAs), have been widely found in species from plants to humans, and it has been realized that miRNAs contribute to a number of biological processes such as cell differentiation and morphogenesis in every aspect [1, 2]. miRNAs are shown to bind to complementary sequences often found in the 3'-untranslated region (3'-UTR) of target messenger RNAs (mRNAs), resulting in the translational repression or destabilization of the mRNAs [3]. Most plant miRNAs bind to target transcripts with perfect or near-perfect complementarity and function like small-interfering RNAs (siRNAs) to guide target RNA cleavage, hinting that the targets of a single miRNA are totally limited [4]. Animal miRNAs usually interact with their mRNA targets with imperfect complementarity and induce their translational repression or destabilization [5, 6]. Because of this imperfect complementarity, a single miRNA has
multiple target sites and hundreds of target genes based on prediction databases, which suggests the
significance of "fine-tuning" this complex gene network far more than we thought.

Recently, with the rapid expansion in the field of miRNA research, it has been shown that
miRNAs somehow contribute to a variety of human diseases, such as lifestyle-related diseases and
cancers. Dysregulations of miRNAs were observed in patients as compared with healthy subjects, and
miRNA expression profiles could be used to classify poorly differentiated tumors [7]. In addition, many
studies have demonstrated that miRNAs are present in body fluids, such as blood, serum/plasma, saliva
and urine, and that miRNAs are contained in exosomes/extracellular vesicles (EVs) that are secreted
from all types of cells as a mediator of cell-to-cell communication [8, 9]. One of the significant
functions of EVs was represented by some studies that EVs and miRNAs in EVs are associated with
cancer progression and distant metastases [10, 11]. It has been suggested that diseases like chronic
hepatitis C virus (HCV) infection would be ameliorated by the silencing of endogenous miRNAs by
antisense inhibitors using locked nucleic acid (LNA)-based oligonucleotides. Furthermore, tumor
growth and metastasis would be inhibited by the systemic delivery of miRNAs to tumor cells in mouse
models using atelocollagen, a neutral lipid emulsion, liposome nanoparticles, or polyethyleneimine.

These studies profoundly reflect the possibilities of miRNAs in EVs and have come under the spotlight
[12-15]. Today, it is fairly easy to monitor disease-related changes in miRNA profiles in the
bloodstream at an early stage of disease [16]. Now many researchers are examining the capacity of
utilizing this miRNA expression pattern as a novel early, specific, and sensitive diagnostic marker, and
miRNA profiles in body fluids are potentially capable of acting as an indicator of health conditions [17].
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In 2016, for instance, it has been suggested that early stage breast cancer could be detected by examining the expression profiles of serum miRNAs, and 13 types of early stage cancers will be diagnosed in this way in the future [18]. In other words, if we could, to some extent, manage and maintain the expression levels and profiles of miRNAs in the body, this approach could contribute to the prevention of diseases and the maintenance of good health.

Certain lifestyles, especially unhealthy eating habits, are associated with an increase in the risk of diseases including cancer [19-22]. It is particularly intriguing how our daily food impacts on the expression of various miRNAs because most people eat three meals a day. However, the relationship between food and miRNAs and the effect on health remain to be elucidated. Studies described in this review have raised the possibility that food ingredients or food-derived natural products that are orally ingested affect miRNA expression in the body. Furthermore, there is the potential that exogenous diet-derived miRNAs would be absorbed intact after oral ingestion and regulate target gene expression in mammals in a cross-kingdom fashion and/or by horizontal transfer of genetic information. Here we focus our discussion mainly on these two topics and present an overview of the current knowledge of the relationship between food-derived natural products and miRNAs in relation to EVs.

2. Natural compounds that have an effect on miRNA expression

For a long time, it has been considered that eating healthy foods prevents and cures illness, which is sometimes referred by the sayings: "you are what you eat" or "an apple a day keeps the doctor away."

We experientially feel that this is somehow correct and that food is one of the most critical factors for
health. Diets provide macronutrients (such as fats, proteins and carbohydrates) and micronutrients (such as vitamins and minerals) that are vital for survival and are also known to contribute to our health condition at a genetic level. In recent years, there have been increasing reports that suggest that some food-derived natural products would be likely to change the expression of miRNAs in a positive way in a specific part of the body. Among these studies, many results have been reported in regard to cancer biology, and in this chapter in our review, we will summarize the natural compounds that regulate the expression of miRNAs associated with cancer research (Table 1 and Figure 1).

2.1. Curcumin (Diferuloylmethane)

Curcumin is a polyphenol derived from the rhizome of turmeric (*Curcuma longa*) that belongs to the ginger family (Zingiberaceae). Curcumin is a major component of turmeric, which is a well-known exotic spice that has been widely used as a food additive, a dietary supplement, and a traditional medicine in some Asian countries. The effects of curcumin were examined in pancreatic, breast, lung, colon, and esophageal cancers. It was suggested that curcumin inhibits cell growth by up-regulating *miR-22* in the BxPC-3 pancreatic cell line [23]. Curcumin promotes apoptosis by up-regulating *miR-15a* and *miR-16* in the MCF-7 breast cancer cell line, and by down-regulating *miR-186* in the A549 lung cancer cell line [24, 25]. Furthermore, in a chicken-embryo-metastasis assay, treatment with curcumin reduced *in vivo* metastasis of the RKO and HCT116 colon cancer cell lines and significantly inhibited pre-*miR-21* and *miR-21* expression in primary tumors from both cell lines [26].

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2.2. Genistein

Genistein is a nontoxic dietary isoflavone mainly found in legume plants including kudzu (*Pueraria lobata*), Indian bread root (*Psoralea corylifolia*), lupine (*Lupinus albus*), fava beans (*Vicia faba*) and soybeans (*Glycine max*) [28]. It was reported that genistein promotes cell-cycle arrest and apoptosis *in vitro* and *in vivo* in breast cancer, lung cancer, head and neck squamous cell carcinoma, leukemia, and prostate cancer [29-36]. In the PC3 prostate cancer cell line, it has been indicated that genistein induces the expression of *miR-34a*, *miR-574-3p* and *miR-1296*, and also reduces the expression of *miR-151*, *miR-221*, *miR-222* and *miR-1260b*, leading to the inhibition of cancer cell proliferation, invasion and metastasis of the cancer [37-42].

2.3. Epigallocatechin-3-gallate (EGCG)

EGCG is the most abundant polyphenol constituent of the dried leaves of green tea (*Camellia sinensis*), and it accounts for approximately 60–70% of the total catechins in green tea [43]. EGCG is suggested to increase apoptosis by up-regulating *miR-16* in human hepatocellular carcinoma HepG2 cells, and by regulating tumor-suppressive miRNAs (*miR-7-1*, *miR-34a*, and *miR-99a*) and oncogenic miRNAs (*miR-92, miR-93*, and *miR-106b*) in human malignant neuroblastoma SK-N-BE2 and IMR-32 cells [44, 45]. Additionally, in a mouse xenograft model of prostate cancer, a significant up-regulation of tumor-suppressive miRNA (*miR-330*) was observed in a tumor xenograft of the CWR22Rv1 cells after EGCG treatment [46].

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2.4. Resveratrol

Resveratrol is a polyphenolic phytoalexin present in grapevines, pines, peanuts, berries, and grapes [48, 49]. As red wine is made from grapes (*Vitis vinifera*) that are rich in resveratrol, resveratrol has been moved into the limelight with "the French paradox," which describes the association between the consumption of red wine and the relatively low incidence of coronary heart disease in French people despite a high dietary fat intake [50]. Along the way, many intensive studies on resveratrol have been carried out, including work on sirtuin genes and longevity, and resveratrol-regulating miRNAs have captured our interest [51-53]. In human breast cancer MCF-7 and MDA-MB-231 cells, with the treatment of resveratrol, the expression of *miR-663* and *miR-744*, or *miR-200c* and *miR-141* is induced respectively, inhibiting cell growth and invasion [54, 55]. In addition to breast cancer, it has been observed that resveratrol affects miRNA expression in prostate, pancreatic, lung, and colon cancers [56-60].

2.5. Quercetin

Quercetin is a dietary flavonoid found in many vegetables and fruits such as onions, tomatoes, apples and green tea [61, 62]. This flavonoid is famous for its strong antioxidant activity and is becoming more well-known due to its high-content in these vegetables [63, 64]. In pancreatic ductal adenocarcinoma cells (MIA PaCa-2, Capan-1, and S2-013), the up-regulation of *miR-142-3p* and the miRNA-induced apoptosis were observed by treatment with quercetin [65]. With regard to the human intestine, in...
colonic myofibroblast CCD-18Co cells, it is suggested that quercetin protects the normal CCD-18Co colon cells from reactive oxidative species and inflammation, partly due to the quercetin-induced expression of miR-146a, which is likely to act as a negative regulator of pro-inflammation [66, 67].

2.6. Sulforaphane, 3,3′-Diindolylmethane (DIM), and Butyrate (Butanoate)

Besides compounds that come from food, it has been considered that active metabolites can work effectively when they are converted by digestion and enzyme reactions after oral intake. For instance, the plant secondary metabolites glucosinolates are contained in vegetables in the Brassicaceae family, such as broccoli, cabbage, and cauliflower. They are converted to isothiocyanates by the endogenous enzyme myrosinase when plant tissues are damaged [68]. Induction of damage via chopping or chewing results in an enzyme reaction: glucoraphanin, one of the major glucosinolates in broccoli, is transformed into an isothiocyanate sulforaphane, which appears to have anticarcinogenic activity [69-71]. Aside from the previous studies, sulforaphane is indicated to have the ability to positively change the miRNA expression in colonic epithelial cell lines NCM460 and NCM356, in human bladder cancer cell T24, and in human breast cancer cell lines MCF10DICS and MDA-MB-231 [72-74].

Additionally, indole-3-carbinol, a metabolite of the glucosinolate glucobrassicin, is also found in cruciferous vegetables (e.g., cabbage, radishes, cauliflower, broccoli, Brussels sprouts, and kale), and is converted into 3,3′-diindolylmethane (DIM) in a low pH environment during digestion in the stomach [75]. Some researches have indicated that DIM could control the expression of miRNAs: in...
human pancreatic cancer cells, DIM contributed to the inhibition of cancer cell proliferation and the promotion of apoptosis through the up-regulation of \textit{miR-146a}, the \textit{miR-200} family and the \textit{let-7} family, and the down-regulation of \textit{miR-221} [76-78].

On the one hand, butyrate (butanoate), a short-chain fatty acid, is an intriguing compound. Butyrate is produced in the colon by microbial anaerobic fermentation of insoluble dietary fiber that is often derived from vegetables. It was reported that, in the HCT-116 colon cancer cell line, butyrate suppresses the expression of the miRNA family (\textit{miR-17, miR-20a, miR-20b, miR-93, miR-106a}, and \textit{miR-106b}) that is increased in human colon cancer tissues [79].

\textbf{2.7. Other naturally occurring products that possibly regulate miRNA expression}

As described above, several studies have shown the potentiality of natural compounds changing the expression of miRNAs for the better; "ellagitannins," a class of polyphenols isolated from symploco plants (Symplocaceae) and used as an antipyretic, an antidote or a hemostatic agent in Chinese medicine [80]; "garcinol," a polyisoprenylated benzophenone derivative found in \textit{Garcinia indica} extract that is potent antioxidant and anti-cancer agent [81]; "matrine," an alkaloid extracted from \textit{Sophora flavescens} and used clinically in China as adjuvant therapy for breast cancer to improve the survival rate and quality of life [82]; and "ursolic acid (3b-hydroxy-12-urs-12-en-28-oic acid)," a pentacyclic triterpene distributed in many fruits and medicinal herbs and known as an anti-inflammatory, antioxidant and anti-tumour compound [83].
Moreover, some natural products have been implied to possess functions to alter miRNA expression; "folate or folic acid (vitamin B9)," one of the B vitamins, which is important for the synthesis of nucleic acids, cell division and growth; "retinoic acid," a derivative of vitamin A, which is required for cell-to-cell signaling during early organogenesis in vertebrates; "1,25-dihydroxyvitamin D (1,25(OH)2D)," an active metabolite converted from vitamin D; and "vitamin E (α-tocopherol)," a fat-soluble antioxidant. Lastly, "selenium," an essential trace element, "oleic acid," an ω-9 (n-9) monounsaturated fatty acid, and "ω-3 (n-3) polyunsaturated fatty acid" could all affect miRNA expression [84].

3. The prospect of miRNA-regulation of natural compounds available for food application.

As we discussed, each naturally occurring product is likely to have its own positive effect on miRNA regulation, although different cancer cell types are used in the different studies. It would be useful for health promotion and preventive strategies to desterilize the natural compounds that potentially help prevention and treatment of diseases. However, there are difficulties with the effective application of these compounds in food, such as whether the compounds can be commercially employed, whether they are suitable for daily intake, and how much compound can be absorbed and metabolized in the body.

The first problem is a business of price or availability of a compound. Secondly, it might be necessary to think about nutrients in the same way as vitamins. For example, a moderate daily
intake of folate is generally recommended. If we eat a well-balanced diet, additional intake is not necessary, which indicates that we should avoid the excessive intake of the vitamin and take into account potential side effects of overingestion. The third problem is an important topic for discussion. A natural compound might show its ability to the fullest in terms of miRNA if it acts directly on tissues and organs such as skin, stomach and intestines. However, if the total amount of the compound is completely metabolized after ingestion, the compound will lose its benefit and will not be able to pass through the bloodstream to the entire body and act at multiple sites. Today, there are a variety of approaches, such as synthetic analogs and nanoparticles (including liposomes, nanoemulsions, and lipid nanoparticles), for the improvement of the solubility, stability, and bioavailability of these compounds for a wide range of applications. From the point of view of food application, whether a compound is hydrophilic or hydrophobic, or whether it is stable during both processing and storage, as well as the proactive ingenuity that will be required from development through production are all potential issues that must be addressed. [85-87] It has been highly anticipated that realizing the development of functional foods would contribute to health promotion worldwide by connecting underexploited but competent natural products to miRNAs as a tool for health management.

4. Do food-derived miRNAs contribute to our health by functioning in a cross-kingdom manner and/or by horizontal transfer?

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4.1. Plant-derived miRNAs

There have been many discussions about exogenous miRNAs that derive from organisms other than human; the exogenous miRNAs could act in a cross-kingdom fashion upon ingestion with certain foods. Food-derived miRNAs have been investigated in animals such as pork, beef, and chicken (Gallus gallus), and in plants such as wheat (Triticum aestivum L.), maize (Zea mays), wild cabbage (Brassica oleracea), rice (Oryza sativa), apple (Malus × domestica), orange (Citrus sinensis (L.) Osbeck), potato (Solanum tuberosum L.), tomato (Solanum lycopersicum), and grapevine (Vitis vinifera) [88-99].

Plant miRNAs are different from animal miRNAs in that their 3'-ends are methylated by the methyltransferase HUA ENHANCER 1 (HEN1) with a 2'-O-methyl modification (2'-O-methylation), which likely protects plant miRNAs from degradation and uridylation [100-103]. For instance, the plant miRNAs MIR156a and MIR166a, which are found in rice, Chinese cabbage, wheat, and potato, can be detected after cooking, even though the level is decreased [104]. Moreover, in the low-pH environment that mimics the gastrointestinal tract, the degradation rate of plant miRNAs such as MIR168a is much lower than mammalian miRNAs and the synthetic form of plant miRNAs without a 2'-O-methyl modification, showing that the stability of plant miRNAs is partly due to the protection of 2'-O-methylated 3'-ends. As vegetables are commonly consumed raw (in salad, fruit, and juice), it seems that vegetables are much more suitable for oral dietary miRNA intake compared with animal products.

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In 2012, it had been previously reported that, after absorption through the gastrointestinal tract, a plant miRNA (MIR168a) in rice (*Oryza sativa*) reduced the expression of low-density lipoprotein (LDL) receptor adapter protein 1 (LDLRAP1) in the liver in a mouse model, resulting in the elevation of plasma LDL-cholesterol levels after 3 days of feeding with fresh rice [104]. Furthermore, next-generation sequencing analysis showed that plant miRNAs are found in the serum of humans whose main diet is rice. These results amazingly have implied the presence of gene regulation in a cross-kingdom manner via exogenous miRNAs. However, although additional studies were performed on food-derived miRNAs, plant miRNAs were not fully detected in the plasma in similar experiments. As there would be various differences in detection methods, detection sensitivity, experimental methods, and experimental environments, this question still continues to be a matter of debate [105, 106].

It was reported in 2015 that MIR2911, a Chinese herb honeysuckle (*Lonicera japonica*)-encoded miRNA, is accumulated in mouse peripheral blood and lung after continuous drinking or gavage feeding of honeysuckle decoction [107]. In traditional Chinese medicine, honeysuckle has a long history of being used to treat influenza infection, and several reports have suggested that honeysuckle decoction suppresses the replication of influenza virus. It was also reported that MIR2911 could survive during the boiling process of honeysuckle decoction, and after ingestion, could pass through the gastrointestinal tract and accumulate in the lung. MIR2911 could suppress the replication of influenza A viruses (H1N1, H5N1 and H7N9) by possibly binding the *polymerase basic protein 2* (*PB2*) and *nonstructural protein 1* (*NS1*) genes in the lung, preventing...
weight loss and death caused by viral infection. This was further examined using synthetic miRNAs and mutant H1N1 virus in a mouse model. It had been confirmed that an anti-MIR2911 antagonim diminished the inhibitory effect of synthetic MIR2911, total RNAs extracted from the decoction, or honeysuckle decoction on viral replication. In addition, the decoction and synthetic MIR2911 were not able to inhibit the replication of mutant viruses where the MIR2911-binding sites in the PB2 and NS1 genes were changed. The results will help us investigate the field of cross-kingdom regulation by miRNAs, but this needs to be viewed from multidimensional perspectives.

The studies described above were performed by the same research group, and the group noted the possibility that plant miRNAs would be encapsulated in microvesicles after being taken up by intestinal epithelial cells and then delivered into distant tissues, although the mechanism remains to be addressed. They also mentioned that the stability of miRNAs would be based on its unique sequence and high GC content. Mutated MIR2911 became susceptible to the boiling process or RNase treatment after the sequence or GC content was altered, and dietary miRNA uptake would be enhanced by the condition of the gastrointestinal tract which was altered by diet or pathology [108].

More importantly, we have to question the presence of foods containing a large amount of miRNAs that are resistant to degradation and beneficial for our health, but these kinds of foods has still not been found. If we aim at controlling the level of miRNAs found in food, this modulation will affect plant development and architecture, and the safety of genetically modified foods could potentially be problematic.
4.2. Milk-derived miRNAs

It is widely acknowledged that milk is an important source of nutrients not only for infants but also for adults. Especially for newborns, breast milk promotes survival and healthy development and reduces the risk of many diseases. In general, milk contains a wide spectrum of nutrients (such as proteins, carbohydrates, fats, minerals, and vitamins) and bioactive components (such as hormones, growth factors and immune factors) [109].

Recently, another interesting component, miRNA-containing EV, was found in milk, and the functions have been discussed. In 2006, it was reported that EVs was secreted into the culture medium when using the mouse mammary epithelial cell line COMMA-1D, and that EV-like vesicles were also found in mouse milk [110, 111]. In human, EVs was confirmed in colostrum (initial milk, usually up to five days after birth) and mature milk (later milk, usually more than ten days after birth) and they contained some exosome-markers such as major histocompatibility complex (MHC) classes I and II, CD63, CD81, and CD86 [112]. In addition, miRNAs were detected by microarray analysis and quantitative PCR in the supernatant of human, bovine, and rat milk, and even in commercially available milk [113-115]. The miRNAs in milk showed resistance to acidic conditions and to RNase treatment, but after detergent treatment, they became subject to degradation [116]. These results suggest that miRNAs in milk are packaged into EVs and EV-like vesicles and that the EVs would remain intact in the gastrointestinal tract. Interestingly, the studies have shown that some immune-
and development-related miRNAs are highly expressed, implying a possible connection with the regulation of the infant immune system by milk.

On the subject of milk-derived EV uptake, several research groups have investigated the interaction between cells and EVs: RNA-containing EVs from human breast milk were taken up by human macrophages [116]; bovine milk-derived EVs were incorporated into RAW246.1 macrophages and human THP-1 macrophages, and miRNAs were not transferred via milk-derived EVs when the physical structure of the vesicles was destroyed by ultrasonication [117, 118]. In addition to observations on macrophage cell lines, bovine milk-derived EVs interacted with human colon carcinoma Caco-2 cells and rat small intestinal IEC-6 cells [118].

Interestingly, some research groups have been investigated the *in vivo* effects of breast milk to understand the functional roles of milk-derived EVs. Gu et al. observed that the amount of immune-related miRNAs in EVs was higher in porcine colostrum than in mature milk, and that miRNAs were increased in the serum of piglets fed only the colostrum compared to those fed only the mature milk [119]. Baier et al. found that *miR-29b* and *miR-200c*, which are rich in cow milk, were present in higher amounts in human plasma until four to six hours following milk consumption. In mouse model, the level of *miR-29b* decreased in the plasma of mice that were fed milk miRNA-depleted diets compared to those that were fed milk miRNA-sufficient diets [120]. These studies have indicated that milk-derived EVs loaded with miRNAs might be transferred into the human body after consumption.

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In contrast, Laubier et al. have pointed out that the horizontal delivery of miRNA after ingestion may not occur in general [121]. By using miR-30b-enriched milk from transgenic mice overexpressing miR-30b in the mammary gland, they checked the amount of miR-30b in the blood and tissues of infant mice fed mild by transgenic mice and detected a high level of miR-30b only in milk itself and in the stomach contents, but not in the liver, small intestine, kidney, lung, or blood.

As is the case with plant-derived miRNAs, it is uncertain whether milk-derived miRNA and EVs could pass through the gastrointestinal tract and function in the body. However, immune- and development-related miRNAs in EVs are present in large quantities in the colostrum, and the question of whether they can modulate the development of the infant immune system should be considered.

5. Concluding remarks

Over the last decade, miRNAs have increasingly played a central role in the study of disease, particularly in the study of cancer. Moreover, in 2007, the discovery of miRNAs in EVs opened new avenues for research into cancer such as diagnosis and drug discovery, and this has generated huge interest in the contribution of miRNAs to health promotion.

In this review, we have discussed the potential of miRNAs, especially focusing on plants and food. First, many studies have unveiled some of the naturally occurring products that regulate miRNAs in a favorable manner, and this is likely to be advantageous for health promotion and preventive strategies. At present, it might be difficult to put the compounds to use directly, and these
compounds currently have application limits. If we look at the effects of natural compounds from another perspective, it will be intriguing to investigate whether the natural compounds have an impact not only on miRNA expression but also on EV production. In cancer, tumor-derived miRNAs in EVs are known to contribute to pre-metastatic niche formation and cancer progression when tumors secrete EVs into body fluids [122, 123]. It was found that tissue-derived miRNAs in EVs could regulate gene expression in distant tissues: adipose-derived circulating exosomal miRNAs controlled the expression level of the target gene in liver tissue, which suggests that circulating miRNAs in body fluids would regulate whole-body metabolism and mRNA translation in various tissues [124]. There is, therefore, considerable potential that functional miRNAs are delivered by EVs throughout the body and that the positive effects would thus be disseminated to all parts of the body. In regard to preventing disease or cancer, a series of studies will need to be conducted from a long-term perspective. This type of research requires patience and persistence, but more extensive studies will solve challenging problems and usher in the coming age of health promotion.

Secondly, some works have highlighted the possibilities of cross-kingdom regulation by plant-derived miRNAs and of horizontal delivery of milk-derived miRNAs. With respect to plant miRNAs, these studies are interesting and epoch-making research that may explain the protective effect of herbal/traditional medicine against influenza infection at the molecular and genetic levels in a mouse model. This kind of study is promising to help understand how foods would be beneficial in many areas of health. Milk miRNAs are a good model for studying the in vivo effects of miRNAs and EVs. Although human breast milk is currently thought to have many additional benefits compared to...
artificial milk (infant formulae produced from processed cows’ milk) [125, 126], precisely why breast-feeding is better for infants is still not completely known. Recently, EVs were identified as another component of milk, and colostrum was shown to be rich in immune- and development-related miRNAs. The relationship between breast-feeding, miRNAs and EVs in milk, and healthy development of infants will certainly present a new and interesting area for investigation and possibly reveal the key mechanisms involved in efficient uptake of food-derived miRNAs and EVs in vivo. In general, however, it is not clear whether a wide variety of food-derived, exogenous miRNAs function effectively in the body after ingestion of food, and this is still a subject of controversial discussion and needs to be investigated.

In the modern age, it has been pointed out that one of the major lifestyle problems is the insufficient intake of fruit and vegetables in many countries, especially in the developed world. Among the topics described above, it is of interest that the expression of miRNAs could respond to butyrate, which is generated in the colon by the microbial fermentation of dietary fiber from vegetables. This might provide new insights into cancer prevention and imply the importance of a well-balanced diet for good health. In practical terms, it is assumed that lack of exercise, smoking, and high alcohol intake might influence the expression level of miRNAs in a negative way, and changes to lifestyle habits, such as food and physical activity, are therefore necessary. As in any lifestyle, food is not medicine that has an immediate effect on the body, and it will take a few months or even years to realize the health benefits from foods and its food-derived compounds. Basically, it is safe to say that food on a daily basis and its ingredients are important for the maintenance of good health.
health. The meals eaten each day will contribute to regulating the expression profiles of miRNAs in the body, and we look forward to further progress in this research field.

In the human body, the gene expression is changed from moment to moment by a variety of factors, and the species and amount of circulating miRNAs in body fluids will be altered correspondingly. For many years, there has been considerable research on the relationship between food and health, but these studies of miRNAs and EVs are still in their infancy. We believe that the field of miRNAs and EVs research will advance more rapidly in the future, and many of the challenges will lead to innovations and improved health promotion around the world.

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Conflicts of interests

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The authors have declared no conflict of interests.
References


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Figure legends

**Table 1.** Food-derived natural compounds regulating the expression of miRNA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Up-regulated miRNA</th>
<th>Down-regulated miRNA</th>
<th>Cancer type</th>
<th>Cell line</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
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Figure 1. Structural formula of natural compounds controlling miRNA
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