

Diet-Derived Exogenous miRNAs As Functional Food Components: Facts and New Perspectives

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Abstract

Exogenous miRNAs derived from dietary substances have been shown to be orally transferred to the mammalian system and proven to remain active to regulate host-gene expression. This way they have become an active area of research as functional food components and aspects for dietary supplementation. They are being studied as a new class of metabolically targeted therapeutics that work through diet manipulation and may hold promise for a therapeutic approach in reducing the risk of life-threatening diseases. However, a substantial amount of evidence also defies this dietary miRNA concept in terms of their absorption, bioavailability, cellular uptake and its physiological effects in the mammalian system. But recent advances in the identification of some unique sequence and structural characteristics of dietary miRNAs and a deeper understanding of their stability in host peripheral blood for its cellular uptake have strengthened the whole concept. The review comprehensively summarizes the mechanism for miRNA extracellular transport, absorption through the gastrointestinal tract (GI), stability in peripheral blood, and cellular uptake in mammalian cells. It recapitulates the shreds of evidence, related to the influence of dietary miRNAs on gene expression based on the source of the origin (plant vs animal), and compares their cross-kingdom behaviour in terms of their unique sequence and stem-loop structure properties that help them to get stabilized in the mammalian system. The review also summarizes the parameters required for maintaining the sustainable uptake and bioavailability of the dietary miRNAs with existing examples of successful in-vivo and in-vitro delivery of dietary miRNA for augmented therapy. Lastly, it provides an overview of the available and required databases, webserver, and tools that can be used for the successful identification of potential dietary miRNA candidates.

Keywords: Functional food components, Dietary miRNA, XenomiRs, dietary supplementation therapy

1. Introduction

Environmental and lifestyle factors, including diet and nutritional habits, have been found to strongly associated with diseases like diabetes, cancer, cardiovascular disorders, etc[1, 2]. Recent evidence indicates that food and diet influence the gene expression and its regulators such as miRNAs, Transcription factors (TFs), epigenetic factors etc[3]. TF are regulatory molecules that regulate gene expression at transcriptional level. miRNAs are small non-coding RNAs that modulate gene expression at the translational level. They are also detected in tissues, blood, and other body fluids with high stability and have a recognized role in the maintenance of tissue homeostasis[4, 5]. The miRNAs in extracellular human body fluids (e.g., serum, plasma, and saliva) are called circulatory miRNAs (cmiRNAs). These cmiRNAs have recently been shown to be associated with various pathological conditions like biomarkers for various cardiovascular disorders, for pancreas cancer, for liver cancer therapy, targeted therapy against hepatitis C virus (HCV) and ischaemia-reperfusion injury etc [6-9]. In circulatory system, these miRNAs are mainly present in extracellular vesicles, such as the exosomes (extracellular vesicles of endosomal origin), and are emerging as the key mediators of intercellular communication[10]. On further screening of these cmiRNAs, some of them were identified to be from exogenous species[11]. These special groups of miRNAs were categorized as xenomiRs. Some of these xenomiRs have found to enter the host through food ingestion; like breast milk from mother to infant may modulate the immune system of new-borns through dietary miRNA present in milk[11]. This gives rise to a new concept of food-derived miRNAs or dietary miRNAs. Zhang et al. were the first group to report that exogenous miRNA miR168a can be absorbed in the GI, enter the peripheral bloodstream, uptake by host cells, and exert LDLRAP1 gene regulation to reduce its expression, leading to elevation of mouse blood LDL levels[12]. Though Witwer et al. and other groups have stated that it is a fantasy-like spotting a unicorn leading to a controversial statement but Zhang et al. have harnessed an incredible volume of data for calling it a silver bullet, not a hoax [13]:[14-16]. The recent introduction of a new discipline called nutrimiRomics have also strengthened these claims. NutrimiRomics is a new discipline which examines the influence of the diet on the modification of gene expression due to miRNAs regulation that can effect disease development[17]. It can be classified as an amalgamation of three sciences, including health, diet, and genetics (Figure 1) [18]. Nutrition is a science that describes the diet in relation to the wellbeing of the body[18].

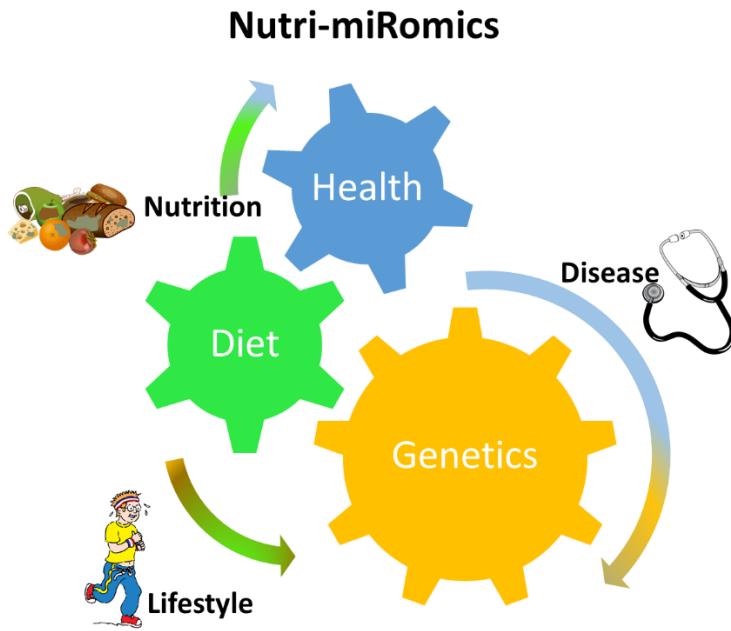


Figure 1: Nutri-miRomics as an amalgamation among Health, Diet, and Genetics.

In broad terms, nutri-miRomics help to understand the relationship between dietary elements and the targeted response of miRNA in the host body. One of the major aspects of this discipline is to study the externally supplied bioactive supplements effecting host miRNA profiles during specific human health condition e.g. externally supplied resveratrol controls expression miRNAs miR-21, -181b, -663, and -30c2[19, 20]. These miRNAs have been found specifically up-regulated in inflammatory response reactions [17, 21]. While Polyunsaturated Fatty Acids (PUFA) downregulates miR-146a, miR-146b, miR-21, miR-125a, and miR-155 to control anti-inflammatory action in cells [22]. Other bioactive compounds such as quercetin have potential health benefits, as quercetin-enriched diets may increase the level of miR-125b and miR-122 levels in the mouse that negatively regulate inflammation[23]. Curcumin, is one of the most potent anti-oxidants, anti-diabetics, anti-cancer and anti-inflammatory naturally occurring compounds that have been found to down-regulate pro-oncogenic miRNAs i.e. miR-17-5p, miR-20a, miR-21, and miR-27a expression[24]. These miRNAs have been found to control important molecular interactions in cancer cell modulation [25].

miRNA from dietary sources has gained momentum because they are accessible, stable and absorbed and bioactive in a cross-kingdom manner (Figure 2) [26, 27].

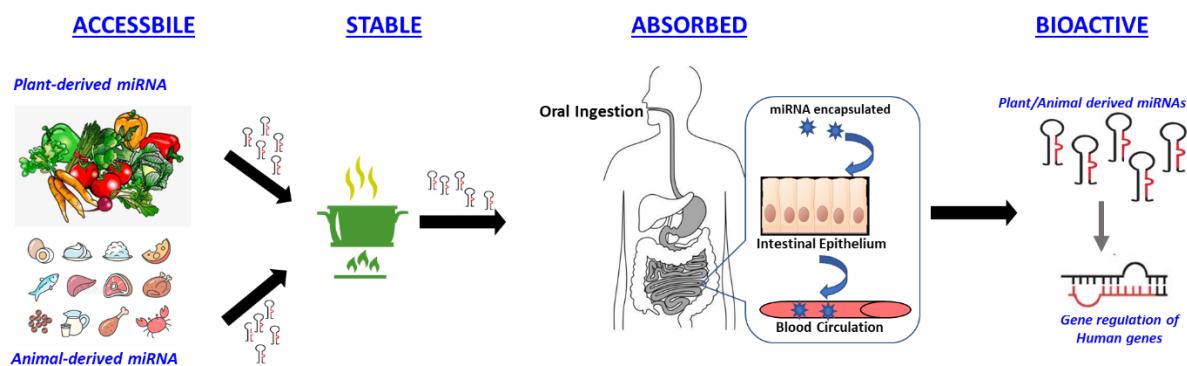


Figure 2: Dietary-derived miRNAs are accessible, stable, absorbed and bioactive.

Recent reports have shown diet supplemented miRNAs could be absorbed by host GI system like minerals, vitamins, micronutrients and can modulate host miRNAs machinery, demonstrating its potential role in maintaining health homeostasis[28, 29]. Though many reports confirm the use of bioactive compounds to regulate the host miRNA expression, however, the use of dietary miRNA as a supplement to control host miRNA machinery is still less explored. Viral and plant xenomiRs have been found in the plasma of healthy individuals through deep sequencing techniques, and subsequently, their homologous ones were deregulated in patients[29]. Recent scientific experiments have shown that some unique characteristics of dietary microRNAs help them maintain steady-state levels of miRNAs in the peripheral blood circulation after the intake by mammals like humans and mice[17, 26, 30]. Thus, exogenous dietary miRNA might substantially contribute to the pool of circulating miRNAs to maintain health status in recipient organisms, opening new perspectives for use of dietary miRNAs in maintaining health and disease. These miRNAs also show tissue-specific behaviour e.g. pab-miR951 is specifically present in breast milk, ata-miR-528-5p in kidney, ppt-miR894, aly-miR168a-3p, ath-miR401 and aly-miR158a-3p in serum/plasma respectively [31]. Some miRNAs like aly-miR166a-3p found in almost all the body fluids/tissues such as bladder, brain, kidney, liver, milk, pancreas etc.[31] The review tries to capitulate all possible information to strengthen the concept of dietary miRNAs and its cross kingdom behaviour. It offers a deeper understanding of the health effects of food borne miRNAs as a dietary supplement.

2.1 Extracellular exports mechanism and GI uptake of xenomiR.

miRNAs follow two different biogenesis mechanism i.e. canonical and non-canonical. Canonical miRNA biogenesis starts with the formation of the pri-miRNA transcript[32]. The microprocessor complex, comprised of two enzymes: Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8), cleaves the pri-miRNA to produce the precursor-miRNA (pre-miRNA)[33]. Exportin5/RanGTP helps to export pre-miRNA to the cytoplasm and process to produce the mature miRNA duplex. Further, the 5p or 3p strand of the mature miRNA duplex binds with the Argonaute (AGO) family of proteins to form an miRNA-induced silencing complex (miRISC)[32]. In the non-canonical pathways, small hairpin RNA (shRNA) is cleaved by the protein complex of Drosha and (DGCR8)[34]. This cleaved RNA is then exported to the cytoplasm via Exportin5/RanGTP. Some of these pre-miRNAs get 7-methylguanine capped (m7G) before exporting to the cytoplasm. Hence, uncapped miRNAs are called mirtrons and capped miRNAs are called 7-methylguanine capped (m7G)-pre-miRNA, differ in their nucleocytoplasmic shuttling. Mirtrons are exported via Exportin5/RanGTP while m7G-pre-miRNA are exported via Exportin1. The maturation of miRNAs is DICER enzyme-dependent[35]. All these mechanisms ultimately lead to a functional miRISC complex. miRISC binds to the target mRNAs to induce translational inhibition, by interfering with the eIF4F complex. The overview of this biogenesis is also represented in (Figure 3).

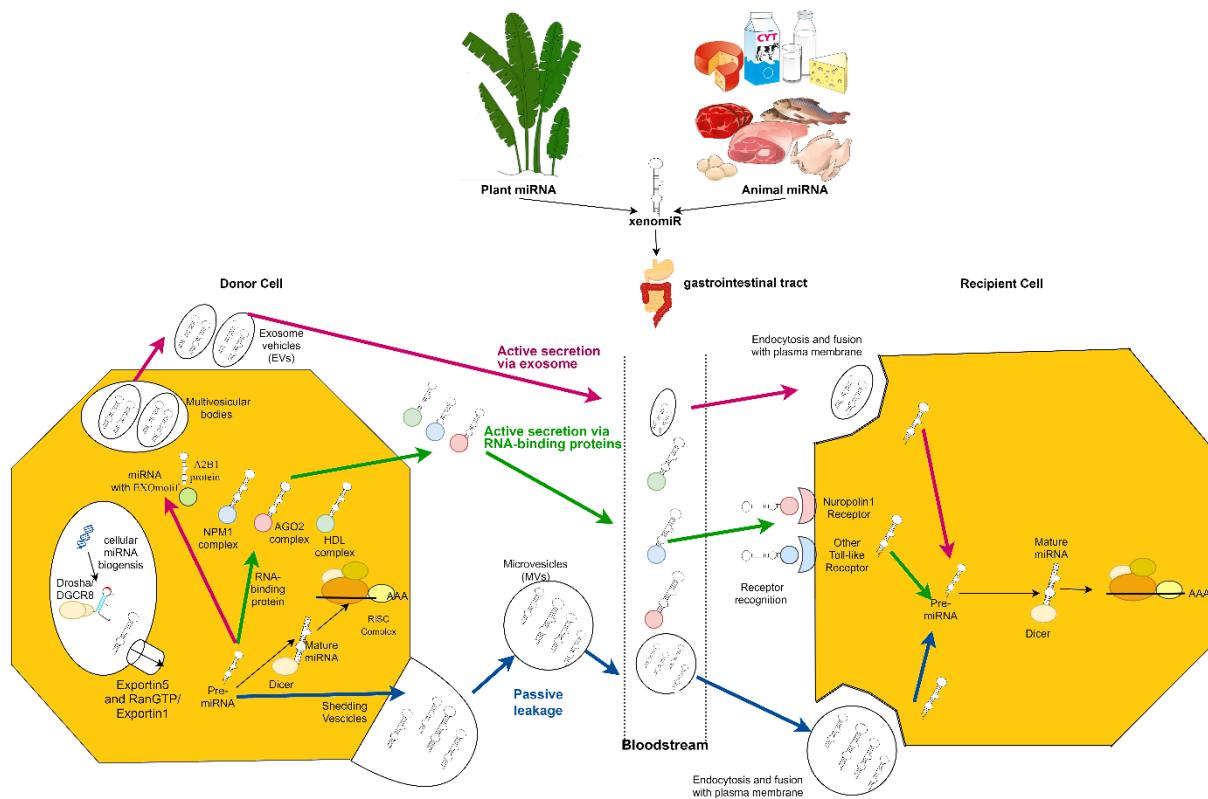


Figure 3: Circulatory miRNA packaging, transport from plant source/animal source/donor cell and uptake by the recipient cell through blood stream. MiRNA biogenesis start from its pre-processing from pri-miRNA to pre-miRNA that enters cytoplasm from nucleus. Depends on requirement miRNA can processed again to mature form and cleaved to form a RISC complex to deregulate the mRNA expression or can enter the extracellular circulation through three pathways: (1) active secretion via EVs shown with pink arrows; (2) active secretion in conjunction with the RNA-binding protein shown with green arrows and (3) passive leakage is shown with the blue arrows. The uptake mechanism of these miRNAs by recipient cells also differs and depend on their extracellular transport. miRNA packaged in EV or MV can enter host cell either through endocytosis or EV fusing with plasma membrane. Whereas miRNA associated with the RNA binding proteins like AGO2, HDL, NMP1 etc. have cell surface receptors like nupr1 or other Toll like receptors that recognise these miRNAs.

Extracellular miRNAs are a new class of cellular messengers, stably existing in most biological fluids. Selectively exported and functional in recipient cells, extracellular miRNAs are now

recognized as regulatory signals in cell-to-cell communication [36]. The miRNAs are released into the extracellular space via three different ways: First, passive leakage from disrupted cells due to inflammation, cell necrosis, and apoptosis, etc. Second, active secretion via RNA-binding protein-dependent manner, RNA-binding protein including Argonaute 2 (AGO2), High-Density Lipoprotein (HDL), Nucleophosmin 1 (NPM1), etc. could bind with miRNAs and transport them outside of cells. Most extracellular miRNAs have been found with AGO2 RNA-binding protein present in blood that is required for their function. AGO2 binding with miRNAs may enhance its resistance against RNase degradation [37, 38]. This protein has also been detected to be significantly enriched in exosomes. In addition, AGO2 could facilitate the packaging of intracellular miRNAs into MVs, and MV-loaded AGO2 is key to the functioning of secreted miRNAs in recipient cells. Third and most common of them are active secretion via membrane-enclosed cell fragments called microvesicles (MVs) or Exosomes vehicles (EVs) that are released by all cell types in normal as well as pathological conditions[39]. The structure of MVs protects miRNAs from degradation by RNases and helps them to remain stable in the extracellular space[40]. Lipids, proteins and nucleic acids, including miRNAs, can be exchanged by MVs, giving them the ability to target the recipient cells [41]. Evidence has accumulated that miRNAs can be packaged into MVs selectively; that is, cells may preferentially select the particular miRNA populations and sort them into MVs[42]. For example, as the response to various stimuli, hsa-miR-150 in human blood cells is selectively packaged into MVs and it is actively secreted in blood[43]. Most of the extracellular miRNAs, including MV-encapsulated miRNAs, are associated with RNA-binding proteins[38]. Recent studies have confirmed that a short sequence motif ‘GGAG’ overrepresented in miRNAs known as EXOmotif is responsible for guiding the loading of these miRNAs into EVs[44]. The protein heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) could recognize the EXOmotif and specifically bind to exosome miRNAs, thus controlling their loading into exosomes[45]. EVs are nano-vesicles present in the circulation that are involved in cell-to-cell communication and regulation of different biological processes [46]. miRNAs are a part of their cargo as they can transport proteins, mRNAs and other bioactive molecules from donor to recipient cells via target cell membrane fusion[46]. These vesicles have recently been recognized as important mediators of interactions between different cells[47]. Endosomal sorting complex, required for transport (ESCRT complex) and relevant proteins helps in EVs biogenesis, uptake and material cargo sorting[48]. The ESCRT complex identify “cargo” protein labelled by ubiquitin and direct it to multivesicular bodies (MVBs)[49]. These MVBs

then separate from the peripheral membrane in a process that is similar to the process of cytokinesis and virus budding[49].

The mechanism of the GI absorption of the xenomiRs involves, transcytosis of miRNAs vesicles or miRNA-protein complexes in the gut, but there are largely unknown [29]. XenomiRs that are protected in vesicles or miRNA-protein complexes can survive for numerous hours in the circulation, long enough to be absorbed by cells throughout the body, possibly via receptor-mediated endocytosis[29]. The mechanism of selection of these miRNAs for absorption is a grey area for research. The same dietary miRNA can have selective absorption in two different but phylogenetically close species like Human and mouse e.g. healthy men and mouse were fed with the same western fruit diet but only in human miR-156a, miR-159a, miR-169a were found upregulated[17, 50]. Cells are thought to be able to recognise specific MVs by their surface receptors and to initiate internalisation of MV miRNAs by phagocytosis, endocytosis, or direct fusion with plasma membrane[51]. These extracellular miRNAs have been demonstrated as participant in various physiological and pathological processes in mammals and have significant roles in fatal-maternal crosstalk and cross-kingdom regulation[43, 52]. Examples are miR168a present in rice is absorbed by forming AGO2 complex in mice and regulates mouse LDLRAP1 gene expression[12]. Orally administered miRNA miR168 source from strawberry have been found to reduce the induced production of IL-1 β , TNF α CD80, CD86, CD83 and act as an immuno-modulator in multiple sclerosis induce mice model[12]. Similarly miR156a, miR157a, miR158a, sourced from watermelon was found to be absorbed largely through encapsulated MVs in mice and maintained body weight homeostasis[53]. Furthermore, the abnormal expression of extracellular miRNAs has been shown to be associated with many diseases, making extracellular miRNAs promising, novel, non-invasive diagnostic markers[54]. These abnormal expression of extracellular miRNAs are extensively studied in cancer, where cancer cells can target their surroundings through extracellular miRNAs like miR-214 and can induce tumorigenesis in nearby cellular environment[55]. This tumor-secreted miR-214 transferred to mouse peripheral CD4+ T cells by MVs, downregulates phosphatase and tensin homolog (PTEN), causing T cell expansion, Immune suppression and enhance tumor growth[55]. Similarly extracellular miR-92a, from the human leukaemia cells, transferred into human umbilical vein endothelial cells (HUEVCs), target integrin α 5, causing cell migration and tube formation resulting in tumorigenesis[56]. Moreover, extracellular miR-155, miR-210 and miR-21 have also been found as promising non-invasive diagnostic markers for diffuse large B-cell lymphoma (DLBCL)[57].

2.2 Animal derived XenomiRs Vs Plant derived XenomiRs.

Plant-derived miRNAs can detect mammalian mRNAs as targets. They were found in body fluids of mammals' e.g. plant-derived miRNAs mir-168a, mir-166a, mir-159a, miR-824, and miR-167a present in high concentration in human and mice serum[12, 58, 59]. At the structural level, it contains 2'-O-methylation on 3'end that enhances its stability to withstand in adverse conditions inside the GI such as RNases, phagocytosis and low pH. Whereas milk-derived miRNAs and other non-veg sources are suggested to be more resistant to enzymatic digestion, due to EV transport[60]. This difference may arise due to different processing of pre-miRNA from pri-miRNAs in animals and plants. In animals it is carried out by Drosha protein, whereas in plants by RNase-III-like protein, Dicer-like 1 (DCL 1)[61]. Due to this processing step, second hydroxyl group of 3'ribose in the last nucleotide undergoes methylation by HEN1 protein that protect miRNA from a 3'-to-5' exonucleolytic activity and uridylation activity in extracellular environment. DCL 1 protein further catalyses pre-miRNA to mRNA:miRNA duplex in nucleus of plants. On the contrary, the pre-processing of animal miRNAs is catalysed by Dicer enzyme in cytoplasm.

There are many differences in animal and plant miRNAs sequences specially in seed regions [62]. Some of these differences effect their uptake and bioavailability in the host system e.g. plant miRNAs contains negligible or low frequency of 'UUCC' 'CCA' and 'CG' repeats in seed regions whereas these are frequently present in animal miRNAs. This sequence property of animal miRNA helps in their interspecies sequence conservation, high stability in body fluids and extracellular vesicle transport[17]. Also, plant miRNAs are comparatively less cysteine 'C' rich than animal miRNAs that also affect their cellular uptake in host system [62].

Dietary uptake, bioavailability, and biological function of plant-originated miRNAs is a debatable topic. Zhang et al report the dietary uptake of plant-originated miRNAs and their function in the host system by giving an example of rice miRNA miR168a regulating LDLRAP1 expression in host mouse model[12]. Dickenson et al. failed to find reliable evidence of dietary uptake of miR168a from rice after replicating Zhang's study[13]. Similarly Tosar et al. use deep sequencing technologies to show that exogenous miRNA miR168a was not transmitted exogenously from other kingdoms and was present due to cross-contamination in the samples[14]. Other studies like Baier et al. have questioned the bioavailability of plant-borne miRNAs through the example of brassica-specific miR824 that are found in undetectable

range in the blood of four randomly selected human participants[63]. Numerous other studies have mentioned low measurable uptake of plant miRNAs in human and other mammalian species, after the consumption of plants or plant products[63]. Therefore, in order to increase the concentration of plant-miRNA in animal cells, food consumption must be substantially higher than normal dietary conditions[50]. In contrast to these controversial information on plant miRNA, report have shown animal miRNAs; miR-29b-3p and miR-200c-3p from bovine milk present in higher circulating levels number in individual who drink bovine milk [64]. Suggesting that animal-derived miRNAs, especially from milk, have been more resistant to enzymatic digestion due to exosome/vesicle transport called milk EV[65]. The presence of mRNA in milk EVs have detected in earlier 1955, however, the first study focusing on milk miRNA have only reported in 2010 that have opened avenues for new research areas on milk EV encapsulated miRNAs[66, 67]. Thereafter, numerous studies have investigated milk EV miRNAs in various conditions and species like mammary gland infections[68], breast cancer[69], lactation periods[70, 71], milk processing[72, 73], and other roles[60]

Experimental studies on plant miRNAs show that mammals humans and mice are unable to retain steady-state levels of these miRNAs in the circulation because of absence of EV encapsulated miRNAs[50]. Though recent discoveries illustrates that plant do contain naturally occurring nanostructures that are similar to EVs called Edible plant-derived exosome-like nanoparticles (EPDELNs)[74]. These EPDELNs can be absorbed in the mammalian GI and can exhibits anti-inflammatory properties that helps to maintain intestinal homeostasis. They also have the potential to transfer miRNAs in host system for intercellular communication e.g. Soybean (MIR-5781, MIR-4996, MIR-5671a), Hami melon (MIR-164a), Orange (MIR-398b), Ginger (MIR-1078) and Tomato (MIR-4995) have been found encapsulated EPDELNs that can regulate inflammatory cytokines IL17A, IL16, IL1A, IL10, IL6, IL5 and IL33 respectively[74]. The thorough investigation of all above reports states that though animal miRNAs have more potential to be used as dietary miRNAs but some plant miRNAs that are encapsulated in EPDELNs in spite of all controversies can also be a good source of dietary miRNAs.

2.3 Dietary xenomiRs and Cross-kingdom effect on health.

The interdependence of health and diet is evident, e.g. during infectious diseases like jaundice, malaria, etc. loss of appetite is a part of their acute cytokine-induced innate immune responses. Other diseases such as diabetes mellitus and cancer, dietary conditions are associated with their

premium risk factors e.g. anorexia, malnutrition, weight loss, cachexia, and wasting[75, 76]. Each of these risk factors are connected to reduced nutrition intake, leading to metabolic changes and negative energy balance. There are a number of studies available in literature that shows diet has effect on miRNAs expression e.g. Le Leu et al showed that a high red meat diet would lead to the upregulation of miR-17-92 cluster and miR-21 in colon mucosa associated with tumor, supporting the link between the diet and variations in miRNA expression[77]. Growing evidences of such research have shed light upon the potential implications of diet derived xenomiRs, identification in human biofluids for their role in cross-kingdom gene regulation and human disease pathogenesis[78]. It started with first evidence of exogenous plant miR-168a that was proposed to be a novel biomarker for its possible functional role in low-density lipoprotein cholesterol (LDL-C) metabolism and atherosclerosis[12]. Another instance of dietary miRNAs is plant xenomiR miR-159 which has been detected in human serum with an anti-proliferative role in breast cancer cell growth[79]. Qi Zhao et al uses metanalysis on 388 public small RNA sequencing data from 11 different human tissue samples to identify 166 types of plant derived miRNAs. About 80% of these miRNAs are abundant in the human samples and represented from 14 plant species only[31]. Humans also absorb miRNAs from the breast milk. Several immune-related miRNAs found abundant in the milk e.g. miR-155, a regulator of T- and B-cell maturation and the innate immune response; miR-181a and miR-181b, regulators of B-cell differentiation and CD4+ T-cell selection; miR-17 and miR-92 cluster: a ubiquitous regulator of B-cell, T-cell and monocyte development, miR-125b, a negative regulator of tumor necrosis factor- α production, activation and sensitivity; miR-146b, a negative regulator of the innate immune response; miR-223, a regulator of neutrophil proliferation and activation; and let-7i, a regulator of Toll-like receptor 4 expression in human cholangiocytes. The expression level of these miRNAs from human breast milk were found higher during first 6 months after the child birth, which is the period before infants receive complementary food[80]. Several tissue-specific xenomiRs, such as miR-122 (liver), miR-216, miR-217 (pancreas) and miR-142-5p and miR-142-3p (hematopoietic cells), were also found in smaller quantities in breast milk[28]. Researchers have also observed these miRNAs in mice experiments, when fed with the specific diet supplements e.g. miR-166a and miR-159a overexpressed in the mice blood, when fed with rapeseed bee pollen[58]. An alternative hypothesis also proposes that some of endogenous miRNAs are glucose-sensitive, so they could respond to dietary intake of any plant or animal material[64]. Subsequently, the diet-associated effects of xenomiRs have drawn researcher's attention since this knowledge may unravel the link of xenomiR, diet and health and ultimately lead to new translational applications.

2.4 Determination of the potential bioavailability of dietary miRNA and their cellular uptake in host cell.

miRNAs have been considered endogenously synthesised for a long time until very recent discoveries show that humans can absorb dietary miRNAs of animal and plant origin[12, 64, 65]. However, debate continues as to whether dietary intake poses a feasible route for such exogenous gene regulators. The selective uptake of miRNAs from different exogenous nutritional sources like rice, milk, honeysuckle etc. to host tissue by unknown mechanism have created an interest to know the criteria behind these selections [62].

Compelling evidence shows the tedious selection process of dietary miRNAs that is absorbed from both plants as well as animal sources by the human host[81]. This has created another fundamental question about the selective nature of the absorption of dietary miRNAs. The difference in the uptake and bioavailability of two different sources of miRNA is due to certain conditions. These conditions are: the sequence conservation between interspecies miRNAs, the stability of miRNAs in body fluids of the host system, plasma level of the exogenous miRNAs should resemble to those complementary host miRNAs that are under-expressed or absent under specific pathological condition. Also, the extracellular vesicle active transport mechanism of miRNAs in the host cell is most preferred mechanism for cellular uptake because encapsulation of miRNAs in extracellular vesicle not only increases its stability in exogenous fluids but also help in its cellular uptake[82]. To know potential bioavailability of dietary miRNA is important because it helps to check whether these miRNAs can survive passage through the mammalian GI system and would be available in sufficient number to be uptake by cell for regulation host genes expression. A recent study by Philip et al. demonstrated that knowing the miRNA levels before supplementation in human diet is essential to assess their bioavailability after ingestion through its simulated human digestion system[83]. The study revealed that some plant miRNAs miR-166, miR-167, miR-168 and Lin-4 from soybean remain significantly bioavailable even after digestion for 75 min because of their high pre-treatment or supplementation level[83] .Also, cooked food contains higher miRNA levels than raw food, suggesting that disintegration of the cell wall structure of the plant leads to possible increase in significant amount of cells free miRNAs responsible for their bioavailability in host system.

A recent study has computationally identified discriminative sequence based features to significantly predict human blood secretory miRNAs i.e. (1) % G+C content in the should be less in precursor miRNA sequence and more in its mature form; (2)Frequency of 'GU' repeats should be less in precursor and more in mature miRNA sequences; 3) Normalized frequency of 'U' on mature miRNA should be less; 4)Number of palindromes (sequence that reads the same backward as forward) with length >3 occurring on precursor sequence should be less preferable[62].

Pre-miR168a was well known to be highly stable in the human serum and tissues[12, 84]. Hence a recent study after thorough examination of secondary structures of pre-miR168a, predicted various structural parameter to screen cross-kingdom miRNAs that could be stable in human serum and tissues such as energy, precursor length, loops and bulges and Adenine-Uracil (AU) percentage[84]. They have used these criteria to successfully predict serum stability of clo-mir-14 of curcumin (*C. longa*). The details of these criteria are (a) miRNA structures with energy greater than -73(kcal/mol) (b) Structures with not more than 9 mismatches, 1-4 bulges and no more than 3 loops were considered; (c) Precursors with sequence length greater than 105nt and with less than 53% AU content were permitted[84]. Overall investigation have shown that integrated genomics and computational analysis can further help to screen the potential transportable cross-kingdom miRNAs. Multiple screening at both structural level and sequence level would increase the potency miRNAs to remain stable in the host system serum and tissues. Finally, higher the pre-supplementation miRNA level in source, it would allow significant bioavailability of miRNA after digestion for cellular uptake and regulation of host gene expression.

2.5 miRNAs in diseases and Cross- kingdom miRNAs as an augmented therapy

Most lifestyle diseases especially different types of cancer have strong correlation with the diet and nutrition e.g. Nutritional habits have a strong relationship to colorectal cancer, unhealthy dietary habits lead to adiposity and chronic inflammation, which is the primary risk factor for colorectal cancer[85, 86]. Similarly, evidence also indicates that improper dietary profile such as alcohol consumption, intake of aflatoxin-contaminated food contribute as a risk factor for liver carcinoma, etc[87]. Superfoods such as coffee have been studied extensively in the prevention of liver carcinoma[88]. Hence, nutrition plays an important role in initiating, modulating and suppressing multifactorial diseases like cancer.

miRNAs can be easily detected in faeces and differential expression of miRNAs may be helpful in the diagnosis of CRC and inflammatory bowel diseases[89]. Since the expression of miRNAs found to have been altered in cancer cells, a minor change in their expression can fine tune the pattern of gene expression of oncogenes and tumour suppressor genes during cancer progression. In addition, the recent reports have shed light on the existence of inter-kingdom miRNA group as an analogous substitute for endogenous miRNAs[90]. In this novel scenario, xenomiRs may represent as key players in the interaction between plant and animal kingdoms, capable of influencing disease onset and outcome. These miRNAs can act as both suppressive as well as therapeutic agents. Hence they can potentially be used not only as an early screening tool for cancer, but also for patients' prognosis during cancer treatment.

Recent reports have presented the effective role of acquired cross-kingdom miRNAs on human gene expression[91]. These reports have also shown that orally acquired exogenous miRNAs can successfully enter digestive and circulatory system and can remain stable, protected inside EV[91]. This resulted in the expansion of gene regulation theories and the role of cross-kingdom miRNAs as a dietary supplement for therapeutics. There are several reports available suggesting cross-kingdom miRNAs as a supplement for the prevention and treatment of diseases in mammals.

E.g. plant miRNA miR2911 from honeysuckle (*Lonicera periclymenum*) has shown the antiviral activity against influenza-A virus[92]. It was observed that through gavage and continuous feeding of honeysuckle extract orally, this miRNA inhibits the viral replication in mouse lungs preventing weight loss and mortality due to infection. This miRNA is found effective against a broad spectrum of Influenza A viruses such as H1N1, H5N1, H7N9, etc. Some reports have mentioned the use of synthetic mimic miRNAs, rather using extract as supplement e.g. plant miRNAs ath-miR-159a (from *Arabidopsis thaliana*), gma-miR159a-3p and gma-miR159e-3p (from soybean or *Glycine max*) were used to treat breast cancer in the mouse model[79]. As all three miRNAs share a common sequence, so miR-159 encapsulated in the EV was given to treat cancer. This miRNA has been found to inhibit TCF7 and suppress breast cancer cell proliferation in mice. This miRNA was also reported to be present in human serum and transported to breast tissues and able to reduce the transcriptional activity of the TCF7 gene in breast cancer cell lines.

Similarly, a recent study has used a viral miR-132-expression system using recombinant adeno-associated viruses (rAAVs) to administer this miRNA in Huntington's brain disease affected

model mice for its treatment[93]. Huntington's brain disease is an intractable neurodegenerative disorder caused by mutant Huntingtin (HTT). The treatment of viral miR-132-expression shows improvement in the motor function and increased lifespan of Huntington's disease mice. This cross-kingdom miRNA relationship was also analysed for the treatment of diseases in human e.g. clo-mir-14 can retain its secondary structure in human serum for a long duration and remain active to regulate crucial targets for the treatment of rheumatoid arthritis[84].

A similar cohort study performed by Hou et al., 2018, aimed to identify stability of plant miRNA in human serum of Chinese individuals having a daily diet of rice, veggies, and meat[94]. The study identified miR-156a, and miR-164a found in green leafy vegetables including cabbage, spinach and lettuce are present at a modest level in human serum of healthy individuals upto 6 hrs. They also associated the MIR156a from green lettuce to be associated with reduced risk of cardiovascular disease (CVD) and can act as cardio protectant[94].

2.6 Databases to study dietary/food-borne miRNAs

As the concept of food borne miRNAs has evolved in last decade, a number of studies related to dietary miRNAs has increased rapidly. Several databases, tools, webserver have been developed to support the identification, prediction, analysis of these miRNAs. Some commonly used database, webserver and tools related to dietary miRNAs have been discussed in this section. The most popular tool for diet derived miRNAs is Dietary MicroRNA Database (DMD). DMD is a collection and analytical tool for food-derived extracellular miRNAs[95]. Currently, this database has fifteen types of dietary miRNAs and related information such as apple, grape, cow milk, and cow fat etc. Out of the total species in the database, 9 originates from plant and 5 from animal sources. DMD collection annotation includes cross-species sequence comparison, hairpin structures, mature sequences, genome locations, of parental pre-miRNAs, disease relevance, and experimentally validated gene targets for each miRNA entry. It can also perform few basic functional analysis i.e. target prediction, pathway enrichment and gene network construction. Xeno-miRNet, a comprehensive platform for analysing both, validated and predicted xenomiRs and its regulatory host targets from 54 different source species[96]. This webserver also allows users to perform network analysis on the XenomiRs-target networks with topological and functional tools such as edge-betweenness, degree distribution, gene ontology, KEGG pathways etc. Apart from these common tools, many plant

miRNAs databases were also developed e.g. PmiREN: a comprehensive encyclopaedia of plant miRNAs [97], PMRD: plant miRNA database, PmiRExAt: : plant miRNA expression atlas database [98], MepmiRDB: : a medicinal plant miRNA database [99] etc. These databases can also be used as knowledge resource for plant based dietary miRNAs [100]. While these databases have successfully captured the knowledge of dietary miRNAs but a growing data of dietary miRNAs require more competent and robust tools having properties to identify and predict these miRNAs. The tools should include use of EXOmotif sequence search and their structural properties such as AGO2, LDL binding pockets to predict dietary miRNAs more comprehensively. The tools should also facilitate the user to perform meta-analysis.

Conclusions

Exogenous miRNAs obtained from dietary source can be orally transferred and absorbed in cross-kingdom host cells. They have unique sequence and structural properties that help them to stabilize in host peripheral blood for their cellular uptake. Bioavailability of exogenous miRNAs in host however depends on the source (Plant or Animal) and its pre-supplementation level in the source. Recent advancement shows exogenous miRNAs can maintain sustainable uptake, bioavailability and can be used for augmented therapy especially against diet associated diseases.

Declaration of competing interest

The authors declare no potential conflict of interest.

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