miRNAs, polyphenols, and chronic disease

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Standard Abbreviations: cardiovascular disease (CVD), carnitine palmitoyltransferase 1A (CPT1a), cholesterol efflux transporter ATP-binding cassette transporter A1 (ABCA1), Epigallocatechin gallate (EGCG), fatty acid (FA), fatty acid synthase (FAS), grape seed proanthocyanidin extract (GSPE), non-alcoholic fatty liver diseases (NAFLD), oxidised LDL (oxLDL), renin-angiotensin-aldosterone system (RAAS), sterol response element binding protein 1 (SREBP1) and 2 (SREBP2), triglyceride (TG), type 2 diabetes (T2D).

Keywords: cardiovascular, insulin, lipid metabolism, microRNA, polyphenols.

Abstract

Mi(cro)RNAs are small non-coding RNAs 18-25 nucleotides in length that modulate gene expression at the post-transcriptional level. Thousands of miRNAs have been described, and it is thought that they regulate some aspects of more than 60% of all human cell transcripts. Several polyphenols have been shown to modulate miRNAs related to metabolic homeostasis and chronic diseases. Polyphenolic modulation of miRNAs is very attractive as a strategy to target numerous cell processes and potentially reduce the risk of chronic disease. Evidence is building that polyphenols can target specific miRNAs, such as miR-122, but more studies are necessary to discover and validate additional miRNA targets.

1. Introduction

MicroRNAs (miRNA) are small non-coding RNAs approximately 21-23 nucleotides in length that modulate gene expression by suppressing translation and/or reducing the stability of their target mRNAs [1-4]. These miRNAs are transcribed from DNA as part of longer precursors (primary transcripts or pri-miRNAs) that fold back on themselves to form distinctive hairpin structures. Pri-miRNAs are cleaved in the nucleus into miRNA precursors (pre-miRNAs) by the Drosha complex. These pre-miRNAs, approximately 70 nucleotides, are exported to the cytoplasm and cleaved by Dicer ribonuclease to generate functional miRNAs. Through association with Argonaute proteins, mature miRNAs are included in the RNA-induced silencing complex (RISC) that binds to the 3'untranslated region (3'UTR) of target mRNA [5-7]. The binding of mature miRNA to the 3'UTR of target mRNA depends on the interaction of a 6- to 8-nucleotide seed sequence at the 5' end of the miRNA with miRNA response elements in the target mRNA [8].

Most of the miRNAs described to date regulate crucial cell processes such as proliferation, differentiation, and apoptosis. Thus, RNAs are involved in normal human development as well as in the initiation of various cancers, where miRNAs have been found to be significant prognostic and predictive markers [9-11]. Furthermore, miRNAs have been reported to regulate several metabolic pathways including insulin secretion and carbohydrate and lipid metabolism [12]. miRNA may influence almost all genetic pathways by targeting transcription factors, secreted factors, receptors and transporters [4]. Moreover, as an epigenetic mechanism, miRNAs may mediate the effects of nutrition and may be causal in the development of many common chronic diseases [13]. Current data indicate that a wide range of dietary factors, including micronutrients and

non-nutrient dietary components such as polyphenols, can modify expression of miRNA [14] [15]. ~~[14]~~

Several hundred unique polyphenols have been identified as secondary metabolites in edible plants. Polyphenols are classified into different groups as a function of their molecular structure: phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids are divided into 6 subclasses: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (monomeric catechins and oligomeric proanthocyanidins) [16]. Epidemiological studies suggest that high dietary intake of polyphenols is associated with a decreased risk of a range of diseases including cardiovascular disease (CVD) and some cancers and neurodegenerative diseases [17]. Flavonoids improve endothelial function, lipid metabolism, and glucose homeostasis and can reduce oxidative stress and blood pressure [18, 19]. Most of the health effects of flavonoids have been attributed to the alteration of gene expression that codes key metabolic proteins. These gene modifications can result from the interaction of polyphenols with signalling cascades and/or with epigenetic factors like miRNAs.

Thousands of miRNAs have been described, and it is thought that they regulate more than 60% of all human cell transcripts that modulate metabolism and are implicated in various diseases [20]. Polyphenolic modulation of miRNAs is very attractive as a strategy to modulate numerous cell processes and reduce the risk of chronic disease. ,

The aim of the present paper is to review miRNAs that are targeted by polyphenols and to discuss the implication of miRNAs in the beneficial health effects of polyphenols on metabolic disease.

2. miRNAs and metabolic control

miRNAs are known to modulate more than 60% of genes [20] and should therefore be implicated in almost all metabolic pathways. However, the specific role of each miRNA in controlling metabolic pathways is still unknown, and most studies have focused on lipid metabolism. For instance, in the two last years, the key roles of miR-33 and miR-122 in lipid metabolism control have emerged. Briefly, miR-122 is expressed primarily in the liver and was the first miRNA to be linked to the regulation of lipid metabolism [21]. It is recognised as vital to hepatitis C virus infection [21]. Another important miRNA in lipid metabolism is miR-33, which has been studied extensively and targets genes involved in cholesterol efflux, fatty oxidation and very low-density lipoprotein (VLDL)-triglycerides [22, 23]. Interestingly, two isoforms of miR-33, miR-33b and miR33a [22, 23], have been identified. These miRNAs are intronic of the sterol response element binding protein 1 (SREBF1) and 2 (SREBF2) genes, respectively [24]. SREBF1 and SREBF2 code for the transcription factors SREBP1 and SREBP2, which regulate all SREBP-responsive genes in both the cholesterol and fatty acid (FA) biosynthetic pathways [25]. Therefore, miR-33 and the SREBP host genes cooperate to control cholesterol homeostasis [26]. Other miRNAs do not directly affect metabolism, but instead target nuclear receptors; for example, miR-613 targets the nuclear liver X receptor (LXRα) [27]. Additional studies have implicated other miRNAs in the regulation of lipid metabolism [22].

Lipid metabolism

Different miRNAs are implicated in the control of each key point in cholesterol homeostasis. For instance, only miR-122 is related to the biosynthesis of cholesterol; miR-122 inhibition in normal mice resulted in reduced plasma cholesterol levels and a decrease in cholesterol synthesis rates [28]. Sequestration of miR-122 represses 3-

hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the key controller of cholesterol biosynthesis, and decreases the pathway activity in liver cells [29]. However, the regulation of HMGCR by miR-122 is postulated to be indirect [29].

No studies of the role of miRNAs regulating native low-density lipoprotein (LDL) uptake by cells have yet been published. Intriguingly, exogenous plant miRNAs in food have been described to target genes related to LDL uptake in humans. Rice miR-168a, which is abundant in the sera of Chinese subjects, targets the human and mouse LDL receptor adapter protein 1 (LDLRAP1) mRNA, inhibits LDLRAP1 expression in liver, and consequently decreases LDL removal from mouse plasma [30]. However, some miRNAs have been implicated in the regulation of scavenger receptors for oxidised LDL (oxLDL) in cells activated with oxLDL. The lectin-like oxLDL receptor-1 (LOX-1) contains a let-7g binding site, and the transfection of let-7g inhibits LOX-1 expression in macrophages [31]. In addition, miR-29a regulates the expression of the scavenger receptor for oxLDL on dendritic cells [32], and miR-146a significantly reduces intracellular LDL cholesterol accumulation in macrophages [33].

The cholesterol efflux transporter ATP-binding cassette transporter A1 (ABCA1) is crucial for reversing cholesterol transport. This transporter is involved in both high-density lipoprotein (HDL) biogenesis in the liver and in cholesterol efflux to HDL in extrahepatic cells such as macrophages [34]. Its expression is under the control of miR-33. In this sense, miR-33 represses ABCA1 expression in liver [35, 36] and extrahepatic tissue, such as pancreatic islets [37] and macrophages [26, 36, 38]. Interestingly, inhibition of miR-33 increases the expression of ABCA1, enhancing HDL biogenesis, and increasing HDL-cholesterol levels in non-human primates [35] and mice [36]. In addition, miR-33 also controls other cholesterol transporters implicated in cholesterol efflux, such as ATP-binding cassette sub-family G member 1 (ABCG1) and Niemann-

Pick C1 (NCP1) [39]. Recently, miR-758 has also been implicated in the repression of ABCA1 levels in several cell types, including macrophages [40]. The key enzyme of bile acid synthesis, 7alpha-hydroxylase (CYP7A1), is controlled by miR-122a and miR-422a in hepatic cells [41].

In addition to cholesterol, miRNAs control FA and triglyceride (TG) metabolism. Key enzymes of FA oxidation are targeted by miR-33 including carnitine O-octaniltransferase (CROT), carnitine palmitoyltransferase 1A (CPT1a) and hydroxyacyl-CoA-dehydrogenase (HADH) in the liver [42]. In this sense, overexpression and inhibition of endogenous miR-33 reduces and increases, respectively, FA oxidation in hepatic cell lines [42]. FA synthesis activity in the liver is also controlled by miR-33, which increases the expression of SREBF1 and genes codifying key enzymes of lipogenesis, such as fatty acid synthase (FAS), ATP citrate lyase (ACLY) and acetyl-CoA carboxylase alpha (ACACA) [35]. As a result of the inverse effects of miR-33 on FA synthesis and oxidation in the liver, miR-33 antagonism has been reported to significantly reduce the plasma levels of VLDL-associated TGs in a non-human primate model [35]. Two other miRNAs controlling FA and TG metabolism in the liver are miR-370 and miR-122. Of these, miR-122 inhibition increases hepatic FA oxidation and decreases hepatic FA synthesis rates in normal mice [28]. Transfection of human liver hepatocellular carcinoma cell lines (HepG2) with sense or antisense miR-370 or miR-122 upregulated and downregulated, respectively, SREBP-1c and the enzymes diacylglycerol acyltransferase-2 (DGAT2), FAS, and acetyl-CoA carboxylase 1 (ACC1) [43]. On the other hand, miR-370 targets CPT1a, decreasing the rate of beta-oxidation. Interestingly, because miR-370 upregulates the expression of miR-122, it has been suggested that some of the effects of miR-370 on FA and TG metabolism in liver are mediated by miR-122 [43].

TG utilisation by tissue and its storage in adipose tissue are also governed by miRNAs. Lipoprotein lipase (LPL), which catalyses the delivery of TG from the TG-rich lipoprotein, is a direct target of miR-29a [32]. Several miRNAs control TG storage in adipocytes. For example, overexpression of miR-378/378*, miR-9*, miR-143, miR-103, or miR-210 induces TG accumulation in adipose cells by increasing adipogenesis [44-46]. Specifically, miR-378/378*, an intronic miRNA located within the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α), increases the expression of fatty acid binding protein 4 (FABP4), FAS, and stearoyl-coenzymeA desaturase (SCD-1) in adipocytes [45]. In addition, some studies have focused on miRNAs that appear to act as negative regulators of adipocyte differentiation and TG accumulation [46]. For instance, miR-27a targets peroxisome proliferator-activated receptor gamma (PPARγ) and represses PPARγ protein levels, thus inhibiting adipocyte differentiation.

Glucose metabolism

Although glucose metabolism has not been studied as extensively as lipid metabolism, some miRNAs have been linked to glucose homeostasis and insulin sensitivity. For example, miR-375, which is highly expressed in pancreatic islets, is required for normal glucose homeostasis. Mice knockouts for miR-375 are hyperglycaemic and exhibit reduced pancreatic beta-cell mass, increased fasting and fed-plasma glucagon levels, and increased gluconeogenesis and hepatic glucose output [47].

Several miRNAs regulate cell insulin sensitivity by acting on various components of the insulin signalling pathway. For instance, the Let-7 family of miRNAs regulates glucose metabolism in multiple organs because it mediates the repression of multiple components of the insulin signalling pathway, including insulin-like growth factor 1

receptor (IGF1R), insulin receptor (INSR), and insulin receptor substrate 2 (IRS2)[48]. Therefore, Let-7 overexpression in mice resulted in impaired glucose tolerance and reduced glucose-induced pancreatic insulin secretion [49]. Let-7 knockdown mice, however, are protected from glucose tolerance impairment in obesity induced by diet [49]. Both miR-143[50] and miR-33[42] target components of the insulin signalling pathway; in contrast, miR-103 and miR-107 regulate insulin sensitivity by targeting caveolin-1, a component that stabilises the insulin receptor [51].

Another key molecule in glucose homeostasis is GLUT4, the insulin-dependent glucose transporter, which is under the control of several miRNAs. GLUT4 expression is repressed by miR-9* and miR-143 in adipocytes [46] and by miR-133 in cardiomyocytes [52], whereas miR-223 increases GLUT4 expression in cardiomyocytes [53]. Moreover, insulin-dependent glucose uptake is regulated by miR-29a and miR-23a in muscle [54].

Studies of the control of glycolysis by miRNAs have only been performed in cancer cells. Hexokinase 2 (HK2), a hexokinase isoform expressed in cancer cells, is the target of miR-143 and acts as a negative regulator [55, 56]. The miRNA miR-155 upregulates HK2 by repressing mir-143 [57].

Amino acid metabolism

A few studies have focused on the role of miRNAs in controlling amino acid metabolism and protein synthesis and degradation, but only miR-23 has been directly implicated in the regulation of amino acid metabolism. In cancer cells, miR-23 targets glutaminase mRNA, inhibiting glutamine utilisation [58].

Ingestion of essential amino acids, which stimulates muscle protein synthesis, increases miR-499, -208b, -23a, -1, and pri-miR-206 levels in human muscle [59]. Moreover,

miR-23a has been associated with protection against muscle atrophy [60]. Further studies are needed to determine whether these miRNA are implicated in amino acid metabolism and/or protein synthesis.

3. miRNA signature in chronic diseases

As previously mentioned increasing evidence shows that miRNAs are involved in almost all biological processes and affect most metabolic pathways. Hence, aberrant deregulation of some miRNAs has been related to metabolic disorders and other diseases such as human immunodeficiency virus (HIV), cancer, hepatitis C, obesity, CVD, non-alcoholic fatty liver diseases (NAFLD) and type 2 diabetes (T2D). In this sense, miRNAs are emerging as potential biomarkers of numerous pathologies and therefore as new therapeutic targets. The present review examines the miRNAs involved in some of the major chronic diseases: CVD, obesity, T2D and NAFLD.

miRNAs in CVD

CVD is the leading cause of human morbidity and mortality in industrialised countries. These diseases are associated with genetic mutations or deregulation of genes essential for cardiac function, which can also be regulated by miRNAs. Multiple miRNAs important for cardiovascular regulation have been identified and are recognised to control a considerable number of cardiac functions. Furthermore, miRNAs are emerging as potential targets for the diagnosis, prevention and treatment of CVD. ~~Numerous~~ Review articles that describe the role of miRNAs in CVD [61-65] have recently appeared in the literature. Although specific patterns of miRNA expression correlate well with cardiovascular disorders (e.g., cardiac hypertrophy, heart failure, myocardial

infarction, cardiac fibrosis, arrhythmia, angiogenesis and vascular remodelling) [66-73], the mechanisms and alteration of CVD are complex, and it is unclear which miRNAs are important. However, evidence is mounting that some specific miRNAs have a major role in cardiac pathologies, including miR-1 and miR-133 in cardiac hypertrophy [74-76] and the miR-29 family in cardiac fibrosis [70].

One of the most common and important cardiovascular health problems is hypertension, which is defined as a constant elevation of systemic blood pressure. Many characteristics of hypertension development at the molecular level are still unknown, but it is evidently a multifactorial disease that involves several genes. In this sense, miRNAs are likely to have a potential role in regulating these main genes [77-81]. Evidence suggests that specific miRNAs are involved in vascular endothelial pathogenesis in hypertension (e.g., miR-126), acting as pro/anti-angiogenic factors [82], interacting with the renin-angiotensin-aldosterone system (RAAS) (e.g., miR-155) [83] or targeting vascular smooth muscle cells (VSMCs) [84] (e.g., miR-143 and miR-145) [73, 85]. Some miRNAs have also been shown to be related to the nitric oxide (NO) and atrial natriuretic peptide (ANP) pathways in VSMCs [86].

miRNAs in T2D

T2D, which has reached epidemic levels worldwide, is a metabolic disorder that is characterised by hyperglycaemia in the context of reduced insulin sensitivity and insulin resistance. T2D is a complex disease whose disorders are not fully understood. However, it appears that insulin resistance has a major role in the development of this pathology. Moreover, insulin resistance and β-cell dysfunction are mainly developed because of deregulation of adipose tissue function and lipid metabolism [87]. Recently, several studies have shown that miRNAs play major roles in insulin production and

secretion, insulin resistance, pancreatic islet development and β-cell dysfunction (Reviewed in [88-90]). Furthermore, miRNAs are also involved in glucose homeostasis and lipid metabolism related to T2D. Most studies have been based on the miRNA microarray analysis of insulin-resistant tissues, such as skeletal muscle, liver, adipose tissue and pancreatic β-cells, in animal models of spontaneous T2D. In these studies, various miRNAs were shown to be deregulated, but it is still not clear which specific miRNAs are important for T2D and what their roles are. However, some research in this area has been reported, including the deregulation of miR-335 in the adipose tissue of obese mice, which has been correlated to adipocyte differentiation and maturation [91]. The deregulation of miR-27b and miR-335 in the liver of T2D rats, has been suggested to contribute to fatty liver and associated pathologies [91, 92]. Some miRNAs are also involved in the adjustment of skeletal muscle to insulin resistance and T2D. For example, a decrease in miR-24 or miR-126 may help muscles to increase insulin-dependent glucose uptake; miRNAs therefore participate in the adaptation of muscle to high glucose levels [93, 94]. Finally, miR-375 and miR-34a may have an important role in T2D in islets [47, 95].

miRNAs in adipogenesis and obesity

Obesity, characterised by increased fat mass and energy storage in adipose tissue, has reached pandemic proportions in recent years. This pathology is related to diseases such as T2D, hypertension, CVD and cancer [96]. miRNAs are important regulators of the development and function of adipose tissue and metabolic functions and therefore have potential roles in obesity and their associated diseases (Reviewed in [46, 97, 98]). Several studies have demonstrated that miRNAs acts as central modulators of normal white and brown adipose tissue differentiation and biology. Many miRNAs that are

downregulated in obesity are upregulated during adipogenesis, and vice versa [44, 99]. In this sense, several miRNAs regulate, enhance and inhibit adipogenesis (e.g., miR-143), suggesting that miRNAs have a potential role in controlling adipocyte number and size. However, miRNAs govern not only mass size but also the metabolic consequences of obesity and adipose tissue metabolism [98]. More evidence for the role of miRNAs in obesity-related diseases is necessary to understand their regulatory roles in modulating energy balance, adipose biology and their potential contribution to obesity [12].

miRNAs in NAFLD

NAFLD is characterised by fat accumulation in the liver without significant alcohol consumption [100]. Clinical manifestations of this pathology include dyslipidaemia, hypertension and insulin resistance. Recently, the involvement of miRNAs in NAFLD has been described (Reviewed in [101, 102]). It has been demonstrated that miRNAs are able to modify lipid droplet accumulation in hepatocytes, which is characteristic of NAFLD [103]. Other studies have showed that some miRNAs target PPARα, a key molecule for NAFLD [104]. Studies in humans have demonstrated altered hepatic miRNAs profiles in steatohepatitis (NASH), in which miR-122 is remarkably downregulated [105].

Circulating miRNAs as biomarkers for chronic diseases

miRNAs are good candidate biomarkers of diseases because they are stable, conserved, tissue-specific, pathology-specific and detectable in serum, plasma and other biological fluids [106]. miRNAs circulating in the plasma are remarkable stable because they circulate packed inside microparticles (microvesicles, exosomes and apoptotic bodies) [80, 107] or associated with RNA-binding proteins (Argonaute2) [108] or lipoprotein

complexes (HDL) (Reviewed in [109, 110]). Because miRNAs circulate with microparticles, they may function in cell-to-cell communication, as suggested by several studies [80, 111]. Because circulating miRNAs are transported from donor cells to surrounding tissue, they alter the genes and functions of recipient cells and therefore have a role in endocrine and paracrine communication (reviewed in [12]). Furthermore, miRNAs can originate from exogenous sources, such as ingested plants [30].

A major challenge for chronic disease research is the identification of reliable biomarkers that can be measured in a non-invasive way using accessible samples such as plasma or serum. Therefore, miRNAs in plasma and serum are beginning to be studied as biomarkers for chronic disease, and altered circulating miRNAs profiles have already been correlated to several diseases states. Circulating liver-specific miR-122 was found to be a good biomarker for hepatic injuries such as NAFLD and NASH [112-114]. Deregulation of circulating miR-223 was correlated with atherosclerosis [109], miR-126 to T2D [115], and Let-7a to hypertension [116], and circulating miR-499-5p was postulated to be a sensitive biomarker for non ST-elevation myocardial infarction [117]. Other examples are miR-17-5p and miR-132, which are differentially expressed in obese and non-obese subjects in peripheral blood, suggesting their potential role as novel metabolic biomarkers [118]. Increased levels of miR-122 and miR-370 in plasma were found in patients with coronary artery disease in hyperlipidaemia [119].

4. Modulation of miRNA levels by polyphenols

Polyphenols have beneficial properties in almost all chronic diseases, and recently polyphenol extracts and polyphenols such as quercetin or resveratrol have been shown to modulate the expression of miRNAs.

Epigallocatechin gallate (EGCG) was evaluated in HepG2 cells using a range of times and concentrations. Using 50 μM EGCG and a 5-h cell treatment, 5 miRNAs were downregulated by EGCG, miR-30b*, miR-453, miR-520e, miR-629 and miR-608 [15]. Using 100 μM EGCG and a 24-h treatment, 13 miRNAs were upregulated, such as let-7a, miR-16 and miR-221, and 48 miRNAs were downregulated, such as miR-18a, miR-34b, miR-193b, miR-222 and miR-342 [120]. Therefore, the number and types of miRNAs deregulated by EGCG depends on the time and polyphenol concentration of the treatment. Furthermore, EGCG treatment of other cell lines, such as lung cancer cells, showed deregulation of other miRNAs, such as miR-210 [121].

Quercetin, which is a major representative of the flavonol subclass of flavonoids, also has been reported to modulate miRNAs. Specifically, quercetin and isorhamnetin, upregulate miR-155 levels in macrophages activated by lipopolysaccharide (LPS). However, quercetin metabolites, such as quercetin-3-glucoronide, do not [122]. *In vivo* studies showed that miR-122 and miR-125b are upregulated in the livers of mice fed with quercetin-enriched diets (2 mg quercetin per g diet), at 61 and 48%, respectively [123].

Resveratrol is the phenolic compound that has been most studied regarding its relationship to miRNAs. In human non-small cell lung cancer cells, line A549, the number of miRNAs that resveratrol modifies depends on the concentration. Some of the miRNAs showed more than a 20-fold change, such as miR-299-5p, miR-194*, miR-338-3p, miR-758, miR-582-3p and miR-92a-2* [124]. Likewise, in a human colon cancer cell line, SW480, resveratrol decreased the levels of oncogenic miRNAs, such as miR-17, miR-21, miR-25, miR-26a, miR-92a-2, miR-103-2, and miR-181a2. Moreover, resveratrol increased the levels of the tumour-suppressor miR-663 [125]. In a transformed human bronchial epithelial cell line, 16HBE-T, miR-622 was upregulated

by resveratrol [126]. These and other studies in cancer cells provide evidence that resveratrol can modulate miRNA expression by downregulating oncomiRs and upregulating tumour-suppressors miRs in cancer cells. In contrast, in a monocyte cell line, THP-1, resveratrol upregulates miR-663 and impairs the upregulation of the pro-inflammatory miR-155 [127]. Interestingly, resveratrol also modulates heart and skeletal muscle functions through miRNAs, such as miR-20b, miR-149, miR-133, miR-21 and miR-27b [128].

Another polyphenolic compound that it has been studied for its effects on miRNAs is the ellagitannin BJA3121. This ellagitannin modulated 25 miRNAs; 17 were upregulated and 8 were downregulated in HepG2 cells. Surprisingly, 7 of the 17 upregulated miRNAs (i.e., miR-526b, miR-373*, miR-518f*-526a, miR-525, miR-519e*, miR-518c* and miR-512-5p) were located in the same cluster. Moreover, 3 of the 8 downregulated miRNAs (i.e., let-7a, let-7f and let-7a) were also located in another cluster. This result suggests that ellagitannin acts on the regulatory region of these gene clusters [129].

A *Hibiscus sabdariffa* phenolic extract was observed to modulate the expression of miRNAs expression in the livers of mice deficient in the LDL receptor. Interestingly, the continuous administration of this extract reversed the effect of a high fat diet, increasing the expression of miR-103 and miR-107. However, miR-122, which was not affected by the diet, was repressed by the polyphenol extract [130]. In contrast, a polyphenol extract from *Ilex vomitoria* leaves upregulates miR-146a, which is a negative regulator of nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), in human colon fibroblast [131]. Finally, proanthocyanidin extracts from cocoa (CPE) and grape seed (GSPE) deregulates different miRNAs in HepG2 cells under the same experimental conditions, and only miR-30b* was downregulated and miR-1224-

3p, miR-197 and miR-532-3p were upregulated by the two extracts [15]. Recently, we have shown that GSPE downregulated miR-122 and miR-33 and their target gene FAS and ABCA1, respectively in liver cells, both *in vivo* and *in vitro* [132]. These results suggest that each polyphenol extract may influence particular miRNAs. Polyphenols can have a variety of structures and the characteristic composition of an extract varies based on its botanical origin [16]. Therefore, a specific polyphenol, or a specific interaction between compounds, may affect a specific miRNA.

Altogether, these results provide evidence of the ability of dietary polyphenols to influence miRNA expression, suggesting a new mechanism of action for polyphenols. However, further studies are needed in humans to elucidate the effects of polyphenolic extracts on miRNAs and the metabolic pathways affected by these small molecules, showing a cause-effect relationship.

5. Relationship between polyphenol miRNA modulation and their health effects on chronic diseases.

The majority of studies involving miRNAs as mediators of polyphenol effects in cells have been performed in cancer cells, and only a few studies have been centred on metabolic diseases. As we indicated in the last section, several research groups have studied the ability of polyphenols to modulate miRNAs using microarray technology. In table 1, we present those miRNAs that are deregulated by polyphenols and are known to regulate metabolism and be involved in chronic diseases other than cancer. From all of the miRNAs described as targets of polyphenols in the literature, only 16 miRNAs are clearly involved in metabolic control and chronic diseases. Furthermore, the referenced polyphenols (or polyphenols extract) do not target all 16 miRNAs, and some of them are only targeted by one polyphenol. This is not surprising because the experimental

conditions (e.g., cell line, tissues, etc.) and treatment conditions (e.g., time, dose, etc.) are different between studies.

Although the identity of miRNAs that are common targets for all polyphenols seems unclear, the liver-specific miR-122 is a clear putative target of polyphenols. miR-122 is targeted by different types of polyphenols (i.e., a polyphenol extract from *Hibiscus sabdariffa*, quercetin, ~~and~~ coffee polyphenols and grape seed proanthocyanidins) in mice livers. ~~Moreover, results from our laboratory indicate that GSPE also targets miR-122, both in rat liver and hepatic cell lines (unpublished results)~~. miR-122 controls cholesterol and bile acid biosynthesis and FA oxidation in liver as well as being related to NAFLD. Interestingly, polyphenol extracts from *Hibiscus sabdariffa* [130], quercetin [133] and coffee polyphenols [134] prevent diet-induced liver steatosis in mice. Moreover, these polyphenols repress the expression of SREBP-1c [133, 134], ACC1 [134], ~~and~~ CYP7A1 [135], and FAS [132] which are also under the control of miR-122 in the liver. Clinical manifestations of NAFLD include dyslipidaemia, hypertension and insulin resistance. Therefore, the health benefits of polyphenols for dyslipidaemia, hypertension and insulin resistance partially could be caused by improvement of the liver metabolism, resulting from the targeting of miR-122. Relating to lipid metabolism proanthocyanidins also repress miR-33, which plays a crucial role in cholesterol homeostasis and lipoprotein levels.

In addition to miR-122, other miRNAs are influenced by specific polyphenols in hepatic cell lines. Specifically, EGCG and ellagitannin modulate the expression of some components of the Let-7 family and miR-210 in HepG2, and both of them are related to insulin sensitivity. The Let-7 family regulates glucose metabolism in multiple organs as result of these miRNAs mediating the repression of several components of the insulin

signalling pathway [48, 49]. In contrast, miR-210 is upregulated in the liver of diabetic rats [88] and downregulated in the fat of obese humans [90].

In addition, the modulation of miRNAs by polyphenols has been studied in monocytes and macrophages. All of the polyphenols studied in macrophages, including resveratrol, quercetin and isorhamnetin, target miR-155. Levels of miR-155 in serum are proposed as a biomarker of CVD [110], and this miRNA is linked to inflammatory responses in macrophages [136]. The inflammatory response of macrophages is a key feature in the pathogenesis of atherosclerosis, and interestingly, quercetin metabolites are accumulated in human atherosclerotic lesions but not in the normal aorta [137, 138]. Activated macrophages show the accumulation of quercetin metabolites, suggesting that this accretion underlies the anti-atherosclerotic activity of this polyphenol [138]. miR-155 has also been related to hypertension through targeting the RAAS [81]. Protection from atherosclerosis and hypertension is a generalised effect of polyphenols [139-142]. However, further studies with other polyphenols are necessary to confirm miR-155 as a real target of polyphenols.

The modulation of miRNAs in the heart by polyphenols has recently been studied using resveratrol, which targets miR-27a. miR-27a is upregulated during cardiac hypertrophy [143]. Furthermore, miR-27a controls the phosphoinositide 3-kinase (PI3K) pathway that regulates physiological hypertrophy and cardiac protection [61]. Resveratrol reduces cardiac hypertrophy in hypertensive animals [144], and several signalling pathways affected by resveratrol (or its analogues) in the heart have been described to influence this effect, including the AKT/PI3K pathway [144, 145].

6. Concluding remarks

A single miRNA can regulate the expression of multiple target mRNAs, and a particular transcript can be modulated by multiple miRNAs. To date, thousands of miRNAs have been discovered, and it is thought that these small molecules may regulate more than 60% of all cell transcripts [20]. Hence, the fact that dietary compounds modulate miRNAs suggests new functions of polyphenols and provides insights into the mechanisms by which these compounds improve health and protect from diseases. However, the information in human is poor and scarce and most of the evidence is only observational and do not show a cause-effect relationship. Therefore, more studies are needed with other polyphenols and different cell types, animal models and more specifically in humans to establish the target miRNAs of polyphenols. However, evidence of these effects is being uncovered, and some clear targets of polyphenols, such as miR-122, can be identified. This research will be important because the modulation of key miRNAs implicated in chronic diseases by natural products, such as polyphenols, has a great potential for dietary applications.

Currently, the molecular mechanism by which polyphenols modulate miRNAs levels is unknown. However, there is evidence that polyphenols can bind to mRNAs and proteins [146, 147]. Therefore, it is possible that they also bind to miRNAs or to some component involved in miRNA biogenesis, such as DICER or RISC. Additionally, some miRNAs are intronic of genes and polyphenols that modify host gene expression, which would also affect the miRNA levels.

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7. References

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Table 1. miRNAs deregulated by polyphenols and involved in chronic diseases and metabolic control.

miRNA	Polyphenol	Up/down regulation	Experimental condition	Metabolic pathway	Chronic disease
Let-7a	EGCG [120]	up	100 μM, 24 h HepG2 cells	Glucose metabolism, insulin sensitivity	Hypertension [61] Heart hypertrophy [61]
	Ellagitannin [129]	up	15 μg/mL, 6 h, HepG2 cells		
Let-7b	EGCG [120]	up	100 μM, 24 h HepG2 cells	Glucose metabolism, insulin sensitivity	Diabetes [88] Obesity [90]
Let-7c	Resveratrol [148]	down	50 μM, 24 h prostate cancer cells	Glucose metabolism, insulin sensitivity	Heart failure [149] Diabetes [88]
	EGCG [120]	up	100 μM, 24 h HepG2 cells		
miR-23a	Resveratrol [125]	down	50 μM 14 h, SW480 colon cancer cells	Insulin-dependent glucose transport	Heart failure [149] Cardiac hypertrophy [64]
miR-27a	Resveratrol [128]	up	5 mg/kg/day for 21 days. Ischemic heart of rat	TG storage in adipocytes	HBV-related HCC [150] (adipocyte hypertrophy) Obesity [46, 98] Cardiac hypertrophy [143] Diabetes [88]
miR-29a	Ellagitannin [129]	up	15 μg/mL, 6 h, HepG2 cells	Lipoprotein lipase, insulin-dependent glucose transport	Liver fibrosis [150] T2D [88, 90] Obesity [89] Insulin resistance [97] Cardiac hypertrophy [88]

miR-33	Grape seed proanthocyanidins [132]	down	250 mg/Kg for 1h, mice liver 25mg/L for 1h, FAO cells	Cholesterol efflux, HDL biogenesis and VLDL levels. Fatty acid metabolism and insulin signaling	Atherosclerosis [36]
miR-103	Polyphenol extract (Hibiscus sabdariffa) [130]	up	28.6 mg/kg.day, 10 weeks Liver hyperlipidaemic mice	TG storage in adipocytes Insulin sensitivity	Obesity [46, 89, 90, 98] Diabetes [12, 88, 90, 115] Insulin resistance [97]
miR-107	Polyphenol extract (Hibiscus sabdariffa) [130]	up	28.6 mg/kg.day, 10 weeks Liver hyperlipidaemic mice	Insulin sensitivity	Obesity [46, 89] T2D [90] Diabetes [12, 115]
	Ellagitannin [129]	down	15 µg/mL, 6 h, HepG2 cells		
miR-122	Polyphenol extract (Hibiscus sabdariffa) [130]	down	28.6 mg/kg.day, 10 weeks Liver of hyperlipidaemic mice	Cholesterol synthesis Bile acid biosynthesis Fatty-acid oxidation	NAFLD and NASH [97, 150]
	Quercetin [123]	up	2 mg/g diet, 6 weeks, mice liver		
	Coffee polyphenols [134]	up	0.5 to 1.0% for 2-15 wk, mice liver 2.5µg/mL 24 h Hepa 1-6 cells		
	Grape seed proanthocyanidins [132]	down	250 mg/Kg for 1h, mice liver 25mg/L for 1h, FAO cells		
	Resveratrol [125]	down	50 µM 14 h, SW480 colon cancer cells	LDL uptake	ALD/NAFLD [150] T2D [90]

miR-146a	polyphenol extract (yaupon holly leaves) [131]		50 μM, 14 h, SW480 colon cancer cells		Heart failure [63] Apoptosis [115] Inflammation [136]
	Ellagitannin [129]	up	15 μg/mL, 6 h, HepG2 cells		
miR-155	Resveratrol [127]	down	50 μM, 14 h Human THP-1 monocytic cells and human blood monocytes	Glycolysis LDL uptake	Inflammation [136, 150] Hypertension [83]
	Quercetin [122]	up	10 μM, 6 h, murine RAW264.7 macrophages		
	Isorhamnetin [122]	up	10 μM, 6 h, murine RAW264.7 macrophages		
miR-206	Resveratrol [124, 125]	down	120 μM, 24 h A549 human non-small cell lung cancer cell	Related to protein synthesis in muscle	Obesity [46, 98] Diabetes [89] Apoptosis [90]
		up	50 μM 14 h, SW480 colon cancer cells		
miR-210	EGCG [120]	down	100 μM, 24 h HepG2 cells	TG storage in adipocytes	T2D [88] Obesity [90]
		up	40 μM, 9h human and mouse lung cancer cells		
	Ellagitannin [129]	up	15 μg/mL, 6 h, HepG2 cells		
miR-223	Ellagitannin [129]	down	15 μg/mL, 6 h, HepG2 cells	GLUT4 in myocytes	Diabetes [89]
miR-370	Ellagitannin [129]	down	15 μg/mL, 6 h, HepG2 cells	Fatty acid oxidation TG synthesis	NAFLD [151]
	EGCG [120]	down		Bile acid biosynthesis	Obesity [97, 98]

miR-422			100 μM, 24 h HepG2 cells		T2D [88]