

Potential Roles of Adipocyte Extracellular Vesicle–Derived miRNAs in Obesity-Mediated Insulin Resistance

Yujeong Kim¹ and Ok-Kyung Kim^{1,2}

¹Division of Food and Nutrition, Chonnam National University, Gwangju, Republic of Korea; and ²Human Ecology Research Institute, Chonnam National University, Gwangju, Republic of Korea

ABSTRACT

Recently, extracellular microRNAs (miRNAs) from adipose tissue have been shown to be involved in the development of insulin resistance. Here, we summarize several mechanisms explaining the pathogenesis of obesity-induced insulin resistance and associated changes in the expression of obesity-associated extracellular miRNAs. We discuss how miRNAs, particularly miR-27a, miR-34a, miR-141-3p, miR-155, miR210, and miR-222, in extracellular vesicles secreted from the adipose tissue can affect the insulin signaling pathway in metabolic tissue. Understanding the role of these miRNAs will further support the development of therapeutics for obesity and metabolic disorders such as type 2 diabetes. *Adv Nutr* 2021;12:566–574.

Keywords: extracellular vesicle, miRNAs, obesity, insulin resistance, insulin signaling

Introduction

Insulin is a hormone that is secreted by pancreatic B cells in response to increased circulating glucose concentrations. It reduces blood glucose concentrations by binding to the insulin receptor (IR) in the cell membrane of tissues such as the liver, skeletal muscles, and in adipose tissue. Insulin stimulates the synthesis of glycogen, lipids, and protein and inhibits glucose production in the liver (1, 2). Although insulin plays many physiological roles, its key function is to regulate glucose homeostasis as it is the only hormone that can lower blood glucose concentrations. Thus, insulin resistance results in systemic hyperglycemia by impairing the ability of insulin to stimulate glycolysis and inhibit gluconeogenesis, which is closely linked to the pathogenesis

of metabolic disorders such as type 2 diabetes, nonalcoholic fatty liver disease, and other metabolic syndromes (3, 4).

Obesity is directly or indirectly associated with the development of insulin resistance. Numerous studies have investigated several molecular mechanisms linking the pathogenesis of obesity to insulin resistance (5, 6). Here, we present a review of several such mechanisms to explain the pathogenesis of obesity-induced insulin resistance, including inflammation and cellular stress. In addition, we provide an overview of the role of extracellular vesicle (EV) microRNAs (miRNAs) as novel metabolic regulators during the development of obesity-induced insulin resistance. In particular, this review covers the effect of EV miRNAs secreted from adipose tissue on the insulin signaling pathway in metabolic tissues including the adipose tissue, muscle, and liver. This review investigates this novel mechanism underlying the development of obesity-mediated insulin resistance and how this could provide a potential avenue for miRNA-based therapeutics for obesity and type 2 diabetes.

Obesity-Associated Insulin Resistance Development

Insulin signaling pathway

Insulin acts by binding to the IR, which is a transmembrane protein comprising 2 extracellular α -subunits and

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Address correspondence to O-KK (e-mail: 20woskxm@chonnam.ac.kr).

Abbreviations used: AMPK, AMP-activated protein kinase; AS160, Akt substrate of 160 kDa; CERK, ceramide kinase; ER, endoplasmic reticulum; EV, extracellular vesicle; FoxO1, forkhead box protein O1; GLUT4, glucose transporter 4; GSK3, glycogen synthase kinase 3; IKK β , I κ B α kinase β ; iNOS, inducible NO synthase; IR, insulin receptor; IRS, insulin receptor substrate; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; KLF4, Kruppel-like factor 4; MCP-1, monocyte chemoattractant protein 1; miRNA, microRNA; mTOR, mammalian target of rapamycin; MV, microvesicle; MVB, multivesicular body; NDUFA4, NADH dehydrogenase (ubiquinone) 1 α subcomplex 4; PI3K, phosphoinositide 3 kinase; PPAR γ , peroxisome proliferator-activated receptors γ ; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SOCS1, suppressor of cytokine signaling 1.

2 intracellular β -subunits. The tyrosine kinase activity of the β -subunits is induced when insulin binds to the α -subunits. This triggers autophosphorylation in the β -subunits as well as activation of docking and tyrosine phosphorylation of intracellular proteins known as insulin receptor substrates (IRSs). Phosphorylation of IRS-1 at a tyrosine residue activates phosphoinositide 3 kinase (PI3K), which comprises a heterodimer consisting of a p110 catalytic subunit and a p85 regulatory subunit. The p85 regulatory subunit is responsible for PI3K activity and is an important factor regulating the insulin signaling pathway (7). Activated PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3), which then activates Akt [also known as protein kinase B (PKB)]. The downstream substrates of Akt include Akt substrate of 160 kDa (AS160), glycogen synthase kinase 3 (GSK3), mammalian target of rapamycin (mTOR), and forkhead box protein O1 (FoxO1) (8–10). AS160 promotes glucose uptake into the muscle and adipose tissues via the translocation of glucose transporter 4 (GLUT4)-containing storage vesicles to the plasma membrane (11). Deactivation of GSK3 by Akt-mediated phosphorylation stimulates glycogen synthesis by dephosphorylating and activating glycogen synthase. mTOR stimulates protein synthesis and inhibits the translocation of the transcription factor FoxO1 into the nucleus via Akt-mediated phosphorylation; it also inhibits the expression of enzymes involved in gluconeogenesis such as phosphoenolpyruvate carboxykinase (PEPCK) (8–10). Thus, this series of intermediary steps, following insulin binding, provides an integrated set of signals for balancing nutrient availability, particularly the promotion of anabolic metabolism. However, when IR is negatively regulated by dephosphorylation, it can lead to insulin resistance via a negative feedback mechanism.

Negative regulators of insulin activity in obesity

In this article, we have comprehensively summarized the negative regulators of insulin activity in obesity-mediated insulin resistance, focusing on inflammatory mediators, cellular stress, and adipocyte factors. Obesity is characterized by chronic low-grade inflammation in white adipose tissue. In low-fat adipose tissue, most adipose tissue-resident macrophages are polarized to M2 macrophages, which contribute to insulin sensitivity by secreting IL-10 and arginine substrates. However, in adipose tissue, adipocytes release monocyte chemoattractant protein 1 (MCP-1) and proinflammatory cytokines, which induce monocyte infiltration and M1 macrophage polarization with increased production of inducible NO synthase (iNOS) and proinflammatory cytokines, further promoting local inflammatory responses in adipose tissue. This obesity-associated inflammation is closely related to insulin resistance and other well-known metabolic abnormalities (12, 13). Kamei et al. (14) reported that, in mice, MCP-1 overexpression caused macrophage accumulation and increased proinflammatory cytokine mRNA levels in the adipose tissue. In addition, suppression of insulin signaling has been observed in both the skeletal muscle and

liver of mice with MCP-1 overexpression, suggesting that MCP-1-induced inflammation in adipose tissue contributes to the development of insulin resistance.

The proinflammatory cytokines, TNF- α , IL-6, and IL-1 β are overproduced in adipose tissue and polarized M1 macrophages in conditions of obesity; these cytokines stimulate the c-Jun N-terminal kinase (JNK) and I κ B α kinase β (IKK β)/NF- κ B pathways not only in adipose but also in other tissues (12, 13, 15). The JNK and IKK β /NF- κ B pathways play an important role in inflammation-induced insulin resistance in obesity. When the proinflammatory cytokines bind the receptors, the JNK and IKK β /NF- κ B pathways trigger the upregulation of target genes and inflammatory mediators such as TNF- α , IL-6, and IL-1 β , and this induces the blocking of IR signaling (13, 15). Aguirre et al. (15) showed that anisomycin, a strong activator of JNK, inhibits insulin-stimulated tyrosine phosphorylation of IRS-1 in vitro. Cai et al. (16) reported that a high-fat diet induced insulin resistance and inflammation, whereas inhibitors of IKK β and NF- κ B reversed insulin resistance. Consequently, we can surmise that the activation of JNK and IKK β /NF- κ B pathways by circulating inflammatory mediators contributes to the progression from inflammation to insulin resistance in obesity.

Obesity-induced insulin resistance is associated with cellular stress signaling such as oxidative stress and endoplasmic reticulum (ER) stress (17, 18). When excess nutrient intake causes an oversupply of fatty acids and glucose in mitochondria, reactive oxygen species (ROS) are overproduced via mitochondrial oxidation. The level of ROS overproduction reaches beyond the threshold, and when the balance between ROS production and the antioxidant defense system is disturbed, oxidative stress occurs (18). Under conditions of oxidative stress, ROS can inhibit insulin signaling by activating the JNK and IKK β /NF- κ B pathways, similar to that in an inflammatory response (19). Wen et al. (20) demonstrated that palmitate induces the activation of the NLRP3-ASC inflammasome for IL-1 β production that is involved in mitochondrial ROS and AMP-activated protein kinase (AMPK) activation. Han (18) reported that ROS is the key regulator of early events in obesity-induced inflammation and that mitochondria-derived ROS lead to the development of insulin resistance as well as inflammation in the late stages of obesity. Therefore, ROS not only inhibits insulin signaling but also induces an inflammatory response, thereby adversely affecting insulin resistance.

Many recent studies have reported that obesity-mediated inflammation is associated with ER stress and affects insulin signaling. The ER plays an essential role in calcium storage, lipid synthesis, and protein folding. Unfolded proteins induce the dissociation of chaperone proteins from each ER transmembrane protein, thereby activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1). The activation of ER transmembrane proteins leads to chaperone production to promote protein maturation; this response is termed the unfolded protein response (UPR). When an imbalance occurs between the cellular demand

for protein folding and the ability of the ER to promote protein maturation, accumulation of unfolded proteins is promoted in the ER lumen; this is defined as ER stress (17). Several studies have demonstrated that ER stress leads to JNK and IKK β /NF- κ B pathway activation and ROS accumulation, which may subsequently induce inflammatory mediator production, and these inflammatory mediators can, in turn, induce ER stress. Many studies have demonstrated that obesity-associated insulin resistance is associated with inflammation and oxidative stress as well as ER stress both in vitro and in vivo (21). Taken together, oxidative stress and ER stress affect each other and lead to mitochondrial dysfunction, thereby adversely affecting insulin resistance.

Adipocytes can secrete proinflammatory cytokines as well as metabolically active proteins such as leptin and adiponectin, the absence of which leads to dramatic metabolic disturbance (22). Leptin is a hormone that regulates appetite and energy balance by acting on receptors in the hypothalamus. In addition, leptin binds the receptor Ob-Rb and phosphorylates Janus kinase (JAK) 2, which leads to IRS and Akt activation, thereby affecting insulin signaling. Leptin in normal muscle tissue stimulates fatty acid oxidation by inhibiting acetyl-CoA carboxylase (ACC) activation by activating AMPK, which helps improve insulin sensitivity. However, in conditions of obesity, leptin resistance develops, and this signal does not function effectively, leading to an increase in serum leptin concentrations. Obesity-induced leptin resistance adversely affects insulin signals, resulting in decreased glucose uptake and glycogenolysis in muscles and increased gluconeogenesis in the liver, leading to the development of insulin resistance (22, 23).

Adipocyte-secreted adiponectin also plays a key role in obesity-related insulin resistance. Achari and Jain (24) reported that the adiponectin signaling pathway directly interacts with insulin receptor substrates. When adiponectin binds to its receptor, it activates protein phosphatase 2A, resulting in the activation of AMPK and dephosphorylation and inactivation of protein kinase C (PKC), which phosphorylates and inhibits the insulin receptor. Thus, adiponectin plays a major role in improving insulin sensitivity. Several studies have shown that a high-fat diet inhibits adiponectin activity and decreases serum adiponectin concentrations relative to those observed in response to a normal diet. Reduced adiponectin concentration may be a major factor as it is an important physiological regulator of obesity-induced insulin resistance (22, 25). Therefore, adiponectin replacement therapy may be effective at treating obesity and insulin resistance.

miRNAs Released from Adipocytes through EVs in Obesity

EVs and miRNA loading

Almost all cell types can produce EVs that act as mediators of cell–cell communication (26). Cells can release different types of EVs, exosomes, microvesicles (MVs), and apoptotic vesicles, which differ in size, biogenesis pathway, and

biological function. Exosomes (40–100 nm in diameter) are secreted from normal or diseased cells and are formed upon the fusion of the multivesicular bodies (MVBs) with the plasma membrane. MVs (100–1000 nm in diameter) are also secreted from normal or diseased cells but differ from exosomes in that they are formed at the cell membrane surface directly by budding. In apoptotic cells (1–4 μ m in diameter), cells shed apoptotic vesicles by outward blebbing of the membrane that can be removed by phagocytosis. Most body fluids, such as urine, blood, breast milk, and saliva, contain MVs or exosomes, which are structurally similar but induce different extrinsic signals (26, 27).

miRNAs are a major class of small noncoding RNAs that regulate gene expression by post-transcriptional gene silencing via binding to specific target mRNAs. A single miRNA can regulate several mRNA targets, so the abnormal expression of 1 miRNA can affect a wide range of biological processes in both normal and pathological conditions. Although most miRNAs regulate gene expression inside cells, extracellular miRNAs can affect gene expression in recipient cells after loading into and circulating within MVs or exosomes (28, 29). miRNAs packaged within EVs are protected from RNase degradation and can be effectively delivered to recipient cells. In addition, circulating miRNAs in EVs contain attractive candidate biomarkers for the diagnosis of disease (28, 29). Chen et al. (30) analyzed miRNA profiles in cell lysates and EVs, including MVs and exosome, from the isogenic colorectal cancer cell line, and demonstrated the selective packaging of cellular miRNAs into MVs and exosomes. In addition, they discovered that the profiles of several miRNAs in MVs and exosomes differ. This result suggests that the sorting of miRNAs into EVs in cells occurs selectively, and that the mechanism by which miRNAs are loaded into MVs and exosomes is different. The mechanism that determines the specific loading of miRNA into EVs is yet to be understood completely. Nevertheless, Villarroya-Beltri et al. (31) have demonstrated major advances in how miRNAs are loaded into exosomes and secreted from cells. They determined that the sumoylated heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) specifically binds exosomal miRNAs and plays an important role in determining the content of exosomal miRNAs. In addition, some studies have suggested that protein argonaute-2 (AGO2) phosphorylation and neutral sphingomyelinase 2 (nSMase2) regulate not only exosome secretion but also secretion of miRNAs (32). However, the research on mechanisms of miRNA loading into EVs and secretion remains challenging.

Expression patterns of extracellular miRNA in obesity

Recently, circulating miRNAs were identified as novel adipokines that can affect pathophysiological mechanisms involved in obesity-induced metabolic syndromes, and thus serve as attractive potential biomarkers. Several studies have demonstrated different extracellular miRNA expression patterns between the normal and obese states and examined the effects of miRNAs secreted from obesity-induced adipocytes

in metabolic disease. Most studies on extracellular miRNA expression patterns in obesity involve isolation of miRNAs from blood, and only a few have studied EVs; however, extracellular miRNAs are abundant in plasma-derived EVs (33–36, 32, 37–61). In this review, we summarize in **Table 1** the obesity-associated changes in circulating miRNAs and their expression patterns.

Expression patterns of circulating miRNAs in obesity.

In 2011, Heneghan et al. (33) investigated blood miRNAs in specimens from individuals with obesity versus those from normal individuals for the first time. They showed that the concentrations of blood-carried miR-17-5p and miR-132 were decreased in omental fat tissue from individuals with obesity compared with those from normal individuals and suggested that extracellular miRNAs are potentially important players in metabolic pathways. Pescador et al. (34) found that serum miR-138, miR-376a, and miR-503 concentrations were decreased and serum miR-15b concentration was increased in obesity [BMI (kg/m²): 42.73 ± 4.67] compared with that in controls (BMI: 22.7 ± 2.43); they further suggested these miRNAs are potential biomarkers in obesity. Wang et al. (35) found that the concentrations of circulating miR-130b were increased in *ob/ob* mice as well as in Chinese individuals with obesity; in addition, they found that adipocytes secrete miR-130b, which regulates muscle metabolism by targeting peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α). Prats-Puig et al. (36) also showed increased concentrations of circulating miR-130b in individuals with obesity compared with those in lean individuals. However, Ortega et al. (32) and Thomé et al. (37) found an opposite result. Ortega et al. (32) showed decreased concentrations of miR-130b in morbidly patients with obesity, and Thomé et al. (37) showed decreased concentrations of miR-130b in patients with obesity and heart failure compared with those in lean patients with heart failure and controls. Additionally, miR-21, miR-423-5p, and miR-532-5p were found in both the increased miRNAs and decreased miRNAs. Therefore, these miRNAs have not yet proven to be biomarkers of obesity. However, the concentrations of circulating miR-122, miR-222, and miR-320a followed the same trend in these 3 papers (36, 32, 37). Increased circulating miR-122 concentrations were associated with childhood obesity, young-adult obesity, and mouse obesity, while increased circulating miR-222 concentrations were associated with childhood obesity and patients with morbid obesity and were correlated with BMI; decreased circulating miR-320a concentrations were also associated with childhood obesity and adult obesity (36, 32, 37). Thus, these 3 miRNAs may be suitable for use as biomarkers for diagnosing obesity.

Expression patterns of EV miRNAs in obesity.

In 2011, Müller et al. (38) showed that the concentrations of miR-16, miR-27a, miR-146b, and miR-222 were increased in MVs from large primary rat adipocytes and older rats compared with those in small adipocytes and younger

rats. Although these results were identified for miRNAs from MVs, this study suggested that obesity affects the secretion of specific extracellular miRNAs. Increased miR-155 concentrations have been identified in MVs from mice, in which obesity was induced by a high-fat diet, and caused M1 macrophage polarization by targeting suppressor of cytokine signaling 1 (SOCS1) and JAK/signal transducers and activators of transcription (STAT) signaling (39).

Exosomes have also shown different patterns of specific miRNAs in obesity. During MVB formation, pre-miRNAs or mature miRNAs can be selected and sorted into the intraluminal vesicles in MVBs, and can then be secreted from cells as exosomes, in the form of secreted intraluminal vesicles. Secreted exosomal miRNA is delivered to target cells, and the physiological functions of exosomes can be largely deduced based on the content of these miRNAs. Exosomes and exosomal miRNAs have been demonstrated to be involved in multiple processes during the development of several diseases. Therefore, exosome-carried miRNAs may play an important role in the therapy and diagnoses of such conditions, and consequently, this has resulted in an expansion of interest in exosomes during recent years (28, 29).

Ferrante et al. (40) demonstrated altered expression of exosomal miRNAs between obesity and lean subjects. They demonstrated upregulation of miR-23b and miR-4429 and downregulation of miR-148b and miR-4269, which are correlated with transforming growth factor β (TGF- β) and Wnt/ β -catenin signaling pathways. We show in **Table 1** that miR-122 in exosomes and miR-222 in MVs are both increased in the obese state. This trend matches that of circulating miRNAs in other obesity studies. Obesity leads to changes in exosomal miRNAs from not only adipose tissue but also from adipose tissue macrophages. Ying et al. (41) demonstrated obesity-induced changes in the expression of exosomal miRNAs from adipose tissue macrophages by deep sequencing of small RNAs. In particular, the increase in exosome miR-155 from obese adipose tissue macrophages in this study displays a similar trend as the increase in miR-155 in MVs in individuals with obesity (39, 41).

Most of the studies that examined changes in exosomal miRNAs in obesity have demonstrated that these affect metabolic regulation, including obesity-mediated insulin resistance, by delivering miRNAs to target cells. This supports the hypothesis that obesity-mediated insulin resistance develops in response to exosomal miRNAs that act as novel adipokines. In this review, we summarize the findings on the potential role played by EV miRNAs released from adipocytes in obesity-mediated insulin resistance.

Function of Adipocyte-Derived Extracellular miRNAs in Insulin Resistance

In the recent paper by Thomou et al. (60), circulating exosomal miRNAs from the adipose tissue of distant tissues were demonstrated to act as regulators of glucose tolerance and expression of hepatic fibroblast growth factor 21 (FGF21). Extracellular miRNAs from adipose tissue have

TABLE 1 Extracellular miRNA profiles differ between obese and normal conditions¹

Increased miRNAs in obesity		Decreased miRNAs in obesity	
miRNAs	Study (first author reference)	miRNAs	Study (first author reference)
Blood			
miR-15b	Pescador 2013 (34), Cui 2018 (51)	miR-15a	Ortega 2013 (32)
miR-16-1	Prats-Puig 2013 (36)	miR-17-5p	Heneghan 2011 (33)
miR-20a	Cui 2018 (51)	miR-17	Williams 2019 (54)
miR-21-5p	Thompson 2017 (49)	miR-21 ²	Murri 2013 (42), Ghorbani 2018 (53)
miR-21 ²	Yang 2020 (55)	miR-23-3p	Goguet-Rubio 2017 (50)
miR-26b	Cui 2018 (51)	miR-27a-3p	Goguet-Rubio 2017 (50)
miR-26b-5p	Iacomino 2016 (46)	miR-27b	Murri 2013 (42)
miR-27	Can 2016 (47)	miR-28-3p	Prats-Puig 2013 (36)
miR-29a-3p	Thompson 2017 (49)	miR-100	Pek 2016 (45)
miR-31-5p	Iacomino 2016 (46)	miR-103	Murri 2013 (42)
miR-34a	Yang 2020 (55)	miR-125b	Prats-Puig 2013 (36), Ortega 2013 (32)
miR-103a-5p	Thompson 2017 (49)	miR-130a-3p	Goguet-Rubio 2017 (50)
miR-122	Prats-Puig 2013 (36), Wang 2015 (44), Jones 2017 (48)	miR-130b ²	Ortega 2013 (32), Thomé 2015 (37)
miR-126	Yang 2020 (55)	miR-132	Heneghan 2011 (33)
miR-130b ²	Prats-Puig 2013 (36), Wang 2013 (35)	miR-136-5p	Iacomino 2016 (46)
miR-140-5p	Prats-Puig 2013 (36), Ortega 2013 (32)	miR-138	Pescador 2013 (34)
miR-142-3p	Prats-Puig 2013 (36), Ortega 2013 (32)	miR-143	Can 2016 (47)
miR-146a	Cui 2018 (51)	miR-155	Murri 2013 (42), Mahdavi 2018 (52)
miR-146b	Cui 2018 (51)	miR-197	Cui 2018 (51)
miR-148a	Yang 2020 (55)	miR-197-3p	Goguet-Rubio 2017 (50)
miR-150-5p	Thompson 2017 (49)	miR-206	Iacomino 2016 (49)
miR-192	Jones 2017 (48)	miR-221	Prats-Puig 2013 (36), Ortega 2013 (32)
miR-222	Prats-Puig 2013 (36), Ortega 2013 (32), Cui 2018 (51)	miR-223	Wen 2015 (43)
miR-223-3p	Thompson 2017 (49)	miR-320a	Iacomino 2016 (46), Goguet-Rubio 2017 (50), Yang 2020 (55)
miR-363	Prats-Puig 2013 (36)	miR-328	Prats-Puig 2013 (36)
miR-370	Can 2016 (47)	miR-335	Can 2016 (47)
miR-378	Can 2016 (47)	miR-361-3p	Iacomino 2016 (46)
miR-423-5p ²	Prats-Puig 2013 (36), Thomé 2015 (37)	miR-376a	Pescador 2013 (34)
miR-486-3p	Prats-Puig 2013 (36)	miR-423-5p ²	Ortega 2013 (32)
miR-486-5p	Prats-Puig 2013 (36)	miR-454	Yang 2020 (55)
miR-532-5p ²	Prats-Puig 2013 (36)	miR-500	Yang 2020 (55)
miR-2355-5p	Iacomino 2016 (46)	miR-503	Pescador 2013 (34)
		miR-520c-3p	Ortega 2013 (32)
		miR-532-5p ²	Ortega 2013 (32)
		miR-758	Can 2016 (47)
		miR-1231	Iacomino 2016 (46)
Microvesicle			
miR-16	Müller 2011 (38)		
miR-27a	Müller 2011 (38)		
miR-146b	Müller 2011 (38)		
miR-155	Zhang 2015 (39)		
miR-222	Müller 2011 (38)		
Exosome			
miR-23b	Ferrante 2015 (40)	miR-15b-5p	Dang 2019 (58)
miR-27a	Yu 2018 (56)	miR-141-3p	Dang 2019 (58)
miR-27a-3p	Castaño 2018 (57)	miR-148b	Ferrante 2015 (40)
miR-27b-3p	Castaño 2018 (57)	miR-151-5p	Dang 2019 (58)
miR-34a	Pan 2019 (59)	miR-351-5p	Dang 2019 (58)
miR-122	Castaño 2018 (57)	miR-365-2	Ying 2017 (41)
miR-149	Ying 2017 (41)	miR-431-5p	Dang 2019 (58)
miR-155	Ying 2017 (41)	miR-449a-5p	Dang 2019 (58)
miR-181a-1	Ying 2017 (41)	miR-511	Ying 2017 (41)
miR-181a-2	Ying 2017 (41)	miR-682	Ying 2017 (41)
miR-181b-1	Ying 2017 (41)	miR-690	Ying 2017 (41)
miR-181b-2	Ying 2017 (41)	miR-692-2	Ying 2017 (41)
miR-192	Castaño 2018 (57)	miR-692-3	Ying 2017 (41)
miR-210	Ying 2017 (41)	miR-874-3p	Dang 2019 (58)
miR-299a-5p	Dang 2019 (58)	miR-1928	Ying 2017 (41)
miR-1945	Ying 2017 (41)	miR-1948b	Ying 2017 (41)
miR-4429	Ferrante 2015 (40)	miR-3968	Ying 2017 (41)
		miR-4269	Ferrante 2015 (40)
		miR-5098	Ying 2017 (41)
		miR-7054	Ying 2017 (41)
		miR-7070	Ying 2017 (41)

¹ miRNA, microRNA.² miRNAs found in both the increased miRNAs and decreased miRNAs list.

been demonstrated to affect the control of gene expression in other tissues that can induce insulin resistance (60–72). Thus, several important extracellular miRNAs play a key role in the development of insulin resistance during obesity.

Extracellular miR-27a

miR-27a is an important extracellular miRNA in the development of obesity-mediated insulin resistance. An increase in extracellular miR-27a in response to obesity results in promotion of insulin resistance via inhibition of insulin signaling. Obesity-induced extracellular miR-27a can accumulate in skeletal muscle cells and adipose tissue cells and inhibits the expression of the peroxisome proliferator-activated receptor γ (PPAR γ) transcription factor. PPAR γ regulates the expression of genes involved in lipid and glucose metabolism and plays an important role in insulin sensitization (56). miR-27a inhibits PPAR γ /PI3K/Akt/GLUT4 signaling, leading to insulin resistance (61, 62). In addition, miR-27a leads to macrophage infiltration and activation via PPAR γ inhibition and via the activation of NF- κ B-mediated transcription (63). miR-27a also targets mitogen-activated protein kinase 14 (MAPK-14), which is mostly expressed in the skeletal muscles and nervous system and activates GLUT4 translocation via IRS-1/Akt/signaling (62, 64). These results show that adipocyte-derived extracellular miR-27a can induce inflammation and insulin resistance and acts as a messenger between adipose tissue and other tissues.

Extracellular miR-34a

Pan et al. (59) demonstrated that the concentrations of exosomal miR-34a were elevated in obesity, and that ablation of adipocyte-derived miR-34a protected against obesity-induced insulin resistance and inflammation. Exosomal miR-34a from adipocytes can be transported to macrophages and targets Kruppel-like factor 4 (KLF4), which acts as a regulator of insulin signaling and cell proliferation, differentiation, and apoptosis. miR-34a-mediated downregulation of KLF4 in macrophages leads to inhibition of M2 polarization, which causes obesity-induced inflammation and insulin resistance (65). In the skeletal muscle, miR-34a can target ceramide kinase (CERK). CERK activation induces the phosphorylation of ceramide to produce ceramide-1-phosphate that stimulates PI3K/Akt, extracellular signal-regulated kinase 1/2 (ERK1/2), and mTOR in skeletal muscle cells. miR-34a-mediated suppression of CERK can induce increased ceramide concentrations that activate the JNK pathways and inhibit the insulin signaling pathway (66). Thus, exosomal miR-34a from adipocytes can serve as a key mediator of obesity-induced inflammation and insulin resistance.

Extracellular miR-141-3p

Exosomes from adipose tissue in obesity can also deliver to, and induce insulin resistance in, hepatocytes. Exosomal miR-141-3p can inhibit insulin sensitivity and glucose uptake

of hepatocytes, but its concentrations are decreased in exosomes from obesity-induced mice compared with those from normal mice (58). miR-141-3p directly targets phosphatase and tensin homolog (PTEN), which acts as a negative regulator of the PI3K/Akt signaling pathway and GLUT4 translocation (67, 68). Thus, obesity-induced suppression of exosomal miR-141-3p led to PTEN overexpression in hepatocyte and skeletal muscle cells, and ultimately to insulin resistance.

Extracellular miR-155

Increased miR-155 concentrations in conditions of obesity were found in both MVs and exosomes. The increased miR-155 concentration in adipocyte-derived MVs from obesity-induced mice induced M1 macrophage polarization by targeting the suppressor of SOCS1, which is involved in anti-inflammatory actions. Moreover, obesity caused an increase in the release of exosomal miR-155 from adipose tissue macrophages, which targets PPAR γ and inhibits insulin signaling in adipocytes, muscle cells, and hepatocytes (39, 41). These results suggest a mechanism in which obesity-mediated increases in extracellular miR-155 affect the development of inflammation as well as insulin resistance.

Extracellular miR-210 and miR-222

Although miR-210 in exosome and miR-222 in MVs are increased in conditions of obesity, there have been no published articles showing that the miR-210 and miR-222 from adipocytes directly affect insulin signaling in obesity. However, studies have indicated that extracellular miR-210 and miR-222 may act as factors regulating the development of obesity-mediated insulin resistance in the insulin signaling pathway (38, 41).

Recently, Tian et al. (69) demonstrated that exosomal miR-210 from high-glucose-induced macrophages inhibited NADH dehydrogenase (ubiquinone) 1a subcomplex 4 (NDUFA4) expression and induced the suppression of glucose uptake in adipocytes. NDUFA4 is one of the subunits of mitochondrial respiratory chain complex IV, and thus it is involved in mitochondrial function (70). Therefore, we suggest that the downregulation of NDUFA4 expression by miR-210 can lead to mitochondrial dysfunction that affects insulin signaling during obesity. Overexpression of miR-222 inhibits the expression of IRS-1 protein, a key molecule in the insulin signaling pathway, by directly binding to IRS-1 untranslated regions (71). Ono et al. (72) found that the concentrations of miR-222 are increased in the livers of individuals fed a high-fat/high-sucrose diet, and that this increase is associated with inhibition of insulin signaling. These findings suggest the role of extracellular miR-222 in obesity-induced insulin resistance by targeting IRS-1.

Conclusions

In this review, we summarized changes in the concentrations of obesity-associated circulating miRNAs and discussed the potential mechanisms involved in the development of

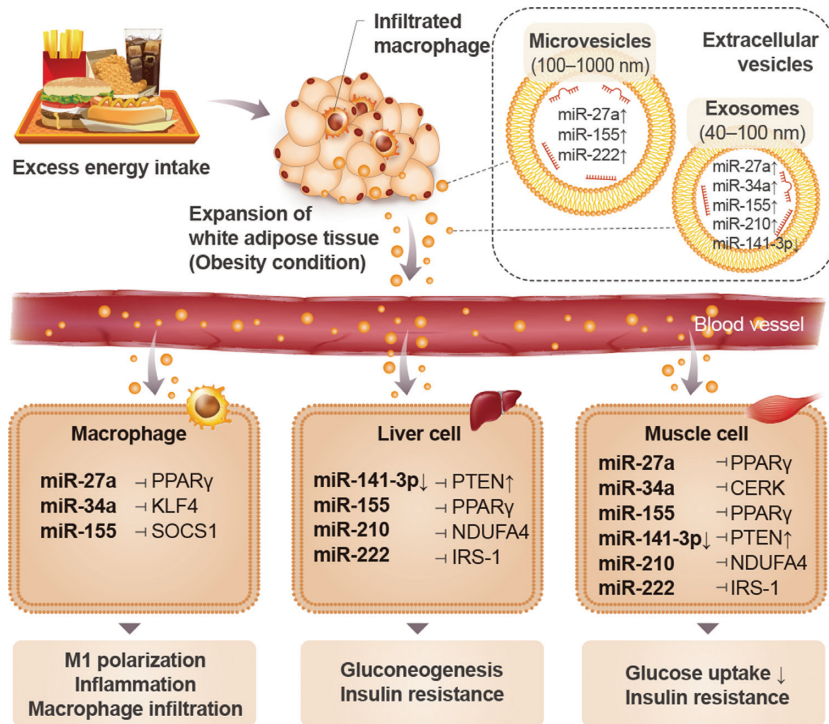


FIGURE 1 Novel mechanisms for the development of extracellular miRNA-associated insulin resistance during obesity. miR-27a, miR-34a, miR-141-3p, miR-155, miR210, and miR-222 in extracellular vesicles secreted from the adipose tissue affect the insulin signaling pathway in macrophage, liver, and muscle cells. CERK, ceramide kinase; IRS-1, insulin receptor substrate 1; KLF, Kruppel-like factor 4; miRNA, microRNA; NDUFA4, NADH dehydrogenase (ubiquinone) 1 α subcomplex 4; PPAR γ , peroxisome proliferator-activated receptor γ ; PTEN, phosphatase and tensin homolog; SOCS1, suppressor of cytokine signaling 1.

extracellular miRNA-associated insulin resistance during obesity. This article discussed how miRNAs, particularly miR-27a, miR-34a, miR-141-3p, miR-155, miR210, and miR-222, in EVs secreted from the adipose tissue can affect the insulin signaling pathway in metabolic tissue (Figure 1). The roles of exosomes and miRNAs in obesity-mediated insulin resistance require further investigation. This review assessed the role played by extracellular miRNAs derived from adipocytes in the development of insulin resistance and potential strategies for the development of therapeutics aimed at obesity and metabolic disorders such as type 2 diabetes.

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