REVIEW

Implication of obesity-induced miR-96 in hepatic insulin resistance

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Obesity is a serious health problem that is caused by an equilibrium shift towards elevated energy intake over expenditure, and is often involved in a range of metabolic diseases. A diet rich in saturated fatty acids (SFA), which is one of the leading causes of obesity and ectopic lipid accumulation in the key organs for metabolic regulation, results in an imbalance of the cellular metabolism and an inadequate response of hepatocytes to insulin, which is known as hepatic insulin resistance. Although endogenous non-coding small microRNAs (miRNAs) play important roles in the post-transcriptional repression of the target genes, the implications of obesity-induced miRNAs in metabolic diseases, particularly in the development of hepatic insulin resistance, are largely unknown. In recent studies, SFA and a high fat diet were found to increase the expression of certain miRNAs significantly in the liver and skeletal muscle. These obesity-induced miRNAs were also up-regulated in human subjects with metabolic diseases. Our recent study highlights a novel mechanism whereby miR-96, which is one of the obesity-induced miRNA, participates actively in the development of hepatic insulin resistance in obesity. Studies focusing on obesity-induced miR-96 have indicated the strong diagnostic and therapeutic importance of miRNAs in insulin resistance and metabolic diseases. This will also help better understand the pathogenesis of insulin resistance and T2DM in obesity, and enable the development of inhibitors against obesity-induced miRNAs as a novel diagnostic and therapeutic strategy for metabolic diseases.

Keywords: Obesity; MicroRNA; Liver; Insulin resistance; Diabetes; Insulin signaling

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Obesity is a serious global health problem resulting from an equilibrium-shift towards elevated energy intake over expenditure, and has been suggested to be involved in the development of a range of metabolic diseases, such as hypertension, dyslipidemia, and type 2 diabetes (T2DM) [1, 2]. A diet rich in saturated fatty acids (SFA), which is one of leading causes of obesity and ectopic lipid accumulation in the liver, causes an imbalance of the cellular metabolism that can lead to an inadequate response of hepatocytes to insulin, also known as hepatic insulin resistance [3, 4]. Accumulating evidence has indicated the molecular mechanisms underlying the hepatic insulin resistance induced by SFA and obesity, such as the overburden of ceramide and diacylglycerol (DAG), increased oxidative stress, inflammation, and

mitochondrial dysfunction [5, 6]. On the other hand, despite the impressive progress with sophisticated investigation, the precise mechanisms for how SFA causes hepatic insulin resistance is not completely understood.

MicroRNAs (miRNAs) consist of approximately 20-25-nucleotides and are highly conserved naturally occurring, small non-coding RNA molecules that regulate the expression of the target genes at the post-transcriptional level [7]. In general, miRNAs bind to the 3' untranslated regions (3'UTRs) of the specific target mRNAs, thereby causing either mRNA degradation or translational suppression of their target genes [7]. Extensive studies have suggested that miRNAs are negatively involved in the expression of a variety of genes in both normal and pathological states, and the inappropriate regulation of miRNAs expression is linked to the pathogenesis of many diseases, such as proliferative, degenerative, and metabolic diseases [7, 8]. Following the discovery that miRNA plays a key role in the amino acid catabolism [9], many studies have reported miRNAs to be important regulators of the hepatic lipid and glucose homeostasis, whose impairment is linked to the pathophysiology of insulin resistance and T2DM [10, 11]. Recently, the expression of certain miRNAs targeting the molecules transducing insulin signaling are regulated aberrantly in SFA-induced obesity, and linked intimately to the pathogenesis of hepatic insulin resistance [10, 12-15]. Furthermore, a high fat diet (HFD) appears to impair hepatic insulin signaling in mice, concomitant with the induction of specific miRNAs targeting the insulin signaling molecules in the liver [12, 16]. Although there have been many advances in studies of the implication of miRNAs in the metabolism, the emerging mechanisms for how obesity-induced miRNAs are responsible for the regulatory roles in hepatic insulin resistance are unclear.

A recent study suggested that the expression of certain miRNAs targeting the mRNAs of insulin signaling molecules is modulated aberrantly in HFD- or SFA-induced obesity, leading to insulin resistance in hepatocytes [17]. Using miRNA expression profiling based on an Affymetrix miRNA array, the miRNAs whose expressions are affected by the development of HFD-induced insulin resistance were identified [17]. Followed by PicTar and TargetScan analysis, the miRNAs that are expected to target 3'UTR of *INSR* were identified and classified because impaired insulin signaling by HFD can be attributed most likely to the reduction of INSR and its downstream target, IRS-1, in the insulin signaling cascade. Twenty miRNAs were selected initially as miRNAs presumably targeting 3'UTR of the *INSR* gene for further experiments based on their targeting score to the 3'UTR of *INSR*, as well as expression abundancy and reliability of the results from the Affymetrix miRNA array. As expected, certain miRNAs, including miR-96, miR-140, miR-151, miR-185, miR-455, miR-532, and miR-874 *etc*., which are presumably targeting 3'UTRs of *INSR*, were increased (> 1.5-fold) in the liver of HFD mice [17]. This is consistent with other independent studies based on obese-type insulin resistance mice models [12, 16, 18-20]. Therefore, these upregulated miRNAs are regarded as potential candidate miRNAs for suppressing INSR expression, but none of these have been validated and confirmed to suppress the expression of INSR by targeting the 3'UTR of *INSR*.

Although the induction of miR-96 in the liver of HFD-fed mice and SFA-treated hepatocytes was reported, there are few reports on the differential expression of miR-96. Similar to previous results, miR-96 was upregulated in the liver of NAFLD patients [21] and obese diabetic animal models, such as *ob/ob* and *db/db* mice [22, 23]. In addition, miR-96 was increased in the liver of mice after an injection of resistin [24], an adipokine associated with obesity-mediated insulin resistance and T2DM [25]. Interestingly, the plasma level of resistin was higher in the HFD-fed mice and its inhibition ameliorated the HFD-induced hepatic insulin resistance [26]. Previous studies suggested an increase in miR-96 as a contributor to the development of hepatic insulin resistance induced by obesity. The SFA-enriched HFD caused an impairment of insulin signaling by the reduction of INSR and IRS-1 expression with the concomitant upregulation of miR-96 in the mice liver, and the 3'UTRs of *INSR* and *IRS-1* were suggested to be a potential target of miR-96 from the *in silico* target analysis. Therefore, a previous study [17] focused on miR-96 to further determine its functional significance in hepatic insulin resistance.

Hsa-miR-96 is located on human chromosome 7q32.2 and belongs to the miR-183 family, which is comprised of three conserved miRNAs (miR-96, miR-182 and miR-183) in various animals [27]. Accumulating evidence suggests that members of the miR-183 family with strong sequence similarity play crucial roles in a wide range of normal processes and pathological conditions, such as cell growth, differentiation, apoptosis, immunity, sensing, and metabolism [27-29]. Among them, miR-96 is involved mainly in oncogenesis and cancer progression in various types of tissues and organs, including the liver, stomach, and breast cancer [29-31]. Therefore, miR-96 is acknowledged as an oncogenic miRNA that facilitates the development of cancers by promoting the proliferation and survival of the malignant cells [32, 33]. Recent studies have shown that miR-96 also regulates the expression of many of the genes involved in the metabolism, including in lipid homeostasis and adipogenesis. The role of miR-96 in the metabolism was first unveiled in the regulation of insulin secretion from pancreatic β-cells [34].

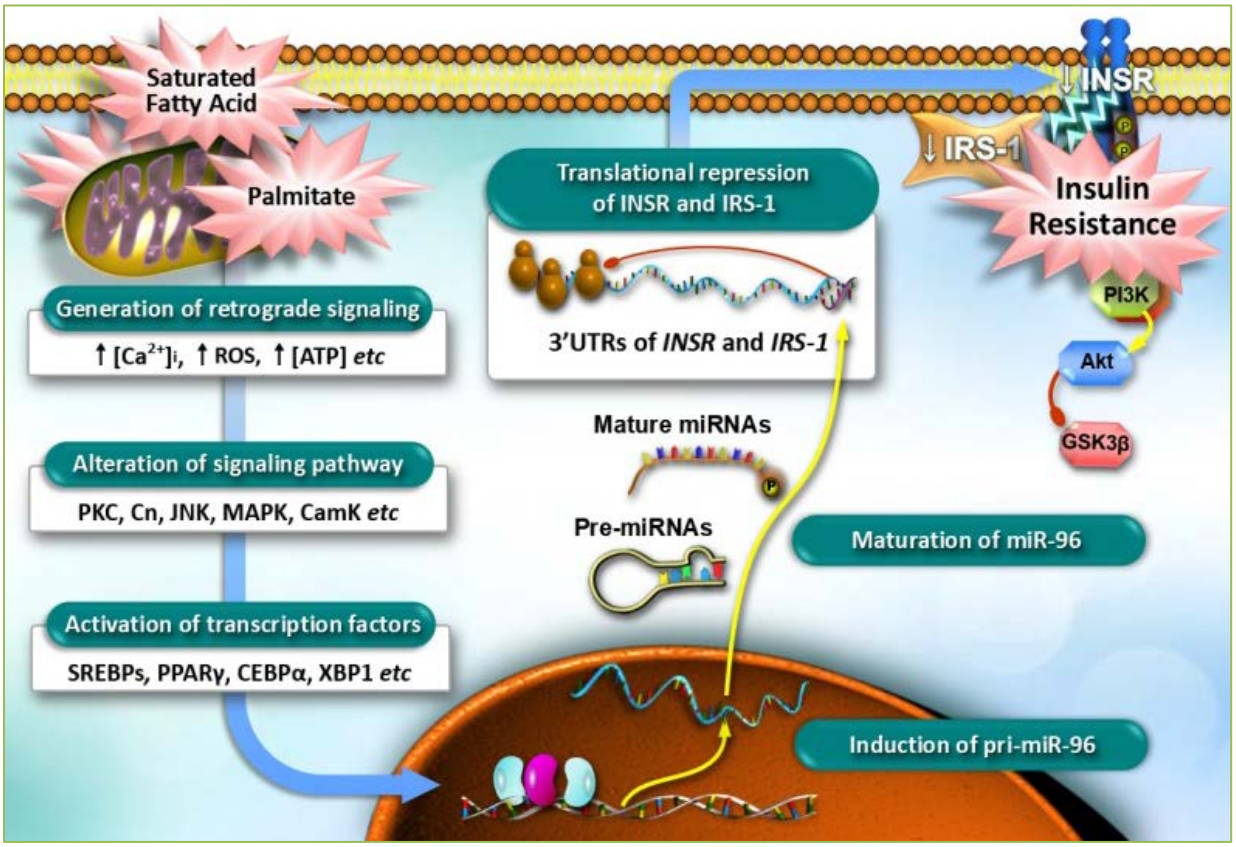

Figure 1. Implication of obesity-induced miR-96 in hepatic insulin resistance.

Lovis P. *et al*. reported that insulin secretion was impaired substantially by miR-96, which increases the expression of granuphilin, a negative modulator of insulin exocytosis, and suppresses the expression of Noc2, a key player in insulin secretion, from pancreatic β-cells [34]. This suggests that miR-96 is involved in the regulation of insulin exocytosis, working as an important modulator in the optimal insulin secretory capacity. The importance of miR-96 in the regulation of lipid homeostasis was also reported. miR-96 increases lipid synthesis and adipogenesis in the mouse liver by downregulating INSIG-2, a regulatory molecule for retaining the SREBP-precursor in the ER [35]. In addition, miR-96 also plays an important role in the cholesterol metabolism by targeting hepatic scavenger receptor class B type I (SR-BI) directly, which promotes the selective high-density lipoprotein cholesterol (HDL-C) uptake [36].

The former study showed that the roles and effects of miR-96 in insulin resistance resulted from a mitochondrial dysfunction [37]. Interestingly, miR-96 was found to be upregulated by the mitochondrial dysfunction and associated with impaired insulin signaling in SK-Hep1 cells, suggesting its role in insulin signaling and insulin resistance [37]. The most important finding from our recent study is that obesity-induced miR-96 is linked causally to the impaired insulin signaling and glycogen metabolism through the down-regulation of INSR and IRS-1 in hepatocytes. miR-96 targets the 3'UTRs of *INSR* and *IRS-1* directly and down-regulates the expression of INSR and IRS-1 at the post-transcriptional level. The ectopic expression of miR-96 impairs insulin signaling and the insulin-stimulated synthesis of glycogen in hepatocytes via the post-transcriptional repression of INSR and IRS-1. Therefore, our study unveiled a novel mechanism where miR-96 participates actively in the pathogenesis of hepatic insulin resistance. Moreover, the upregulation of miR-96 by SFA is proposed as a casual factor in the pathogenesis of hepatic insulin resistance.

The underlying mechanism for how the expression of miR-96 is upregulated by SFA or HFD is not completely understood. Although there is insufficient evidence elucidating the molecular mechanism underlying the regulation of miR-96 expression in the liver by obesity, the induction of miR-96 might be initiated by certain transcription factors activated by palmitate or a HFD in hepatocytes. Therefore, the transcription factor binding sites on the promoter of miR-96 were predicted further by *in silico* analysis because several tentative transcription factors activated by obesity or HFD might bind to the specific binding sites on the promoter. This type of analysis allows a

prediction of several potential mechanisms that could contribute to the induction of miR-96 in obesity. Among the handful of transcription factors identified to be activated by a HFD or obesity, the intensely studied transcription factors have been SREBPs, PPARγ, and C/EBPα. SREBPs play a key role in the biosynthesis of fatty acids and cholesterol, and their activation is associated closely with diabetic fatty liver and hyperlipidemia [38]. A recent study showed that SREBP targets the promoter of miR-96 on mouse chromosome 6 directly [35]. Furthermore, miR-96 can increase the nuclear translocation of SREBPs and lipid synthesis rapidly in a mouse liver via the suppression of INSIG-2, a key molecule for retaining the SREBP-SCAP complex in the ER [35]. Therefore, the induction of miR-96 by palmitate or HFD reported in a previous study [17] is most likely to be caused by the activation of SREBPs, suggesting that the activation of SREBPs by SFA is a substantial induction mechanism of miR-96 expression in the liver. Similar to the role of SREBPs, PPARγ also has been shown to be an important nuclear receptor protein that regulates the expression of several genes involved in the lipid metabolism and lipid biosynthesis [39]. Based on the global expression analysis of miRNAs, miR-96 was upregulated in differentiating 3T3-L1 adipocytes by PPARγ activation [40]. PPARγ was found to colocalize with C/EBPα [41], which is also found as an adipogenic transcription factor activated by HFD [42]. The activation of PPARγ facilitated with C/EBPα could play a substantial regulatory role for an increase in miR-96 in the liver under obese conditions. In addition, XBP1 is activated by ER stress, such as a HFD and obesity, and activates the expression of C/EBPα by binding to its specific sites located on the promoter [43, 44]. Therefore, further study is warranted, but miR-96 might be a key regulator in the vicious cycle between hepatic lipid accumulation and insulin resistance. Several lines of evidence imply the upregulation of miR-96 through XBP1-dependent mechanisms under ER stress-induced by HFD.

In summary, Fig. 1 presents a possible mechanism for how obesity-induced miR-96 is implicated in the development of hepatic insulin resistance. An excess amount of dietary SFA or palmitate triggers various stresses generating retrograde signals to the nucleus [45]. These signals regulate several corresponding kinases, and subsequently certain transcription factors that are influenced by a retrograde signal may induce the production and processing of obesity-induced miRNAs targeting the 3'UTR of *INSR* or *IRS-1*, leading to impaired insulin signaling and hepatic insulin resistance by suppressing the expression of INSR or IRS-1. Further studies of the signals and transcription factors involved and how they modulate obesity-induced miRNAs and miRNA-mediated insulin resistance will be the next interesting challenge. Over the past decade, the prognostic and therapeutic importance of obesity-induced miRNAs in insulin resistance and T2DM have been suggested from solid molecular evidence. These studies revealed the novel role of obesity-induced miR-96 as a causal factor for hepatic insulin resistance. Overall, miR-96 can target a range of genes, in which each gene can be regulated by several other metabolic regulators. Studies of the regulation mechanism in miR-96 and other obesity-induced miRNAs as well as their crosstalk with key metabolic regulators are currently underway. It will increase the knowledge of the pathogenesis of obesity-induced insulin resistance and T2DM, and enable the discovery of miRNAs as clinically potential diagnostic and therapeutic applications. In addition, global expression analysis of miR-96 and other obesity-induced miRNAs in body fluids, such as urine and blood, will also facilitate the development of new diagnostic strategies for metabolic disease.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Author contributions

All authors (K.M., Y.S., W.Y., W.L.) contributed to the concept development and experimental design. K.M., Y.S., W.L. wrote the manuscript.

Abbreviations

C/EBPα: CCAAT/enhancer binding protein alpha; GSK3β: glycogen synthase kinase 3 beta; Has: human; PPARγ: peroxisome proliferator activated receptor gamma; SREBP: sterol regulatory element binding protein; XBP1: X-box binding protein 1.

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