REVIEW

Obesity-induced microRNAs and type 2 diabetes

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Obesity is a growing health problem and causally linked to the development of type 2 diabetes mellitus (T2DM). T2DM is a metabolic disorder caused by peripheral insulin resistance as well as suboptimal insulin production. Although endogenous non-coding small microRNAs (miRNAs) have been shown to play an important role in the post-transcriptional repression of target genes, the implication of obesity-induced miRNAs in metabolic disorders particularly in the development of insulin resistance is largely unknown. Recent studies have revealed that saturated fatty acid (SFA) palmitate and high fat diet (HFD) significantly increase the expression of certain miRNAs, such as miR-29a and miR-195, in myocytes and hepatocytes. These obesity-induced miRNAs are also up-regulated by SFA treatment in cultured myocytes and hepatocytes. MiR-29a targets IRS-1 3'UTR directly and down-regulates IRS-1 expression at the post-transcriptional level. The induction of miR-29a impairs insulin signaling and the insulin-stimulated glucose uptake in myocytes. MiR-195 down-regulates the mRNA and protein expression of INSR without apparently changing IRS-1 expression in hepatocytes. The ectopic expression of miR-195 reduced significantly the insulin-stimulated phosphorylation of INSR and its downstream molecules in hepatocytes. Moreover, the ectopic expression of miR-195 reduced the insulin-stimulated glycogen synthesis. Taken together, these results clearly suggest that the induction of miR-29a and miR-195 by SFA are causally linked to the pathogenesis of insulin resistance and T2DM in obesity. These evidences suggest novel molecular mechanisms of insulin resistance, and implicate SFA-induced miRNAs in T2DM and metabolic diseases.

Keywords: microRNA; miR-29a; miR-195; palmitate, insulin resistance; IRS-1; saturated fatty acid

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Introduction

MicroRNAs (miRNAs) are short endogenous non-coding RNAs with high evolutionary sequence conservation that regulate gene expression at post-transcriptional level [1, 2]. Mature miRNAs bind to specific complementary sequences within the 3' untranslated regions (3'UTRs) of the target genes, thereby eventually triggering either the suppression of translation or degradation of the target mRNAs [1, 2]. Although the molecular targets and roles of individual miRNAs are largely unknown, there is growing evidence suggesting that deregulated expression of miRNAs is closely linked to various pathological states, such as cancer, neurodegeneration and cardiovascular diseases [1, 3]. Since the first discovery that miRNA is involved actively in metabolism, such as amino acid catabolism [4], several recent publication have suggest that miRNAs play significant roles in metabolic homeostasis and are implicated in the pathogenesis of insulin resistance and type 2 diabetes mellitus (T2DM) [3, 5].

Obesity is a growing public health problem worldwide, and the accumulation of excess lipid in non-adipose tissues causes lipotoxicity, which is known as dysregulation of the

cellular metabolism and apoptosis [6,7]. In particular, the accumulation of excess saturated fatty acid (SFA) in the skeletal muscle and liver has been linked to the pathogenesis of insulin resistance [8,9]. Insulin resistance is a state of reduced cellular responsiveness to insulin, and plays an important role in the development of T2DM, hypertension and metabolic diseases [8, 9]. In the skeletal muscle and liver, the excess SFA results in the accumulation of diacylglycerol and provoking oxidative stress and mitochondrial dysfunction, leading to dysregulation of the cellular metabolism and insulin resistance [9-11].

Recently, it was hypothesized that the expression of certain miRNAs targeting 3'UTR of the insulin signaling intermediates' mRNAs is modulated by the SFA-induced obesity, which participate actively in the development of insulin resistance [3, 5]. Moreover, the plasma or tissue levels of those miRNAs might be associated with the insulin sensitivity of humans, thereby allowing the development of miRNAs as potential new diagnostic and therapeutic targets in insulin resistance and T2DM [3, 5]. Previous our studies unveiled that the certain miRNAs, such as miR-29a and miR-195, were increased in the skeletal muscle and liver of high fat diet (HFD)-fed mice with insulin resistance [12,13]. Hence, we investigated whether these miRNAs could be induced by SFA, and whether these miRNAs might actively participate in SFA-induced insulin resistance.

MiR-29a, which is located in human chromosome 7q32.3 [14], is a member of the miR-29 family comprised of three mature forms, miR-29a, -29b and -29c [15]. Thus far, approximately 50 target genes of the miR-29 family members have been validated experimentally from thousands of predicted target genes [15]. However, it is unclear if and how miR-29a is linked to the regulation of insulin sensitivity in the skeletal muscle. We have found that the expression of miR-29a was up-regulated in the skeletal muscle, liver and serum of HFD-fed mice (60% kcal from fat for 14 weeks) [12]. In addition, the cellular treatment of SFA palmitate in myocytes increased the level of miR-29a expression [12]. *In silico* target analysis indicates that the insulin receptor substrate-1 (IRS-1) 3´UTR contains the two putative binding sites for miR-29a and is highly conserved in various species. According to the reporter gene assay, the luciferase activities of IRS-1 3´UTR containing the miR-29a binding site were reduced significantly by miR-29a, whereas the luciferase activities of IRS-1 3´UTR containing miR-29a binding site mutants were unaffected. Moreover, the ectopic expression of miR-29a reduced IRS-1 protein expression significantly without an apparent change in the IRS-1 mRNA level in myocytes [12]. Therefore, miR-29a targets IRS-1 3'UTR directly and down-regulates IRS-1 expression at the post-transcriptional level. Moreover, the ectopic expression of miR-29a reduced the insulin-stimulated tyrosine phosphorylation of IRS-1 and its downstream molecules, such as Akt2 and glycogen synthase kinase-3β (GSK3β) in myocytes [12]. In addition, the ectopic expression of miR-29a reduced the insulin-stimulated glucose uptake in myocytes [12]. Overall, the ectopic expression of miR-29a impairs insulin signaling and the insulin-stimulated glucose uptake in skeletal muscle cells through the post-transcriptional repression of IRS-1.

The mechanisms of SFA-induced and HFD-induced miR-29a expression in myocytes are unclear. Nevertheless, several reports have suggested that the expression of miR-29a induced several transcription factors, such as NF-κB, c-Myc, CEBPA, and p53 through binding the miR-29a gene promoter regions [15-18]. These transcription factors are up-regulated in HFD-fed mice [15-18]. Therefore, the up-regulation of these transcription factors by SFA and HFD might increase the expression of miR-29a. It is suggested that obesity induced by SFA generates the signals related to oxidative stress, inflammation and ER stress, and activates several transcriptional factors, such as p53 and CEBPA. These changes might up-regulate the expression of miR-29a, leading to the post-transcriptional repression of IRS-1 and subsequent insulin resistance.

Hsa-miR-195 is an intronic miRNA of a hypothetical protein, LOC284112, located on chromosome 17p13.1 [19]. Based on the expression profiling and target validation analysis, the deregulation of miR-195 expression is associated with multiple diseases, such as cancer, cardiac hypertrophy and heart failure [20]. An increase of miR-195 expression by SFA also causes apoptosis via targeting Sirt1 and Bcl-2 in cardiomyocytes [21]. The other report suggests that miR-195 expression was increased in the liver of a T2DM animal model, Goto-Kakizaki rat [22]. Previously, we have found that HFD increased the miR-195 expression in the liver with a concomitant decrease in insulin receptor (INSR) and IRS-1 protein expression, suggesting that miR-195 participates in the development of hepatic insulin resistance in diet-induced obesity (DIO) mice [13]. Moreover, miR-195 expression was also found to be up-regulated significantly in palmitate-treated hepatocytes [13]. According to TargetScan and PicTar analysis, the 3'UTRs of the INSR and IRS-1 genes contain the predicted conserved binding sites for miR-195. Based on the dual luciferase reporter gene assay, the ectopic expression of miR-195 showed a significant decrease in INSR mRNA concomitant with the substantial suppression of the INSR protein in HepG2 cells [13]. On the other hand, the ectopic expression of miR-195 did not affect the mRNA and protein levels of the insulin signaling molecules, such as IRS-1, Akt2 and GSK3β in HepG2 cells, indicating that miR-195 down-regulates the

mRNA and protein expression of INSR without apparently changing IRS-1 expression in hepatocytes [13]. Furthermore, the ectopic expression of antimiR-195, an antagomiR of miR-195, partially but significantly rescued the palmitate-induced suppression of INSR in HepG2 cells, indicating that the up-regulation of miR-195 contributes to palmitate-induced INSR suppression in hepatocytes [13]. Previous study also examined whether the up-regulation of miR-195 impairs insulin signaling in hepatocytes. The ectopic expression of miR-195 impairs the insulin-stimulated phosphorylation of INSR and its downstream molecules in hepatocytes [13]. Moreover, the ectopic expression of miR-195 decreased the glycogen synthesis stimulated by insulin [13]. These results clearly show that the up-regulation of miR-195 impairs insulin signaling and the insulin-stimulated glycogen synthesis in hepatocytes through post-transcriptional repression of INSR.

The mechanisms of SFA-induced and HFD-induced miR-195 expression in hepatocytes are unclear. *In silico* analysis, however, suggested that several transcription factors, such as SREBPs, PPARs, CEBPA and p53, might bind to the specific sites on the miR-195 gene promoter regions. Because the activation of SREBPs are essential for diabetic hepatic steatosis and carbohydrate-induced hyper-triglyceridemia [23], the miR-195 level in the liver may be up-regulated by SFA or HFD through SREBPs activation. PPARs are also well-known transcription factors activated by HFD that have been implicated strongly in T2DM and metabolic syndrome [24]. Another adipogenic transcription factor, CEBPA, is activated by high-energy nutrients [25]. Furthermore, the levels of CEBPA and p53 expression are up-regulated in DIO mice [18, 25]. Although further study will be needed, the induction of miR-195 in hepatocytes might be triggered by the activation of certain transcription factors regulated by SFA or DIO.

Over the last decade, studies focusing on the role of obesity-induced miRNAs in metabolism have suggested the strong prognostic and therapeutic importance of miRNAs in insulin resistance and T2DM. Previous studies have implicated obesity-induced miRNAs, such as miR-29a and miR-195, with the development of insulin resistance and T2DM. Because miR-29a and miR-195 can target several genes and each gene can be regulated by several other metabolic regulators, we are currently investigating the regulation mechanism in these miRNAs and their crosstalk with metabolic regulators. Such studies will help better understand the pathogenesis of obesity-induced insulin resistance and T2DM, and enable the development of obesity-induced miRNAs as novel diagnostic and therapeutic targets for diabetes. In addition, expression profiling of miR-29a and miR-195 in body fluids, such as plasma and urine, will facilitate the development of new diagnostic methods by differentiating between normal and diabetic characteristics. Moreover, obesity-induced miRNAs might even find applications in therapeutics as either single agents or in combination therapies.

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