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RESEARCH HIGHLIGHT

MicroRNA links obesity and impaired glucose metabolism

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The current obesity pandemic, which is in part a consequence of social trends toward higher energy intake and reduced energy expenditure, is expected to result in type 2 diabetes (T2DM) and considerable downstream morbidity, mortality and incremental costs to healthcare systems around the world [1]. The number of T2DM cases is increasing at a worrisome rate and is expected to double within the next 20 years. However, the molecular mechanisms that underlie individual changes in the predisposition to obesity and T2DM remain obscure.

The identification of DNA variants influencing disease predisposition will deliver clues to the processes involved in disease pathogenesis. This would not only spur translational innovation but also provide opportunities for personalized medicine through stratification according to an individual's risk and more precise classification of the disease subtype. Linkage analysis and large-scale association studies have revealed many loci that confer susceptibility to T2DM [2, 3].

Because microRNAs (miRNAs) have been shown to be another layer of gene regulation, it is not surprising that they are also involved in the development of obesity-induced insulin resistance and T2DM [4]. miRNAs are endogenous, single-stranded, small, ~22-nucleotide noncoding RNAs. miRNAs are generally regarded as negative regulators of gene expression by inhibiting translation and/or promoting mRNA degradation by base pairing to complementary sequences within the 3′ untranslated region (3′UTR) of protein-coding mRNA transcripts [5]. However, recent studies have suggested that miRNA-binding sites are also located in 5′UTRs or open reading frames (ORFs), and the mechanism(s) of miRNA-mediated regulation from these sites has not been defined [6, 7]. The first miRNA assigned to a specific function in metabolism was identified in *Drosophila melanogaster*, where loss of miRNA (miR)-14 increases body fat content [8]. Since then, a variety of miRNAs have been discovered in the regulation of glucose and lipid metabolism [9-12]. The fact that T2DM susceptibility genes discovered by whole-genome association studies were located in noncoding regions supports the possibility that noncoding RNAs, such as miRNAs, may also contribute to the development of obesity and T2DM [13, 14].

In order to understand the function of miRNAs in obesity and T2DM, the Brüning's group searched for miRNAs upregulated in liver, which were associated with specific feeding conditions [15]. Upregulation of miR-143 was also confirmed in the liver of db/db mice and diet-induced obese mice. miR-143 and miR-145 form a small gene cluster on mouse chromosome 18 and miR-145 was also upregulated in the liver and pancreas of obese mice, whereas the pattern of regulation differs in other tissues, including white and black adipose tissue.

To investigate whether increased hepatic miR-143 expression contributes to the metabolic phenotype arising from obesity *in vivo*, Jordan and colleagues [15] generated time-controlled, doxycycline (DOX)-inducible miR-143 transgenic mice. Although after 4 weeks of DOX treatment miR-143 transgenic mice (miR-143^{DOX}) showed conditional overexpression of miR-143, predominantly in liver and brown adipose tissue, there was no change in body weight, fat mass or circulating plasma leptin concentrations. However, overexpression of miR-143 did result in a dysregulation of glucose metabolism. DOX-treated miR-143^{DOX} mice exhibited impaired glucose and insulin tolerance without any change in β-cell function.

To further investigate the molecular basis of insulin resistance in miR-143-^{DOX} mice, the authors analyzed insulin-stimulated signaling in liver and skeletal muscle. Conditional miR-143 overexpression impairs insulin-stimulated AKT activation in the liver but not in skeletal muscle.

To confirm the involvement of miR-143 in insulin resistance and inhibition of insulin-stimulated AKT activation,

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the authors investigated whether miR-143 deficiency can protect against obesity-induced insulin resistance *in vivo*. miR-143-145-deficient and control mice were exposed to high-fat feeding, and glucose metabolism and hepatic insulin signaling were compared in these mice. Both glucose tolerance and insulin sensitivity were significantly improved in obese miR-143-145-deficient mice compared with controls. Insulin sensitivity was paralleled by increased insulin-stimulated phosphorylation of AKT in the liver of obese miR-143--145-deficient mice compared with controls, which was consistent with the results from conditional miR-143 overexpression.

The recent work of Jordan and colleagues represents an important breakthrough in the determination of the molecular target of miR-143 by employing *in vivo* stable isotope labeling of amino acids (SILAC) for quantitative-mass-spectrometry-based analysis of hepatic protein expression in miR-143^{DOX} mice. They analyzed the relative abundance of 2 043 proteins and detected peptide fragments of 1 463 proteins, of which 214 proteins were upregulated and 48 were downregulated by 25% or more in liver extracts of miR-143^{DOX} mice compared with controls. They further analyzed the 48 downregulated proteins for predicted miR-143 binding sites in their 3′UTR. Five proteins showed predicted 3′UTR binding sites for miR-143 and one of them, oxysterol-binding-protein-related protein (ORP) 8, had 3 predicted miR-143 binding sites. The functional significance of the potential binding sites in the 3′UTR of ORP8 was confirmed by luciferase reporter assays. Hepatic ORP8 protein expression was downregulated by 50% in miR-143^{DOX} mice and upregulated by 1.9-fold in miR-143-145 deficient mice compared with controls. ORP8 depletion by a short interfering RNAs (siRNAs) technique in HepG2 cells revealed a significant reduction of insulin-stimulated AKT activation and downstream forkhead O-family (FOXO) phosphorylation.

This study showed increased hepatic miR-143 expression in two different obesity mouse models and demonstrated that it affects hepatic insulin action and systemic glucose homeostasis *in vivo* by overexpression and knockout mouse models. The present study further provides evidence for miR-143-ORP8-dependent regulation of AKT signaling in obesity (Figure 1), and thus it is now time to examine miR-143, ORP8, and their regulated molecules as future therapeutic targets for obesity-associated insulin resistance and diabetes.

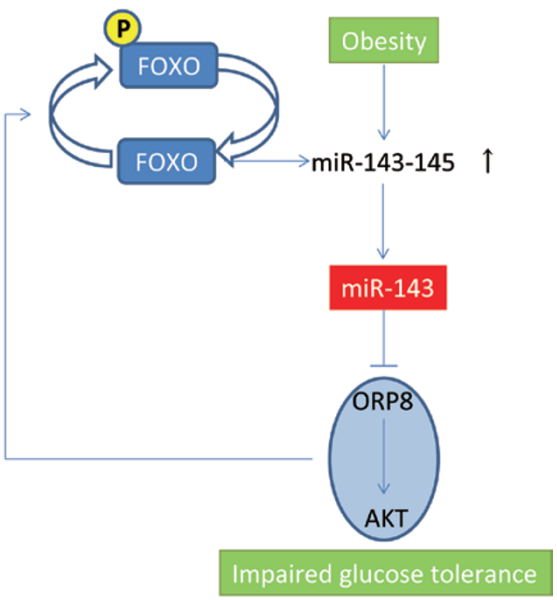

Figure 1 Schematic overview of the regulation of hepatic miR-143. Obesity results in induced expression of miR-143-145 in the liver. The involvement of miR-145 in glucose metabolism is unclear; however, upregulated miR-143 suppresses the translation of ORP8, which reduces AKT activation and impairs glucose homeostasis. The putative promoter region of the miR-143-145 cluster possesses numerous predicted consensus binding sites for FOXO transcription factors. Thus, there may be an autoregulatory loop in obesity-induced miR-143 expression, which is enhanced through the FOXO-dependent upregulation of hepatic miR-143 under the situation of insulin resistance/AKT inhibition.

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