



A Novel Link between Plasma MicroRNA-33b Levels and Lipid Disorders in Diabetes Mellitus

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MicroRNAs (miRNAs, miRs) are single-stranded non-coding RNAs of ~22 nucleotides in length that repress the expression of specific target mRNAs by binding to complementary sequences in their 3' untranslated regions¹⁾. miRNAs have been detected in the blood stream and other body fluids, raising interest in their potential use as markers for specific disease conditions. Indeed, distinct expression patterns of circulating miRNAs have been linked with a variety of cardiovascular diseases²⁾.

miRNAs are released into the circulation in membranous structures known as apoptotic or necrotic bodies during cell lysis after organ injury. There is also evidence of regulated secretion of miRNAs from intact cells. Most of these miRNAs are associated with AGO2 in nuclease-resistant complexes or included in exosomes, which are small membrane vesicles³⁾. High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol particles are also known to contain miRNAs by unknown mechanisms⁴⁾. It is possible that secreted miRNAs can be transferred and taken up by distant cells/organs. However, it remains unclear whether or how they exert their specific functions in other cells.

Kimura *et al.* first demonstrated that plasma miR-33b/miR-16 levels positively correlated with plasma insulin, serum C-peptide, and triglyceride levels, but not with HDL-C in patients with type 2 diabetes mellitus (T2DM)⁵⁾. They also showed that blood levels of miR-33a were around 1/150th of that of miR-33b and were not correlated with the above parameters. The authors hypothesized that insulin may

stimulate the expression of *SREBF1c* and miR-33b, which resulted in the suppression of ABCA1 and HDL-C. Thus, miR-33b/miR-16 may be useful as new metabolic biomarkers of dyslipidemia in patients with T2DM.

The intriguing aspect of this article is that circulating miR-33b may reflect the intracellular metabolic condition of the liver. It is very hard to perform liver biopsies in patients with T2DM and blood tests are ideal as a substitute for biopsies. It has been shown that *SREBF2* levels in the liver are significantly less than those of *SREBF1*⁶⁾, which is consistent with the authors' finding that plasma miR-33a levels were lower compared with miR-33b levels. However, it remains unknown how these miRNAs are released into the circulation and why plasma miR-33a concentrations were a hundred times lower than that of miR-33b. As mentioned above, circulating miRNAs can be released when tissue is damaged. Thus, measurement of the levels of AST, ALT, and other liver enzymes may provide clues to the mechanism of release of these miRNAs. However, it is unlikely that ongoing liver damage exists in patients with T2DM. Other mechanisms such as active transport via exosomes, lipoproteins, and other microvesicles should also be taken into consideration (**Fig. 1**).

There are several questions to be elucidated in the future. First, quantification of miRNAs is conducted by RT-PCR. In the RT step, cDNA is reverse transcribed from total RNA by use of target-specific stem-loop reverse transcription primers⁷⁾. The efficiency of this primer depends on the unique sequence of each miRNA and it is difficult to compare the precise expression levels of different miRNAs. Therefore, in order to compare the levels of miR-33a and miR-33b, it is required to establish a novel method to directly measure the copy number of small RNAs. Second, the authors measured plasma miRNAs concentration in their study. Heparin is known to inhibit enzymatic steps such as cDNA synthesis and PCR⁸⁾. Currently, there is no reliable way of removing heparin

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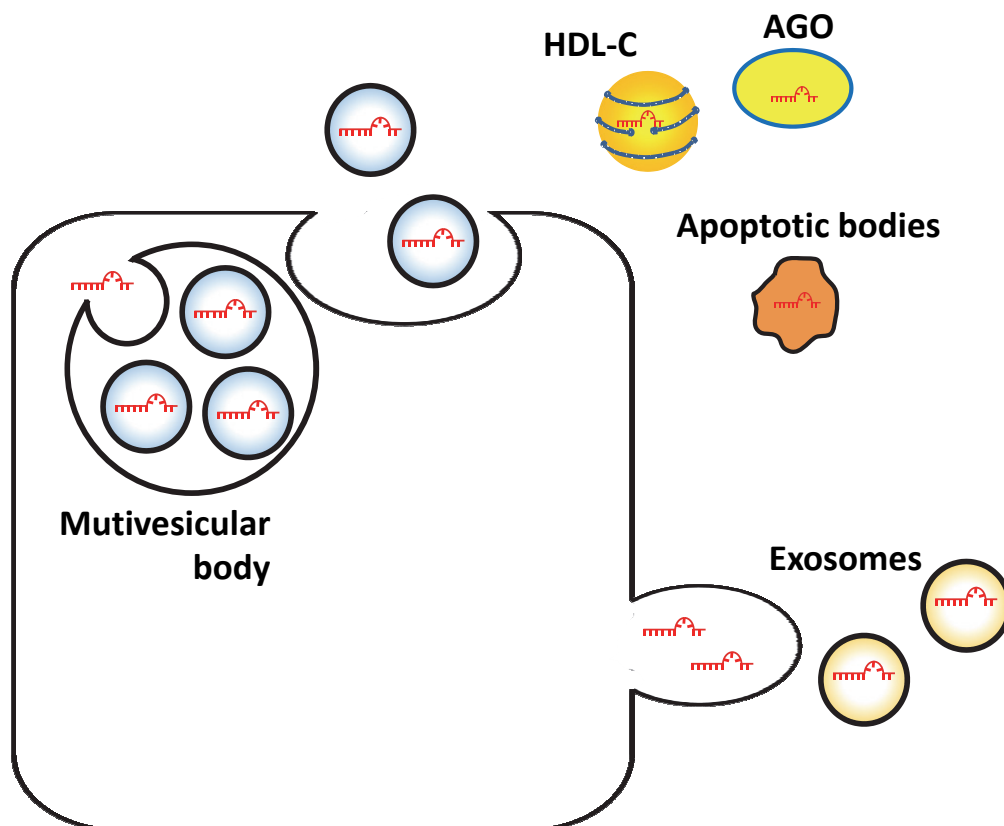


Fig. 1. Multiple ways to produce circulating miRNAs.

from RNA samples or from the original blood plasma samples. Comparison of the results obtained from serum and those from plasma may add more information. Finally, as already mentioned by the authors, miR-33b levels should also be measured in age-matched controls. Because such controls do not have insulin resistance, a clear relationship between insulin and miR-33b levels can be observed.

Conflicts of Interest

None.

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