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A standard operation procedure to effectively detect dietetically absorbed plant miRNAs

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In a new study published in the *Journal of Nutritional Biochemistry*, Chen-Yu Zhang, Xi Chen and Ke Zen's group at Nanjing University systematically characterized the kinetics of plant microRNAs (miRNAs) in human plasma after healthy volunteers drank watermelon juice or ate fruits.

miRNAs are a class of noncoding RNAs with lengths of approximately 22 nucleotides that bind to target messenger RNAs to inhibit protein translation. In previous studies, the same group has found the stable presence of food-derived plant miRNAs in mammalian plasma and organs and reported the potential cross-kingdom regulatory functions of these exogenous miRNAs in mammalian cells. However, the kinetics of dietetically absorbed plant miRNAs in human and animals has not been rigorously studied ever since this discovery. Therefore, it is urgently to solve the essential problems of detecting uptake of plant miRNAs from the diet. In this newest study, the group effectively and quantitatively measured the kinetics of plant miRNAs in plasma after healthy volunteers drank watermelon juice or ate fruits. The group also addressed some critical technical problems, such as the lack of a standard method for RNA extraction and the lack of proper internal controls that may cause inconsistent results in measuring exogenous plant miRNAs.

The choice of "right" plant miRNAs to be detected is the most essential factor that needs to be considered in quantitative detection of plant miRNAs in human plasma. Currently, the quantitative RT-PCR (qRT-

PCR) assay is the most common and sensitive and method to determine the levels of miRNAs. However, due to the limitation of qRT-PCR, non-specific signals (e.g., CT value of no-template control) are often obtained. Furthermore, a specific standard curve for individual plant miRNAs in plasma is indispensable for showing the linear quantification range. Among the 18 plant miRNAs they tested, 2 had high background noise and 6 were outside the dynamic quantification range, suggesting that these plant miRNAs can not be accurately measured by qRT-PCR analysis in plasma. Along this line, the failure to detect these plant miRNAs in human plasma is not strange, although they are highly abundant in plant. Therefore, to find the "right" plant miRNAs to be detected by qRT-PCR, a screening process may be required, and a specific standard curve and a no-template control are absolutely necessary.

In this study, the group finally identified 6 plant miRNAs (MIR156a, MIR162a, MIR168a, MIR172a, MIR390a and MIR528) to show dynamic physiological patterns and typical kinetic absorption curves in human plasma after the volunteers drank watermelon juice. The absorption rates ranged from 0.04% to 1.31%. Further calculation indicated that these plant miRNAs are present in human plasma in a similar concentration range to endogenous miRNAs. Moreover, unlike circulating endogenous miRNAs, which were present both in microvesicles (MVs) and the MV-free fractions, plant miRNAs were found to be largely encapsulated in MVs and nearly undetectable in the MV-free fraction of human plasma. Since MVs can mediate intercellular communication by transporting bioactive miRNAs between cells, these plant miRNAs are present in the plasma in a in a form that can be easily absorbed by other cells. Therefore, although the concept of plant miRNAs as a new type of nutrition remains to be elucidated, plant miRNAs have the full capacity to exert physiological functions and may be as important as endogenous miRNAs.

As an important extension of this study, the group validated the qRT-PCR results by a Northern blot analysis using LNA-modified oligonucleotide probes. The results obtained by Northern blotting were in accordance with those by qRT-PCR, which showed that plant miRNAs were readily detected in human plasma after healthy volunteers drank watermelon juice. Thus, plant miRNAs in human plasma can be efficiently detected and reliably compared by common molecular techniques. This study may serve as a guideline to clarify the controversial issues in the quantification of exogenous plant miRNAs in plasma and other bodily fluids.

The detection of exogenous plant microRNAs in human/animal plasma/sera lies at the foundation of exploring their biological functions. This study established a standard operation procedure to effectively detect dietetically absorbed plant miRNAs and thus promoted further research in this nascent field. Future studies should focus on investigating the dietary uptake mechanism of plant miRNAs.

More information: *Journal of Nutritional Biochemistry*, DOI: 10.1016/j.jnutbio.2014.12.002