JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 1 (2014) 126-127

Letter to the editor

The Weight of The Evidence: Eating Food Plants and Food RNA Is Not Detrimental to Health

Dear Editor

In a recent editorial article published by Salehi (2014) in *Journal of Food Quality and Hazards Control* (1: 93), the author cited two studies of plant microRNAs (miRNAs) as supporting "adverse health consequences" of genetically modified foods (Zhang et al., 2012a; Zhang et al., 2012b). The first of these studies reported that a conserved, ubiquitous, and high-abundance plant microRNA could regulate expression of the mammalian gene LDLRAP1 (Zhang et al., 2012a). On the other hand, analysis of public RNA expression datasets (Zhang et al., 2012b) found little evidence for uptake of plant dietary miRNAs, or "xenomiRs" (Witwer, 2012) by ingesting animals. Strikingly, and of some potential concern, it was suggested that the foods examined in these two studies (plants without specific modifications) are safe, while a food category not examined in the studies (genetically modified plants) represents a health risk. It might be useful to examine the two cited studies more closely, as well as to consider the more recent literature on the topic of xenomiRs.

The first issue to resolve is that Zhang et al. (2012b) have not claimed that "double-stranded RNAs (dsRNAs) generated in genetically modified plants...can create biosafety risks" indicated by mentioned article. Instead, they reported that MIR168a, a ubiquitous and abundant mature endogenous plant miRNA (not dsRNA), can affect the expression of a mammalian gene in the liver (Zhang et al., 2012b). This result was questioned in light of new evidence from a subsequent study (Dickinson et al., 2013) that included important additional experimental controls. Had the initial finding been validated, it would still have had no specific relevance to genetically modified food as opposed to plants in general. If adverse consequences could be inferred from the original study, the logical conclusion would be "to avoid the hazards of..." eating any and all plants, not just genetically modified foods. Genetic modification, in this scenario, would have provided useful tools to reduce the expression of the putatively harmful MIR168a and thus allow safer consumption of plant material. The point is moot, however, since no xenomiR-based harm has been established for modified or unmodified plants.

Secondly, a subsequent independent publication (Zhang et al., 2012b) did not state that dietary xenomiRs "enter the serum and plasma of humans and animals." Quite the opposite: examining around 80 public datasets, the authors found that meager, highly variable evidence of xenomiR detection was most consistent with contamination rather than actual uptake. Sources of environmental plant RNA abound, especially in labs that study plant sequences. Contamination is easily detected with sensitive high-throughput techniques (Tosar et al., 2014) and gives a possible explanation for previous, apparently positive results.

The history of safe plant consumption demonstrates that hypothetical xenomiR-based regulatory mechanisms are not a health risk. Indeed, essential human genes contain many binding sites for plant RNA products, without evidence of adverse consequences (Jensen et al., 2013). Since interactions of these genes with foreign xenomiRs or other RNA interference effectors would have resulted in negative selection, we must assume that the interactions do not occur. One reason why xenomiRs do not affect genes in the ingesting animal—and contrary to the statement that "food-derived small RNAs and dsRNAs can enter the circulation"—is that they are not normally present in circulation and cannot reach copy numbers in mammalian cells that would be consistent with regulatory influence. An increasing number of investigators have found no significant uptake of plant xenomiRs into mammalian blood (Baier et al., 2014; Dickinson et al., 2013; Snow et al., 2013; Witwer et al., 2013), even when the most abundant miRNAs in the food sources are examined. None has reported uptake of dsRNA. The many barriers to uptake and function of dietary RNA have been reviewed elsewhere (Petrick et al., 2013; Witwer and Hirschi, 2014). Considering the mounting evidence against uptake and function of even high-abundance, ubiquitous plant xenomiRs, there is currently no scientific reason to discourage ingestion of genetically modified plants on the basis of small RNA considerations.

References

Baier S.R., Nguyen C., Xie F., Wood J.R., Zempleni J. (2014). MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, hek-293 kidney cell cultures, and mouse livers. *The Journal of Nutrition*. 144: 1495-1500.

Dickinson B., Zhang Y., Petrick J.S., Heck G., Ivashuta S., Marshall W.S. (2013). Lack of detectable oral bioavailability of plant microRNAs after feeding in mice. *Nature Biotechnology*. 31: 965-967.

Jensen P.D., Zhang Y., Wiggins B.E., Petrick J.S., Zhu J., Kerstetter R.A., Heck G.R., Ivashuta S.I. (2013). Computational sequence analysis of predicted long dsRNA transcriptomes of major crops reveals sequence complementarity with human genes. *GM Crops and Food*. 4: 90-97.

Petrick J.S., Brower-Toland B., Jackson A.L., Kier L.D. (2013). Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: A scientific review. *Regulatory Toxicology and Pharmacology: RTP*. 66: 167-176.

Salehi R. (2014). Probable adverse health consequences through alteration of circulating free micro- and small-RNAs following consumption of genetically modified foods. *Journal of Food Quality and Hazards Control*. 1: 93.

Snow J.W., Hale A.E., Isaacs S.K., Baggish A.L., Chan S.Y. (2013). Ineffective delivery of diet-derived microRNAs to recipient animal organisms. *RNA Biology*. 10: 1107-1116.

Tosar J.P., Rovira C., Naya H., Cayota A. (2014). Mining of public sequencing databases supports a non-dietary origin for putative foreign miRNAs: underestimated effects of contamination in NGS. *RNA*. 20: 754-757.

Witwer K.W. (2012). XenomiRs and miRNA homeostasis in health and disease: Evidence that diet and dietary miRNAs directly and indirectly influence circulating miRNA profiles. *RNA Biology*. 9: 1147-1154.

Witwer K.W., Hirschi K.D. (2014). Transfer and functional consequences of dietary microRNAs in vertebrates: Concepts in search of corroboration: Negative results challenge the hypothesis that dietary xenomiRs cross the gut and regulate genes in ingesting vertebrates, but important questions persist. *Bioessays: News and Reviews in Molecular, Cellular and Developmental Biology*. 36: 394-406.

Witwer K.W., McAlexander M.A., Queen S.E., Adams R.J. (2013). Real-time quantitative PCR and droplet digital PCR for plant miRNAs in mammalian blood provide little evidence for general uptake of dietary miRNAs: Limited evidence for general uptake of dietary plant xenomiRs. *RNA Biology*. 10: 1080-1086.

Zhang L., Hou D., Chen X., Li D., Zhu L., Zhang Y., Li J., Bian Z., Liang X., Cai X., Yin Y., Wang C., Zhang T., Zhu D., Zhang D., Xu J., Chen Q., Ba Y., Liu J., Wang Q., Chen J., Wang J., Wang M., Zhang Q., Zhang J., Zen K., Zhang C.Y. (2012a). Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Research*. 22: 107-126.

Zhang Y., Wiggins B.E., Lawrence C., Petrick J., Ivashuta S., Heck G. (2012b). Analysis of plant-derived miRNAs in animal small RNA datasets. *BMC Genomics*. 13: 381.

Dr. K.W. Witwer

Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, USA

E-mail: kwitwer1@jhmi.edu