The doubts about dietary RNA

Scientists are grappling with the radical idea that microRNAs from food could directly affect human gene expression. **By Kristina Campbell**

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Huang-Ge Zhang has shown that nanoparticles from grapes can deliver therapeutic RNA.

Until September 2011, Janos Zempleni's main focus was working out how the bodies of mammals use chemical compounds such as vitamins. But new research published online at the time changed that.

Zempleni, a molecular nutritionist at the University of Nebraska–Lincoln, like many others in the field, was struck by the findings of an astonishing study published in *Cell Research* suggesting that food could provide something other than nutrients – information from ingested plants could switch mammalian genes on and off[1]. In the study, researchers reported that microRNAs (miRNAs) – very short fragments of non-coding RNA molecules – originating from plants such as rice had been found in the bloodstream of mice, cows and humans. And in a mouse model, one particular rice-derived miRNA seemed to reach the liver, where it directly inhibited the expression of a gene that normally serves to clear 'bad' low-density lipoprotein cholesterol from the blood. After learning about the work, Zempleni was keen to follow up on the possible transfer of genetic material from dietary components, and to determine how extensive this phenomenon might be.

When Kenneth Witwer, a molecular biologist at Johns Hopkins University School of Medicine in Baltimore, Maryland, read the paper, he immediately realized the potential significance of the work. "I thought, wow, this is amazing. I want to do this, too." He remembers thinking, "maybe this is some evolutionarily conserved way that we can extract something else from our food other than just nutrition." He corralled some of his lab's resources and set about trying to verify the findings in a small animal study of his own.

But misgivings about the *Cell Research* study soon began to surface. Not only were Witwer and several others unable to reproduce the findings, but some of its basic premises were also called into question. Scientists doubted that diet-derived miRNAs could make it into the systemic circulation of animal hosts at sufficient levels to have a meaningful impact. Follow-up work[2] also revealed the strong possibility that the 'diet-derived' miRNAs were actually the result of contamination.

Initial excitement about the possible health effects of rice-derived miRNAs gradually tapered off. Some researchers, including Witwer, gave up studying it altogether. But others persevered with the idea that what we eat can directly affect gene expression. What's at stake is a clearer understanding of how humans relate to, and derive benefit from, their food.

A tall glass of exosomes

Zempleni, after a brief and disappointing spell looking for broccoli-specific miRNAs in humans, turned his attention to miRNAs in milk. "We settled on milk because of the importance for infant nutrition and because Americans consume lots of milk," he says.

Zempleni wondered whether the miRNAs in milk go beyond the gastrointestinal tract. But he quickly encountered a problem: the miRNA molecules themselves rapidly degraded in the gut. "We realized what matters is really not just the miRNAs," Zempleni says. "What's at least equally important is the shell in which these miRNAs are packaged." This shell is a bubble-like vessel called an exosome. "In order for miRNAs to be bioavailable and to be absorbed from the gut, they have to be encapsulated in these exosomes," Zempleni says. As others had shown, fragile miRNAs need to be protected in these containers to be transported from cell to cell.

The exosomes accounted for how the miRNAs could remain intact in the host's digestive tract, but the next challenge was to work out how they end up in different places in the body. As a way of testing whether the milk miRNAs could go beyond the mouse gut, Zempleni and his colleagues devised a method for labelling the miRNAs contained in cow's milk exosomes with fluorescent compounds. These could then be tracked in animal models. "This technology confirmed that these microRNAs, if encapsulated in exosomes, accumulate in various tissues," he says – mainly the brain, liver and intestinal mucosa[3].

This established that the miRNAs could reach not just local sites (the gut wall), but also distant ones. Turning, then, to the question of how the miRNA-containing exosomes were affecting host health, Zempleni carried

out various experiments in which he gave mice a diet deficient in both free miRNAs and miRNA-containing exosomes, and compared them with other mice consuming a diet that had normal levels of each. He found a range of effects, including a decrease in the cognitive performance[4] of mice receiving the depleted diet, a decrease in fecundity[5] and changes in muscular growth[6].

Zempleni is now tackling the question of whether these health effects are conferred by the dietary miRNAs or something else, such as the entire exosome or a component of the exosome besides miRNAs. He and his colleagues are looking at a group of mice engineered to lack miRNAs in their milk. Initial unpublished results show that their offspring, whose diet consists only of their mother's milk, have numerous health and developmental problems. If confirmed, this would specifically implicate the diet-derived miRNAs as major players in health – at least, those in milk during early life.

Zempleni says that "miRNAs and exosomes are way more bioavailable in milk than in plants". He speculates that this might have evolutionary underpinnings: "Nature may have made them to be bioavailable because of infant nutrition," he says (see page S12). Zempleni is investigating other foods of animal origin, and, as part of an ongoing study, he is looking at whether he can track how dietary chicken-egg exosomes deliver miRNA cargo to mouse tissues.

A gut feeling

Some of Zempleni's animal-model work is based on the idea that exosomes interact with the gut microbiota – the community of microorganisms involved in the health effects conferred by a host's diet. This led to the hypothesis that the gut microbiota might mediate cell-to-cell communication between milk exosomes and mammalian hosts.

It's in this realm that Witwer predicts much of the progress in the field will occur over the next few years. "We can shift our focus from the circulation and the tissue of the animal, to the gut," says Witwer. He thinks that interactions of diet-derived exosomes with gut epithelial cells or particular gut microbes hold promise.

The gut has also been a central focus for researchers studying the extra-nutritional health effects of dietary plants. Immunologist Huang-Ge Zhang at the University of Louisville in Kentucky is pursuing the question of how plant foods, such as grapefruit, carrots and mushrooms, might affect specific cells. He studies the plant equivalent of exosomes, entities called exosome-like nanoparticles, which are protective vesicles with similar precious cargo inside: protein, lipid and RNA. In 2018, Zhang reported how ginger exosome-like nanoparticles are stable in the intestine, and how they regulate gut bacterial composition[7].

According to Zhang, when introduced into mammals, exosome-like nanoparticles can home in on different cells in the intestine with remarkable specificity. He has shown, for example, that exosome-like nanoparticles from grapes are taken up by gut stem cells[8], and that nanoparticles from grapes, ginger, carrots and grapefruit target gut-associated macrophages[9].

Zhang's view is that the miRNAs in these exosome-like nanoparticles might have been incorrectly singled out in earlier work as responsible for host health effects. Because exosome-like nanoparticles consist of numerous proteins, lipids, RNAs and polysaccharides, says Zhang, they might do many things at once. "Multiple factors carried by a single nanovesicle can be taken up by the same cells," he says. "Therefore, we can see multiple molecules as regulating multiple pathways."

Zhang hopes that, by learning which host cells (in the gut and elsewhere) preferentially take up different plant-derived exosome-like nanoparticles, researchers could assemble new nanoparticles for use as drug-delivery vehicles to very specific cell types in the body. Having abandoned his own studies on milk exosomes around 2008, he says that plant nanoparticles have several distinct advantages over exosomes of animal origin. Not only are exosome-like nanoparticles safer because they avoid possible transfer of cow-derived pathogens, but they are also more versatile – drug developers looking to target a particular cell type can explore the exosome-like nanoparticles derived from thousands of different types of plant, each with its own target in the host. Furthermore, Zhang says, purification of milk exosomes is particularly challenging, and large quantities of exosomes are more expensive to produce than are plant nanoparticles.

Molecular biologist Jiujiu Yu, also at the University of Nebraska–Lincoln, became interested in the therapeutic potential of plant-derived vesicles because they could be extracted in large numbers from various plant foods. In particular, she wanted to know how vesicles affected metabolic inflammation and obesity. Her lab developed a cell-culture system to screen dietary exosome-like nanoparticles from ginger or mushrooms to find out how they affected the cells implicated in inflammatory processes related to metabolic disease.

Yu is focused on identifying the part of the exosome-like nanoparticle responsible for anti-inflammatory effects. Her latest work, which has not yet been published, has shown that only in rare cases is the RNA component necessary for the anti-inflammatory effects of the vesicles. She wants to explore the possibility that, for a given food, any part of the exosome-like nanoparticle could be responsible for a health effect. "People try to focus on miRNA because it's a new component," Yu says. "Protein and lipids are not that exciting. But we should try to study all these components of the vesicles, not just focus on something that catches the eye."

"If you load milk exosomes with cancer drugs, you could deliver them to tumour sites in cancer patients."

Yu thinks there is much more still to learn before exosome-like nanoparticles from plants are put to therapeutic use in humans. Her lab has found that ginger purchased from different grocery stores contains different exosome-like nanoparticles that yield different results[10]. The vesicles can have strong or mild anti-inflammatory effects, or even promote inflammation. "There's inconsistency, so we need to be very careful if we want to just use those dietary vesicles for therapeutic use," she says. "I really want to identify the active molecule."

Zempleni, meanwhile, sees applications for milk exosomes on the horizon. "If you load milk exosomes with cancer drugs, you could deliver them to tumour sites in cancer patients – even if the drugs themselves are not very bioavailable or not very stable," Zempleni says. "That's a big story these days." Indeed, PureTech Health of Boston, Massachusetts, in collaboration with pharmaceutical giant Roche, is already working to advance technology that uses milk exosomes for drug delivery.

The ultimate goal is to learn the language in which our food speaks to us – and to discover whether miRNAs might serve as a Rosetta Stone.

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