MicroRNAs in breast milk might have a role in regulating development in babies.

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Unravelling the mysteries of microRNA in breast milk

A decade after microRNAs were found in mother's milk, scientists are still trying to work out why they are there and how they affect health. **By Tien Nguyen**

A mother's milk is a comestible marvel. It's packed with nutrients and other benefits for babies' health. A steady stream of research has linked breast-feeding to lower risk of infection, obesity, diabetes and respiratory disease in infants.

Scientists are busy investigating how exactly breast milk provides these protective effects. Researchers have a good grasp of how many of the nutrients it contains, such as fats and vitamins, affect infant health, says Steven Hicks, a paediatrician at Pennsylvania State University College of Medicine in Hershey. But breast milk is a complex blend of numerous bioactive molecules, and the contribution of all components is not yet clear.

"Our knowledge base about breast milk and how it provides health benefits is constantly evolving," says Hicks. "Every time we think that we've got it figured out, a scientist comes along and finds a new molecule that we didn't know was there", or that we didn't realize was important, he says.

One such molecule is microRNA (miRNA) — short, fragile strands of RNA made up of around 22 nucleotides and found inside protective extracellular vesicles called exosomes. Once overlooked as genetic junk, miRNA is now attracting attention as an important player in regulating gene expression. By attaching to matching strands of messenger RNA, which is involved in protein synthesis, miRNA can effectively turn mRNA off and on, and alter what proteins are made.

It was not until 2010 that miRNAs were found in breast milk[1]. Researchers suspect that the molecules have a role in regulating important aspects of infant development, such as immune function. If this is true, miRNAs could be added to infant formula so babies fed this way don't miss out on the health benefits. But before this can happen, researchers must answer basic questions about the molecules — including whether miRNA can even survive in the gut.

Food versus function

Bo Lönnerdal, a biochemist at the University of California, Davis, has spent decades studying the bioactive components of breast milk. When Lönnerdal learnt that researchers had found miRNAs in breast milk, he remembers wondering what the molecules were doing there. There must be a reason why these seemingly random bits of RNA are present in milk, he recalls thinking.

To explain their existence, researchers came up with two theories. The first, known as the nutritional hypothesis, proposes that miRNAs are just convenient packages of nutrients — much like one of breast milk's major proteins, serum albumin — that are broken down in the gut. The second, dubbed the functional hypothesis, suggests that miRNAs have a regulatory role and affect an infant's gene expression.

One way to work out whether miRNA is more than just molecular baby food is to determine whether it is broken down during digestion or if it survives to influence cells in the gut wall and beyond. In 2017, Lönnerdal and his colleagues explored this question by exposing miRNA-containing exosomes from breast milk to acidic conditions that mimic those in the infant gut and observing how the packages fared[2].

"They survive quite well," Lönnerdal says — the exosomes protect the otherwise vulnerable miRNA from being degraded. The team also found that when the exosomes were incubated with human cells, the miRNAs made their way into the nuclei of cells, where the molecules could affect gene expression.

Not everyone agrees with the conclusion that miRNAs can survive the conditions in the stomach. Researchers at the Swiss Federal Institute of Technology Zurich (ETH Zurich) found that miRNAs were not present in the

digestive organs or the bloodstream of mice at biologically relevant levels[3]. But it has been argued that miRNA could be active in very low amounts, and further research has supported the idea that miRNAs can withstand acidic conditions. To resolve the matter once and for all, researchers, including Hicks, are designing clinical studies of breastfed infants. A study to detect intact miRNA in babies' stool is already under way.

Cataloguing mother's milk

The issue of whether miRNAs survive aside, so far scientists have mostly been identifying common miRNAs in breast milk, and then working out which biological processes they might affect. The first part is fairly easy: miRNAs can be isolated from breast-milk samples. After that, "it's a bit of a guessing game", Hicks says.

Once researchers have read the 22 or so base pairs of a strand of miRNA, they can match it to a complementary mRNA, he explains. That mRNA might code for a protein involved in a key biological process, such as immune function or metabolism.

The issue, however, is that mRNAs are much bigger than miRNA. So a single miRNA might match 20–50 mRNAs, Hicks says. Scientists are using software algorithms to whittle down their results – weeding out imperfect matches that are off by one or two base pairs, or searching the literature to find previously reported matches between a specific miRNA and mRNA.

So far more than 1,400 miRNAs have been identified in breast milk, according to a 2019 review[4]. Several studies have linked the major miRNAs present in breast milk to regulation of immune responses.

One article reported that miRNA-148a is highly expressed in mother's milk[5]. This particular miRNA has been shown to suppress the activity of genes in tumour cells involved in proliferation, leading the authors to speculate that miRNA-148a has a protective effect against cancer in infants. One of the paper's authors, Regina Golan-Gerstl at Hadassah Medical Center in Jerusalem is now investigating whether miRNA-containing exosomes can travel beyond the gut to reach other organs through the bloodstream. Using fluorescent labelling, her team has detected exosomes in the liver and brain of mice, suggesting that miRNA can reach cells in other parts of the body and possibly regulate their gene expression. Another study has already shown that miRNAs in cow's milk can reach the liver and brain in mice[6].

Hicks' team has found that the miRNA strands found in the breast milk of mothers who give birth at full-term differ from those seen in the milk of mothers who give birth to premature babies. The researchers showed that milk from mothers who delivered early is richer in the miRNAs that target mRNA involved in metabolism[7]. This suggests that the miRNA composition of a mother's milk changes to help her baby grow faster in order to catch-up growth, Hicks says. Research has show that milk from mothers with preterm infants has a higher concentration of macronutrients such as protein and fat.

Such findings could have implications for the health of preterm babies, Hicks says. Mothers of premature babies sometimes can't breastfeed because their bodies have not yet started producing milk. Preterm babies in intensive care units are often given donated breast milk from a milk bank. The banks include full-term and preterm milk, but donations are currently not labelled with this distinction. Hicks suggests that changing this practice so that physicians can consider giving premature babies milk donated by mothers who had a preterm birth could make a difference to the development of these infants.

Baby steps

Establishing a direct connection between miRNA and infant health could pose ethical challenges. A conventional clinical trial normally includes a control group, meaning that some infants would receive miRNA and some would not. Although scientists don't know for sure that miRNAs improve babies' health, Lönnerdal says, it's hard to imagine an ethics panel approving a trial that withholds potentially beneficial molecules from a cohort of infants.

Instead, researchers might be limited to observational studies. In February, Hicks' team enrolled its 185th mother–baby pair in a clinical study funded by the non-profit Gerber Foundation in Fremont, Michigan. The study will ultimately include more than 200 participant pairs. The trial, which started in 2018, measures levels of miRNA found in breast milk, infant saliva and infant stool over 12 months. It also tracks the health of infants – specifically whether they develop food allergies, eczema or asthma.

Hicks says that his group is looking for associations between high levels of particular strands of miRNA in breast milk that survive digestion and protective effects against such conditions in babies. The group's results could be a first step towards singling out miRNAs that affect babies' health. Confirming miRNA's mechanism of action will require more basic science, Hicks says.

If the evidence shows that miRNAs are beneficial, he says, the last step would be to add the molecules to baby formula. But given that the formula industry's consumers are infants, introducing additives into products will be difficult. Indeed, two ingredients – the human milk oligosaccharides 2′-fucosyllactose and lacto-*N*-neotetraose – that first showed infant health benefits more than a decade ago, including improving gut health, were added to formula in the United States only in 2016 and in Europe in 2017.

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Lönnerdal predicts that it will be harder for molecules such as miRNAs to gain acceptance because of their origin in cancer research – miRNA dysregulation is linked to certain types of cancer. "If you google microRNA, which a lot of parents will do, you will get cancer, cancer, cancer," he says. Although the formula industry has expressed some interest in miRNA research at scientific meetings, he says, the market appeal of these molecules could impact its decision to move ahead with research.

If formula companies did decide to add miRNA, these molecules could be isolated from animal sources, says Golan-Gerstl. Research from her group has shown that around 90% of miRNAs found in human milk are also found in that of cows and goats[5]. Given many people's preference for products labelled as natural, an animal-derived molecule might be more acceptable to the public than synthetic versions, and stand a better chance of approval, she says.

But putting miRNA into formula does not have to be the only focus for the field, Golan-Gerstl says. Uncovering the health benefits of milk miRNA could be valuable information in and of itself. Breast milk could hold more surprises for us still.

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