Key factor in neonatal zinc deficiency may impact lactation and breast cancer

April 30 2014, by Sara Lajeunesse

Graduate student Steve Hennigar peers at a female mouse. Credit: Patrick Mansell

(Medical Xpress)—It started when her son was just two months old with a diaper rash that would not go away. The blisters eventually spread across his body, including his face. He soon began to suffer from

chronic ear infections, unusual for an exclusively breastfed infant who was not in day care. By the time he was six months old, his mother noticed that he was not as active as other babies his age, and he was also failing to gain weight.

"The pediatrician told me that my son was allergic to my breast milk and that I should wean him onto infant formula," she says. "I refused to stop breastfeeding, because I knew 'breast is best.' Instead, I eliminated all possible allergens from my diet. Only organic foods crossed my lips. Out went cow's milk, all things soy, eggs, gluten, and the dreaded peanut, but nothing helped."

That's when the woman contacted Shannon Kelleher, associate professor of nutritional sciences.

"This mother had read about our work on breast milk composition, and she asked us if we could figure out what was wrong with her milk," says Kelleher. "It turned out her milk contained only 25 percent of the zinc that it should and as a result the baby was severely zinc deficient. He was quickly put on zinc supplementation and within a week, his symptoms began to improve dramatically."

Kelleher has made a career of studying zinc's role in breast development, lactation, and involution—the process whereby the majority of breast epithelial cells rapidly undergo programmed cell death once an infant is weaned. She also investigates the consequences when these processes go awry, as they can in breast cancer and obesity.

Got Zinc?

According to Kelleher, zinc is one of the most important micronutrients in breast milk: Infants need large quantities of it during the first months of their lives to support growth, immune function, and cognitive

development. Yet despite zinc's importance, pediatricians aren't trained to detect the symptoms of its deficiency.

Now Kelleher and her colleagues may have found a way to recognize women who are at risk of having low milk zinc levels. The team has identified a mutation in a particular zinc transporter—called ZnT2—that cause defects in the milk-secreting mammary epithelial cells. This mutation causes women to have severe zinc deficiencies in their breast milk—about a 75-percent reduction. "The woman who came to me with her zinc-deficient son actually had this mutation," she says.

To investigate ZnT2, Kelleher and her colleagues use mouse and human mammary epithelial cells, various mouse models, and clinical populations of breastfeeding women.

"Some 60 to 80 percent of women across the world are thought to be marginally zinc deficient just because of their diets," Kelleher says. "One of our important findings is that in mice, it takes only a slight zinc deficiency to have profound consequences on mammary gland development, the ability to lactate, and the effectiveness of involution. Now we're trying to understand why that is."

According to Kelleher, the amount of zinc in your diet does not affect the amount of zinc that makes its way into breast milk. "There is no evidence that milk zinc concentration can be affected by your diet, so taking zinc supplements won't help," she says. "Our research suggests that milk zinc levels may have more to do with whether or not you have genetic variation in ZnT2, or factors that affect the ability of ZnT2 to function properly."

Graduate student Steve Hennigar and his advisor, nutritional scientist Shannon Kelleher, study changes in lactation-related cellular processes in mice fed on high-fat and low-fat diets. Through their research, Kelleher and Hennigar have advanced understanding of zinc's role in breast development, lactation, and involution. Credit: Patrick Mansell

A cancer link

The same zinc transporter that influences milk zinc levels may influence a woman's predisposition to breast cancer.

"A higher breast density predisposes a woman to breast cancer," explains Kelleher. "Our zinc-deficient mice and our mice without ZnT2 all have dense breasts. They actually lay down collagen, fibrotic tissue in their mammary glands."

Kelleher and her colleagues are investigating whether women who are zinc deficient, particularly girls who are zinc deficient during puberty

and into young adulthood, are also laying down more collagen in their breasts. The researchers want to find out if low zinc intake is associated with an inflammatory response in girls' breasts that results in the creation of fibrotic tissue, an increase in breast density, and thus an elevated risk of developing breast cancer. They also want to know if the genetics of ZnT2 or other zinc transporters increase a person's risk for breast density and cancer.

In addition to heightening the risk of breast cancer by contributing to dense breasts, ZnT2 may also do so by decreasing the length of time a woman is able to breastfeed.

"Scientists have long known that women who breastfeed have a lower risk of developing breast cancer than women who do not breastfeed," Kelleher says. "There seems to be something protective about breastfeeding, and this protection becomes greater the longer a woman breastfeeds."

Her team is investigating how ZnT2 influences involution following weaning. They have found that ZnT2 is critical for this process.

"About 80 percent of the cells in your breast die within 72 hours of weaning an infant," Kelleher says. "It's the most dramatic example of programmed cell death in biology. It's totally orchestrated, it's tightly regulated, and one hypothesis is that it may clean out all of the pre-malignant cells. So the more you breastfeed and the more fully you activate involution, the more fully you may remove those premalignant cells and eliminate them from becoming malignant in the future.

"Our mice with defects in ZnT2 function have delayed involution," she continues. "So we suspect that women who have defects in ZnT2 might not involute well either, and this may increase their risk for breast cancer."

Lactation and obesity

Just how does ZnT2 affect involution? Kelleher's graduate student, Steve Hennigar, aims to find out.

Previous work by Kelleher's team showed that during lactation, ZnT2 is normally found on small organelles in the cell called vesicles. ZnT2 imports zinc into these vesicles, and the vesicles in turn secrete zinc into milk. Hennnigar has found that during the early phase of involution, a cytokine called TNF-alpha, a protein involved in inflammation, stimulates the redistribution of ZnT2 from vesicles to lysosomes, organelles that are involved in programmed cell death.

"We have found that when zinc begins to accumulate in lysosomes it causes the cell to die," says Hennigar. "This is the mechanism that initiates the normal process of mammary epithelial cell death and involution following lactation."

Yet according to Hennigar, the process can go haywire when a mother is overweight or obese.

"Two-thirds of women of reproductive age in the U.S. are overweight or obese," he says. "These women tend to have difficulties initiating and maintaining lactation. We are investigating the possibility that zinc and ZnT2 might have something to do with this."

In an experiment, Hennigar and Kelleher fed mice either a high-fat or low-fat diet. They found that about 75 percent of the obese mice were unable to maintain their litters past lactation day five, suggesting a lactation defect. Those obese mice that were able to lactate had increased levels of the TNF-alpha in their mammary glands, increased ZnT2 and zinc in their lysosomes, and increased markers of lysosome-mediated cell death. As a result, these mice underwent premature

involution.

Obesity is generally associated with increased inflammatory cytokines, Hennigar notes.

"Fat cells release cytokines, which attract immune cells, which then secrete more cytokines," he says. "It's a vicious cycle of inflammation that never gets resolved. We are currently trying to figure out how TNF-alpha redistributes ZnT2 to lysosomes. If we can figure that out, we can start looking at ways to prevent premature involution in overweight and obese women, perhaps by introducing dietary interventions aimed at reducing inflammation in the first place."

One of Kelleher's major motivations for conducting this work is her desire to help increase the number of breastfeeding women in the United States.

"The World Health Organization and American Academy of Pediatrics recommend exclusive breastfeeding through six months of age, but only about 14 percent of women meet this goal," she says. "There is some evidence that breastfeeding improves immune function and cognitive development in babies, and also reduces their risk of diabetes, obesity, and cardiovascular disease when they become adults."

"If we can better understand genetic and dietary factors that affect the ability of the mammary gland to work then we can design some interventions to increase the number of breastfeeding women in the United States and, by doing so, improve both infant and maternal health."

Provided by Pennsylvania State University