Botched production of insulin molecule may lead to diabetes

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Picture a pretzel factory production line, with conveyer belts carrying the dough, formed into unbaked pretzels, down to the oven to be cooked.

Now imagine what would happen if pretzel dough started to overflow the mixer and oozed as a blob onto the conveyor, misshapen, and sticking fast to the dough of the other fully formed, unbaked pretzels. The result: a mess. And if that mess could no longer be conveyed into the oven, the backup of messy dough in the system would get worse and worse, and might eventually shut down the whole factory.

That's essentially what might be happening in a much smaller kind of factory: the cells that make insulin in the body of people with diabetes.

According to new findings by a team from the University of Michigan Medical School, those tiny factories may shut down because of glitches in the production of a molecule called proinsulin — the precursor, or "dough", out of which insulin is made.

The insulin factories are called beta cells, and they normally churn out large quantities of insulin within the pancreas. This insulin supply can be released into the bloodstream as needed, to help the body turn sugars from food into energy for cells.

But in people with diabetes, the beta cell factories don't keep up with the demand for insulin, and sugar builds up in the blood, wreaking havoc on nerves, blood vessel walls and kidneys. And just like a factory that can't

fill a growing number of orders for a hot product, the situation just keeps getting worse and the diabetes progresses.

Scientists have been working to understand why insulin production falters in people with diabetes, and the U-M team has focused on the production and folding of the proinsulin molecule deep within the beta cell. Using a tag that can make proinsulin glow green, they have now found a way to watch proinsulin being made within animal cells, and folded into a shape that can then be turned into insulin. Of course, this also allows them to study what happens when that process goes awry.

In the new paper, published online before print publication in the Proceedings of the National Academy of Sciences, the team details its findings and proposes that proinsulin 'blobs' might lead to beta cell dysfunction and death, which in turn can lead to the start, or progression, of diabetes.

Senior author Peter Arvan, M.D., Ph.D., says, "We believe that in the insulin production factory, misfolded copies of newly-made proinsulin can gum up the works in several ways. This paper shows that one of the first things that can happen is that misfolded proinsulin can stick to other proinsulin in the very first stages of production within the endoplasmic reticulum," the area of the cell where proteins are made.

Arvan, who is chief of Metabolism, Endocrinology and Diabetes at the U-M Medical School and director of the Michigan Comprehensive Diabetes Center, explains that this chain reaction can start with just a few misfolded proinsulin molecules. It can then lead to beta cell shutdown and an insulin shortage. "The misfolded proinsulin does not get exported from the factory, and neither does the normally folded proinsulin made after it," he says. "Pretty soon, pancreatic beta cells are running out of insulin to secrete in response to the customer's demand for the product – that is, an increase in blood glucose." And that is a key

hallmark of diabetes.

Arvan, who is the William and Delores Brehm Professor of Type 1 Diabetes Research, and first author Ming Liu, M.D., Ph.D., led the research team in developing the techniques needed to visualize proinsulin production and then study problems with the process by following misfolded molecules through the production pathway.

First, the team engineered the gene for human proinsulin to insert a tag that makes the protein fluorescent, but does not interfere with the production, function or secretion of insulin. They inserted the human gene into rat pancreas cells, which allows them to see the human proinsulin being made in live rat cells under the microscope.

Next, the team introduced a mutation into the tagged human insulin gene that causes the proinsulin molecule to fold incorrectly. This allowed them to see what happened when the misfolded human proinsulin and the normal rat proinsulin were produced together inside the same cell.

What they saw was misfolded fluorescent proinsulin getting stuck in the endoplasmic reticulum, so it could not move along normal ‘conveyor belt’ to make insulin. Simultaneously, this blocked the traffic of the normal proinsulin in the same cells. This ‘protein mess’ in the endoplasmic reticulum directly inhibits insulin production in the beta cells, even including insulin production that comes from the otherwise normal rat proinsulin. The beta cells begin to suffer from this, and they ultimately die.

The Arvan lab is also collaborating with other groups to identify new mutations in the proinsulin gene of people with congenital diabetes, and to understand how these mutations may cause a similar “protein mess.”

These mutations are apparently the second most common genetic cause

of congenital diabetes, which is a relatively rare genetic illness. Congenital diabetes differs from Type 1 diabetes because congenital diabetes is not caused by an attack by the immune system on the body's own beta cells, and because it is passed down from parent to child. Arvan and his team suspect that congenital diabetes in babies mirrors the proinsulin misfolding seen in their new study, and in a strain of mice known as Akita mice, which develop diabetes spontaneously after birth.

"The big question -- still to be determined -- is how much of the more common forms of diabetes also involve proinsulin misfolding in beta cells that are stressed to the max to make all the insulin they can," Arvan notes. "This is a question that we are actively pursuing."

Source: University of Michigan Health System