RESEARCH FRONTLINE

The JDRF Research Newsletter

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Investigating the Epigenetic Basis of Diabetic Complications

Two new JDRF-funded studies offer important insights into the role of epigenetics in the development of diabetic vascular complications such as heart disease and small blood vessel damage in the eyes and kidneys. The findings could open up new avenues in treating and preventing these common complications.

Epigenetics is a biological mechanism by which our cells adapt to changes in the environment—for good or for bad. The best example of epigenetics may be the process of cell differentiation: As an embryo grows and divides, many of its cells become specific cell types like skin cells or muscle cells. Epigenetic factors promote this process by ensuring that certain genes are turned off and others on—a necessity for cells to specialize since each contains the same complement of genes.

In the new studies, researchers have identified epigenetic mechanisms within our cells that lead to an over-expression of genes that set the stage for vascular complications.

According to Helen Nickerson, Ph.D., scientific manager of the Complications Program at JDRF, the importance of these findings is twofold: "Firstly, they may help to explain the step-by-step development of diabetic complications in terms of sustained effects, such as inflammation or other cellular changes. A second, more intriguing outcome from these studies, which potentially could have a very large impact, is that they point to possible pathways by which metabolic memory, or hyperglycemic memory, might be explained."

Metabolic memory was apparent in the landmark Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) clinical trials, Dr. Nickerson said, when patients with early, tight control of their diabetes were better protected from developing complications like retinopathy than were patients who achieved tight control later on: "Despite eventually achieving similar A1c levels, the latter group still showed an increased incidence and progression of complications."

In the longer term, she said, JDRF may be able to apply a better understanding of metabolic memory toward not just treating the complications of type 1 diabetes, but reversing them. Large clinical trials show that metabolic memory exists, and this new research is offering clues that may open the field to further study.

"Epigenetics is a novel area for exploration in diabetes," she added, emphasizing that there are likely to be a number of different epigenetic mechanisms that lead to changes in gene expression. "It has been well explored in other diseases, but is essentially uncharted territory in diabetes and its complications."

Spikes in Blood Glucose Cause Persistent Epigenetic Changes

One study, published in the *Journal of Experimental Medicine* and led by Michael Brownlee, director of the JDRF International Center for Diabetic Complications Research at Albert Einstein College of Medicine in New York City, has pinpointed an epigenetic mechanism that could explain the link between changes in blood-sugar levels and the increased risk for cardiovascular complications in diabetes patients. The research is the first to show that spikes of high blood sugar cause longlasting epigenetic changes in a specific gene, and that the increased expression of that gene launches a cascade of events ending with the activation of several inflammatory genes. These changes persisted even during subsequent periods of normal blood-sugar levels.

From a practical perspective, the study has two important implications for type 1 patients:

- First, tight blood glucose control aimed not just at reducing average blood sugar levels but also at avoiding spikes in blood sugar is important in preventing cardiovascular complications. To achieve this, some researchers have suggested that closer blood-sugar monitoring is needed on a day-to-day basis. Others expect that new therapeutic interventions—such as the development of an artificial pancreas—and increased patient compliance will be required.

- Second, researchers may be able to target the epigenetic changes as a way to minimize inflammation and limit vascular damage.

The international team of researchers launched their study in part to explain why many patients who lower their average blood-sugar levels still develop diabetic complications. They hypothesized that specific epigenetic changes occur during high blood-sugar levels and that these changes persist even after a return to normal levels.

To simulate high-blood sugar spikes, Dr. Brownlee and his colleagues put cells from the aorta in a high-glucose medium for 16 hours, then returned them to a normal medium for six days.

The cells showed increased expression of a gene called p65 and greater activity of a protein that drives inflammatory gene expression. All of these changes—both the epigenetic changes and the gene expression changes—persisted for the six days that the cells were at normal glucose levels. However, by blocking the protein that initiated this chain reaction, the researchers were able to prevent all of the negative outcomes.

Epigenetic Mechanisms in Diabetes and Inflammation

The second study, published in the *Journal of Biological Chemistry*, also identified an epigenetic role in diabetes complications. JDRF scientists from the Beckman Research Institute of the City of Hope in Duarte, Calif., found that DNA modification induced by the same enzyme activated in the Brownlee experiment promotes proinflammatory events within a type of white blood cell.

The researchers explained that since white blood cell binding to the lining of blood vessels is a key step in the development of inflammation and diseases, such as atherosclerosis, or hardening of the arteries, the results suggest a critical role for the enzyme in these diseases.

Lead scientist Rama Natarajan, Yan Li, and colleagues set out to determine the DNA mechanisms linking specific enzymes and the activation of proinflammatory genes associated with diabetes and its complications. Their findings suggest that high blood sugars are very much involved in the sequence of events. For example, in white blood cells that no longer showed activity of the enzyme studied, the researchers saw significantly reduced proinflammatory gene activation through the RAGE molecule, which has been linked to diabetic retinopathy, nephropathy, neuropathy, and heart disease, as well as nondiabetic conditions such as Alzheimer's disease and cancer.

As a result, they suggest that the enzyme may be a novel therapeutic target for inflammatory diseases, including diabetes and certain metabolic disorders.

Epigenetics Research and Initiatives at JDRF

To ensure that these studies and related research advance the ultimate goal of finding a cure for type 1 diabetes and its complications, JDRF is funding a range of projects looking at the epigenetic basis of complications. JDRF recently held an in-house workshop to address "the need for collaborative crosstalk between the type 1 diabetes complications and epigenetics biology research communities."

Key Point:

Some of the common complications of type 1 diabetes may have an epigenetic basis. JDRF is actively funding this promising field of study because it may provide new approaches to the prevention and reversal of diabetes complications.

New Regeneration Therapy Reverses Diabetes in Mice

A short treatment with two drugs partially restores the number of insulin-producing beta cells in the diabetic mouse pancreas and also slows their autoimmune destruction—enough to restore normal blood-sugar levels and reverse the disease.

The study, published in the journal *Diabetes*, was funded by grants from JDRF and Transition Therapeutics, Inc., one of JDRF's Industry Discovery and Development Partners.

According to the investigators, led by Alex Rabinovitch, M.D., at the University of Alberta in Edmonton, the findings suggest that the two drugs work together to target both the cellular mechanisms that promote beta-cell growth and survival, as well as the immunologic mechanisms that destroy beta cells in type 1 diabetes. As a result, the use of both drugs offers a "promising strategy" for reversing beta-cell loss in people with the disease.

Patricia Kilian, Ph.D., director of the Regeneration Program at JDRF, described the study results as "exciting and unexpected," in that the combination therapy produced positive effects related to both regeneration and autoimmunity. The study is unique, she said, because it is the first to test the two-drug combination (gastrin and glucagon-like peptide 1, or GLP-1) in mice with diabetes, and to show that it reverses diabetes, not just prevents it.

"While much more needs to be determined," Dr. Kilian said, "the study supports, at the preclinical level, the use of gastrin combination therapy in a human clinical trial, making it highly relevant to JDRF's mission."

Background and approach

GLP-1 and gastrin are among the most promising beta-cell growth factors being tested for therapeutic use in people with diabetes. GLP-1 is a protein produced by intestinal cells after food is ingested; one of the things it does is stimulate insulin production by the pancreas. In mice, it has been shown to promote beta-cell function, survival, and growth. Gastrin, also a protein produced by the gastrointestinal tract, also stimulates insulin production, but it causes new insulin-producing cells to form from pancreatic duct cells.

Within three to six days after the onset of diabetes, Dr. Rabinovitch and colleagues treated the mice with GLP-1 and gastrin, administered alone or together. They measured blood-sugar levels once a week during the treatment period and for five weeks after treatment ended. At the end of eight weeks, the researchers measured pancreatic insulin content and assessed whether insulin-producing cells had grown or declined in quantity.

Major Findings

The two-drug combination restored normal blood sugar levels in 11 of 13 mice, and as a result, the mice gained weight as they aged at rates similar to what one would see in non-diabetic mice. In contrast, neither drug alone restored normal blood sugars in a significant number of mice. "The surprise," Dr. Rabinovitch said, "was that no immunosuppressive drugs were required. The GLP-1 and gastrin were sufficient, in combination, to restore pancreatic beta-cell mass and stop the autoimmune destruction of beta cells in the diabetic mice."

Combination therapy had a pronounced effect on the amount of insulin produced by the pancreas. For example, low doses of GLP-1 plus gastrin increased insulin content threefold. In mice given high doses of GLP-1 plus gastrin, pancreatic insulin content was fivefold higher—about 74 per cent of normal.

The combination therapy also increased beta-cell mass. Examination the pancreas revealed that the islets were abundant with insulin-containing beta cells. These changes, however, were found to be less about replication of existing insulin-producing cells, and more about decreased beta-cell death (attributed to GLP-1) and increased production of new beta cells from pancreatic duct cells (attributed to gastrin).

Other findings suggested that the combination therapy had altered the autoimmune response against the beta cells. For instance, autoantibodies directed at insulin were undetectable in mice receiving GLP-1 and gastrin, whereas these antibodies were present before treatment and rose to very high levels in the control mice. In an "adoptive transfer" experiment, the researchers combined diabetes-causing immune cells with immune cells from the mice given the two-drug treatment, and then transferred both cell types into mice that were expected to quickly develop diabetes. What they found was that onset of diabetes was delayed by as much as 107 days. "This suggested," the scientists said, an "activation of immunoregulatory cells" in the mice treated with the two drugs.

In another set of experiments in which insulin-producing cells were transplanted into mice, even when those transplants were set up to fail, they did not do so in the majority of mice (seven of eight) that received the two-drug combination immediately following transplantation. This again suggests that the treatment is somehow regulating the autoimmune response against the beta cells.

Especially important, according to the researchers, was the finding that although white blood cells had infiltrated the transplanted islets in the mice treated with the two-drug therapy, beta cells were not destroyed. The researchers will need to determine the basis of this effect to establish the combination treatment's full therapeutic potential.

Practical applications and next steps

The clear application of this intervention within regeneration is to treat type 1 patients with gastrin combination therapy to promote new beta-cell growth within the patient and to restore functional beta-cell mass.

JDRF is also working with Dr. Rabinovitch to develop alternative strategies leading to beta-cell regeneration. One such approach, for which Dr. Rabinovitch was awarded a two-year project grant, is to elevate the body's natural gastrin and GLP-1 levels using next-generation regenerative products.

"Instead of giving the large protein gastrin," Dr. Kilian said, "another possible strategy to achieve the same endpoint is through the small molecule approach," elevating GLP-1 and gastrin indirectly through next generation compounds. Preclinical data presented by Dr. Rabinovitch last year showed that diabetic mice could be cured by a combination of two newer drugs, both of which "are safe for human consumption and can be taken in pill form," he said.

Next steps will be to advance these observations to proof-of concept clinical trials in type 1 patients.

There are also several theoretical applications of these findings, with a number of them supporting JDRF efforts in replacement. For instance, one might incubate the two growth factors *in vitro* with isolated human islets, with pancreatic duct cells that might otherwise be discarded, or both, in order to increase the total number of beta cells available for islet transplantation; one could also administer the two agents to islet transplant recipients after islet transplantation to promote new beta-cell growth in the graft.

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Gastrin's potential as a regenerative agent continues to be investigated on other fronts as well. Transition Therapeutics' exploratory phase IIa clinical trial of gastrin in combination with the growth factor EGF showed improvements in important measures of blood-sugar control in both type 1 and type 2 patients. Transition Therapeutics has subsequently partnered with the pharmaceutical company Eli Lilly to develop gastrin-based therapies using Transition's proprietary technology.

Key Point:

Combination therapy with GLP-1 and gastrin normalizes blood-sugar levels in diabetic mice by increasing beta-cell mass and reducing the autoimmune response. These findings support the use of the two-drug therapy in human clinical trials.

Type 1 Diabetes And Celiac Disease Linked Through Shared Genetic Markers

Type 1 diabetes and celiac disease appear to share a common genetic origin, scientists at the University of Cambridge and Barts and The London School of Medicine and Dentistry have confirmed.

Their findings, reported in the *New England Journal* of Medicine, identified seven chromosome regions that are shared between the two diseases. The research suggests that type 1 diabetes and celiac disease may be caused by common underlying mechanisms such as autoimmunity-related tissue damage and intolerance to dietary antigens (foreign substances that prompt an immune response).

Type 1 diabetes is an autoimmune disorder that causes the body to attack the insulin-producing cells of the pancreas. Celiac disease, also an autoimmune disorder, attacks the small intestine and is triggered by eating gluten, a protein found in wheat, barley, rye, and cereal grains. The development and anatomy of the small intestine and pancreas are closely related, and the gut immune system shares connections with the pancreatic lymph nodes.

In order to assess the genetic similarities and differences between the two inflammatory disorders, the researchers studied approximately 20,000 DNA samples from people with type 1 diabetes, celiac disease, or without either condition (the latter served as controls). The researchers, who were funded by JDRF, the Wellcome Trust, and Coeliac UK, believe that the shared chromosomal regions may regulate how the body's immune system attacks the insulin-producing cells in the pancreas and the small intestine. Their results suggest that type 1 diabetes and celiac disease not only share genetic causes but could have similar environmental triggers as well.

Professor David van Heel, from Barts and The London School of Medicine and Dentistry, said: "These findings suggest common mechanisms causing both celiac disease and type 1 diabetes—we did not expect to see this very high degree of shared genetic risk factors."

Professor John Todd, from the University of Cambridge, said: "The next step is to understand how these susceptibility genes affect the immune system, and to keep exploring environmental factors that might alter the risk of type 1 diabetes, which results from an incredibly complex interaction between nature and nurture."

Richard A. Insel, M.D., executive vice president of research at JDRF, said: "These studies demonstrate that type 1 diabetes and celiac disease share far greater genetic overlap than had been appreciated, which helps explain the high prevalence of both diseases occurring simultaneously in an individual. They provide new avenues for understanding the cause and mechanisms of both diseases."

Type 1 diabetes and celiac disease together affect about one per cent of the population.■

Key Point:

With seven chromosomal regions in common, type 1 diabetes and celiac disease have a similar genetic fingerprint, suggesting a shared underlying cause.