Mouse pancreas cells grown in chimeric rats cure diabetes in mice

Mouse pancreases grown in rats generate functional, insulin-producing cells that can reverse diabetes when transplanted into mice with the disease, according to researchers at the Stanford Institute for Stem Cell Biology and Regenerative Medicine and the Institute of Medical Science at the University of Tokyo.

The recipient animals required only days of immunosuppressive therapy to prevent rejection of the genetically matched organ rather than lifelong treatment.

The success of the interspecies transplantation suggests that a similar technique could one day be used to generate matched, transplantable human organs in large animals like pigs and sheep.

To conduct the work, the researchers implanted mouse pluripotent stem cells, which can become any cell in the body, into early rat embryos. The rats had been genetically engineered to be unable to develop their own pancreas and were thus forced to rely on the mouse cells for the development of the organ.

Once the rats were born and grown, the researchers transplanted the insulin-producing cells, which cluster together in groups called islets, from the rat-grown pancreases into mice genetically matched to the stem cells that formed the pancreas. These mice had been given a drug to cause them to develop diabetes.
We found that the diabetic mice were able to normalize their blood glucose levels for over a year after the transplantation of as few as 100 of these islets,” said institute member Hiromitsu Nakauchi, MD, PhD. “Furthermore, the recipient animals only needed treatment with immunosuppressive drugs for five days after transplantation, rather than the ongoing immunosuppression that would be needed for unmatched organs.”

People suffering from diabetes could benefit from this approach. Diabetes is a life-threatening metabolic disease in which a person or animal is unable to either make or respond appropriately to insulin, which is a hormone that allows the body to regulate its blood sugar levels in response to meals or fasting. The disease affects hundreds of millions of people worldwide and is increasing in prevalence. The transplantation of functional islets from healthy pancreases has been shown to be a potentially viable option to treat diabetes in humans, as long as rejection can be avoided.

The researchers’ current findings come on the heels of a previous study in which they grew rat pancreases in mice. Although the organs appeared functional, they were the size of a normal mouse pancreas rather than a larger rat pancreas. As a result, there were not enough functional islets in the smaller organs to successfully reverse diabetes in rats.

In the current research, the scientists swapped the animals’ roles, growing mouse pancreases in rats engineered to lack the organ. The pancreases were able to successfully regulate the rats’ blood sugar levels, indicating they were functioning normally. Rejection of the mouse pancreases by the rats’ immune systems was uncommon because the mouse cells were injected into the rat embryo prior to the development of immune tolerance, which is a period during development when the immune system is trained to recognize its own tissues as “self.” Most of these mouse-derived organs grew to the size expected for a rat pancreas, rendering enough individual islets for transplantation.

Next, the researchers transplanted 100 islets from the rat-grown pancreases back into mice with diabetes. Subsequently, these mice were able to successfully control their blood sugar levels for over 370 days, the researchers found.

Because the transplanted islets contained some contaminating rat cells, the researchers treated each recipient mouse with immunosuppressive drugs for five days after transplant. After this time, however, the immunosuppression was stopped.

After about 10 months, the researchers removed the islets from a subset of the mice for inspection. “We examined them closely for the presence of any rat cells, but we found that the mouse’s immune system had eliminated them,” said Nakauchi. “This is very promising for our hope to transplant human organs grown in animals because it suggests that any contaminating animal cells could be eliminated by the patient’s immune system after transplant.”

Importantly, the researchers also did not see any signs of tumor formation or other abnormalities caused by the pluripotent mouse stem cells that formed the islets. Tumor formation is often a concern when pluripotent stem cells are used in an animal due to the cells’ remarkable developmental plasticity. The researchers believe the lack of any signs of cancer is likely due to the fact that the mouse pluripotent stem cells were guided to generate a pancreas within the developing rat embryo, rather than coaxed to develop into islet cells in the laboratory. The researchers are working on similar animal-to-animal experiments to generate kidneys, livers and lungs.

Although the findings provide proof-of-principle for future work, much research remains to be done. Ethical considerations are also important when human stem cells are transplanted into animal embryos, the researchers acknowledge.
Institute updates

Navdar Sever resigned from the Beachy Lab
Wan-Jin Lu left her position as a postdoc and became an instructor in the Beachy Lab

Robert Hsieh moved from a postdoc position to an instructor position in the Clarke Lab
Maddalena Adorno moved from an instructor position to an ASR position in the Clarke Lab
Maider Zabala Ugalde became a visiting scholar in the Clarke Lab

Jonathan Villanueva has become a temporary LSRP 1 in the Nakauchi Lab

Rachel Salomon has become an LSRP 1 in the Loh Lab
G One Ahn will become a visiting professor in the Weissman Lab at the beginning of March
Fangfang Zhu moved from a postdoc to an ASR position in the Weissman Lab
Jinyi Xiang became a postdoc in the Weissman Lab

Rachelle Riley was hired to be the administrative assistant for the Wernig Lab
Soham Chanda moved from a postdoc to an ASR position in the Wernig Lab
Katie Schaukowitch became a postdoc in the Wernig Lab

Stephen Weber was hired to replace Jennifer Ho as an LSRP 2 in the FACS core

and my former mentors have all contributed to the success of my research. The research and collaborative environment at Stanford and the long-term support from the Howard Hughes Medical Institute have also been fantastic. I see this award as a great honor for the entire community.”

The Breakthrough Prizes, initiated in 2013, honor paradigm-shifting research and discovery in the fields of life sciences, fundamental physics and mathematics. In total, about $25 million was awarded this year. A black-tie, red-carpet ceremony will be held in San Francisco on December 5 to honor the winners.

Recent CIRM Grants

Three institute researchers have recently been awarded grants by the California Institute for Regenerative Medicine (CIRM) to promote the discovery of potential stem cell-based therapies.

Roel Nusse, PhD, was awarded $1.7 million to investigate ways to grow liver stem cells in the laboratory while also maintaining their regenerative capacity. These cells could potentially be used to treat severe liver disease or to alleviate the shortage of donor organs.

Matt Porteus, MD, was awarded $2.2 million to investigate ways to use gene editing to correct cystic fibrosis mutations in airway stem cells.

Rosa Bacchetta, MD, was awarded $1.1 million to use a gene-editing technique to repair blood stem cells from patients with a rare but fatal genetic autoimmune disease called IPEX.
Matthew Porteus named senior investigator in the new Chan-Zuckerberg Biohub

After a fiercely competitive selection process, institute scientist Matthew Porteus, MD, PhD, was named one of seven senior investigators in the newly formed Chan Zuckerberg Biohub.

The CZ Biohub is an independent nonprofit medical research organization that has the ambitious goal of harnessing the power of science, technology and human capacity to cure, prevent or manage all human disease. It is funded through a $600 million commitment by the Chan Zuckerberg Initiative, which was created by Facebook founder Mark Zuckerberg and his wife Priscilla Chan, MD.

Researchers in the CZ Biohub will have access to advanced technologies and will get research grants of up to $1.5 million over five years. About 700 researchers from around the Bay Area applied to become CZ Biohub investigators. In addition to the 7 senior investigators from the Stanford School of Medicine Investigators, the Chan Zuckerberg Initiative also named 8 junior investigators from the Stanford SOM, 4 investigators from other schools at Stanford, 15 investigators from UCSF and 13 from UC Berkeley.

Porteus uses genome editing as curative therapy for genetic diseases, as exemplified by his correction of the mutation in sickle cell disease in hematopoietic stem and progenitor cells. He is now combining genome editing with synthetic biology to engineer cells having new phenotypic properties, such as engineering resistance to HIV and enhancing wound healing.
Researchers turn leukemia cells back into iPS cells

The question sounds more like sociology than biology: What would happen if you could take a cell gone bad—a cancer cell—bring it back to its infancy, before it turned to the dark side, and let it grow up again? Would it become cancerous again? What if you raised it in a different environment? Stanford Professor Ravi Majeti, MD, PhD, and his colleagues posed this simple question about a leukemia cell. And the answer they got gave them a new set of tools for studying leukemia and designing better therapies against it.

Ten years ago, Japanese scientist Shinya Yamanaka discovered that researchers could chemically push the reset button on mature cells, turning them into something very close to embryonic stem cells, which he called induced pluripotent stem (iPS) cells. If genetic instructions are like the recipes in a cookbook, epigenetic markers are like the notations and sticky notes that accumulate over time in that cookbook as the chef gains experience and annotates which recipes should be modified, ignored or are favorites. Mature cells of different types may have the same DNA (the same recipes) as embryonic cells, but how they look and behave is different because the epigenetic annotations are different. Yamanaka’s process for creating iPS cells is like taking out all the notes in a cookbook—all the modifications and annotations are wiped out and the cell reverts to its naïve, embryonic state. These iPS cells could then be grown back up into mature cells of various types.

Over the last decade, researchers around the world have successfully turned mature cells of various types into iPS cells, but no one had yet succeeded in creating iPS cells from leukemia. When Majeti and Chao figured out how to create an iPS cell out of a leukemia cell they were curious to see what would happen. After all, although the epigenetic annotations had been wiped out, the cells still had the genetic mutations that the leukemia cells contained. When they grew the leukemia iPS cells up into other kinds of cells like heart cells or neurons, the cells behaved completely normally. But when the cells grew into blood cells, they once again became cancerous.

“This was super surprising to us,” says Majeti, who is a member of the Stanford Ludwig Center in addition to being a member of the Institute for Stem Cell Biology and Regenerative Medicine. “What this tells us is that context matters. Those leukemic gene mutations only cause cancer when they exist in the context of a blood cell.”

The ability to make lots of leukemic cells from a single iPS cell also provides a number of important tools. “We can now grow up a lot of leukemia cells,” Majeti says. “Getting a large number of cancer cells to study can be a limiting issue since patient cells are difficult to grow.” Creating iPS cells from a single leukemia cell can also help researchers study the natural variations that occur among leukemia cells. “Leukemia cell populations actually contain a number of subclones that have different genetic mutations,” Chao says. “Before this there was no good way to separate out and expand the different subclones, since some can be very rare. But now we can use this technology to create and study leukemias from each subclone within a patient’s cancer.”

This can be useful if physicians want to know how to treat a particular leukemia. If a patient has leukemia, pure populations of various subclones can be grown and tested to see how well they react to various chemotherapy agents. If a leukemia reacts well to chemotherapy but one subclone is less affected, that may set the patient up for relapse. In fact, using this technique, Majeti and Chao were able to verify that this is what happened in one particular patient. By analyzing blood samples stored over the course of the patient’s treatment, they found that one subclone was more resistant to chemotherapy, and that this subclone ended up being the source of the patient’s eventual relapse.

This technology now offers a platform to more accurately investigate how the genetics of individual cells contribute to leukemic disease. Majeti and Chao hope that these findings will lead to a better understanding of how leukemia evolves and to the development of better personalized therapies.
Diabetes impairs fracture repair by inhibiting bone stem cell activity in mice

Institute researchers found that activating bone stem cells helps repair fractures in diabetic mice. Applying a protein to the fracture site increased the expression of key signaling proteins and enhanced healing in the animals.

Bone fractures in diabetic mice heal better in the presence of a protein that stimulates the activity of skeletal stem cells, according to a study by researchers at the Institute for Stem Cell Biology and Regenerative Medicine.

The protein counteracts a decrease in stem cell activity that the researchers observed both in mouse models of diabetes and in bone samples from diabetic patients who had undergone joint replacements. The researchers hope the discovery will lead to ways to help people with diabetes heal more efficiently from broken bones.

“We’ve uncovered the reason why some patients with diabetes don’t heal well from fractures, and we’ve come up with a solution that can be locally applied during surgery to repair the break,” said institute co-director Michael Longaker, MD.

“Diabetes is rampant worldwide, and any improvement in the ability of affected people to heal from fractures could have an enormously positive effect on their quality of life.”

Longaker shares senior authorship of the study with Charles Chan, PhD, an instructor at the institute. Postdoctoral scholar Ruth Tevlin, MD, is the lead author.

Diabetes mellitus is a metabolic disease characterized by the inability to either produce or to respond appropriately to insulin. It affects hundreds of millions of people worldwide and is increasing in prevalence. In addition to causing dangerous swings in blood sugar levels after meals, the condition leads to many other debilitating symptoms, including an impaired ability to heal soft tissue injuries and skeletal fractures. The precise molecular reason behind this impaired bone healing has been unknown, however.

Longaker, Chan and Tevlin built on previous research in which they and colleagues in the laboratory of institute director Irv Weissman, MD, identified and described a population of cells in the bones of mice that serve as skeletal stem cells, or SSCs. These adult stem cells can become all components of the skeletal system, including bone, cartilage and a part of the bone marrow known as the stroma. They subsequently showed that fracture healing in mice was severely impaired when these stem cells were depleted. That finding got them thinking.

The researchers hope the discovery will lead to ways to help people with diabetes heal more efficiently from broken bones.

“We wanted to apply what we knew about skeletal stem cells to the problem of impaired bone healing in people with diabetes,” said Chan. “Does the disease affect fracture healing by somehow modulating the activity of these stem cells?”

The researchers used a mouse model of Type-2 diabetes, in which the disease arises when the animals are about 4 weeks old. Prior to the development of the disease, the prediabetic mice were able to heal leg bone fractures as effectively as wild-type mice, the researchers found. In contrast, after the disease had manifested itself, the repaired bone was significantly weaker and less dense than the bone in the control animals. When they compared the numbers of SSCs in the healing bone seven days after fracture, they found that the diabetic mice had significantly lower numbers of these cells than did the control animals.

A series of experiments ruled out a systemic reason for this reduction in stem cell numbers, and also confirmed that the cells themselves were fully
functional. That left only a potential problem with the signals the cells were receiving from the surrounding environment, or niche. When Tevlin and her colleagues analyzed that environment, they found that the diabetic animals produced significantly lower levels of a family of signaling proteins called hedgehog that are known to play a critical role in many biological processes, including embryonic development and tissue regeneration.

We’ve looked to stem cells to learn why people with diabetes don’t heal bone fractures properly, and come up with an approach that we are excited to try in the clinic.

The researchers collaborated with institute researcher Philip Beachy, PhD, to test whether artificially blocking the hedgehog signaling pathway could impair bone healing in nondiabetic mice. They found that control mice exposed to a molecule that blocked the pathway regrew bone that was weaker and more brittle — just like the diabetic animals.

“Next we had to test whether adding the hedgehog signaling proteins back into the local environment in diabetic animals restored their ability to heal fractures,” said Longaker. The researchers collaborated with co-authors Fan Yang, PhD, assistant professor of bioengineering and orthopaedic surgery, and postdoctoral scholar Xinming Tong, PhD, to devise a biologically friendly hydrogel into which the hedgehog signaling proteins were embedded. The gel was applied directly to the fracture site. “And these animals healed just like normal mice,” said Longaker, who holds the Deane P. and Louise Mitchell Professorship in the School of Medicine.

Finally, the team reached out to co-author Stuart Goodman, MD, PhD, professor of orthopaedic surgery, to obtain bone samples from patients with diabetes who were undergoing joint replacement for osteoarthritis. They compared the expression of proteins important to the hedgehog signaling pathway from these samples with others obtained from non-diabetic patients. Normally this tissue would be discarded by the surgeon, but in this case it held important clues.

“What we saw in these human samples completely echoed what we saw in the mice,” said Chan. “The bones from the diabetic patients displayed significantly reduced expression of these important signaling proteins.”

Longaker, Chan and Tevlin believe the inhibition of the hedgehog signaling pathway arises from diabetes-associated inflammation that causes high levels of a molecule called tumor necrosis factor alpha. TNF-alpha levels are known to be elevated in patients with diabetes, and the researchers observed a corresponding increase in their mouse models of the disease. They also showed that these increased levels of TNF-alpha inhibited the expression of some hedgehog family members. Directly inhibiting all TNF-alpha activity, however, could have other dire consequences for an animal or a human patient because TNF-alpha plays many important biological roles.

“Here we’ve devised a feasible strategy for reversing a tissue-specific pathology — the inability to heal skeletal fractures efficiently — in a complex metabolic disease like diabetes,” through the local application of a compound to stimulate the activity of adult stem cells,” said Longaker said. “We anticipate that hedgehog-mediated molecular therapies that directly target stem cells in human patients could be therapeutic.”

More research is necessary before trying this approach in humans, but the scientists are hopeful that local application of hedgehog proteins will be shown to be both safe and effective. Their findings further validate the idea that tissue-specific stem cells are likely to play vital roles in tissue regeneration and response to injury.