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Resetting the clock in diabetes

By Lev Osherovich, Senior Writer

A Columbia University team has evidence that pancreatic islet β cells do not die in mouse models of type 1 diabetes and type 2 diabetes but rather dedifferentiate into stem cell-like precursors.¹ The findings could explain why diabetes rapidly resolves in obese patients who undergo bariatric surgery and could argue for developing strategies to turn these dedifferentiated cells back into β cells to restore insulin secretion in diabetic patients.

β Cells secrete insulin, which promotes the packaging of sugar into glycogen and results in the build-up of energy reserves. In both forms of diabetes, the inability to produce insulin leads to numerous metabolic, vascular and neurological problems.

Although the two forms of diabetes have different root causes—autoimmunity in the case of type 1 and excess metabolic activity in the case of type 2—both culminate in β cell dysfunction and degeneration.

It is generally accepted that once β cells are lost, the only way to boost insulin levels is to regularly inject the hormone—the standard of care for type 1 diabetes—or to restore missing β cells with regenerative therapies.

Now, a team led by Domenico Accili, professor of medicine and director of the Columbia University Diabetes and Endocrinology Research Center, has found that rather than dying off, β cells can actually remain intact in mouse models of advanced diabetes, albeit in a quiescent state.

“The key result here, which is applicable to both type 1 and type 2 diabetes, is that β cells aren't dead,” said Accili. “They're present in the pancreas under a different guise.”

Devolution

Accili's team made the discovery while characterizing a mouse with knockout of *forkhead box O1* (*Foxo1*), a transcription factor involved in stress response in multiple tissue types. Those mice had multiple abnormalities in food intake and glucose homeostasis.²

In the new study, Accili's team examined how pancreas-specific deletion of *Foxo1* affected disease progression in mouse models of diabetes.

Initially, pancreas-specific *Foxo1* knockout mice had normal pancreatic activity. However, as the animals aged they showed loss of β cell mass, lower insulin secretion and higher levels of the glucose-releasing hormone glucagon compared with wild-type controls.

Fluorescent labeling studies of the *Foxo1* knockouts' pancreas indicated that the β cells were still present but had reverted to an



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undifferentiated state. These dedifferentiated cells expressed markers associated with pluripotent or stem cell-like characteristics but were unable to secrete insulin.

Over time, the dedifferentiated cells converted into a variety of pancreatic cell types other than β cells. The team found similarly dedifferentiated β cells in two other models of type 2 diabetes—an obese mouse and a mouse lacking insulin receptors in key tissues—and in a chemically induced model of type 1 diabetes.

Results were reported in *Cell*.

Are we not mice?

Accili's findings raise the possibility that dedifferentiated β cells may be present in the pancreas of patients with diabetes. The challenge will be finding such cells and showing that they are a result of disease and can be reverted to their normal state.

"This paper makes people rethink whether cell death is the main cause of diabetes or whether there is something going on with these weird dedifferentiated cells," said Douglas Melton, professor in the Department of Stem Cell & Regenerative Biology at Harvard University and co-director of the Harvard Stem Cell Institute.

"Accili's group provides compelling evidence that dedifferentiation of functional β cells into a quiescent state leads to reduction of β cell mass and function," said Hui Tian, VP of research at NGM Biopharmaceuticals Inc. "It remains to be tested whether this phenomenon can be observed in other animal models, and, more importantly, in humans."

"It remains to be tested whether this phenomenon can be observed in other animal models, and, more importantly, in humans."

—Hui Tian,

NGM Biopharmaceuticals Inc.

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NGM is conducting discovery-stage research to identify targets to regenerate β cells in type 1 and type 2 diabetes.

Melton and Tian said determining whether dedifferentiated β cells are present in humans will require developing imaging tools that detect changes in β cells *in vivo*.

One way to find dedifferentiated cells would be histological studies of pancreatic biopsies from patients, but intact *ex vivo* tissue samples are difficult to obtain.

“The challenge for human studies has always been the availability of high-quality pancreatic specimens with enough sample size,” said Tian.

“We lack the ability to interrogate the status of β cells in a living person,” said Melton. “You can’t do a biopsy because the pancreas is an extremely fussy organ, and you can’t just go in there and remove tissue. You need some kind of imaging method to see cells undergoing dedifferentiation.”

Whip into shape

If β cell dedifferentiation indeed occurs in humans, the challenge will be to reverse the process. Accili suspects this could be done by manipulating metabolic conditions or developmental cues known to affect β cell differentiation.

One hypothesis is that changes in diet or food absorption could be enough to restore β cell functioning.

“We’ve known since the 1970s that type 2 diabetes is reversible by fasting,” said Accili. His team hopes to test whether restoration of β cell identity plays a role in recovery from diabetes brought about by severe dietary restriction.

The study raises the possibility that the recovery of β cells from a dedifferentiated state could account for the rapid restoration of insulin secretion in obese type 2 diabetes patients after bariatric surgery.

“It’s a tantalizing hypothesis that these patients may have dedifferentiated progenitor cells present that are now reactivated by bariatric surgery,” noted Tian.

Separately from its work on β cell regeneration, NGM is conducting discovery-stage research to elucidate the mechanisms underlying

remission of diabetes following bariatric surgery and identify protein and peptide hormones involved in the process.

Accili said another idea is that developmental pathways such as wingless-type MMTV integration site (WNT) and NOTCH signaling that induce β cells from undifferentiated precursors during embryogenesis could be manipulated to restore β cell identity.

His team is now conducting cell culture screens for compounds that promote redifferentiation of β cell precursors and has filed a patent on these screening methods.

Melton noted that in type 1 diabetes, any efforts to restore β cells from a dedifferentiated state will still need to be accompanied by immunomodulatory therapy to prevent the autoimmune attack.

He said dedifferentiation in type 1 diabetes might in fact be beneficial, hiding β cells from complete destruction by the immune system during an early stage of disease. It may, he said, be desirable to accelerate the process of dedifferentiation to protect the β cells until autoimmunity can be brought under control.

“If you can make cells dedifferentiate quickly, you might not attract the attention of immune cells during the so-called honeymoon period” before the onset of a full autoimmune assault on β cells, said Melton.

According to Accili, Columbia has filed for patents covering biomarkers of dedifferentiated β cells. Those patents are available for licensing.

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Suppressing AML through SIRPA

By Kai-Jye Lou, Staff Writer

Researchers at **The Hospital for Sick Children, University Health Network** and **University of Toronto** have shown that targeting SIRPA disrupts its interaction with CD47 on macrophages and could help eliminate acute myelogenous leukemia stem cells.¹ The data provide validation for a SIRPA-Fc fusion protein being developed by **Trillium Therapeutics Inc.** that antagonizes the SIRPA-CD47 interaction—the biotech hopes to submit an IND in 2014.

CD47 is a membrane protein broadly expressed across tissues. It interacts with multiple proteins, including SIRPA (signal regulatory protein- α).

Normal cells express CD47 to protect themselves from phagocytosis—recent studies by researchers at **Stanford University** have shown that

AML stem cells can do the same.^{2,3} The Stanford group also showed that an anti-CD47 mAb promoted macrophage-mediated phagocytosis of AML stem cells and prevented their engraftment in mouse models. AML stem cells are resistant to chemotherapy and have been linked to disease relapse.⁴

Although the Stanford researchers did suggest that the SIRPA-CD47 interaction mediated the phagocytosis-promoting effect, they were not able to provide direct evidence to show that this was indeed the case because the mAb they used may block CD47's interaction with additional known binding partners.^{5,6}

The Toronto team decided to focus on SIRPA based on a study it published in 2007 showing that polymorphisms in the gene encoding SIRPA affected CD47 binding and the survival of engrafted normal human hematopoietic stem cells in mice.⁷

“We wanted to provide clarity as to what key molecule was interacting with CD47 to promote the phagocytosis of leukemia stem cells,” said Jean Wang, an assistant professor in the Department of Medicine at the University of Toronto and a staff hematologist in the Division of Medical Oncology and Hematology in the Department of Medicine at the University Health Network.

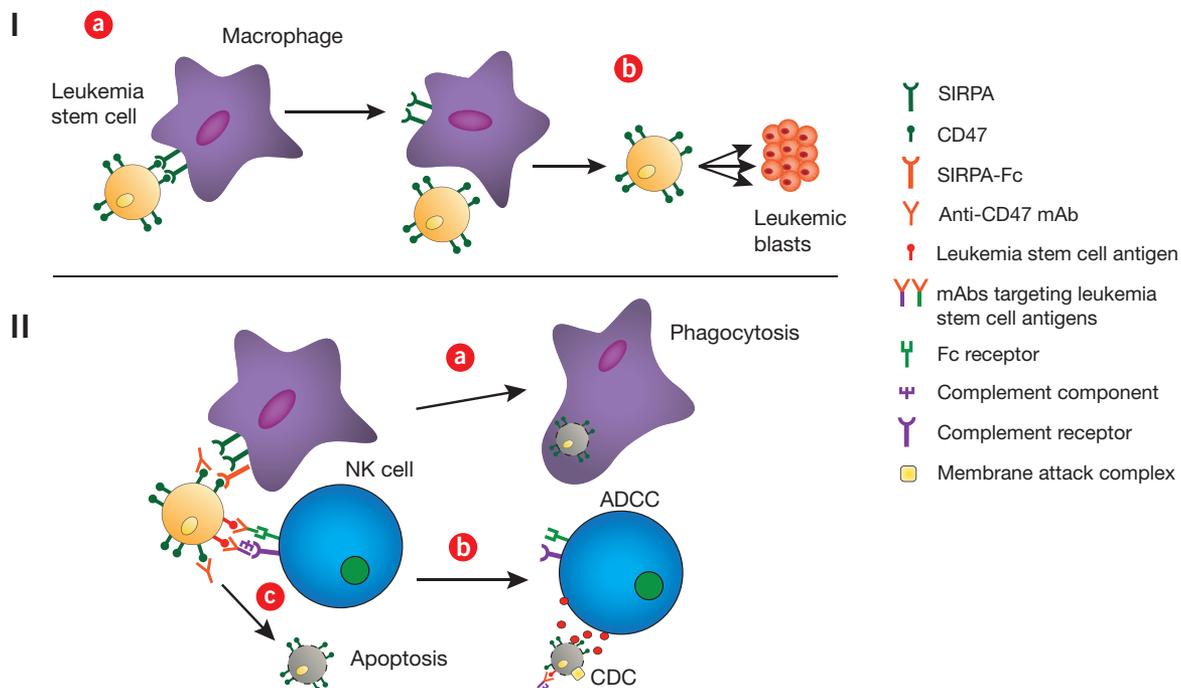


Figure 1. Immune escape by leukemia stem cells and potential therapeutic strategies. Upregulation of CD47 expression is a strategy leukemia stem cells employ to escape the immune system.

(I) CD47 on leukemia stem cells engages with signal regulatory protein- α (SIRPA) on macrophages to convey a ‘don’t eat me’ signal (I[a]). These leukemia stem cells engraft into host bone marrow, where they are able to give rise to leukemic blasts (I[b]).

(II) As reported in Theocharides *et al.*, using a SIRPA-Fc fusion protein to disrupt the interaction between CD47 and SIRPA promotes macrophage-mediated phagocytosis of acute myelogenous leukemia (AML) stem cells and impairs their engraftment (II[a]). Other groups have shown that anti-CD47 mAbs also could have a similar effect.

Disrupting the CD47-SIRPA interaction also has the potential to enhance the killing of leukemia stem cells via Fc-dependent mechanisms (II[b]). For example, disrupting the CD47-SIRPA interaction might improve the effect of mAbs that promote antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).

In ADCC, immune cells such as NK cells interact with the mAb and release factors (red circles) that lyse the leukemia stem cell. In CDC, binding of a mAb activates the complement cascade, which results in the formation of the membrane attack complex that causes cell lysis.

Another strategy could be to use mAbs that promote apoptosis of leukemia stem cells upon binding to CD47 (II[c]).

Wang's team used a series of mice with polymorphisms in the gene encoding Sirpa and showed that disruption of Cd47's interaction with Sirpa is the key to promoting macrophage-mediated phagocytosis of AML stem cells.

The researchers confirmed their results with a SIRPA-Fc fusion protein. In primary AML cells, the SIRPA-Fc fusion protein increased macrophage-mediated phagocytosis of AML cells compared with the control IgG4-Fc fusion protein. In a mouse model of human AML, using the fusion protein to block the SIRPA-CD47 interaction decreased leukemic engraftment and growth compared with using a control IgG4-Fc fusion protein (see Figure 1, "Immune escape by leukemia stem cells and potential therapeutic strategies").

Importantly, disrupting the SIRPA-CD47 interaction using the SIRPA-Fc fusion protein enhanced macrophage-mediated killing of AML stem cells without enhancing the killing of healthy hematopoietic cells.

Results were published in *The Journal of Experimental Medicine*.

"What our work contributes that was not well established previously is that SIRPA is the key binding partner of CD47 and that this is the key interaction responsible for the survival and engraftment of leukemia stem cells," said corresponding author Wang.

"They also show that disrupting the SIRPA-CD47 interaction using a SIRPA-Fc fusion protein is well tolerated in animals and does not trigger phagocytosis of normal hematopoietic stem cells," added Robert Uger, VP of R&D at Trillium. "This suggests that there is a therapeutic window where we will be able to enhance macrophage-mediated killing of leukemia stem cells while sparing normal hematopoietic stem cells."

Ravi Majeti, an assistant professor of medicine in the Division of Hematology at Stanford, said induction of phagocytosis by the immune system is a new therapeutic mechanism to consider for AML.

"These studies take off from previous work with anti-CD47 targeting antibodies and really utilize the same mechanism of blocking the SIRPA-CD47 interaction. In that way, this study validates the mechanism and target," he said.

Trillium teams up

The Toronto group and Trillium have a SIRPA-Fc fusion protein in preclinical development to treat AML.

Uger said the Toronto group's 2007 study caught the company's initial attention. "We were very intrigued by the group's previous work exploring the role of the CD47-SIRPA interaction in the survival of normal hematopoietic stem cells," he told *SciBX*.

Indeed, in 2010 Trillium launched a preclinical program to develop a CD47-Fc fusion protein that agonizes and a SIRPA-Fc fusion protein that antagonizes the SIRPA-CD47 interaction and thus would help promote the engraftment of hematopoietic stem cell transplants.

"What our work contributes that was not well established previously is that SIRPA is the key binding partner of CD47 and that this is the key interaction responsible for the survival and engraftment of leukemia stem cells."

—Jean Wang,
University of Toronto

However, Uger noted that the company is currently prioritizing the SIRPA-Fc program. He declined to disclose specific details.

"We wanted to move into the cancer stem cell space," he said. "The cancer stem cell story is best defined in AML, so it was a natural place for us to start."

Wang noted that the SIRPA-Fc fusion protein works by enhancing the immune system's ability to recognize leukemia stem cells, which sets it apart from targeted agents that disrupt specific regulatory pathways and targets on leukemia stem cells themselves.

"Patients could have leukemia subclones that have evolved and are dependent on different mechanisms to enhance growth and survival," she told *SciBX*. "Thus, targeted agents may not be able to eliminate subclones that don't depend on the particular targeted pathway."

In contrast, Wang noted that disrupting the CD47-SIRPA interaction acts on macrophages and enhances their ability to recognize all leukemia stem cells irrespective of heterogeneity in their intrinsic survival pathways.

Wang and Uger said Trillium's SIRPA-Fc fusion protein will most likely be used in combination with standard therapies for AML, such as chemotherapy, to help eliminate surviving leukemia stem cells and prevent disease relapse.

Wang also said the tissue distribution of SIRPA is more restricted than that of CD47, so disrupting the interaction from the SIRPA end could potentially have lower toxicity.

Uger said the company hopes to submit an IND in mid-to-late 2014.

The Hospital for Sick Children and University Health Network has filed a patent application covering compositions and methods for treating hematological cancers by targeting the SIRPA-CD47 interaction. Trillium licensed the IP in 2010.

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COMPANIES AND INSTITUTIONS MENTIONED

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Humanizing malaria mice

By **Tim Fulmer**, Senior Writer

U.S. researchers have developed the first mouse model that recapitulates the human stages of the malarial parasite *Plasmodium falciparum* life cycle in one animal.¹ The academics plan to make additional modifications to the mice to improve their utility, but pharma already want to use the animals in early stage research.

The life cycle of *P. falciparum* has three distinct stages. The liver and blood stages occur in the host, whereas the sexual stage occurs in the mosquito gut.

During the asymptomatic liver stage, sporozoites migrate from the mosquito bite site to the host's liver, where they form merozoites within liver cells. In the blood stage, the merozoites enter circulation and invade erythrocytes, which rupture and release new merozoites and gametocytes. The latter then are transmitted to a mosquito during feeding.

Because *P. falciparum* only infects humans and primates, studying the parasite in rodents has been impossible, leaving researchers with only two options for early stage *in vivo* studies of the disease.

One is to use malarial strains such as *P. berghei* and *P. yoelii* that infect rodents as proxies for *P. falciparum*.² Although this has allowed the study of both host stages in the same animal, rodent strains are evolutionarily distant from *P. falciparum*, and as a result those strains lack many *P. falciparum* proteins that could be therapeutic targets or vaccine candidates in humans.

The second option is to humanize mice to make them susceptible to *P. falciparum* infection. This commonly involves engrafting human red blood cells (RBCs) or hepatocytes into immunocompromised mice.³ Although separate humanized models of blood-stage and liver-stage infection exist,^{4,5} no humanized models combining both liver and blood stages have been disclosed.

Researchers at the **Seattle Biomedical Research Institute**, **The Rockefeller University** and **Yecuris Corp.** decided to develop a humanized malaria mouse model that develops liver-stage *P. falciparum* infection and transitions to blood-stage infection.

Yecuris was founded in 2007 to commercialize transgenic mouse technology developed by Markus Grompe and colleagues at the **Oregon Health & Science University**.

As a starting point for the new malaria model, the researchers used one of the mouse models marketed by Yecuris—the *FRG* (*Fah-Rag2-Cd132*) triple-knockout mouse.^{6,7}

In *FRG* mice, knocking out *recombination activating gene 2* (*Rag2*) and *Il-2 receptor γ -chain* (*Cd132*) leads to severe immunodeficiency, whereas knocking out *fumarylacetoacetate hydrolase* (*Fah*) leads to death of hepatocytes from excess buildup of the metabolite fumarylacetoacetate. The resulting animals are then engrafted with functional human hepatocytes to generate animals with human liver tissue.

Until now, *FRG* mice have primarily served as models of human liver disease and hepatitis infection. However, the researchers hypothesized that it might be possible to establish a liver-stage *P. falciparum* infection in the *FRG* mice as a first step in the development of a two-stage model of human malaria infection.

To test that idea, the researchers injected *FRG* mice intravenously with mosquito salivary gland *P. falciparum* sporozoites and euthanized the animals three, five, six and seven days postinfection. At each time point, liver tissue was analyzed for signs of progression of liver-stage *P. falciparum* infection.

At day three, liver-stage parasites were detectable in the engrafted human hepatocytes. Between days three and seven, the parasites developed into schizonts, which are mature forms of the parasite that contain merozoites. At day seven, aggregates of merozoites budded off from the liver cells into surrounding tissues, completing the liver stage.

Next, the researchers set out to test whether the *FRG* mice would also allow the engraftment of human RBCs to study the blood stage of *P. falciparum* infection.

A first round of experiments showed that the *FRG* mice rapidly cleared engrafted human RBCs, suggesting that additional genetic modifications would be required. The researchers backcrossed the *FRG* mice with nonobese diabetic (NOD) mice, a strain that typically shows reduced clearance of engrafted human RBCs.

The resulting *FRG* NOD mice were injected intravenously with human RBCs at six days post-sporozoite injection. Analysis of blood removed on day seven revealed blood-stage parasites that grew in culture at a rate similar to that of the parent *P. falciparum* strain used to generate the initial sporozoites.

Thus, the *FRG* NOD mice supported the establishment of the liver stage of *P. falciparum* infection and the transition of the disease from liver stage to blood stage.

The findings were published in *The Journal of Clinical Investigation*. “The key advance in the present study is that they obtained the complete transition from the *P. falciparum* hepatic stages to infected red blood cells *in vivo* in the same mouse,” said Dominique Mazier. “The model opens the way to assess the effect of a drug throughout hepatic development including in the presence of infected erythrocytes, and this is a clear step forward.”

Mazier and colleagues have developed *P. falciparum*-infected primary human hepatocyte cultures for compound screening.⁸ Mazier is professor of parasitology and mycology at the **Pierre and Marie Curie University** and head of the malaria drug and vaccine target identification laboratory at **Institut National de la Santé et de la Recherche Médicale** (INSERM).

Other potential uses of the *FRG* NOD mice, according to Iñigo Angulo-Barturen, include “looking at the effects of mutations on the liver-stage development

of the parasite as well as doing proteomic/metabolomic analysis of the parasite under different physiological conditions.” Angulo-Barturen is chief scientist of the Therapeutic Efficacy unit at **GlaxoSmithKline plc**.

“The model opens the way to assess the effect of a drug throughout hepatic development including in the presence of infected erythrocytes, and this is a clear step forward.”

**—Dominique Mazier,
Pierre and Marie Curie University**

Next steps

“We are already discussing with companies, including Novartis and GSK, how our mice might be used to test the efficacy of antimalarial compounds,” corresponding author Stefan Kappe told *SciBX*.

Kappe is professor and malaria program director at the Seattle Biomedical Research Institute and affiliate professor of global health at the **University of Washington**.

Indeed, GSK “hopes to set up the new model in-house, or an adapted version of it useful for drug discovery, in order to test compounds that kill liver stages of *P. falciparum* and, hopefully, *P. vivax*,” said Angulo-Barturen. *P. vivax* “produces quiescent liver stages that can be present in liver tissues for months or years until reactivation provokes a new malaria episode.”

The mice could replace the current nonhuman primate model of *P. vivax* infection, said Tim Wells, CSO of **Medicines for Malaria Venture (MMV)**.

Much of the focus of both GSK and MMV has been on developing compounds that target the blood stages of the parasite.

Since the mid-1990s, GSK has collaborated with Leonard Shultz and colleagues at **The Jackson Laboratory** to develop two humanized mouse models of blood-stage *P. falciparum* infection. The models are based on NOD-SCID mice that are deficient in either Cd132 or β_2 microglobulin (B2m).^{9,10} The resulting animals are regularly injected with human RBCs and used for testing the efficacy of antimalarial compounds, according to Angulo-Barturen.

MMV primarily uses cell-based phenotypic screens to look at the effects of compounds directly on the malaria parasite and has screened over five million compounds over the past five years, said Wells.

He added that the most advanced compound to result from the screen is the spiroindolone-based molecule NITD609,^{11,12} which is being developed by **Novartis AG** in collaboration with MMV. The compound entered Phase II testing this year.

Novartis did not respond to requests for comment.

“The mice will be useful for doing genetic crosses of multiple *P. falciparum* strains to identify genomic modifications in the parasite that confer drug resistance,” said Kappe.

“Also, we should be able to use the mice to measure the attenuation levels of *P. falciparum* sporozoites used in vaccines,” said Alexander Ploss, a principal investigator on the *JCI* paper. “This will allow us to better determine whether a given strain is attenuated at sufficiently safe levels before testing it in human volunteers.” Ploss is assistant professor of virology and infectious disease at Rockefeller University.

Finally, additional work can still be done on the mice to establish a more robust blood-stage infection, said Ploss.

“There are two general ways we are considering doing that,” he said. “One approach is basically to combine the liver chimeric mouse we have

now with the humanized blood-stage mice used by GSK and others. Another, more ambitious approach is to modify our mice into a double transplant model, where we transplant not only human hepatocytes for liver-stage studies but also human hematopoietic stem cells for blood-stage studies. In that scenario, the animals don’t require regular injections of human RBCs but instead endogenously produce the entire human erythrocyte lineage.”

Ploss said he and Kappe are collaborating with Richard Flavell to develop those mouse models. Flavell is professor of immunology at the **Yale School of Medicine**.

The *FRG* and *FRG* NOD mice used in the *JCI* paper are covered by patents and are available for purchase from Yecuris.

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Medicines for Malaria Venture, Geneva, Switzerland
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Oregon Health & Science University, Portland, Ore.
Pierre and Marie Curie University, Paris, France
The Rockefeller University, New York, N.Y.
Seattle Biomedical Research Institute, Seattle, Wash.
University of Washington, Seattle, Wash.
Yale School of Medicine, New Haven, Conn.
Yecuris Corp., Portland, Ore.

Slipping therapeutics to the mitochondria

By Tracey Baas, Senior Editor

Researchers from **The University of Georgia** have developed a polymeric nanoparticle technology that can selectively deliver small molecules to the mitochondria of cultured cells.¹ The researchers now are evaluating the nanoparticles in animal models of Alzheimer's disease, cancer and metabolic disorders.

Mitochondrial dysfunction plays a role in multiple diseases, including cancer, neurodegenerative and neuromuscular diseases, obesity and diabetes. However, targeting mitochondria has been difficult because there are no platforms to deliver drugs directly into the organelles, and nonspecific delivery can lead to toxicity in other parts of the cell.

Thus, two University of Georgia researchers, graduate student Sean Marrache and Assistant Professor of Chemistry Shanta Dhar, reasoned that mitochondria-targeting nanoparticles could be a way to selectively deliver therapies.

The duo conjugated a poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG) copolymer to a triphenylphosphonium (TPP) cation, which is known to enter the mitochondrial matrix.² Dhar had previously adapted the PGLA-PEG nanoparticle system to target prostate cancer cells.³

The Georgia team blended the resulting PGLA-PEG-TPP copolymer with a nontargeting PGLA-PEG copolymer to create the nanoparticles in a single-step process. This approach helped reduce the likelihood of unwanted variation in nanoparticle properties that normally occurs with an increase in the number of processing steps.

Finally, the researchers generated a series of nanoparticles with different sizes and surface charges by varying the relative amounts of targeting and nontargeting copolymers.

In a human cervical cancer cell line, nanoparticles containing the targeting copolymer showed greater uptake in mitochondria than nanoparticles that lacked the targeting copolymer. Nanoparticles 80–100 nm in diameter showed maximum uptake by mitochondria.

The researchers also showed that nanoparticles with positively charged surfaces had higher cellular and mitochondrial uptake as the surface charge increased, with maximum cellular uptake at 22 mV and maximum mitochondrial uptake at 34 mV.

Collectively, the data suggested the nanoparticles specifically targeted the mitochondria and that trafficking could be optimized by varying diameter and surface charge.

The scientists also tested the nanoparticles' ability to deliver payloads in cell culture and to alter disease phenotypes.

In human neuroblastoma cells treated with β -amyloid ($A\beta$) to model AD, curcumin-loaded, targeting nanoparticles increased cell viability compared with empty targeting nanoparticles or curcumin alone. Curcumin inhibits $A\beta$ and the associated mitochondrial oxidative stress.

In human cervical cancer cells, lonidamine-loaded, targeting nanoparticles increased cell death compared with loaded, nontargeted nanoparticles or free compound, showing a fivefold lower

IC₅₀ value than the nontargeting nanoparticles. Lonidamine inhibits mitochondrial glycolysis.

Finally, in preadipocytes cultured under differentiation conditions to model metabolic disease, 2,4-dinitrophenol (2,4-DNP)-loaded, targeting nanoparticles suppressed preadipocyte differentiation and decreased lipid accumulation compared with the loaded, nontargeted nanoparticles or free compound, and did not affect cell viability.

The mitochondrial decoupler 2,4-DNP underwent clinical testing by **Stanford University** researchers as a weight loss agent in the 1930s but proved toxic at high doses.

Results were reported in the *Proceedings of the National Academy of Sciences*.

"The two advantages of these nanoparticles over others are the fact they are composed of clinically validated materials, PLGA and PEG, and are produced using a single-step processing method," said Omid Farokhzad, associate

professor at **Harvard Medical School**, founder of **Blend Therapeutics Inc.** and cofounder of **Bind Biosciences Inc.** and **Selecta Biosciences Inc.**

Bind's BIND-014, a polymeric nanoparticle that targets prostate-specific membrane antigen (PSMA; FOLH1; GCPII) and contains docetaxel, is in Phase I testing to treat advanced or metastatic solid tumors.

Dhar and Marrache next plan to use their nanoparticle system *in vivo*. "We will first test curcumin, lonidamine and 2,4-DNP in animal models," Dhar told *SciBX*. "If our results look promising, we will expand to other mitochondrial-acting therapeutics."

Getting into the mitochondria

Although the data in the *PNAS* paper show a marked improvement in mitochondrial targeting with the nanoparticles, actual selectivity and the mechanism underlying this effect remain to be determined.

"These nanoparticles, as creative as they are, have the main limitation that they will enter into all cells that they come into contact with," noted Erkki Ruoslahti, professor at the **Sanford-Burnham Medical Research Institute** and at the Center for Nanomedicine of the **University of California, Santa Barbara**. "After a systemic injection, the uptake would be mostly limited to the endothelial cells in blood vessels and the reticuloendothelial system. Getting the nanoparticles into extravascular tissue, where the target cells reside, will require additional solutions."

Ruoslahti said his lab has been working on nanoparticles that selectively target the mitochondria of tumor endothelial cells and tumor cells by incorporating a tumor-homing peptide. Compared with his approach, he said "Dhar's nanoparticles are more broadly applicable, which is both an advantage and a disadvantage."

Other researchers still had questions about the actual mechanism by which the nanoparticles interacted with mitochondria.

The researchers did not conclusively show "whether the nanoparticle passes across mitochondrial membranes," said Yuma Yamada, assistant professor of pharmaceutical sciences at **Hokkaido University**. "The

"The two advantages of these nanoparticles over others are the fact they are composed of clinically validated materials, PLGA and PEG, and are produced using a single-step processing method."

**—Omid Farokhzad,
Harvard Medical School**

nanoparticle most likely binds to the mitochondria selectively and releases the cargo at the mitochondrial site, with the compounds entering the mitochondrial compartment.”

Mike Murphy, group leader of the Mitochondrial Biology Unit at the **Medical Research Council**, agreed. “The work has some very nice aspects, especially enhanced endosomal escape, but the researchers have not demonstrated actual targeting into mitochondria. Their characterization methods, such as confocal microscopy and cadmium assays, are inadequate to show this. They are probably seeing adsorption to the mitochondrial surface and not penetration to the mitochondrial matrix.”

He added that Dhar’s group would “need to show with isolated mitochondria that they are getting uptake into the matrix and then extend this to cell culture to show the same. There are well-established techniques to do this.”

“We believe the particles are entering the mitochondria through the outer membrane and that the particles are located inside the mitochondrial matrix because the matrix-targeting particles are found in the mitochondrial fractions of cell culture–based assays,” said Dhar. “Detailed *in vitro* studies are ongoing in the lab to study the nanoparticles’ entry process and exact location in the mitochondria.”

Alternatively, if the nanoparticles do not enter the matrix, Murphy said the system could potentially be “very useful because their biodegradable nanoparticles adsorb to the mitochondria, where they slowly break down to release the compounds.”

In fact, according to Murphy, the nanoparticles described by Dhar could be used to deliver proteins or peptides near the mitochondria and thus “provide a breakthrough for slow delivery inside cells in general.”

The University of Georgia has filed for a patent covering the work, and the IP is available for licensing.

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Contact: Shanta Dhar, The University of Georgia, Athens, Ga.
e-mail: shanta@uga.edu
2. Smith, R.A.J. *et al. Proc. Natl. Acad. Sci. USA* **100**, 5407–5412 (2003)
3. Kolishetti, N. *et al. Proc. Natl. Acad. Sci. USA* **107**, 17939–17944 (2010)

COMPANIES AND INSTITUTIONS MENTIONED

Bind Biosciences Inc., Cambridge, Mass.
Blend Therapeutics Inc., Watertown, Mass.
Harvard Medical School, Boston, Mass.
Hokkaido University, Sapporo, Japan
Medical Research Council, Cambridge, U.K.
Sanford-Burnham Medical Research Institute, La Jolla, Calif.
Selecta Biosciences Inc., Watertown, Mass.
Stanford University, Stanford, Calif.
The University of Georgia, Athens, Ga.
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This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in therapeutics includes important research findings on targets and compounds, grouped first by disease class and then alphabetically by indication.

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer				
Acute myeloid leukemia (AML)	FMS-like tyrosine kinase 3 (FLT3; CD135)	<p><i>In vitro</i> and mouse studies suggest statins could help treat <i>FLT3</i>-mutant AML. In mouse bone marrow cells expressing mutant Flt3, Lescol fluvastatin prevented Flt3 glycosylation and cell surface translocation, which is required for FLT3-driven oncogenesis, and decreased proliferation compared with no treatment. In mice, fluvastatin decreased engraftment of mutant <i>Flt3</i>-expressing bone marrow cells and increased survival compared with saline. Next steps could include evaluating statins in combination with FLT3 inhibitors in mouse models of Flt3-driven AML. Novartis AG markets Lescol, an HMG-CoA reductase inhibitor, to treat hypercholesterolemia and prevent cardiovascular disease. At least 10 companies have FLT3 inhibitors in clinical and preclinical testing to treat various cancers.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.999 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>Williams, A.B. <i>et al. Blood</i>; published online Aug. 27, 2012; doi:10.1182/blood-2012-01-403493 Contact: Donald Small, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: donsmall@jhmi.edu</p>
Melanoma	Cyclin dependent kinase 4 (CDK4); MEK; neuroblastoma Ras viral (v-Ras) oncogene (NRAS)	<p>Mouse studies suggest combined inhibition of CDK4 and MEK could help treat NRAS-mutant melanomas. In an inducible mouse model of mutant <i>Nras</i>-driven melanoma, gene network analysis predicted that combined inhibition of CDK4 and MEK would mimic the loss of mutant NRAS expression. In the same mice, the MEK inhibitor trametinib and the CDK4 inhibitor PD-0332991 caused greater tumor growth inhibition than either compound alone. Next steps include evaluating the MEK and CDK4 inhibitor combination in patients with NRAS-mutant melanomas and identifying additional biomarkers that could help better determine whether a patient will respond to the combination therapy. Trametinib, a small molecule inhibitor of MAP kinase kinase 1 (MAP2K1; MEK1) and MEK2 (MAP2K2) from Japan Tobacco Inc. and GlaxoSmithKline plc, is under review to treat melanoma. Onyx Pharmaceuticals Inc. and Pfizer Inc. have PD-0332991, an oral small molecule CDK4 and CDK6 inhibitor, in Phase II testing or earlier to treat various cancers.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1000 Published online Sept. 27, 2012</p>	Patent disclosure filed; available for licensing	<p>Kwong, L.N. <i>et al. Nat. Med.</i>; published online Sept. 16, 2012; doi:10.1038/nm.2941 Contact: Lynda Chin, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: lchin@mdanderson.org</p>

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Multiple myeloma (MM)	Insulin-like growth factor-1 receptor (IGF1R; CD221); insulin-like growth factor-1 (IGF-1)	<p><i>In vitro</i> and mouse studies suggest antagonizing IGF1R could help prevent resistance to Velcade bortezomib in patients with MM. In bortezomib-resistant MM cells, secretion of IGF-1 and activation of IGF1R were greater than those in bortezomib-sensitive cells. In MM cells, small hairpin RNA against IGF1R increased sensitivity to bortezomib compared with scrambled shRNA. In MM cells and in mouse xenograft models of bortezomib-resistant MM, an IGF1R and insulin receptor inhibitor increased the antiproliferative and tumor-suppressive effects of bortezomib compared with bortezomib alone. Next steps could include testing the combination in additional models of MM.</p> <p>At least 14 companies have IGF1R antagonists or antibodies in development stages ranging from preclinical to marketed to treat various indications.</p> <p>Takeda Pharmaceutical Co. Ltd. and Johnson & Johnson market Velcade, a small molecule dipeptide boronic acid proteasome inhibitor, to treat MM and mantle cell lymphoma (MCL).</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1001 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>Kuhn, D.J. <i>et al. Blood</i>; published online Aug. 29, 2012; doi:10.1182/blood-2011-10-386789 Contact: Robert Z. Orlowski, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: rorlowsk@mdanderson.org</p>
Multiple myeloma (MM)	Nicotinamide phosphoribosyltransferase (NAMPT)	<p>Cell culture and mouse studies suggest inhibiting NAMPT could help treat MM. In MM cell lines and patient-derived cells, including cell lines resistant to current MM therapies, an NAMPT inhibitor decreased cell viability compared with no treatment. In mice with MM cells, the NAMPT inhibitor decreased tumor volume and increased survival compared with vehicle. Next steps could include optimizing the NAMPT inhibitor.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1002 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>Cea, M. <i>et al. Blood</i>; published online Sept. 5, 2012; doi:10.1182/blood-2012-03-416776 Contact: Kenneth C. Anderson, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Mass. e-mail: kenneth_anderson@dfci.harvard.edu</p>
Cardiovascular disease				
Thrombosis	Polyphosphate	<p><i>In vitro</i> and mouse studies identified inhibitors of polyphosphate that could help prevent thrombosis. In <i>in vitro</i> assays, polyphosphate inhibitors blocked the interaction between thrombin and polyphosphate and doubled the clotting times in human plasma compared with no inhibitor. In mouse models of venous and arterial thrombosis, the inhibitors prevented platelet and fibrin accumulation in the developing thrombi. Next steps include using this approach to identify a therapeutic candidate.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1003 Published online Sept. 27, 2012</p>	Patent applications filed covering polyphosphate and its effects on hemostasis, thrombosis and inflammation; available for licensing	<p>Smith, S.A. <i>et al. Blood</i>; published online Sept. 11, 2012; doi:10.1182/blood-2012-07-444935 Contact: James H. Morrissey, University of Illinois at Urbana-Champaign, Urbana, Ill. e-mail: jhmorris@illinois.edu</p>

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Endocrine/metabolic disease				
Metabolic syndrome; obesity	MicroRNA-378 (miR-378); miR-378*	<p>Mouse studies suggest inhibiting miR-378 could help prevent obesity and metabolic syndrome. In mice, deletion of miR-378 and miR-378*, which both originate from the same miRNA precursor, increased resistance to diet-induced obesity compared with normal expression of the miRNAs. In miR-378 and miR-378* knockout mice, energy expenditure and mitochondria oxidative capacity were greater than those in wild-type controls. Next steps could include developing compounds to inhibit miR-378 signaling.</p> <p>miRagen Therapeutics Inc. has a preclinical program to validate miR-378 as a target for cardiometabolic diseases.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1004 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>Carrer, M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Sept. 4, 2012; doi:10.1073/pnas.1207605109</p> <p>Contact: Eric N. Olson, The University of Texas Southwestern Medical Center, Dallas, Texas</p> <p>e-mail: eric.olson@utsouthwestern.edu</p>
Obesity	<i>PTEN (MMAC1; TEP1)</i>	<p>Human studies suggest <i>PTEN</i> mutations could help predict obesity risk. Twenty-one nondiabetic individuals with loss-of-function <i>PTEN</i> mutations had greater insulin sensitivity and BMIs than healthy subjects with wild-type <i>PTEN</i>. A meta-analysis of published genomics data from healthy individuals identified associations between <i>PTEN</i> SNPs and high plasma levels of fasting insulin and low levels of fasting glucose. Ongoing work includes investigating the mechanisms by which insulin sensitivity contributes to obesity in individuals harboring <i>PTEN</i> mutations.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1005 Published online Sept. 27, 2012</p>	Unpatented; available for partnering	<p>Pal, A. <i>et al. N. Engl. J. Med.</i>; published online Sept. 13, 2012; doi:10.1056/NEJMoa1113966</p> <p>Contact: Anna L. Gloyn, University of Oxford, Oxford, U.K.</p> <p>e-mail: anna.gloyn@drl.ox.ac.uk</p>
Hepatic disease				
Liver disease	IL-1 receptor (IL-1R); IL-1 receptor antagonist (IL-1RA)	<p>Mouse studies suggest IL-1RA could help treat alcoholic liver disease. In a mouse model of alcohol-induced liver injury, knockout of molecules involved in IL-1β activation or signaling, including IL-1R, decreased liver steatosis and damage compared with normal expression. In the model, recombinant IL-1RA also prevented liver damage. Next steps could include testing IL-1R antagonists in additional models of alcoholic liver disease.</p> <p>Orthogen AG's Orthokine, an injectable form of IL-1RA, is in Phase III testing to treat pain.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1006 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>Petrasek, J. <i>et al. J. Clin. Invest.</i>; published online Sept. 4, 2012; doi:10.1172/JCI60777</p> <p>Contact: Gyongyi Szabo, University of Massachusetts Medical School, Worcester, Mass.</p> <p>e-mail: gyongyi.szabo@umassmed.edu</p>

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Infectious disease				
Leishmaniasis	Dihydrofolate reductase (DHFR); pteridine reductase (PTR1)	<p>Cell culture studies identified piperidine-pteridine-based PTR1 inhibitors that could help circumvent antifolate drug resistance associated with <i>Leishmania</i> infection. <i>Leishmania</i> can overcome DHFR inhibition by overexpressing PTR1. In two strains of <i>Leishmania</i>, members of the PTR1 inhibitor series increased pyrimethamine-mediated growth inhibition compared with pyrimethamine alone. Next steps include testing optimized versions of the identified PTR1 inhibitors in combination with DHFR inhibitors in mouse models.</p> <p>Pyrimethamine is a generic DHFR inhibitor used to treat protozoan infections.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1007 Published online Sept. 27, 2012</p>	Patent pending; available for licensing from Tydock Pharma srl	<p>Cornona, P. <i>et al. J. Med. Chem.</i>; published online Aug. 14, 2012; doi:10.1021/jm300563f</p> <p>Contact: Maria Paola Costi, University of Modena and Reggio Emilia, Modena, Italy e-mail: mariapaola.costi@unimore.it</p>
Influenza virus	Granulocyte macrophage colony-stimulating factor (GM-CSF; CSF2)	<p>Mouse studies suggest increasing GM-CSF signaling in airway epithelial cells could help promote recovery from influenza virus infection. In mice infected with a strain of influenza A, isolated airway epithelial cells showed greater Gm-csf expression than cells from mock-infected mice. Mice that only expressed Gm-csf in airway epithelial cells showed higher survival following influenza virus challenge than virus-challenged wild-type mice. Next steps include setting up a pilot study to evaluate nebulized GM-CSF in patients with pneumonia-associated acute respiratory distress syndrome.</p> <p>Leukine sargramostim, a yeast-derived GM-CSF from Bayer AG and Sanofi, is marketed to treat acute myelogenous leukemia (AML).</p> <p>At least seven other companies have GM-CSF-based compounds in Phase II testing or earlier to treat various cancers or neutropenia.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1008 Published online Sept. 27, 2012</p>	Patent status undisclosed; unavailable for licensing	<p>Unkel, B. <i>et al. J. Exp. Med.</i>; published online Sept. 10, 2012; doi:10.1172/JCI62139</p> <p>Contact: Susanne Herold, University of Giessen Lung Center, Giessen, Germany e-mail: susanne.herold@innere.med.uni-giessen.de</p>
Influenza virus	Influenza A virus hemagglutinin (HA)	<p><i>In vitro</i> and mouse studies suggest the HA-targeting mAb CO5 could help prevent or treat influenza infection. <i>In vitro</i>, CO5 neutralized influenza virus subtypes H1, H2, H3 and H9 by binding the HA globular head domain rather than the stem region targeted by most broadly neutralizing antibodies. In mouse models of H1N1 and H3N2 influenza virus infection, CO5 given 24 hours before lethal challenge resulted in 100% survival. In the same models, CO5 given up to 3 days after infection also resulted in 100% survival. Ongoing work includes developing stable cell line production of the antibody.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1009 Published online Sept. 27, 2012</p>	Covered by issued and pending patents; available for licensing	<p>Ekiert, D.C. <i>et al. Nature</i>; published online Sept. 16, 2012; doi:10.1038/nature11414</p> <p>Contact: Ian A. Wilson, The Scripps Research Institute, La Jolla, Calif. e-mail: wilson@scripps.edu</p> <p>Contact: Ramesh R. Bhatt, Sea Lane Biotechnologies LLC, Mountain View, Calif. e-mail: ramesh.bhatt@sealanebio.com</p>

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Tuberculosis	Unknown	<p><i>In vitro</i> studies suggest a modified oxyphenbutazone (OPB) could help treat tuberculosis. Nonreplicating <i>Mycobacterium tuberculosis</i> (<i>Mtb</i>) can become resistant to therapeutics that target replicating <i>Mtb</i>. In a high throughput screen, the generic anti-inflammatory drug OPB killed nonreplicating <i>Mtb</i> but not replicating <i>Mtb</i> under acidic conditions. In liquid culture, a 4-hydroxylated OPB decreased growth of both replicating and nonreplicating <i>Mtb</i> compared with vehicle and acted synergistically with other antimicrobials. Next steps include clinical trials.</p> <p>OPB is a generic NSAID.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1010 Published online Sept. 27, 2012</p>	Findings unpatented; licensing status not applicable	<p>Gold, B. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Sept. 10, 2012; doi:10.1073/pnas.1214188109</p> <p>Contact: Carl F. Nathan, Weill Cornell Medical College, New York, N.Y. e-mail: cnathan@med.cornell.edu</p>
Other				
Hearing loss	Not applicable	<p>Gerbil studies suggest stem cell-based therapy could help treat hearing loss caused by auditory neuropathy. Reprogrammed human embryonic stem cells showed morphological features of auditory neural progenitor cells. In a gerbil model of chemical-induced auditory neuropathy, progenitor cells transplanted to the cochlea up to two weeks after neuronal injury engrafted to form synaptic connections to the central auditory neural pathway. In this model, ears that received the transplant showed greater sensitivity to sound volume and frequencies than the untreated ear of the same animal. Next steps could include testing the efficacy of cell transplants in the models three or more weeks after neuronal injury.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1011 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>Chen, W. <i>et al. Nature</i>; published online Sept. 12, 2012; doi:10.1038/nature11415</p> <p>Contact: Marcelo N. Rivolta, The University of Sheffield, Sheffield, U.K. e-mail: m.n.rivolta@sheffield.ac.uk</p>

This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
Disease models			
Cystic fibrosis transmembrane conductance regulator (CFTR) knockout ferrets as models for cystic fibrosis-related diabetes	<i>Cftr</i> knockout ferrets could be useful models for cystic fibrosis-related diabetes. <i>Cftr</i> knockout ferrets had mild pancreatic pathology at birth and developed progressive pancreatic inflammation and exocrine pancreas destruction by the first month. The <i>Cftr</i> knockout animals also showed impaired glucose homeostasis and insulin regulation. Next steps include identifying islet cells that express CFTR and characterizing how CFTR modulates insulin secretion.	Model unpatented; available for licensing	Olivier, A.K. <i>et al. J. Clin. Invest.</i> ; published online Sept. 17, 2012; doi:10.1172/JCI60610 Contact: John F. Engelhardt, The University of Iowa, Iowa City, Iowa e-mail: john-engelhardt@uiowa.edu
	SciBX 5(38); doi:10.1038/scibx.2012.1012 Published online Sept. 27, 2012		
Dedifferentiation and restoration of islet β cells in diabetes	Diabetic mice show dedifferentiation of pancreatic islet β cells and could be used to screen compounds to restore islet β cells. In multiple mouse models of type 1 and type 2 diabetes, pancreases showed lower β cell mass but higher levels of α cells and undifferentiated β cell precursors than healthy control pancreases. Lineage-tracing studies showed that the undifferentiated cells were derived from β cells. Next steps include testing whether dedifferentiated β cells are present in human diabetes patients and screening for compounds to reverse dedifferentiation (<i>see Resetting the clock in diabetes, page 1</i>).	Patent pending; available for licensing	Talchai, C. <i>et al. Cell</i> ; published online Sept. 14, 2012; doi:10.1016/j.cell.2012.07.029 Contact: Domenico Accili, Columbia University, New York, N.Y. e-mail: da230@columbia.edu
	SciBX 5(38); doi:10.1038/scibx.2012.1013 Published online Sept. 27, 2012		
<i>Dj-1</i> (<i>Park7</i>)-deficient mice as models for Parkinson's disease (PD)-associated neurodegeneration	<i>Dj-1</i> -deficient mice could be useful models for PD. The <i>Dj-1</i> -deficient animals were generated by backcrossing <i>Dj-1</i> -null mice with C57BL/6J mice. The resulting <i>Dj-1</i> -deficient mice showed unilateral loss of dopaminergic neurons in the substantia nigra pars compacta region of the brain starting as early as eight weeks that gradually progressed to bilateral degeneration as the animals aged. Whole-exome sequencing identified five coding regions that could be potent modifiers of the observed model phenotype. Next steps include refining the model and using it to identify and evaluate therapeutic targets.	Unpatented; model available for licensing	Rousseaux, M.W.C. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Sept. 10, 2012; doi:10.1073/pnas.1205102109 Contact: David S. Park, University of Ottawa, Ottawa, Ontario, Canada e-mail: dpark@uottawa.ca
	SciBX 5(38); doi:10.1038/scibx.2012.1014 Published online Sept. 27, 2012		
Humanized mouse model for liver-stage malaria infection	Mice with humanized livers could be useful for identifying compounds that target the liver stage of human malarial strain <i>Plasmodium falciparum</i> . In the mice, <i>recombination activating gene 2</i> (<i>Rag2</i>) and <i>IL-2 receptor γ-chain</i> (<i>Cd132</i>) were deleted to generate immunodeficiency, while <i>fumarylacetoacetate hydrolase</i> (<i>Fah</i>) was deleted to kill liver cells. The mice were then repopulated with human hepatocytes. Following injection of <i>P. falciparum</i> sporozoites, the triple-knockout mice developed liver-stage infection. When the triple-knockout mice were crossed with nonobese diabetic (NOD) mice, the resulting mice supported transplantation of human red blood cells and allowed study of liver-stage to blood-stage infection dynamics. Next steps include using the mice to test compounds targeting liver-stage parasites (<i>see Humanizing malaria mice, page 6</i>).	Patented; available for purchase from Yecuris Corp.	Vaughan, A.M. <i>et al. J. Clin. Invest.</i> ; published online Sept. 10, 2012; doi:10.1172/JCI62684 Contact: Stefan H.I. Kappe, Seattle Biomedical Research Institute, Seattle, Wash. e-mail: stefan.kappe@seattlebiomed.org
	SciBX 5(38); doi:10.1038/scibx.2012.1015 Published online Sept. 27, 2012		

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Drug delivery			
Activation of sphingosine 1-phosphate receptor to improve drug delivery across the blood brain barrier (BBB)	<p>Sphingosine 1-phosphate receptor agonists could help improve drug delivery across the BBB. In rat brains, sphingosine 1-phosphate receptor agonists increased BBB penetration of the opioid receptor agonist loperamide and the chemotherapeutic paclitaxel compared with delivering the two compounds without a sphingosine 1-phosphate receptor agonist. In the rat model, the sphingosine 1-phosphate receptor agonists decreased P glycoprotein (Mdr1; Abcb1; P-gp; Cd243)-mediated drug efflux compared with no agonist. Next steps include studies to elucidate the mechanism by which sphingosine 1-phosphate receptor agonists modulate P-gp-mediated drug efflux.</p> <p>Mitsubishi Tanabe Pharma Corp. and Novartis AG market the sphingosine 1-phosphate receptor agonist Gilenya fingolimod to treat relapsing forms of multiple sclerosis (MS).</p> <p>At least eight other companies have sphingosine 1-phosphate receptor-targeting compounds in Phase II testing or earlier to treat various diseases.</p> <p>Loperamide is a generic drug for diarrhea.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1016 Published online Sept. 27, 2012</p>	Unpatented; licensing status not applicable	<p>Cannon, R.E. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Sept. 4, 2012; doi:10.1073/pnas.1203534109</p> <p>Contact: Ronald E. Cannon, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, N.C. e-mail: cannon1@niehs.nih.gov</p>
Mixed mitochondria-targeted polymeric nanoparticles for drug delivery	<p>Nanoparticles consisting of a mix of polymeric molecules could help improve drug delivery to the mitochondria. A mitochondria-targeting poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG) copolymer was blended with nontargeted polymers to generate nanoparticles with optimized size and surface charge. In a human cervical cancer cell line, nanoparticles containing the mitochondria-targeting copolymer showed greater uptake in mitochondria than nanoparticles that lacked the copolymer. In human and murine cell lines, the targeted nanoparticles increased the potency of mitochondria-acting compounds compared with nontargeted nanoparticles. <i>In vivo</i> studies to evaluate the polymeric nanoparticles are underway (<i>see Slipping therapeutics to the mitochondria</i>, page 8).</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1017 Published online Sept. 27, 2012</p>	Patent pending; available for licensing	<p>Marrache, S. & Dhar, S. <i>Proc. Natl. Acad. Sci. USA</i>; published online Sept. 18, 2012; doi:10.1073/pnas.1210096109</p> <p>Contact: Shanta Dhar, The University of Georgia, Athens, Ga. e-mail: shanta@uga.edu</p>
Markers			
Markers of squamous cell lung cancer	<p>Genomic studies have identified recurrent mutations in squamous cell lung cancer samples that could be useful markers or therapeutic targets. Genetic analysis of 178 lung squamous cell carcinoma samples identified recurrent mutations in 11 genes, including previously unreported loss-of-function mutations in <i>major histocompatibility complex class I A (HLA-A)</i>. The analysis showed that 96% of the tumors had mutations in potentially druggable targets such as tyrosine kinases, serine/threonine kinases and <i>phosphoinositide 3-kinase (PI3K)</i> subunits. Next steps could include using the data to define specific markers or gene signatures that could help identify squamous cell lung cancers that respond to existing targeted therapies.</p> <p>SciBX 5(38); doi:10.1038/scibx.2012.1018 Published online Sept. 27, 2012</p>	Patent and licensing status unavailable	<p>The Cancer Genome Atlas Research Network. <i>Nature</i>; published online Sept. 9, 2012; doi:10.1038/nature11404</p> <p>Contact: Matthew Meyerson, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: matthew_meyerson@dfci.harvard.edu</p>

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