CiRA Reporter

Center for iPS Cell Research and Application,
Kyoto University

Vol.14
April 2018

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Publisher
International Public Communications Office
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Stem cell 'twins' to study disease

The Woltjen lab reports a new gene editing method that can modify a single DNA base in the human genome with absolute precision.

Single mutations in DNA, also known as single nucleotide polymorphisms (SNPs), are the most common type of variation in the human genome. More than 10 million SNPs are known, many of which are associated with ailments such as Alzheimer's disease, heart disease, and diabetes.

Proving that a SNP is responsible for a disease requires very strict comparisons to genetically matched (or isogenic) iPS cells. The ideal cells are what Associate Professor Knut Woltjen describes as isogenic "twins," cells whose genomes differ only by one SNP.

Dr. Shin-Il Kim, a Specially-appointed Assistant Professor in the Woltjen lab and co-first author on the study, says that creating these twins is not trivial.

"Usually we need to add a gene for antibiotic resistance along with the SNP to overcome low efficiency. Since that adds another change to the genome, we also need a way to remove it," Kim said.

To create isogenic twins, the Woltjen laboratory has developed new genome editing technology with which they inserted a SNP modification. They also inserted a fluorescent reporter gene, on the which left and right sides had a short duplicated DNA sequence known as a microhomology and unique target sites for CRISPR/Cas9. These features allowed the researchers to exploit an endogenous DNA repair system in the cell called microhomology-mediated end joining (MMEJ) in order to precisely remove the reporter gene, leaving only the modified SNP behind. By arranging the mutant SNP in one microhomology and the normal SNP in the other, the method efficiently generates isogenic twins.

Woltjen, who conceived the study, called the new gene editing method MhAX, or Microhomology-Assisted eXcision. Woltjen's inspiration came from observing naturally occurring MMEJ repair in response to DNA damage.

"To make MhAX work, we duplicate DNA sequences which are already present in the genome. We then let the cells resolve this duplication. At the same time, the cells decide which SNPs will remain after repair," he said. "One experiment results in the full spectrum of possible SNP genotypes."

The study shows how MhAX creates SNPs in the HPRT and APRT genes, mutations that are associated with gout and kidney disease, respectively. Collaborating with researchers in Japan and Canada, the lab is now using MhAX to investigate the genetic cause of severe diabetes in juvenile patients.

"Our goal is to generate gene editing technologies which improve our understanding of disease mechanisms, and ultimately lead to therapies," said Woltjen. "We're confident that MhAX will have broad applicability in current human disease research, and beyond."

Reference

Kim SI, Matsumoto T, Kagawa H et al. (2018) Microhomology-assisted scarless genome editing in human iPSCs. *Nat Commun* 9(1):939. DOI: 10.1038/s41467-018-03044-y

A new technology to model disease mutations in cells

The Hotta lab invents CRONUS, easy-to-use technology for introducing randomized mutations in human genomes.

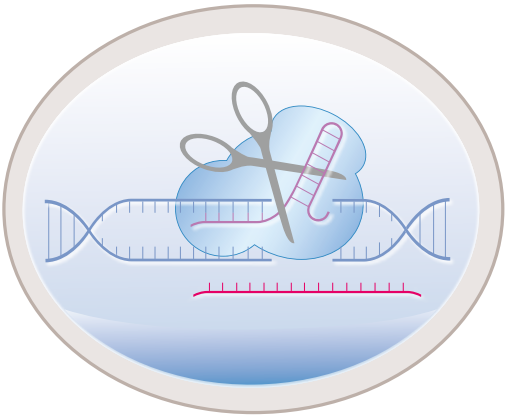
Human DNA contains 3 billion base pairs, but a change in just one can have a profound effect. In fact, the majority of genetic diseases can be attributed to a single base pair mutation. Gene editing technology allows scientists to test the effects of these mutations. However, gene editing just a single base is technically difficult. In response, CiRA researchers have developed CRONUS to simplify gene-editing experiments for disease study.

CRONUS, or CRISPR/Cas9 regulated transcription and nuclear shuttling, takes advantage of the CRISPR/Cas9 system, a bacterial immune system that was first repurposed for genome editing less than a decade ago. When CRISPR/Cas9 is activated, it cuts DNA at a specific region. Natural DNA repair machinery in the cell then fixes this damage. During the repair, scientists can manipulate the machinery to insert a desired mutation, thus permanently changing the DNA. There are two predominant types of DNA repair machinery in human cells, homologous recombination (HR) and non-homologous end joining (NHEJ), but HR is preferred when studying gene function.

"HR gives specific mutations. If you are looking for new function of a gene, HR is preferred because you can dictate the type of mutations that are introduced," said Dr. Peter Gee, who co-authored the study.

CRONUS functions by using two drugs to regulate CRISPR-Cas9 activity.

"We designed CRONUS so that adding doxycycline expresses Cas9 protein and dexamethasone transports Cas9 into the nucleus," where it cuts the DNA, explained Junior Associate Professor Akitsu Hotta. The two-drug approach significantly improves the accuracy by controlling the Cas9 activity to cleave DNA.



CRONUS provides a simple experimental method to cut genes precisely.

To demonstrate CRONUS, the lab inserted single-stranded oligodeoxynucleotides in iPS cells to create mutations at the cut site. CRONUS was confirmed using multiple genes, with HR occurring in about one third of cases.

"In some systems you are lucky if you get over 3%. iCRISPR gives similar efficiency as CRONUS, but establishing the system takes a couple of months longer," said Gee.

The magnitude improvement in efficiency means one tenth the number of cells for experiments, saving time and effort for the scientist.

Reference

Ishida K, Xu H, Sasakawa N et al. (2018) Site-specific randomization of the endogenous genome by a regulatable CRISPR-Cas9 *piggyBac* system in human cells. *Scientific Reports* 8(1): 310. DOI: 10.1038/s41598-017-18568-4

Chemical compound A-674563 protects chondrocytes from deterioration

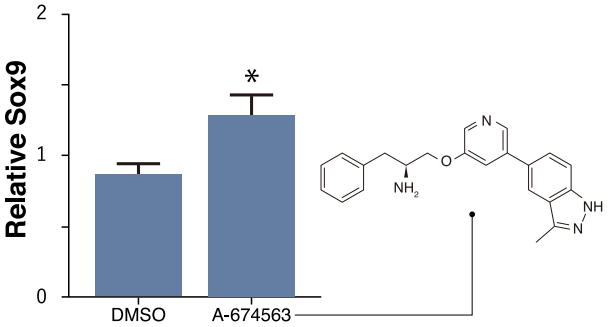
The Tsumaki lab finds A-674563 preserves the expression of an essential protein for cartilage function in mouse cells.

A drug screening of over 5000 compounds by CiRA scientists has discovered a drug compound that could improve current regenerative medicine for cartilage damage. The new study shows A-674563 protects the degradation of Sox9, the protein most important for the identity of chondrocytes, the cells responsible for articular cartilage production.

Arthritic knees and other joint pain is often the result of eroding articular cartilage that covers the ends of bones and lubricates the joint. Cartilage does not naturally heal, so any substantial damage is permanent without medical care. For cases where physical therapy and drug treatments are not enough, patients will undergo cartilage transplants. Autologous chondrocyte implantation (ACI) is a standard procedure in which a biopsy of undamaged articular cartilage is taken from the patient and moved to the damaged area.

"The cartilage we take is never enough to fill the damaged area. We expand the chondrocytes and implant the expanded chondrocytes into the defect," explained CiRA scientist and orthopedic surgeon Tomohito Kobayashi, who first authored the study.

The expansion increases the number of chondrocytes and amount of cartilage to fill the defect. Unknown to doctors, however, is why the expanded chondrocytes change their character from articular to fibrocartilaginous, a process known as dedifferentiation. Fibrous cartilage is not as lubricious as articular cartilage, thus compromising the recovery. Kobayashi had joined CiRA in hopes of findings ways to preserve chondrocyte character as articular cartilage for better ACI outcomes.



The addition of A-674563 to the chondrocyte differentiation protocol enhanced the expression of Sox9.

"Several factors have been reported to inhibit dedifferentiation. The discovery of A-674563 suggests a new mechanism that could sustain chondrocyte character," he said.

Overall, the study suggests that adding A-674563 to ACI protocols could increase Sox9 protein levels and reduce dedifferentiation, but Professor Noriyuki Tsumaki, who managed the study, stressed more work needs to be done before concluding any effectiveness for patient care.

"We need to further analyze mechanisms and to investigate crosstalk with other signals, which would contribute to comprehensive understanding of cartilage biology," he said.

Reference

Kobayashi T, Fujita K, Kamatani T et al. (2018) A-674563 increases chondrocyte marker expression in cultured chondrocytes by inhibiting Sox9 degradation. *Biochem Biophys Res Commun.* 495(1): 1468-1475. DOI: 10.1016/j.bbrc.2017.11.180

Cheap production of liver cells for disease study and treatment

The Osafune lab reports a low-cost protocol for the production of hepatocytes from iPS cells for affordable study of liver disease and therapies.

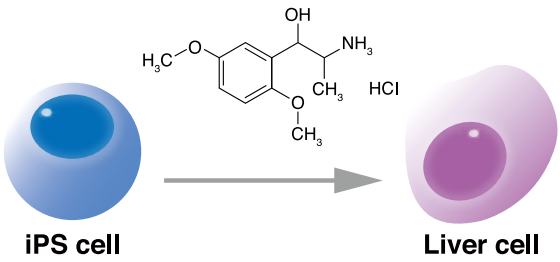
The only effective cure for chronic liver disease is liver transplantation, the first of which in modern medicine dates back more than 50 years. However, the lack of available donors leaves the majority of patients in need of a liver transplantation underserved. Furthermore, even though new technologies now allow for only portions of a liver from a living donor to be used for the transplant, the operation puts the donor at risk, which discourages potential donors from coming forward. As an alternative to liver transplantation, Professor Kenji Osafune is using iPS cells to prepare hepatocytes to study and treat liver disease. His lab's newest study reports a protocol that uses cheap chemicals to lower the cost of the cell preparation.

One factor holding back new cell therapies is limited understanding of how to convert iPS cells into hepatocytes.

"We try to mimic the development process of liver. We induce each stage of development with certain growth factors," said Osafune.

The problem with growth factors is that they are expensive and difficult to standardize for clinical use. Osafune hypothesized that because they are cheaper and easier to manufacture, drugs and other chemical compounds are preferred for differentiating iPS cells into hepatocytes.

His team screened a drug library of over 1,000 compounds and found one compound, methoxamine hydrochloride, has a positive effect on the differentiation of iPS cells to hepatocytes. In fact, the efficiency of the differentiation to hepatocytes using methoxamine hydrochloride was equal to that using expensive growth factors.



Methoxamine hydrochloride significantly lowers the cost of preparing heaptocyte-like cells in the laboratory.

Methoxamine hydrochloride is an adrenergic receptor agonist. Adrenergic receptor agonists and antagonists are known to cause muscle contraction and relaxation, respectively. Beta blockers are the most famous and used to reduce blood pressure.

"We found that methoxamine hydrochloride binds to α1-adrenergic receptors and activates STAT3 signaling to promote the generation of hepatocytes," said Dr. Maki Kotaka, who first-authored the study.

Osafune expects the study to clarify how the liver forms at smaller expense.

"Detailed understanding of the signals that differentiate iPS cells to hepatocytes will help us produce cells at higher efficiency for future cell therapies. We think our method can lower costs significantly," he said.

Reference

Kotaka M, Toyoda T, Yasuda K et al. (2017) Adrenergic receptor agonists induce the differentiation of pluripotent stem cell-derived hepatoblasts into hepatocyte-like cells. *Scientific Reports* 7(1):16734. DOI: 10.1038/s41598-017-16858-5.

Highly purified cells for heart therapies

The Yamashita lab discovers a biomarker that identifies cells with the capacity to nearly exclusively produce cardiomyocytes.

If a heart attack fails to kill its victim, it will succeed at killing heart cells, leaving a weaker heart that is more prone to failure in the future. Cell therapies can remedy damaged hearts by transplanting into them cardiovascular progenitor cells (CPCs). Successful therapies depend on the CPCs differentiating into cardiomyocytes, but the efficiency of the differentiation has been substandard. A new study by the Jun K. Yamashita laboratory uses iPS cells to find a type of CPC that when transplanted into mice almost exclusively produces cardiomyocytes, giving these cells great promise for future heart cell therapies.

"CPCs have been characterized by the expression of several transcription factors and surface markers. However, they differentiate to cardiomyocytes and other cell types," said Dr. Masafumi Takeda, a scientist in the Yamashita lab.

These other cells include endothelial cells, pericytes and mesenchymal cells, all of which are essential for the heart and vasculature, but not suitable for replacing the cardiomyocytes following a heart attack.

"For cell therapies, we want CPCs that differentiate to cardiomyocytes at 100%," he continued.

Takeda considered whether there exists a specific subset of CPCs that exclusively differentiates into cardiomyocytes.

CPCs themselves exist sparsely in the adult body but can be acquired from iPS cells. By differentiating iPS cells and following protein expression patterns, Takeda found the differentiation potential of CPCs could be distinguished by the expression of CD82. He transplanted CD82+ CPCs, which he called cardiomyocyte-fated progenitors, into the hearts of live mice and found that almost all differentiated into cardiomyocytes.

"We found the differentiation efficiency was consistently over 95%. I do not know of any other progenitor that differentiates into cardiomyocytes at such a high rate," said Takeda.

CD82 is a glycoprotein that was first identified to have a suppressive role in prostate cancer. Since then, it has been found to have function in cell adhesion and cell motility. It also regulates exosomes, which led Takeda to the molecular mechanism through which CD82 enhances cardiomyocyte commitment.

"CD82 contributes to the inhibition of Wnt signaling through exosome activity. It is known that in normal heart development, Wnt inhibition is crucial for cardiomyocyte commitment," he said.

Takeda says the finding of CD82 has important implications not only for heart therapies but also heart development.

"The high differentiation efficiency is exciting for cell therapies, because it means we can produce a pure population of cardiomyocytes. It also means we can study heart development more accurately," he added.

Reference

Takeda M, Kanki Y, Masumoto H et al. (2018) Identification of Cardiomyocyte-Fated Progenitors from Human-Induced Pluripotent Stem Cells Marked with CD82. *Cell Reports* 22(2):546-556. DOI: 10.1016/j.celrep.2017.12.057

People with low energy have low immunity

The Megumu Saito lab uses patient iPS cells to find a molecular mechanism behind the disease, reticular dysgenesis.

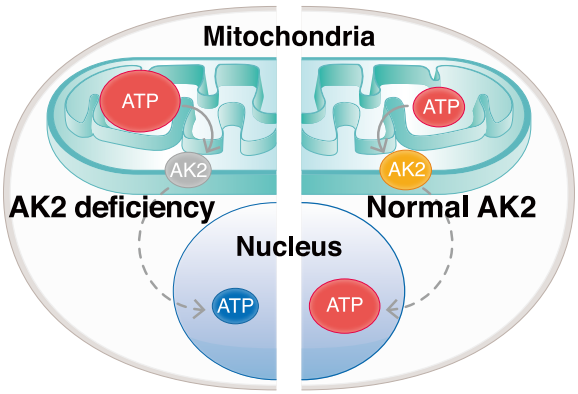
Reticular dysgenesis is considered the most extreme form of severe combined immunodeficiency (or "bubble boy" disease). It is a hereditary disease, and patients will die shortly after birth if untreated. A number of ailments will inflict the patient, but all are attributed to compromised immune function.

Mutations in adenylate kinase 2 are responsible for reticular dysgenesis. All adenylate kinases are responsible for balancing the levels of ATP, ADP and AMP in the cell, making these kinases a gatekeeper of energy levels. Proper energy levels are crucial for the development of blood and immune cells from hematopoietic stem cells, notes Associate Professor and Pediatrician Megumu Saito.

"The transition of the metabolic status is linked to the fate of hematopoietic stem cells. It has been hypothesized that the adenylate kinase 2 mutation in reticular dysgenesis impairs the differentiation, but this is only a hypothesis," he said.

To test this hypothesis, Saito and his team of researchers prepared iPS cells from reticular dysgenesis patients and healthy controls and then differentiated the cells to hematopoietic lineage. The scientists found that the patient iPS cells were defective in their differentiation capacity to immune cells.

More specifically, the development was compromised at the stage of hemoangiogenic progenitor cells, which represent one of the earliest stages in blood development and are responsible for producing both endothelial cells and hematopoietic cells. The result was a severe inability of these



Using iPS cells from patients with reticular dysgenesis, CiRA researchers find the causative mutation leads to an imbalance in ATP levels between mitochondria and the nucleus.

cells to differentiate into immune cells. Further analysis found the cause to be deviations in the ATP distribution in the cells.

"There was much more ATP in the mitochondria of patient cells and much less in the nuclei. There was also a large difference in the genes expressed between patient and normal cells," explained Saito. Many of the genes poorly expressed by the differentiated patient iPS cells were related to blood development.

Besides giving clarity to a devastating disease, the findings also show key molecular events that must happen for proper development to occur.

"Our data suggest that the intracellular redistribution of ATP is important for controlling the cell fate," said Saito.

Reference

Oshima K, Saiki N, Tanaka M et al. (2018) Human AK2 links intracellular bioenergetic redistribution to the fate of hematopoietic progenitors. *Biochem Biophys Res Commun* 497(2): 719-725. DOI: 10.1016/j.bbrc.2018.02.139.

High throughput drug screening to treat muscular dystrophy patients

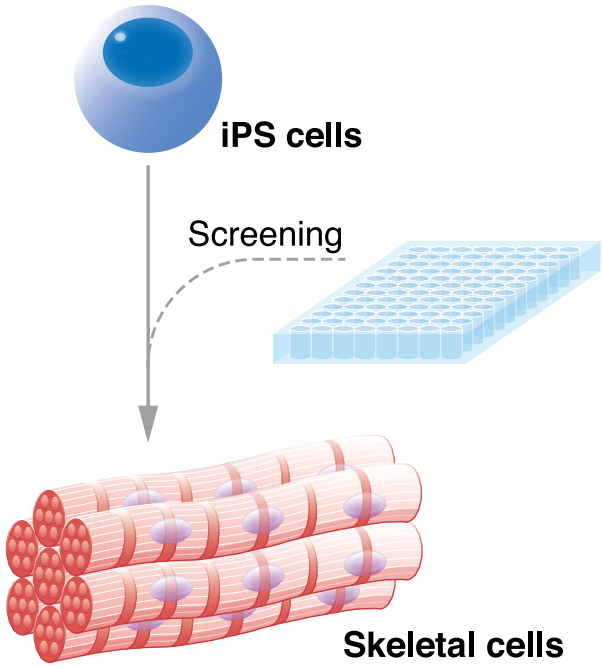
The Sakurai lab presents a new protocol that improves the survival of stem cell-derived muscle cells for drug screening.

The advent of patient iPSCs has opened the door to a new frontier of personalized medicine. High throughput screening, which greatly lowers the cost and labor of drug discovery, can be done using cells differentiated from patient iPSCs to evaluate experimental compounds. However, some cell types derived from the patient iPS cells, such as muscle cells, fail to survive in the high throughput screening process. A new protocol by Junior Associate Professor Hidetoshi Sakurai and his team uses "replating" to present a solution to this problem. The protocol was confirmed by preparing muscle cells from the iPSCs of a patient with muscular dystrophy.

High throughput screening involves the culturing of cells on plates holding dozens or hundreds of wells. This setup allows a number of experimental drugs and doses to be tested on the cells at one time. However, according to Dr. Tomoya Uchimura, a post-doctoral scientist in the lab who designed the protocol, the differentiation of stem cells to muscles is not compatible with high throughput screening.

"Cells suffer from variability of wells and edge effects like evaporation. Certain steps of the differentiation protocol are affected by edge effects," he said.

In standard protocols, iPS cells are kept in 96-well plates throughout the differentiation process. Uchimura found that by first placing the iPS cells in a large dish and then moving them to 96-well or even 384-well plates midway in the differentiation protocol (i.e. replating), a much larger number of myogenic differentiated cells differentiated to myogenic lineage survived.



A new protocol for the differentiation of iPS cells to myocytes is applicable to high-throughput screening using 96-well plates.

"I am not 100% sure why, but my assumption is that proliferation might be largely affected by variability of wells or edge effects in small wells," he said, adding that iPS cell proliferation is a first step to any differentiation procedure.

Importantly, besides enhancing survival, Uchimura found less variability between cell populations in different wells, making it easier to compare the effects of the drug and dose.

Reference

Uchimura T, Otomo J, Sato M et al. (2017) A human iPS myogenic differentiation system permitting high-throughput drug screening. *Stem Cell Res* 25: 98-106. DOI: 10.1016/j.scr.2017.10.023

Greetings from the
Jun Takahashi Lab

Dept. of Clinical Application

It is estimated that by the time a patient is diagnosed with Parkinson's disease, more than 50% of dopaminergic neurons, the primary neurons afflicted by the disease, will have degenerated. Current treatments are only symptomatic, and there is little hope for the patient besides replacing the lost cells with cell transplantation for a full recovery. In this way, iPS cells are an ideal technology, because they can be used to make a large number of dopaminergic neurons. Further, dopaminergic neurons are located in a specific region of the brain, which makes the neurosurgery relatively straightforward. Recognizing these points, we aim to be the first lab to clinically apply iPS cells to treat a neurodegenerative disease. In fact, we plan to begin recruiting patients for our clinical trial this year.

Parkinson's disease makes an ideal test case for an experimental iPS cell therapy against neurodegenerative diseases because of the success seen using fetal cells. There are records of patients showing benefits even 20 years after having dopaminergic neurons prepared from these other cell sources transplanted in the brain. What has limited these cell therapies from becoming mainstream, however, is the difficulty in procuring the cell source. It is difficult to collect fetal tissue of a homogeneous standard, because of their scarce availability. Consequently, there is large variability in cell therapy outcomes for Parkinson's disease using these cells. On the other hand, working with FiT (Facility for iPS Cell Therapy) at CiRA, we can acquire iPS cells of a homogeneous quality. We expect that cells from FiT will contribute to developing a procedure that is optimal for all patients.

In addition to using iPS cells from FiT, we are conducting exhaustive experiments to identify which differentiated cell types are best for the transplantation. This includes identifying surface markers (antigens) and intracellular markers (miRNA) for the cell purification, and irradiating the cells to reduce the risk of tumorigenicity. Based on these studies, we have already confirmed best conditions, including the levels of immunosuppression, in primate models that received human or monkey iPS cell-derived neurons.

Jun Takahashi in his office

The anticipation building with our Parkinson's project has led us to explore the possibility of cell therapies for other neuronal damage, namely stroke. Strokes often occur in an isolated region, but unlike in Parkinson's disease, that region can cover a much wider part of the brain. For this reason, a wider range of neuron types could be affected by the stroke. The brain has an incredible capacity to adapt to damage. In the case of stroke, new neural networks form to compensate for the lost cells. To understand this formation and to advance cell therapies for it, we are also accelerating our work on 3D brain organoids.

Clinic websites mislead about stem cell therapies

by Assistant Professor Taichi Hatta, Uehiro Research Division for iPS Cell Ethics

Continuing advances in stem cell research has added to the expectation of these cells becoming a common form of therapy used in the clinic. However, society demands scientific proof before an experimental therapy is used on humans. While this proof is accumulating, many voices obfuscate or exaggerate. This is a particular problem on the web, where language is not regulated and may persuade patients to select a treatment that is not scientifically proven.

An official investigation by the Japanese government in 2015 found a noteworthy increase in the number of patients requesting cosmetic medical treatments and attributed this rise to information distributed on the providers' websites. In response, Japan amended a law regarding the regulation of medical advertising. The law adds greater responsibility to clinics or hospitals that offer cell therapies for the information they disseminate on their website. The question is how effective are these new measures in terms of providing accurate information for a patient to make an educated decision about the treatment?

The responsibility of the provider with regards to the information given to a patient has been ambiguous in Japan. A law passed four years ago requires providers make public the documents shown to the patients in the clinic about the therapy before informed consent is received. These documents can be found on Japanese ministry websites and describe the potential therapeutic effects of the therapy along with the side effects and other possible risks. It is quite obvious that the details provided in these forms are more elaborate than the details clinics make available on their websites. In this scenario, patients may have already convinced themselves to select the therapy and will be unpersuaded by any information at the final stages such as the signing of the consent forms no matter how discouraging the new information is. It is hoped that the availability of these documents will help patients make informed decisions.

At CiRA, researchers at the Uehiro Research Division for iPS Cell Ethics is studying the effects of the most recent laws on medical advertising. Our goal is not only to evaluate these laws, but to also provide recommendations for their amendment, so that patients are better informed when making decisions about scientifically proven or unproven treatments.

Taichi Hatta at a public speaking

Marathons

For the past six years, the roads of Kyoto are closed one February Sunday to host the Kyoto Marathon. CiRA has always used marathons as a form of fundraising, with more faculty contributing each year. However, only two faculty members joined the 2018 Kyoto Marathon, Professors Shinya Yamanaka and Jun Takahashi. The number of non-faculty CiRA runners outnumbered the faculty members. Takahashi, who ran his first marathon in 2016, was an inspiration to many of his lab members, including Yoshifumi Miyawaki, Takafumi Shimogawa, and Sadaharu Torikoshi, who also joined the race but wisely finished behind their boss. Dr. Hiroki Ikeda and student Thomas Luc Maurissen ran their first marathons. “Training to run that distance is the challenge. It took a lot more mental preparation than I had expected,” said Maurissen. This year Yamanaka did not run the entire marathon. Instead, he and 2016 Paralympian medalist Misato Michishita ran a relay, with Michishita doing the first two thirds and Yamanaka doing the last.

Misato Michishita (left) and Shinya Yamanaka

Charity Concert

Needless to say, CiRA Director Shinya Yamanaka has many fond memories of the Nobel Prize Ceremony in 2012. One unexpected memory is the performance by the Royal Stockholm Philharmonic Orchestra. He was especially touched by the music of violinist Ray Chen. “It was a spectacular performance and remains one of my highlights of the ceremony,” he said.

Chen is the youngest soloist to ever perform at a Nobel Prize Concert. That meeting groomed a friendship between the two, which is why Chen accepted to perform at a charity event for CiRA on January 31.

The event was sponsored by the Nippon Music Foundation and held at Kioi Hall in Tokyo. The 800-seat auditorium was full for the two hours, the first of which had Yamanaka speak about iPS cells and his first encounter with Chen in Stockholm. In the second half, Chen performed with the accompaniment of pianist Eri Hayashi. For the performance, the Foundation loaned Chen from its collection a violin* once owned by Joseph Joachim, a violinist famous for his work in the 19th Century. The iPS Cell Research Fund collected nearly 8 million yen from the concert.

Although the two rarely get to meet, Yamanaka and Chen both describe each other as friends, and Yamanaka was clear to Chen that he would be always be welcome to CiRA where “it would be my privilege to show you our research.”

Ray Chen (left) and Shinya Yamanaka
Photo provided by Nippon Music Foundation
*Stradivarius 1715 Violin “Joachim”

Knowledge in the heart of Osaka

In 2013, the city of Osaka, a 30-minute train ride from Kyoto, opened Knowledge Capital as a hub to share innovation among specialists and the general public. CiRA has used this space to give lectures about iPS cells in Japan's second most populated metropolis. Because of the gains in drug discovery that has come from iPS cell research, such as the clinical trial that came from collaborative research from the Toguchida and Ikeya laboratories (see Vol. 13), CiRA scheduled a series of four talks over the winter beginning with Professor Junya Toguchida in January, who spoke about his use of iPS cells to develop new medical therapies for bone-related disease. Also participating were Associate Professors Hidetoshi Sakurai and Megumu Saito, who respectively spoke about their use of iPS cells to find cures for muscle diseases and for hematological and immunological diseases. In the last talk, held March 14, Assistant Professor Mitsujiro Osawa spoke about the importance of building an iPS cell bank from healthy donors for research.

"One woman told me about her 107-year old relative. She was never sick and only went to the hospital for a dental check. We don't get many healthy 100-year old donors," said Osawa.

Junya Toguchida

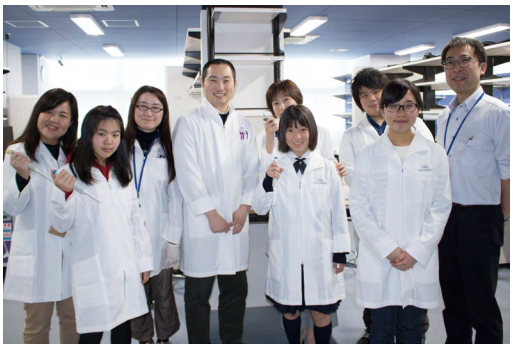
Hidetosh Sakurai

Megumu Saito

Mitsujiro Osawa

Public events at CiRA

CiRA opened its doors to the public on two days in March to learn about iPS cells. For the first event, March 2, four CiRA faculty including Professors Hirohisa Inoue and Kenji Osafune, and Associate Professors Megumu Saito and Yoshinori Yoshida spoke about how iPS cells are advancing drug discovery in their organ systems of interest. A fifth faculty member, Professor Akira Ohta of the Drug Discovery Technology Development Office, spoke more generally about how scientists use patient iPS cells for drug discovery. On March 17, CiRA held its first Family Day, inviting the family of CiRA staff to see the facilities and have an opportunity to conduct simple iPS cell experiments.



CiRA staff and their families

CiRA retracts a 2017 paper

A research paper published by a specially-appointed assistant professor at CiRA has been officially retracted in February for research misconduct.

The report described an experimental model of the blood-brain barrier (BBB) manufactured using iPS cell technology. The BBB prevents drugs from passing from the blood to the brain and is one of the most difficult obstacles hampering drug development for brain-related diseases. The study claimed its BBB model could predict the known permeabilities of 10 clinically used drugs, suggesting it would be an invaluable tool for evaluating whether an experimental drug could pass to the brain from the blood.

The paper was published online in February 2017, but by the summer, CiRA had commenced a six-month investigation on suspected research misconduct. On January 22, 2018, following the conclusions of a committee consisting of internal and external investigators, Kyoto University announced that it had found Dr. Kohei Yamamizu, both the first and corresponding author of the study, had fabricated data for almost all the figures. No other researchers were found to have contributed to the fabrications. The paper was officially retracted February 13, 2018, from Stem Cell Reports. Yamamizu was fired for his actions.

During a press conference on the misconduct held on January 22, CiRA Director Shinya Yamanaka said, “I feel a strong responsibility for not having been able to prevent research misconduct at our institute and sincerely apologize to all who support us and our research activities.”

CiRA is reevaluating its training program for professional research conduct. It is also implementing stronger preventative measures this month.

Awards

Associate Professor Makoto Ikeya was recognized by the Murao Educational Foundation, who awarded him their 35th annual Science Excellence award on March 10. The Foundation recognizes exceptional scientific research that benefits Hyogo Prefecture, which includes the city of Kobe. Research done by Ikeya in collaboration with Professor Junya Toguchida used iPS cells from a Hyogo patient with fibrodysplasia ossificans progressiva to reveal a new molecular mechanism that causes the disease. Further work on this project by the two researchers has led to clinical drug trial.

Within CiRA, Dr. Misao Fujita and Dr. Takuya Yamamoto were officially promoted to the titles of Professor and Associate Professor, respectively, this month.

Misao Fujita

Takuya Yamamoto

The Temples and Shrines of Kyoto

Nanzen-ji

Nanzen-ji lies at the very end of the mountains that border the east side of Kyoto and separates the city from Lake Biwa, the largest lake in Japan. The grounds were originally selected not for any religious purpose, but to serve as a villa for an emperor. That changed after an exorcism was done to free the grounds of mischievous ghosts, convincing its inhabitants that they should reward the priests with a portion for the construction of a temple. Nanzen-ji holds several gardens, the largest of which is named Hojo. The walk in Hojo follows the inside of the main quarters and passes through many small rock and pond gardens. It also provides a tea room in which patrons can enjoy traditional green tea. Yet it is the temple's Sanmon that easily makes the greatest impression. Sanmon are gates that stand at the entrance of many temples in Japan. Nanzenji's is unusual in that it lies about 100 m inside the grounds and stands at 22 m high. The grounds have the added feature of a large aqueduct, which ends Kyoto's old canal, Sosui, and connects to Biwa. The canal provides a unique walk back into the city that follows an abandoned railway.

Sanmon

Hojo Garden

CiRA Internships

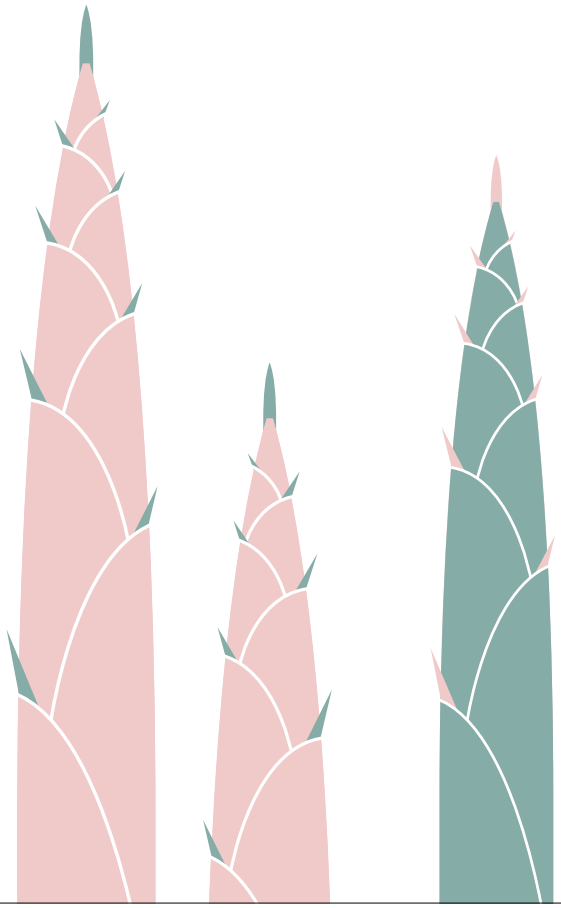
For its third year, CiRA is inviting undergraduate and graduate students to study at the institute for a maximum of 8 weeks. Ultimately, the purpose of the summer internship program is to recruit future graduate students and post-doctorates. CiRA pays for travel and stay through the iPS Cell Research Fund, and each lab may invite one foreign and one domestic student per year. In order to process visas, the application deadline for the foreign cohort comes in March, whereas domestic students can submit their documents in May. This year, CiRA received that largest number of foreign applicants yet, with more than 40 received.

As a testament to the benefits of the program for all, Julia Alexandra Kudryashev, an intern from 2016, was one of the authors on the most recent paper from the Hotta lab (see page 4).

Clinical trials

When will you start them? they ask

Hopefully this year



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Vol.14 | April 2018