CiRA Reporter

Center for iPS Cell Research and Application,
Kyoto University

Vol.16
October 2018

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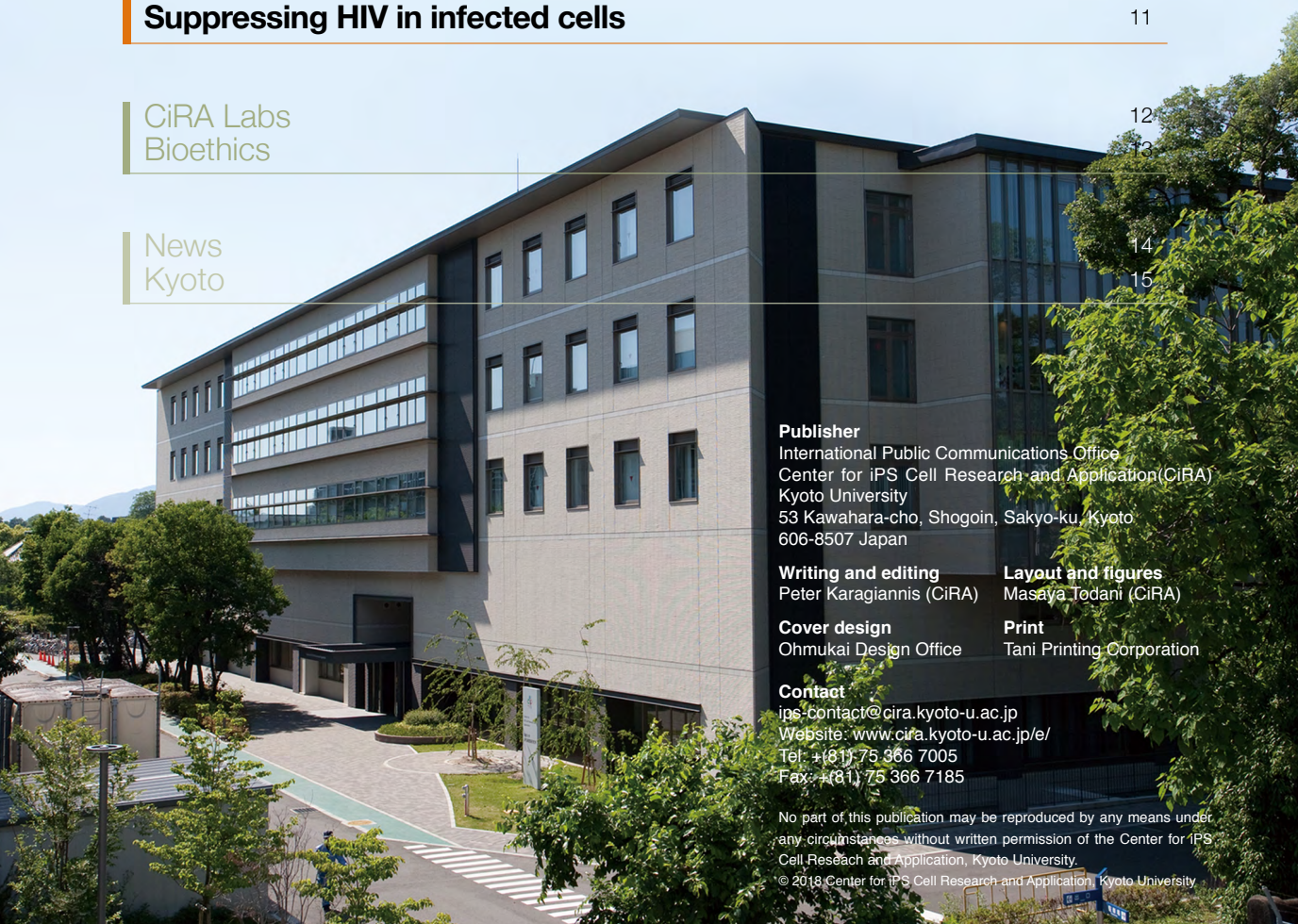

Publisher
International Public Communications Office
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CiRA researchers announce the worlds' first iPS cell-based therapy for brain

In what had been long anticipated, CiRA announced the first iPS-cell based clinical trial to come from one of its faculty. The trial will be conducted at Kyoto University Hospital under the supervision of two neurosurgeons, Ryosuke Takahashi, Professor at the Department of Neurosurgery, Kyoto University Hospital, and Jun Takahashi, Professor at CiRA. The therapy involves the differentiation of iPS cells from the iPS Cell Stock for Regenerative Medicine at CiRA into dopaminergic neurons, which will then be transplanted into the patients. The project is currently at the patient recruitment stage.

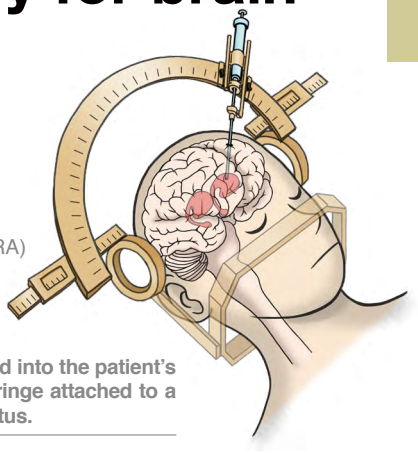
The use of dopaminergic neurons as a cell therapy for Parkinson's disease dates back decades. Originally, fetal tissue was used to generate the transplanted cells. Some patients have responded well, showing improvement for over 20 years. Others, however, have been far less fortunate, and the benefits begin to dissipate after only a few years if they occur at all. A major problem is the availability of tissue.

"Fetal tissue is not easy to acquire. We have to take what we can get. This gives large variability in the quantity and quality of the dopaminergic neurons, which could explain different patient outcomes," says Jun Takahashi.

The cell source shifted to ES cells with their discovery in 1998, and more clinical trials for Parkinson's disease using ES cell products than iPS cell products are anticipated in the near future. This popularity reflects the fact that ES cell research has had a ten-year head start, but, Takahashi notes, iPS cells have an advantage when considering wide patient care.

"Many people donate blood, but few donate embryos. We can get enough blood donors that match the entire population, but maybe not embryo donors," he continues.

Illustration by
Misaki Ouchida (CiRA)



Cells will be injected into the patient's brain through a syringe attached to a customized apparatus.

Cell therapies normally require immune-matching donor and host cells. Although it ultimately aims to provide iPS cells that cover most of the Japanese population, the iPS Cell Stock for Regenerative Medicine currently covers only 30% of the population. This time, donor-patient matching will not be a criterion in the Parkinson disease study. Instead, an immunosuppressant will be used to minimize the risk of cell rejection, and the trial will evaluate the effectiveness of iPS cell-derived dopaminergic neurons on Parkinson's disease and the effectiveness of the immunosuppressant, tacrolimus, in an iPS cell-based therapy.

One determinant of patient eligibility will be the stage of the disease.

"At the early stage of the disease, medical treatment is effective. But along the progression of the disease, it becomes difficult to control the symptoms by medical treatment alone. It is the best time for a cell-based therapy. If the disease progresses to the severe stage when the neurons do not respond to dopamine, it is too late," says Jun Takahashi.

The clinical trial is only the latest in a decades-long journey for Jun Takahashi.

"I have been researching stem cells for a cure since the 1990s. I feel like I am receiving a verdict for my work," he says

Two new studies from the Megumu Saito Lab show the versatility of iPS cells

The lab prepares sympathetic neurons and reports a new model for immune diseases from iPS cells.

iPS cells have been a boon for the study of neurodegenerative diseases, because researchers can reprogram patient cells to observe how the disease develops in its early stages, well before the patient is symptomatic. Moreover, motor neurons made from human iPS cells are the basis of a clinical trial on a drug to treat ALS, and dopaminergic neurons made from human iPS cells are the basis of a clinical trial on a cell therapy to treat Parkinson's disease. However, far less progress has been made converting iPS cells into sympathetic neurons.

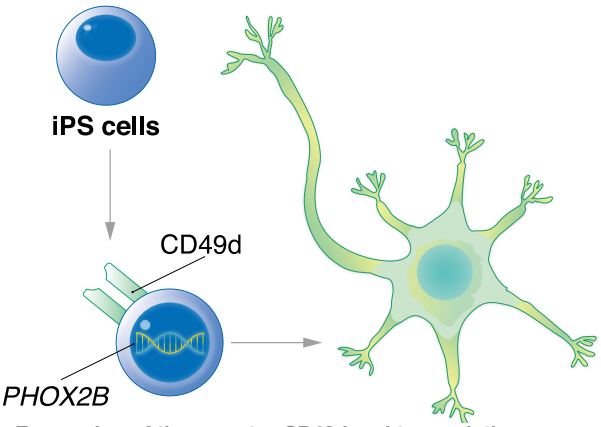
"Sympathetic neurons cannot be differentiated efficiently from iPS cells. Sympathetic neurons regulate many unconscious activities like heart rate, blood pressure, and sweating," says CiRA Associate Professor Megumu Saito.

To solve this problem, his team of scientists investigated the expression of the gene *PHOX2B* during the differentiation of iPS cells.

"Animal studies have shown progenitor cells that express *PHOX2B* are committed to sympathetic neurons," says Saito.

Scientists have previously identified several intermediate stages in the development of sympathetic neurons. Pediatric surgeon Dr. Kosuke Kirino, the first author of the study, aimed to capture these intermediate cells.

"Sympathetic neurons are derived from trunk neural crest cells, which arise from neuromesodermal progenitor cells," he says.



Expression of the receptor CD49d and transcription factor PHOX2B marks cells with potential to differentiate into sympathetic neurons.

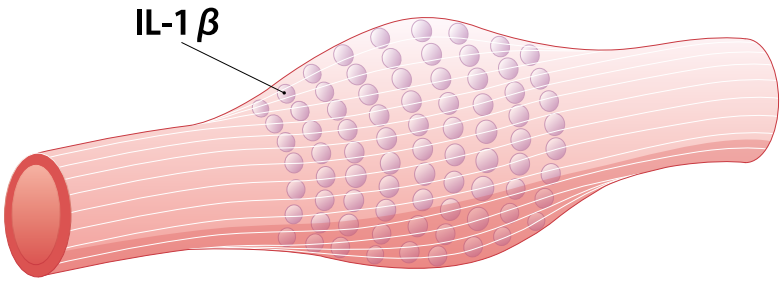
The generation of neuromesodermal progenitor cells from human iPS cells and of neural crest cells from neuromesodermal progenitor cells is well established. However, the generation of neural crest cells leads to other cell types that contaminate the cell population. Therefore, to get a pure population of neural crest cells, Kirino looked for another marker in combination with *PHOX2B*.

"Neural crest cells express CD49d," he notes.

Indeed, capturing cells that expressed both CD49d and *PHOX2B* during the iPS cell differentiation resulted in the generation of sympathetic neurons at levels not seen previously.

Saito believes this discovery could open the door to new studies on poorly understood diseases.

"For many diseases of the sympathetic nervous system, there are no good human models. We hope our differentiation method can be used," he says.



IL-1β alone is enough to prime and activate the inflammasome in vascular muscle cells, causing inflammation.

In a second study, the lab used iPS cells to study causes of inflammation in various diseases. The immune system serves to combat almost any illness or infection. While its intention is to cure, the immune system can also cause discomfort through inflammation. Indeed, many diseases are accompanied or even caused by chronic inflammation. The study provides a new explanation for the common inflammation seen in vascular diseases like atherosclerosis.

"The inflammasome is one of the most critical machineries for regulating the innate immune response," says Saito. "It operates through two sequential signals, a priming signal and an activating signal."

Although the inflammasome is generally believed to require two signals, there are known exceptions.

"One signal is enough to produce interleukin-1β (IL-1β) from monocytes," which are a type of white blood cell, he continues.

Vascular smooth muscles make up the walls of almost all blood vessels and are responsible for controlling the diameter of the vessels. Unexpected observations in preliminary experiments led Saito to wonder if these cells can also activate the inflammasome with just one signal like monocytes.

To test this theory, his group primed vascular smooth cells and monocytes with the destruction of lysozymes, a type of enzyme found inside cells and whose loss stimulates inflammation but also cell death.

Interestingly, the destruction of lysozymes caused monocytes to die, but vascular smooth cells survived to secrete IL-1β.

"Our results show that one signal is enough to both prime and activate the inflammasome in vascular smooth cells. We don't know the reason why the cells responded differently," says Saito.

Saito adds that the findings suggest a unique signal could trigger inflammation in blood vessels.

"Classically, the vasculature does not inflame until it is invaded by immune cells. Our findings suggest the inflammation can occur before the invasion," he says.

Reference

Kirino K, Nakahata T, Taguchi T et al. (2018) Efficient derivation of sympathetic neurons from human pluripotent stem cells with defined condition. *Scientific Reports* 8(1):2081 DOI: 10.1038/s41598-018-31256-1

Ono H, Takada H, Niwa A et al. (2018) Lysosomal membrane permeabilization causes secretion of IL-1β in human vascular smooth muscle cells. *Inflammation Research* 67:879-889. DOI: 10.1007/s00011-018-1178-z

Making new cartilage from reprogrammed cells

The Tsumaki Lab reports how its iPS cell technology, iPS-Cart, binds together to form cartilage.

Cartilage is the tissue that gives joints their mobility. It is extraordinarily robust and built to last a lifetime. Ironically, while durable, unlike other tissue in the body, cartilage is incapable of repairing on its own. CiRA Professor and Orthopedic Surgeon Noriyuki Tsumaki is using iPS cell technology to produce a surgical option that repairs cartilage defects. In the newest study from his lab, he reports how his iPS-Cart adhere to one another to form new cartilage.

"Our iPS-Cart are small particles 1-3 mm in diameter. We plan to transplant about 100 iPS-Cart depending on the size of the cartilage defect," he says.

Once transplanted, iPS-Cart integrate with the surrounding cartilage of the patient and also with each other to form one continuous cartilage that Tsumaki expects to function as well if not better than prior to the defect.

"The best surgical option for cartilage defects is transplanting cartilage tissue. Our iPS-Cart show good integration into pig and other animals. We do not know how they integrate with one another," says. Dr. Xike Chen, a Ph.D. student in the Tsumaki lab and the first author of the study.

iPS-Cart can be divided into an interior that constitutes cartilage and an exterior that constitutes what Chen calls a "perichondrium-like membranous tissue."

"The perichondrium surrounds the cartilage of developing bone. Perichondrium also functions in the growth and repair of cartilage," she says.

She found that two iPS-Cart would first adhere at their perichondrium-like membranous tissue, but after less than two months the adhesion would

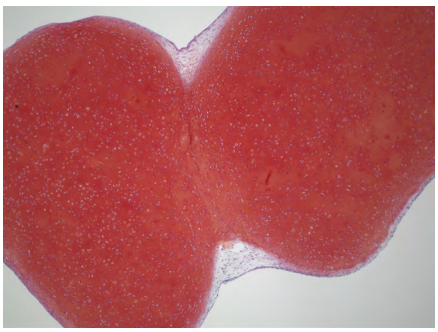


Photo of two iPS-Cart adhering at the cartilage component (red).

reach the interior cartilage, purging the perichondrium-like membranous tissue to leave a structure that resembles one large iPS-Cart.

Chen then looked for biological factors naturally found in cartilage that could promote the adhesion.

"We found FGF18 was expressed more in the perichondrium-like membranous tissue than cartilage of iPS-Cart. We added FGF18 to see if it accelerates the integration," she says.

Indeed, adding FGF18 shortened the adhesion time of the perichondrium-like membranous tissue in iPS-Cart, suggesting that combining FGF18 treatment with an iPS-Cart transplant could quicken the recovery.

Tsumaki explains that understanding how iPS-Cart adhere and what conditions are best for the adhesion is a key step to using iPS cells for cartilage therapies.

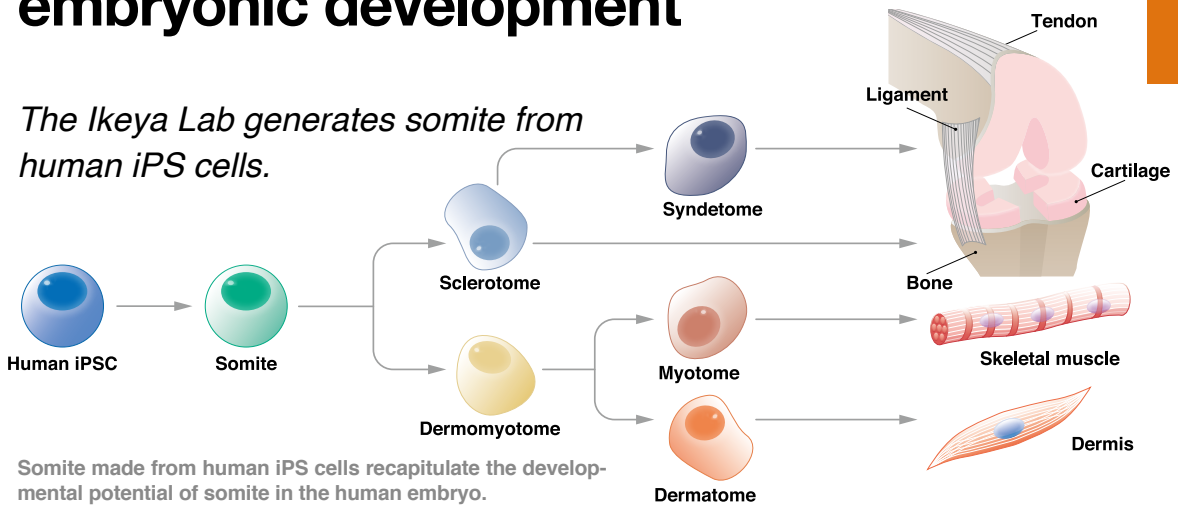
"My goal is to provide good treatment options to patients with cartilage damage. We are developing iPS-Cart to be used in patients," he says.

Reference

Chen X, Yamashita A, Morioka M et al. (2018) Integration capacity of human induced pluripotent stem cell-derived cartilage. *Tissue Engineering Part A.* DOI:10.1089/ten.TEA.2018.0133

iPS cells can form a key stage in embryonic development

The Ikeya Lab generates somite from human iPS cells.



Somite made from human iPS cells recapitulate the developmental potential of somite in the human embryo.

During human development, the embryo quickly partitions into distinctive layers that eventually leads to a fully-grown body. While human iPS cells can be used to make almost all cell types of the human body, certain intermediate stages during embryo development have not been achieved, leaving doubt on whether iPS cells follow the same path as the embryo during development. One example is the somite, a structure that creates repeated structures like the ribs and spine.

"The somite gives rise to sclerotome, myotome, dermatome and syndetome. No one has made dermatome or syndetome from pluripotent stem cells," says Taiki Nakajima, who first-authored the study.

Sclerotome is a precursor of bone, myotome is a precursor of skeletal muscle, dermatome is a precursor of skin, syndetome is a precursor of tendons and ligaments. While researchers have successfully acquired sclerotome and myotome from iPS cell-somite, no one has successfully acquired dermatome or syndetome, bringing question into just how closely iPS cell-somite resembles somite in the embryo. Using the development of mouse and chick embryos as a roadmap, Nakajima identified a series of molecular signals that could achieve all four intermediates from human iPS cell-somite.

"This is strong evidence that iPS cells can be used to model embryo development," said Nakajima.

To demonstrate the application of their iPS cell-somite to disease, the researchers turned their attention to fibrodysplasia ossficans progressiva, an extremely rare disease in which soft tissue like tendon turns to bone.

They prepared iPSC-somite from FOP patients and then made sclerotome, but did not find any characteristics consistent of the disease. However, adult mesenchymal stem cells made from patient iPS cell-somite did.

"The disease appears in late childhood or early adulthood. Because sclerotome is embryonic and because patients show no or little abnormality in their bone and cartilage at birth, it is natural that sclerotome from our somites did not show the disease phenotype. Capturing the disease phenotype with mesenchymal stem cells shows that our iPS cell-somite can be used to model disease," said Associate Professor Makoto Ikeya.

Reference

Nakajima T, Shibata M, Nishio M et al. (2018) Modeling human somite development and fibrodysplasia ossificans progressiva with induced pluripotent stem cells. *Development* 145(16) DOI: 10.1242/dev.165431

Making spinal cord from iPS cells

The Takahashi Lab reports a new protocol that produces 3D spinal cord organoids from human iPS cells.

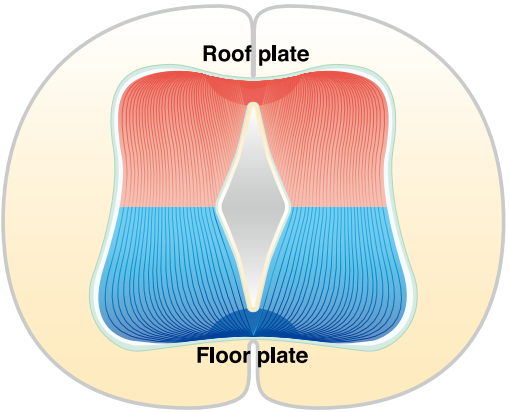
Organoids are three-dimensional organs made in the lab using pluripotent stem cells. Rather than using stem cells to make individual cell types and then assembling them together to make the organ, organoids form all the cells simultaneously in a way that mimics the natural development of the organ both in terms of time and space. Recreating this development is believed to better recapitulate diseases of the organ, because it considers how different cell types interact during development. Applying organoid technology to the brain, CiRA researchers report a spinal cord organoid made from human iPS cells.

The spinal cord consists of cells with two distinctive functions. Somatosensory cells are those that inform the brain of sensations like touch, while primary motor neurons and motor-related interneurons are how the brain informs the body to move in response to those sensations.

"Somatosensory neurons are mostly derived from dorsal side of the spinal cord. On the other hand, primary motor neurons and motor-related interneurons are from the ventral side," says neurologist and lead author of the study, Dr. Hideya Sakaguchi. This spatial organization has a crucial role in spinal cord function, but has not been recreated in the laboratory.

A major reason, explains Sakaguchi, is that scientists have been unable to generate the spinal cord's organizing centers, from which the cells organically assemble in their proper order.

"The roof plate is responsible for organizing the dorsal spinal cord and the floor plate is responsible for organizing the ventral spinal cord," he says.



Differentiated iPS cells form the roof plate and floor plate on their way to becoming spinal cord neurons.

By modifying existing protocols for spinal cord motor neuron differentiation, Sakaguchi successfully formed plates in the organoids that spatially organized as they do when the spinal cord forms in the embryo.

Takahashi believes the ability to recreate not only spinal cord cells, but the spatial organization of these cells from human iPS cells will provide a new way to study diseases of this fundamental structure.

"Many diseases are the result not of defective cells, but defective cell interactions. Organoids are well suited for studying these interactions and finding drugs that can cure them," he says.

Reference

Ogura T, Sakaguchi H, Miyamoto S et al. (2018) Three-dimensional induction of dorsal, intermediate, and ventral spinal cord tissues form human pluripotent stem cells. *Development* 145. DOI: 10.1242/dev.162214

Reprogrammed patient cells explain how the environment triggers muscular dystrophy

A new study by the Sakurai Lab suggests oxidative stress could promote facioscapulohumeral muscular dystrophy.

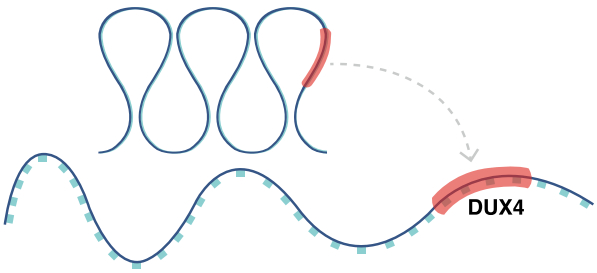
Muscular dystrophy describes a set of diseases of muscle weakness and eventually muscle death. In most cases, all muscles are affected, but facioscapulohumeral muscular dystrophy (FSHD) is unusual in that muscles primarily in the face (facio-), shoulder blades (scapulo-) and upper arms (humeral) are victim. FSHD comes in two forms, FSHD1 and FSHD2, but both involve mutations associated with chromosome 4. These mutations open the chromosome to express the gene *DUX4* at abnormally high levels. A new study by CiRA researchers suggests that patients with these mutations are at higher risk when their cells are exposed to oxidative stress.

"If we can close the chromatin structure, we could stop the expression," hypothesizes CiRA Ph.D. student Mitsuru Sasaki-Honda, who was the first author on the study.

However, CiRA Associate Professor Hidetoshi Sakurai says that because patients show a variety of clinical symptoms, something besides the mutation also contributes to the disease.

"The varying clinical characteristics of the disease strongly support the existence of exogenous factors that modulate *DUX4* expression."

To find these exogenous factors, Sasaki-Honda prepared muscle cells from the iPS cells of FSHD1 and FSHD2 patients. Although these muscle cells naturally expressed higher levels of *DUX4* than did muscle cells from iPS cells of healthy donors, the differences were significantly amplified when exposing the cells to hydrogen peroxide, a molecule that simulates oxidative stress on the cell.



In FSHD, a change in the chromatin structure results in abnormal expression of the *DUX4* gene.

"Oxidative stress leads to an imbalance in free radicals in the cell. It is associated with many diseases," says Sasaki-Honda.

Correcting the mutation using CRISPR-Cas9 gene editing technology attenuated the hydrogen peroxide effect by modifying the chromatin structure in a way that prevented the *DUX4* expression.

"The chromatin is in a relaxed state in patients. In healthy people, it is in a heterochromatic state," says Sakurai.

Further study showed that oxidative stress increased *DUX4* expression by activating the molecule ataxia-telangiectasia mutated kinase.

However, ataxia-telangiectasia mutated kinase is involved in DNA repair, which suggests treatment modifying its behaviour is unwise. Until a cure is found, ways to slow down the disease by reducing oxidative stress, such as lifestyle changes like diet and exercise, should be considered.

Reference

Sasaki-Honda M, Jonouchi T, Arai M et al. (2018) A patient-derived iPSC model revealed oxidative stress increases facioscapulohumeral muscular dystrophy-causative *DUX4*. *Human Molecular Genetics*. DOI: 10.1093/hmg/ddy293

As the thymus shrinks, strong immune cells expand

The Hamazaki Lab reports CXCR3$^+$ naïve phenotype T cells could explain how immune function is maintained with age, but also why the risk of inflammation increases.

The thymus is a vital yet unusual organ. Vital in that it is responsible for producing immune cells; unusual in that it is largest at childhood and progressively shrinks after puberty. The result is less T cell production, which should lead to a higher risk of infection or cancer. However, this is not the case.

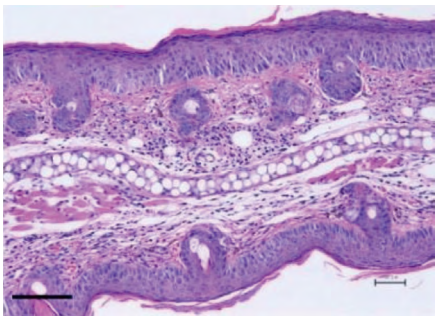
"The thymic output of naïve T cells progressively decreases with age because of thymic involution. However, our immune system tries to compensate the decreased number of naïve T cells by homeostatic proliferation," explains CiRA Professor Yoko Hamazaki.

To study the effect of homeostatic proliferation on naïve T cells, she and her research team induced an accelerated form of homeostatic proliferation in adult mice by removing the thymus. To their surprise, they found that despite the drop in naïve T cell number, the proportion of one specific subtype of T cells increased.

"Mice with accelerated homeostatic proliferation showed a decrease in naïve T cells, but naïve phenotype T cells that expressed CXCR3 stayed constant, meaning that CXCR3$^+$ fraction was relatively increased," says scientist Aiko Kato, who was the first author of the study.

CXCR3 signals recruit T cells to the infected site. The study found that maintaining the number of CXCR3$^+$ naïve phenotype T cells could keep the immune system operating efficiently despite the loss of overall naïve T cells.

However, the relative increase of CXCR3$^+$ naïve phenotype T cells also had a detrimental effect; when the scientists transferred these cells into mice with a preexisting skin condition, the mice showed excessive inflammation compared with the transfer of other T cells. Consistently, these cells tended to express two known proinflammatory molecules, IFNγ and TNFα.



Inflamed mouse tissue following the transfer of CXCR3$^+$ naïve phenotype T cells.

Importantly, the researchers also found that CXCR3$^+$ naïve phenotype T cells exist in humans, but the proportion of these cells among all T cells was different between individuals.

Hamazaki speculates that this difference could explain why some people are predisposed to higher levels of inflammation.

"As people age, they show more chronic inflammation, which can lead to metabolic diseases and autoimmune diseases. Increase of CXCR3 naïve phenotype T cells may be one cause," she says.

Reference

Kato A, Takaori-Kondo A, Minato N et al. (2018) CXCR3^{high} CD8$^+$ T cells with naïve phenotype and high capacity for IFN-γ production are generated during homeostatic T-cell proliferation. *European Journal of Immunology*. DOI: 10.1002/eji.201747431

Suppressing HIV in infected cells

By inserting small hairpin RNA into iPS cells, the Kaneko Lab saves HIV-infected cells.

For the past two decades, better education has consistently dropped annual HIV infection rates, while better treatment has consistently extended the live span of infected patients. However, current treatment does not clear the virus, and patients must undergo regular antiviral therapy to keep the virus dormant. Using iPS cell technology, scientists at CiRA report a new treatment strategy based on transcription gene silencing that can halt HIV replication in infected cells without regular treatment.

As a retrovirus, HIV is made of RNA, but it tricks an infected cell to convert the RNA into DNA, which then incorporates into the cell's own DNA. Thus, as the cell expresses its DNA, it so too expresses HIV DNA. CiRA Associate Professor Shin Kaneko, who led the study, has been investigating molecules that can be used to stop this expression.

"Transcription gene silencing uses short hairpin RNA (shRNA) to bind to a DNA promoter to prevent gene expression," he explains.

shRNA are found in all cells. Their function is to change the structure of DNA or RNA, thus preventing expression of the gene. By transfecting shRNA that bind to HIV DNA, Kaneko and his research team could prevent HIV expression in infected cells without affecting the expression of other genes.

"We transfected iPS cells with shRNA for the HIV promoter and differentiated them to macrophages. HIV DNA was present in the macrophages, but HIV did not replicate," he says.

Macrophages are one of two types of immune cells that HIV infects. By transfecting iPS cells and not macrophages directly, the scientists could take advantage of the high proliferation rate of iPS cells to make a large number of transfected macrophages for observation.

Unlike those macrophages, macrophages made from infected iPS cells that did not receive the shRNA transfection acted like HIV factories, allowing the virus to replicate and thrive.

Further study revealed how the HIV DNA was modified in the transfected cells.

"There was an enrichment of H3K27me3 and failure of enrichment of H3K9Ac," says Kaneko, describing changes in the methylation and acetylation of the DNA, two common modifications that a cell naturally uses to regulate whether a gene is expressed.

Importantly, the shRNA did not bind to other DNA, assuring that only the HIV DNA was not expressed and suggesting macrophage function was unaffected.

"Many gene therapies aim to prevent HIV infection. Our method suggests a therapy for immune cells already infected by HIV," says Kaneko.

Reference

Higaki K, Hirao M, Kawana-Tachikawa A et al. (2018) Generation of HIV-resistant macrophages from iPSCs by using transcriptional gene silencing and promoter-targeted RNA. *Molecular Therapy Nucleic Acids* 12
DOI: 10.1016/j.omtn.2018.07.017

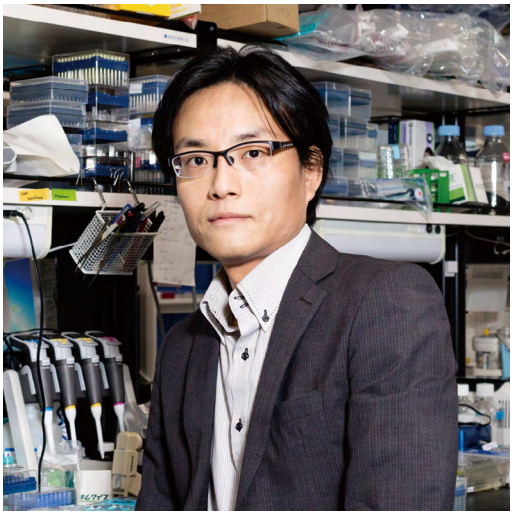
Greetings from the
Akitsu Hotta Lab

Dept. of Clinical Application

Globally, it is estimated that over 10,000 human diseases are caused by mutations in a single gene. Patients suffering from these diseases often have very limited treatment options. The few that are available have poor efficacy, are generally expensive, and must be taken for the duration of the patient's life. Our lab is using genome editing technology to create a new generation of treatments that can expand the number of treatable diseases and increase the efficacy of the treatment.

With the discovery that CRISPR, originally a DNA nuclease utilized by bacteria to combat invading viruses, can be reprogrammed to target and cut a desired DNA sequence of choice, it is now possible to correct mutated genomic DNA in patient cells in the laboratory setting. This ability provides a proof-of-concept for the application of CRISPR to gene therapies. However, for these therapies to reach the clinic, we must enhance the delivery system.

Accordingly, our lab seeks to develop novel gene editing and delivery technologies with CRISPR to modify mutations involved in rare monogenetic diseases such as Duchenne muscular dystrophy (DMD). DMD occurs in boys (1 in 3500) and is caused by mutations in the dystrophin gene, which is one of the largest genes in the human genome and is critical for muscle cell stability. Without it, patients suffer from severe muscle degeneration and usually do not live past the age of 30 years old. Previously, we reported that CRISPR can restore dystrophin protein expression in DMD patient iPS cells differentiated into skeletal muscle cells, providing evidence that CRISPR could potentially benefit these patients in a clinical setting.



Akitsu Hotta

Thus, in the hope of applying CRISPR technology to an in vivo system, we are currently developing several protein- or RNA-based CRISPR delivery platforms for transient delivery into cells, which could prove to be safer than conventional DNA-based delivery methods and reduce any unwanted activity. One of our strategies serves as a "molecular ambulance" and utilizes a virus structural protein derived from a part of HIV to encapsulate the CRISPR protein by chemically inducing dimerization for efficient packaging and delivery into various types of cells (iPS cells, muscle cells, T-cells, monocytes, etc.). We call this system "NanoMEDIC (nanoparticle membrane-derived extracellular vesicles for delivery of cargo)" and believe it could be further engineered to load different cargo and target cells at exceptional specificity.

We are testing our novel delivery platforms on several CRISPR orthogonal systems to expand the clinical application of CRISPR technology.

Patient motivation and informed consent

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

Hierarchies play a very important role in Japan. Traditionally, subordinates have been deferential to their superiors, rarely if ever challenging decisions. The impact of this culture can be seen in all interactions throughout Japan, including in the clinic. What might strike many today as unethical, it was only in the late 20th century when Japanese doctors began to regularly inform patients of certain medical conditions like cancer. Before then, it was acceptable for doctors to recommend a treatment strategy without revealing to the patient the disease. Instead, the patient's family would be informed.

This led to a culture of *omakase*, which roughly translates to "it's up to you," where the patient would defer with complete confidence to the doctor. Since 1990, the patient-physician relationship has changed from the *omakase* model to a participatory model, in which patients are more engaged with the decision making. It was hypothesized that the participatory model would lead to a more motivated patient, which would affect informed consent for a treatment plan. Informed consent serves two purposes. First, it verifies that the medical practice follows ethical standards. Second, it builds the patient-physician relationship, which is very important when considering long-term care.

I and colleagues sought to investigate this hypothesis by surveying 20 cancer patients and conducting a crossover analysis of the findings. Motivation was assessed by the Achievement Motivation Index for Medical Treatment (MTI), which we established in 2016.

In Japan, doctors consult their patients using conversation that can be described as *ki-shou-ten-ketsu* (起承転結) style. This style is common in East-Asian languages. Briefly, each term refers to a component in the conversation. *ki* normally provides the diagnosis. *shou* corresponds to more detail about the condition and treatment options. *ten* gives attention to a subtheme. Commonly, this is when the patient and physician discuss non-clinical matters that can influence the decision, such as impact on work or family. *ten* can have tremendous influence on consent. Finally, *ketsu* refers to the conclusion from the conversation.

Surprisingly, we found that *ketsu* (or consent) was not affected by patient motivation. Motivation did have an effect in the first two stages, *ki-shou*. Based on these findings, it would appear that *ten*, which is when the patient has an opportunity to introduce personal issues into the conversation, has a significant impact on informed consent.

Anecdotally, physicians found "difficult patients" much more cooperative following *ten*. It seems that *ten* allowed the patient to express his or her primary concerns, which are not always health, but lifestyle. Once this concern was articulated, the patient developed a more cooperative stance.

Another point of the study I want to highlight is the application of crossover analysis. Making objective conclusions from a mixture of qualitative and quantitative data is not trivial. Our team has been working on this task for many years, and we hope that the study provides a new analysis tool for similar patient analyses.

Reference

Hatta T, Narita K, Yanagihara K et al. (2018) Crossover mixed analysis in a convergent mixed methods design used to investigate clinical dialogues about cancer treatment in the Japanese context. *JMMR* DOI: 10.1177/1558689818792793

Inspiring the next generation

CiRA was in Tokyo this past August. In the first week, it visited the National Museum of Nature and Science to give a workshop to elementary school students. About 60 people attended. Then two weeks later it returned to take part in MIRAI SUMMER CAMP, a week-long event organized in cooperation with MIT Media Lab.

CiRA has been visiting Tokyo every summer to participate in children's science events. This year, however, CiRA also took the workshop to Iwate Prefecture, a region badly hit by the tsumani and earthquake of 2011. Communications Officer Ayaka Sasaki is from the area. She joined CiRA in 2017, and when learning that CiRA had yet to host an event in the area was eager to organize something.

"I made a proposal to the Morioka Children's Museum of Science. Our games were intended for elementary school students," she said.

Finally, on July 24, 40 middle school students from Kyoto and nearby Otsu attended a lecture from Prof. Noriyuki Tsumaki. Following a general description of iPS cells, Tsumaki explained how he is using cell reprogramming to regenerate damaged cartilage. Students were given the opportunity to view cartilage cells under a microscope. Many were impressed, with one leaving to say, "I hope to do iPS cell research in the future."

Children learning about iPS cells in Tokyo

Ayaka Sasaki at Morioka City

Students learning about cartilage at CiRA

Marathon Running

The Osaka Marathon will be run on Nov. 25, and CiRA will have four participants, Professors Junya Toguchida, Jun Takahashi and Hirohide Saito along with Yuta Mishima, a scientist in the Shin Kaneko Laboratory. They along with 120 other charity runners will donate all their proceeds to CiRA. CiRA Director Shinya Yamanaka will also take part, but as an ambassador of the marathon, all donations to him will be given to various non-profit groups who are assisting with regions affected by the numerous natural disasters that occurred in Japan this year, including floods and earthquakes.

The Temples and Shrines of Kyoto

Honen-In

Philosopher's Path takes its name from the daily promenades of Japanese Philosopher Kitaro Nishida, who was born in the late 19th Century. The path provides a respite from the hurried pace of the city, but its reputation makes it a popular destination among tourists, and it is now as crowded as anywhere else in Kyoto. There are some escapes, however, as the path is dotted by much older temples, including Honen-In.

The temple is dedicated to Priest Honen, who was known for his precocity. Although he lived in the 12th Century, the temple's current design was only constructed in 1680, following the rise of the Tokugawa Shoguns. For a city full of temples, the architecture of Honen-In would be unremarkable if it did not blend in so well with the flora surrounding it. Sitting at the edge of the eastern mountain enclosing Kyoto, the trees filter out the sight and sounds of the crowds passing along Philosopher's Path. And while its colours do little to distinguish the temple, that is not the case of the sand gardens, which have designs leaving as much wonder about how they were made as to what they mean.

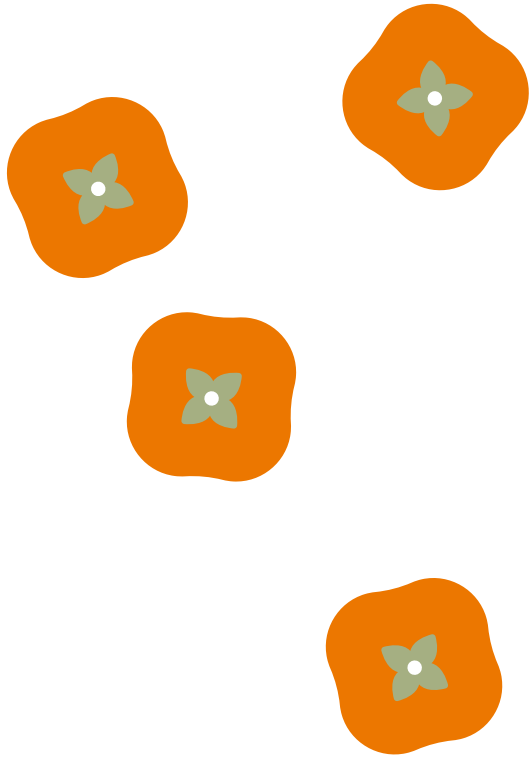
Photos by Robert Milweski

Kyoto University wins a Nobel Prize

Kyoto University Professor Tasaku Honjo was awarded the Nobel Prize in Physiology or Medicine, sharing it with James Allison, for findings key mechanisms that allow cancer cells to evade the immune system. Honjo's discovery, first reported in 1992, was of PD-1, which acts to suppress T-cell activity and has become the primary target in a number of cancer therapies. He became the 10th scientist affiliated with Kyoto University to win a Nobel Prize and sixth Japanese scientist after Shinya Yamanaka won one for his discovery of iPS cells in 2012.

The win created a fervor at the university and left Yamanaka answering media questions until midnight on the day of the announcement.

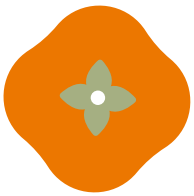
"His discovery has been a source of hope for cancer patients around the world and is a prime example of how basic research can lead to breakthrough medical applications," he said.



CiRA therapies

Are beginning, more to come

Anticipation



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Vol.16 | October 2018