KEIO UNIVERSITY GRADUATE SCHOOL OF MEDICINE

GLOBAL COE PROGRAM

EDUCATION AND RESEARCH CENTER FOR STEM CELL MEDICINE

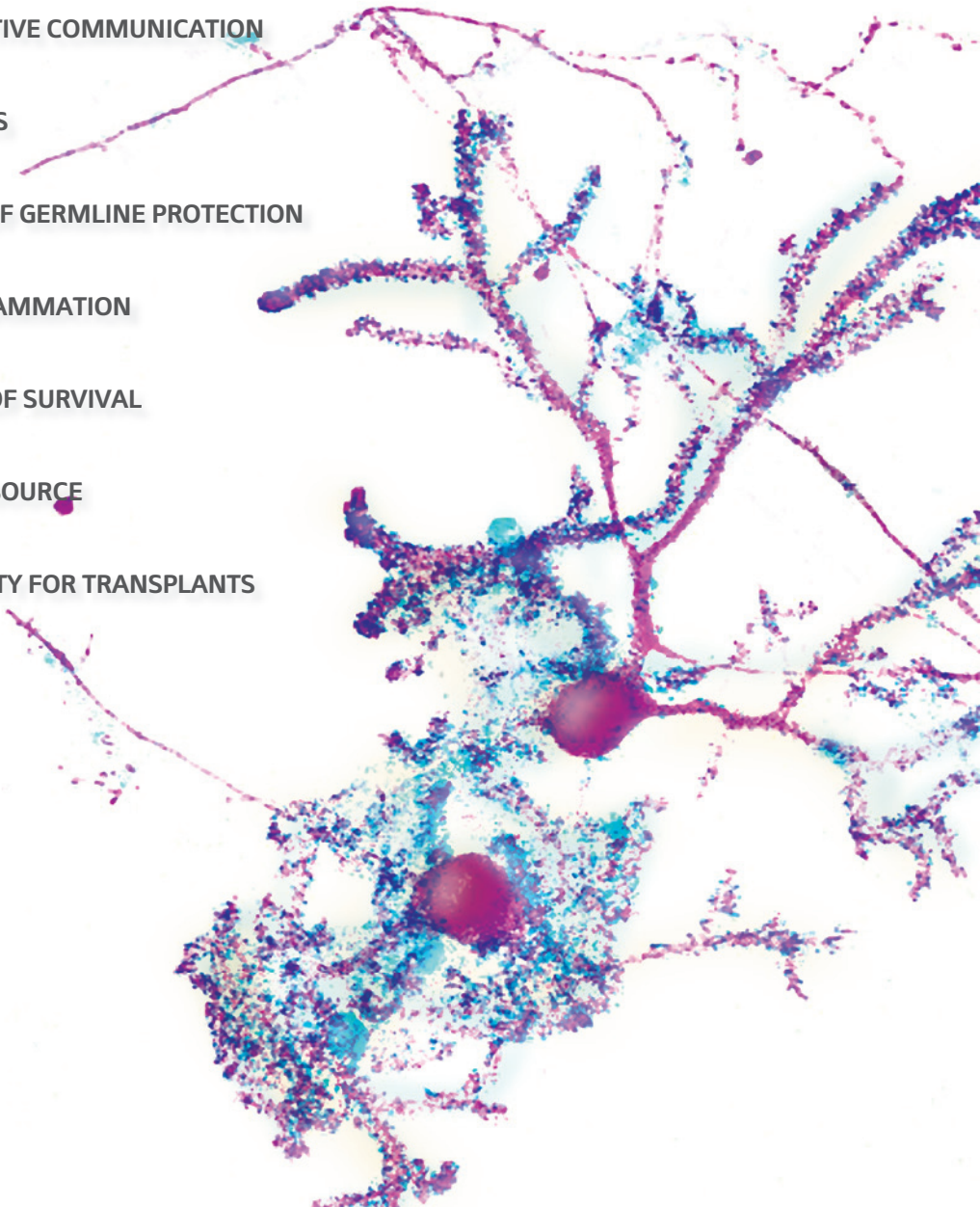
Keio University

1858

CALAMVS GLADIO FORTIOR

# RESEARCH HIGHLIGHT COLLECTION 2011





KEIO UNIVERSITY GRADUATE SCHOOL OF MEDICINE
GLOBAL COE PROGRAM, EDUCATION AND RESEARCH CENTER FOR STEM CELL MEDICINE

# Advancing stem cell research

**Keio University's Education and Research Center for Stem-Cell Medicine and its Global Center of Excellence (GCOE) program provide a cutting-edge research environment that recognizes the growing importance of stem cell research in furthering our understanding of human health, aging and cancer. The Center's overarching goal is to create a world-class training program for the next generation of stem cell research leaders.**

Stem cell research has become a vital part of modern medicine and health science. The ubiquitous nature of stem cells, coupled with their ability to differentiate into any cell type found in the body, offers an invaluable way to explore fundamental questions in biology, such as uncovering the mechanisms of cellular growth and regulation. Recent advances in stem cell research are providing unprecedented opportunities for developing new treatments and therapies based on tissue engineering and regenerative medicine, heralding a new era of biomedical research and drug discovery.

Since its establishment in 2008, Keio University's Education and Research Center for Stem-Cell Medicine has grown from strength to strength, and up to 100 graduate students participate in the GCOE program every year. Program Leader Hideyuki Okano says, "We strongly believe that the creation of an education and research program focused on stem-cell medicine will dramatically contribute to the overall improvement of education and research activities in the doctoral program of the Graduate School of Medicine, because stem cells essentially exist in all organs, spanning the lifetime of an organism — from the early embryonic stage to its death — and because they are closely linked to the pathogeneses and treatments of many diseases including degenerative disorders and cancer."

The GCOE program includes training in immunology, molecular physiology and genetics to investigate the mechanisms that regulate neural stem cell generation, which may contribute to the prevention and treatment of neurological disorders. As stem cell research encompasses all aspects of an organism's life cycle, the potential outcomes and applications of the research are profound and wide-ranging.

In order to integrate research in basic and clinical medicine based on a comprehensive understanding of stem cells, the Center focuses primarily on five key research areas: tissue stem-cell regulation and in vivo experimental medicine; inflammation/immunological regulation and tissue regeneration; the development of new cancer treatments targeting cancer stem cells and epithelial-mesenchymal transition; the development of regenerative medicine for intractable diseases, and the practical implementation of feasible regenerative medicine. "By taking a lead in these five research areas, we aim to create an outstanding and internationally competitive education and research center that trains pioneering leaders in these fields," says Okano.

The Keio Research Highlight Collection 2010–2011 introduces outstanding papers from each of these five research areas, and for the first time, the selected articles represent some of the very best research conducted by young investigators at the Keio University Graduate School in 2010 and 2011.

Young researchers taking part in the GCOE program benefit from a research structure that actively encourages collaboration between group leaders, members of the GCOE program and overseas collaborators, and encourages mentorship between more experienced students and new students. The program is committed to developing a high-calibre, diverse student body with a strong international outlook.

The GCOE has conducted more than 25 collaborative research projects with leading international institutes including Harvard University, the Johns Hopkins University and the M. D. Anderson Cancer Center at the University of Texas in the United States, the Queensland Brain Institute in Australia, the Institut Jacques Monod in France, and the Karolinska Institute in Sweden.

Fostering young scientists and promoting excellence in basic research remain top priorities for Keio University's Education and Research Center for Stem-Cell Medicine as it continues to build on its successes and create an internationally competitive research environment that encourages pioneering scientific discoveries and technological innovation. ■

**http://www.gcoe-stemcell.keio.ac.jp/**

# Spinal cord injury treatment stands up to the test

## Stem cells reprogrammed from adult human cells can encourage tumor-free tissue regeneration in mice with spinal cord injuries

Medical treatments based on stem cells could relieve or even cure a wide variety of serious illnesses and injuries, but they remain controversial in many countries because the cells are often cloned from human embryos. By inducing the expression of certain genes to produce so–called human-induced pluripotent stem cells (hiPSCs), it is now possible to avoid ethical concerns because these cells are cloned from adult human cells rather than embryos.

Pluripotent stem cells, including hiPSCs, have the ability to change into any one of the three germ layers in the body—those related to the stomach and lungs, the blood and muscles, and the nervous system. Unfortunately, research has revealed the potential for treatments based on hiPSCs to cause tumor formation in humans. Medical researchers are therefore striving to find safe ways of using hiPSCs.

Satoshi Nori and colleagues at Keio University's Graduate School of Medicine, working in collaboration with researchers in Japan have now shown that mice with spinal cord injuries can recover motor function after being treated with a particular clone of hiPSCs[1]. Significantly, the clone—called 201B7—was the only one of several types trialed that did not produce tumors over the four-month test period.

The comprehensive experiment carried out by Nori and colleagues involved meticulous tests on over 60 test mice with spinal cord injuries. "It involved a great deal of hard work," says Nori. "However, my hopes of curing patients with spinal cord injury helped me to overcome many obstacles."

The researchers derived neurospheres—spherical groups of cells that occur naturally—from different clones of hiSPCs and then transplanted them into immunodeficient mice. The mice were immunodeficient to prevent their immune systems from attacking the human-derived neurospheres when they were grafted. The researchers observed the mice for four months to assess their motor functions and record any tumor growth.

© iStockphoto.com/comotion_design

Of the clone types trialed, 201B7 was the most successful in healing the injured spinal cord: it promoted blood vessel and nerve regrowth, aided the recovery of the nervous system and even communicated with host mouse cells. But the fact that this clone did not produce tumors in the mice is a crucial breakthrough in this area of hiPSC research.

By testing the treatments on spinal cord injuries in primates, Nori and his colleagues are now determining whether or not hiPSCs can be used in species other than mice.

1. Nori, S., Okada, Y., Yasuda, A., Tsuji, O., Takahashi, Y., Kobayashi, Y., Fujiyoshi, K., Koike, M., Uchiyama, Y., Ikeda, E., Toyama, Y., Yamanaka, S., Nakamura, M. & Okano, H. Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice. *Proceedings of the National Academy of Sciences USA* **108**, 16825–16830 (2011).

# Helping stem cells get a fresh start

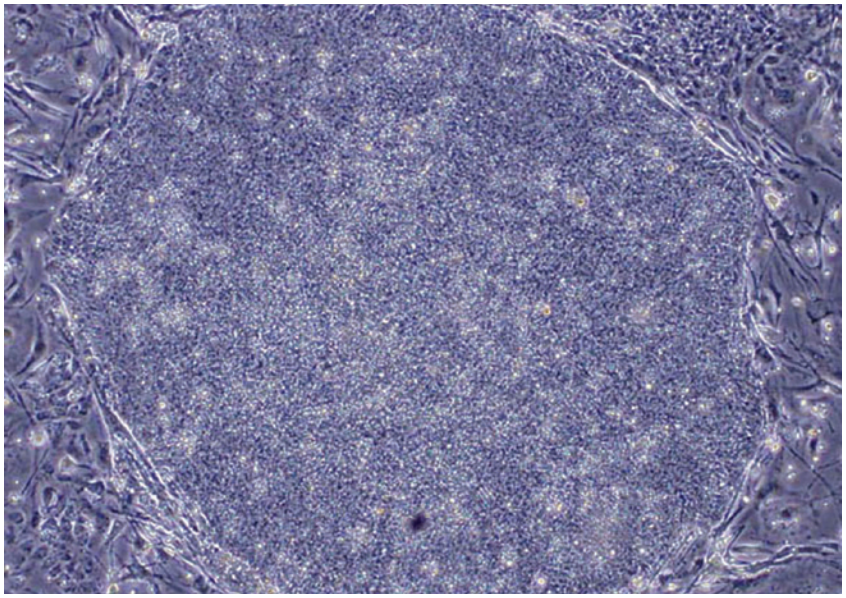
## By cultivating 'reprogrammed' adult cells, scientists may obtain superior surrogates for embryonic stem cells

Studies using induced pluripotent stem cells (iPSCs) could offer a promising alternative for scientists hampered by the legal and scientific challenges associated with embryonic stem cell (ESC) research. Induced pluripotent stem cells are derived by expressing selected genes within fully-differentiated adult cells, essentially 'reprogramming' them into a pluripotent state that closely resembles ESCs.

It remains unclear, however, whether the resemblance is strong enough for iPSCs to replace ESCs. For example, chromosomes are chemically marked in ways—such as DNA methylation—that strongly affect gene expression via so-called 'epigenetic' effects. These marks can vary dramatically between ESCs and adult cells, but some studies have suggested that iPSC epigenetic marks may not resemble those from either cell type.

To resolve this and other questions, a team led by Akihiro Umezawa at Keio University, Japan, recently examined DNA methylation patterns in a diverse array of iPSC lines[1]. "Epigenetic modifications such as DNA methylation are considered to be critical to the reprogramming of iPS cells from somatic cells," explains co-author Koichiro Nishino from the University of Miyazaki. "We wanted to know whether human iPSCs generated from various types of cells are dissimilar from each other, and how continuous cultivation influences the differences between iPSCs and human ESCs."

The researchers examined 22 human iPSC lines derived from five different cell types, and compared their DNA methylation patterns relative to the 'parent' tissues from which they were generated and five independent ESC lines. Strikingly, the DNA from iPSCs exhibited a significantly greater extent of methylation relative to other cells. The researchers determined that this hypermethylation occurred in a highly dynamic and seemingly unregulated manner in the early aftermath of reprogramming, after the resulting iPSCs had undergone several rounds of cell division via subculturing, or 'passaging'.



An iPSC derived via the genetic reprogramming of a fully differentiated adult cell, which recapitulates many of the key features of an ESC



"The aberrant hypermethylation in iPSCs occurs randomly throughout the genome," says Nishino. "Even so-called 'inherited' methylations, which are considered to have come from the parental cells, are non-synchronous and stochastic, much like the other aberrant methylations." Importantly, the methylation process appears to stabilize in subsequent passages, and iPSCs eventually acquire methylation patterns that closely resemble those observed in ESCs (see image).

Given the breadth of the analysis performed by Nishino, Umezawa and colleagues, these results appear to reflect a general characteristic of iPSCs and could facilitate future efforts to obtain ESC analogues for regenerative medicine applications. "Induced pluripotent stem cells have to be standardized for clinical use," says Nishino. "The number of aberrant, differentially methylated regions could be used as a validation index for iPSC identity." ■

1. Nishino, K., Toyoda, M., Yamazaki-Inoue, M., Fukawatase, Y., Chikazawa, E., Sakaguchi, H., Akutsu, H. & Umezawa, A. DNA methylation dynamics in human induced pluripotent stem cells over time. *PLoS Genetics* **7,** e1002085 (2011).

# Preparing the brain for effective communication

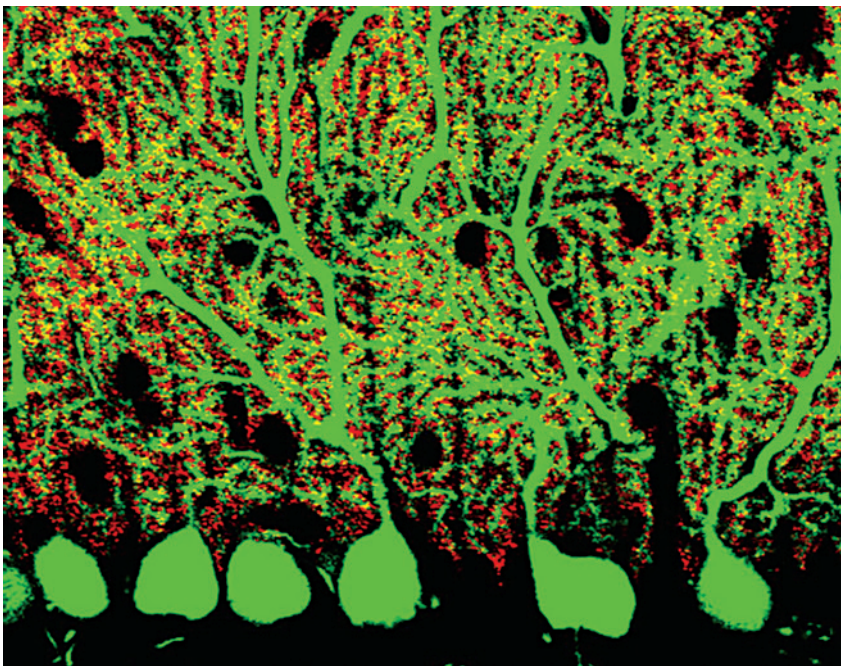
## The formation of special junctions in the cerebellum is regulated by a master protein

Synapses, the specialized junctions that form between neurons, facilitate rapid transfer of messages in the brain. In a finding that will further neuroscientists' understanding of how synapses are formed, a research team led by Michisuke Yuzaki from the Keio University Graduate School of Medicine, Japan, has found that the protein Cbln1, which is secreted from granule cells, can induce the generation of synaptic specializations on both sides of the synaptic junction[1].

In the cerebellum, granule neurons are known to form synapses with other neurons called Purkinje cells. These synapses play an essential role in motor coordination. Previous work by Yuzaki and his team demonstrated that, compared to normal mice, Cbln1-deficient mice have a lower number of synapses between granule neurons and Purkinje cells. However, little has been known about how Cbln1 acts to modulate cerebellar synapse numbers.

An important clue came from a separate line of research which revealed that mice lacking the glutamate receptor δ2 (GluD2) protein also had a lower number cerebellar synapses between granule neurons and Purkinje cells. Yuzaki and his colleagues therefore tested whether Cbln1 could serve as a binding partner for GluD2.

In cell culture experiments, the researchers found that Cbln1 could bind to cells expressing GluD2 or normal Purkinje cells taken from mice. They found naturally occurring Cbln1 on normal Purkinje cells, but not ones lacking GluD2. Typically, granule neurons can form synaptic junctions with non-neuronal cell lines that express GluD2. However, granule cells lacking Cbln1 could not form synapses with these cell lines. As the granule cell is the 'sender' of neuronal messages, it forms the presynaptic side of the synapse so that it is specialized for sending information. Since the Purkinje cell is the 'receiver', it forms the postsynaptic side so that it is specialized for receiving messages.





The amount of Cbln1 (red) found naturally with Purkinje cells (green) in a brain slice taken from the cerebellum of a normal mouse was almost absent from the Purkinje cells of mice lacking GluD2.

To monitor synaptic generation, the researchers took beads that could exhibit proteins on their surface, coated them with Cbln1 and cultured them with granule neurons. The beads allowed them to observe the formation of presynaptic specializations. Similarly, the researchers observed the formation of postsynaptic specializations when they cultured Cbln1-coated beads with Purkinje cells. "In a subsequent study, we found that Cbln1 binds to a protein called neurexin on the presynaptic side of the synapse to induce presynaptic specialization," explains co-author Keiko Matsuda.

The ability of Cbln1 to bind to GluD2 and neurexin on respective sides of the synapse suggests that Cbln1 may be a bidirectional organizer of synapse formation in the cerebellum. ■

1. Matsuda, K., Miura, E., Miyazaki, T., Kakegawa, W., Emi, K., Narumi, S., Fukazawa, Y., Ito-Ishida, A., Kondo, T., Shigemoto, R., Watanabe, M. & Yuzaki, M. Cbln1 is a ligand for an orphan glutamate receptor δ2, a bidirectional synapse organizer. *Science* **328**, 363–368 (2010).

# Stabilizing stressed stem cells

## A new mechanism that maintains stem cell quiescence could lead to improvements in bone marrow transplants

A protein that responds to changes in cellular oxygen levels is essential for maintaining the stem cells in mammalian bone marrow that produce all types of blood cells, according to research led by Keiyo Takubo of the Keio University Graduate School of Medicine, Japan[1]. Biologists considered that these so-called hematopoietic stem cells (HSCs) were restricted to hypoxic, or oxygen-deprived, niches of bone marrow, but the mechanism that maintained them in this environment was unclear.

After Takubo and his colleagues from Japan and the USA confirmed in normal mice that HSCs are hypoxic, they found that HSCs are associated with elevated levels of the transcription factor hypoxia-inducible factor-1α (HIF1α). They created a strain of mice lacking the *HIF1α* gene in blood-forming tissues and organs and found that these animals had a normal blood-cell count and that the differentiation status of their HSCs was identical to that of wild-type mice.

However, when the researchers transplanted bone marrow from their new mouse strain into normal mice, maintenance of the HSC population was defective in the recipients. Four months after transplantation, Takubo and colleagues found that HSC numbers had decreased, but levels had increased for the cell cycle inhibitor *Ink4a*, which is associated with cellular aging and loss of stem cell characteristics. They successfully reversed the depletion of HSCs by blocking *Ink4a* expression.

The researchers then transplanted into normal mice HSCs lacking the gene encoding the von Hippel-Lindau (VHL) protein, which labels HIF1α for destruction. Maintenance of the HSC population was again defective in the recipient animals, owing to abnormally high levels of HIF1α. Further experiments revealed that the lack of VHL likely suppressed cell division, increased cell death and nullified the HSCs' ability to navigate to the hypoxic niches of bone marrow. In contrast, cells containing one copy of the *VHL* gene remained in the non-dividing phase of the cell cycle, leading to an improved outcome for recipient animals after transplantation.

The team's results indicate that precise regulation HIF1α is essential for determining the cell division status of HSCs and maintaining their stem cell capacities—under normal hypoxic conditions as well as stressful conditions such as transplantation and cellular aging.

"We believe that appropriate modification of the hypoxia signal in humans will be beneficial for the improvement of bone marrow transplants in humans," says Takubo. "We are now defining the exact molecular mechanism downstream of HIF1α, and have started to investigate the contribution of other bone marrow cell types."

© iStockphoto.com/Cabodin

Bone marrow transplants may improve with the identification of a protein involved in a mechanism that maintains hematopoietic stem cells

1. Takubo, K., Goda, N., Yamada, W., Iriuchishima, H., Ikeda, E., Kubota, Y., Shima, H., Johnson, R. S., Hirao, A., Suematsu, M. & Suda, T. Regulation of the HIF-1α level is essential for hematopoietic stem cells. *Cell Stem Cell* **7**, 391–402 (2010).

# Unraveling the complexities of germline protection

## An RNA–protein complex which helps to protect genome integrity follows a complicated path on its way into the nucleus

The genome is littered with sequences known as retrotransposons, which generate duplicates of themselves that can later insert into other genomic sites. This process can disrupt the function of essential genes, a risk that is particularly serious for the 'germ cells' that serve as the starting point for sperm and ovum production.

In the fruit fly *Drosophila*, the Piwi protein partners with small RNA molecules known as 'piRNAs' to keep retrotransposons from wreaking germline havoc. "Mutations in the *Piwi* gene result in reactivation of retrotransposons and loss of germline stem cells," explains Kuniaki Saito of the Keio University Graduate School of Medicine, Japan. "This indicates that the Piwi–piRNA complex is involved in gene silencing of retrotransposons."

In a recent study, Saito and his Keio University colleagues Hirotsugu Ishizu, Haruhiko Siomi and Mikiko C. Siomi gained important new insights into the process of piRNA production, which could help illuminate the regulation of retrotransposon silencing[1].

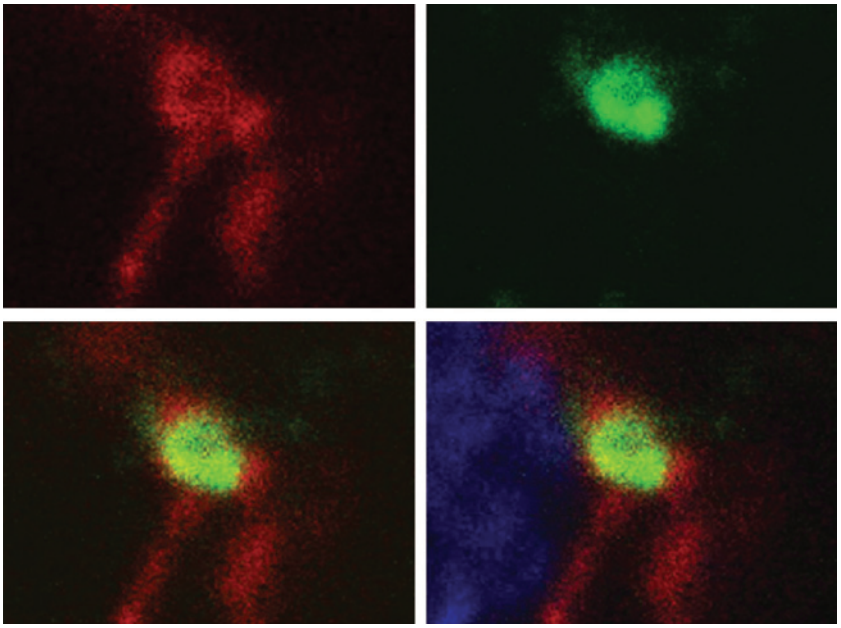
Since Piwi is predominantly expressed in germ cells and the adjacent somatic cells that support them, the researchers used a fly ovarian somatic cell (OSC) line to identify novel components of the piRNA processing pathway. By performing a series of genetic manipulations in these OSCs, they determined that the protein Armitage plays a central role in piRNA production. Subsequent experiments revealed that Armitage appears to act as a tether that links Piwi to cytoplasmic structures known as 'Yb bodies', where Piwi associates with piRNAs.

Saito and colleagues found that full maturation depends on an enzyme called 'Zucchini'. Surprisingly, Zucchini is primarily localized in the mitochondria, an organelle with a central role in producing the ATP molecules that power cellular functions. Imaging by the researchers confirmed co-localization of Yb bodies and mitochondria.

"Our data suggest that mitochondria have a hitherto unidentified role for piRNA biogenesis besides ATP synthesis," says Saito.

Previous work from these researchers has indicated that Piwi–piRNA acts in the nucleus, even though they have now demonstrated that Piwi's presence in the cytoplasm is essential to piRNA production. One possibility is that this represents a quality control mechanism, keeping Piwi from entering the nucleus until it has been loaded with piRNA.

The researchers hope to address this possibility along with other questions in the near future. "We are interested in how these newly identified protein factors are involved in piRNA biogenesis, and why piRNA biogenesis requires these specialized cytoplasmic organelles, the mitochondria and Yb-body," says Saito. ■



Fluorescence labeling of the mitochondria (red; top left), which contain the enzyme Zucchini, and the Yb bodies (green; top right), where Armitage and Piwi assemble, reveals that the two co-localize (bottom left). For reference, the nucleus is stained with the blue dye DAPI (bottom right).



1. Saito, K., Ishizu, H., Komai, M., Kotani, H., Kawamura, Y., Nishida, K. M., Siomi, H. & Siomi, M. C. Roles for the Yb body components Armitage and Yb in primary piRNA biogenesis in *Drosophila*. *Genes & Development* **24**, 2493–2498 (2010).

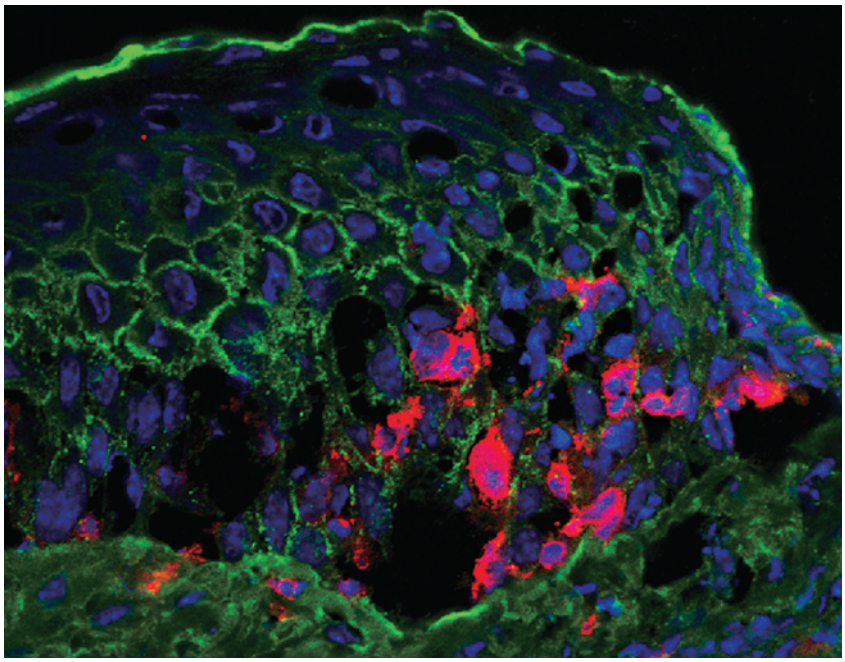
# Getting under the skin of inflammation

## A distinct subset of immune cells targeting a skin-cell protein can cause two types of autoimmune diseases in mice

Interface dermatitis (ID) is an inflammatory disease in which immune cells called T lymphocytes infiltrate and damage the cells in the outermost skin layer. In a study revealing that T lymphocytes trigger ID in mice by targeting the skin-cell protein desmoglein 3 (Dsg3), a research team in Japan led by Masayuki Amagai of the Keio University Graduate School of Medicine has provided a potentially valuable animal model to investigate ID and similar skin diseases[1].

Pemphigus vulgaris (PV), for example, is an autoimmune disease that causes blistering of the skin and mucous membranes, such as the mouth. It is caused by autoantibodies that stop Dsg3 from keeping skin cells strongly attached to each other. Recently, Amagai's team and others showed that T lymphocytes play an important role in Dsg3 autoantibody production in mice and humans with PV. In some patients with blood growth abnormalities called neoplasms, PV can co-occur with ID, in a syndrome called paraneoplastic pemphigus (PNP). Amagai and colleagues therefore investigated whether T lymphocytes that react against Dsg3 would lead to PV, ID, or both, when transferred into mice.

The researchers found that if the Dsg3-specific T lymphocytes developed in the presence of Dsg3, they could induce only ID in mice. However, if the cells developed in the absence of Dsg3, they caused both PV and ID, similar to the development of PNP in patients with neoplasms. This indicated that the presence of Dsg3 during T lymphocyte development somehow blocks the ability of Dsg3-specific T lymphocytes to aid in autoantibody production. Therefore, the environment in which a T lymphocyte is generated could determine whether it can cause PV or ID (see image).





Immunofluorescence staining of tissue from the palate of a mouse with PV and ID shows antibody deposition that is characteristic of PV (green) and T cell infiltrates that are characteristic of ID (red). Cell nuclei are blue.

T lymphocytes can express various inflammatory proteins called cytokines that contribute to different autoimmune diseases throughout the body. By testing T lymphocytes lacking particular cytokines, Amagai and colleagues found that Dsg3-specific T lymphocytes needed to express the cytokine interferon-γ in order to induce ID.

Interestingly, Dsg3-specific T lymphocytes could induce ID without the cytokine interleukin-17A, which is involved in the condition of multiple sclerosis, a debilitating autoimmune disease of the brain. "Our findings indicate that antigen-specific T cells can directly attack the skin cells expressing the antigen," explains Hayato Takahashi, the first author of the study. "At least a subset of ID in humans might also be mediated by this autoimmune mechanism," he adds.

The mice with Dsg3-specific T lymphocytes that exhibit ID may therefore be a valuable model for human ID or for other inflammatory skin diseases. ■

1. Takahashi, H., Kouno, M., Nagao, K., Wada, N., Hata, T., Nishimoto, S., Iwakura, Y., Yoshimura, A., Yamada, T., Kuwana, M., Fujii, H., Koyasu, S. & Amagai, M. Desmoglein 3-specific CD4+ T cells induce pemphigus vulgaris and interface dermatitis in mice. *The Journal of Clinical Investigation* **121**, 3677–3688 (2011).

# Uncovering cancer's secrets of survival

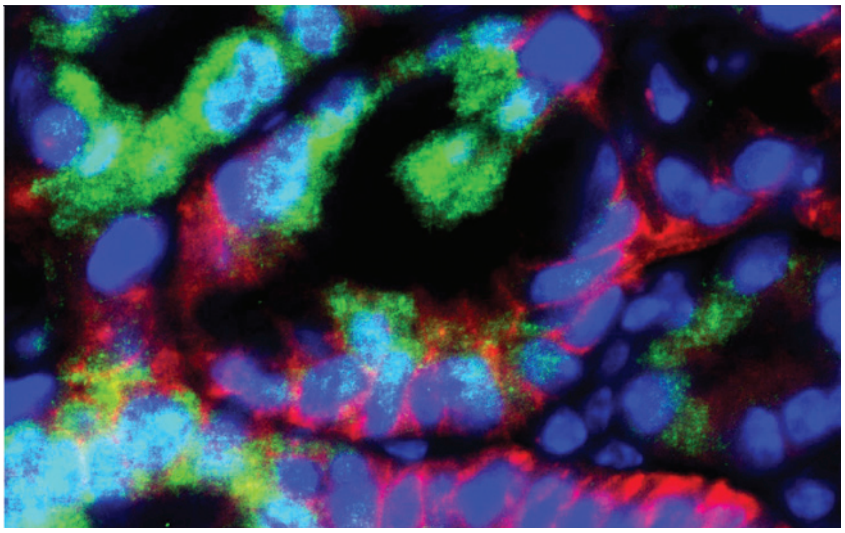
## Investigating the way that tumor stem cells purge harmful chemicals may lead to better chemotherapeutic interventions

Cells produce potentially toxic molecules known as 'reactive oxygen species' (ROS) as a normal byproduct of metabolism, but specialized enzymes eliminate these compounds before they damage proteins or DNA. However, this defense mechanism can backfire: cancer stem cells (CSCs), a subpopulation of immature cells believed to be foundational for tumor growth, often increase their ROS elimination activity to protect against the lethal effects of chemotherapeutic agents.

Osamu Nagano and co-workers at the Keio University Graduate School of Medicine, Japan, have found that these CSCs can often be distinguished by the appearance of CD44v, a variant form of the CD44 protein, a CSC marker. "Increased CD44v expression is associated with cancer progression in several types of cancer, including colon and gastric cancer," says Nagano.

The team of researchers have now characterized the apparent link between CD44v expression and ROS clearance in CSCs, and found that this protein variant contributes to tumor survival[1]. "CD44v is not only a marker for CSCs, but plays a functional role in the maintenance and expansion of immature CSCs *in vivo*," explains Nagano.

On examining a variety of gastric and colorectal tumors, the researchers noted that cells expressing high levels of CD44v also tended to contain low levels of ROS. When they experimentally depleted CD44v, however, these cells generated higher levels of ROS and showed activation of ROS-responsive signaling pathways. Cells typically produce molecules of a substance called reduced glutathione (GSH) that prevents ROS-inflicted damage. Nagano and colleagues found striking evidence that CD44v directly affects GSH production: specifically, they showed that CD44v contributes to the stabilization of xCT, a membrane protein that transports cystine, a precursor required for the production of GSH, into the cell.





Mouse gastric tumor cells expressing high levels of CD44v (red) tend to express the lowest levels of the ROS-accumulation marker phospho-p38$^{MAPK}$ (green).

When the researchers deleted the gene encoding CD44 in a gastric-cancer-prone mouse strain, the animals developed considerably smaller, low-grade tumors relative to their CD44-expressing counterparts. These tumors exhibited marked activation of ROS-responsive pathways and low levels of xCT. They had also reduced cellular proliferation and greater levels of tumor differentiation, suggesting that ROS accumulation leads to relative depletion of CSC populations. A final experiment, in which mice with transplanted human colorectal tumors were treated with both a chemotherapeutic agent and xCT inhibitor sulfasalazine, demonstrated the clinical importance of these findings. Sulfasalazine potently enhanced the tumor-shrinking effects of treatment in this model, and Nagano hopes to exploit this same pathway in humans.

"We are now planning to conduct a Phase I clinical trial using sulfasalazine for patients in Japan with CD44v-positive gastric cancer," says Nagano.

1. Ishimoto, T., Nagano, O., Yae, T., Tamada, M., Motohara, T., Oshima, H., Oshima, M., Ikeda, T., Asaba, R., Yagi, H., Masuko, T., Shimizu, T., Ishikawa, T., Kai, K., Takahashi, E., Imamura, Y., Baba, Y., Ohmura, M., Suematsu, M., Baba, H. & Saya H. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of System $xc^-$ and thereby promotes tumor growth. *Cancer Cell* **19**, 387–400 (2011).

# Tapping a plentiful stem cell source

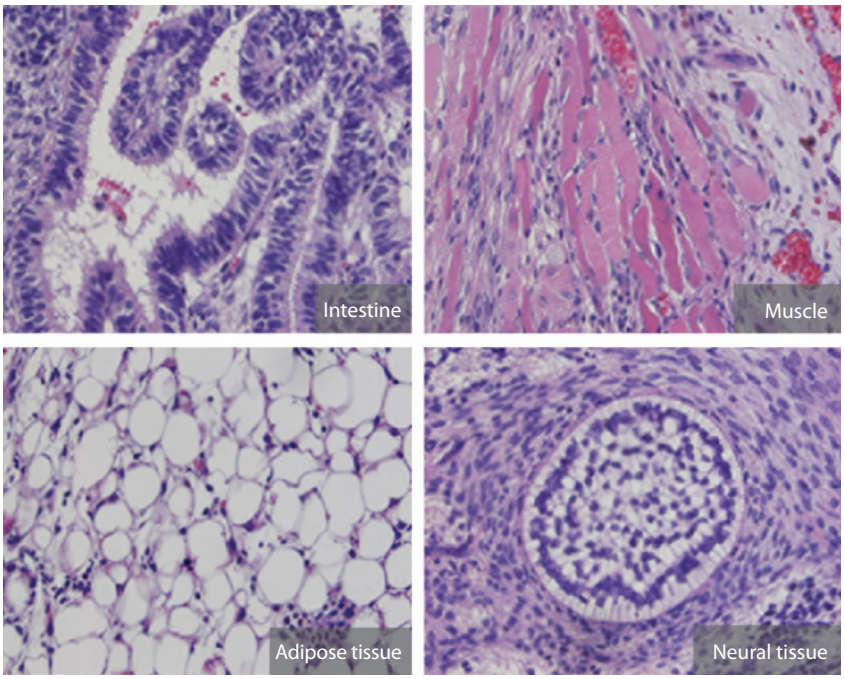
## An improved method to efficiently and safely transform immune cells into stem cells could advance regenerative therapies

Stem cells can mature into any cell type in the human body. This 'pluripotency' means stem cells are useful in regenerative therapies for a variety of human diseases. Easy access to a plentiful source for pluripotent stem cells (PSCs), however, is important for the advancement of such therapies. A research team from Japan has identified a unique solution to this problem. Led by Keiichi Fukuda at the Keio University Graduate School of Medicine, the team has shown that the addition of four stem-cell specific transcription factors can induce mature immune cells called T lymphocytes to become PSCs in one month[1].

Previously, other groups had shown that the same transcription factors—OCT3/4, SOX2, KLF4 and c-MYC—can drive skin cells called fibroblasts to become PSCs. Fibroblasts, however, are more difficult to obtain than human-blood-derived T lymphocytes that are easily extracted and grown from a small, routine blood draw. Other groups had also expressed the four factors in skin cells using methods in which the genes integrate into the host genome. Unfortunately, there is a risk that integration occurs within genes that inhibit tumor formation, which may lead transplanted cells to become cancerous.

To make the process safer, Fukuda and colleagues used a gene therapy approach in which the vector—called Sendai virus—that carries the genes into the cells does not integrate into the genome, and whose expression they could control by regulating the cell temperature.

Previous methods of PSC generation from fibroblasts did not allow for keeping tabs on the cells after transplantation. However, T cells have a unique property that other cells do not have that would enable tracking of T cell-derived PSCs and their progeny.




Human T cells derived from induced pluripotent stem cells in (clockwise from top left) intestine, muscle, neural tissue and adipose tissue.



Mature T lymphocytes respond to particular antigens because they express antigen-specific T cell receptors (TCRs), which are generated during a special process called VDJ recombination that occurs in the T cell genome. Each T cell that reacts to a particular antigen has a different VDJ recombination event to generate the TCR that is specific for that antigen. Therefore, T lymphocytes—and their PSC progeny—can be tracked by identifying cells that have a VDJ recombination event in common. Since this is the first technique that could enable cell tracking after transplantation, the technique has a clear advantage in regenerative medicine.

"We hope that the benefits of our method will mean that it can eventually be applied to clinical practice," says Tomohisa Seki, the first author of the study.

1. Seki, T., Yuasa, S., Oda, M., Egashira, T., Yae, K., Kusumoto, D., Nakata, H., Tohyama, S., Hashimoto, H., Kodaira, M., Okada, Y., Seimiya, H., Fusaki, N., Hasegawa, M. & Fukuda, K. Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. *Cell Stem Cell* **7**, 11–14 (2010).

# Evaluating stem cell suitability for transplants

## Neural stem cells from genetically manipulated skin cells show potential for tumor-free use in treatments for spinal cord injury

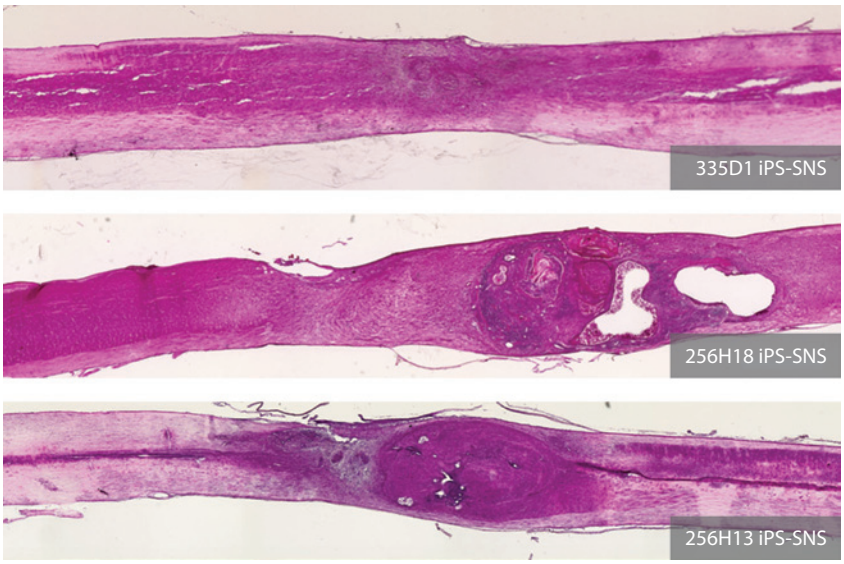
Embryonic stem cells induced to become neural stem cells can differentiate into neurons and other functionally important cell types found in the central nervous system. Despite their therapeutic potential, the patient's immune system can reject these cells, and the clinical use of material derived from embryos raises ethical issues.

"A better and more acceptable approach may be to use the patient's own cells that have been genetically induced to become neural stem cells with specific differentiation potentials," explains Osahiko Tsuji from the Keio University Graduate School of Medicine, Japan. "However, it is important to show that these induced pluripotent stem (iPS) cells are effective and safe to use."

Using a mouse model, a research team led by Tsuji showed that mouse neural stem cells, established from embryo and adult tissue and pre-evaluated for safety, could be effective in the treatment of spinal cord injury[1]. Tsuji's collaborators at Kyoto University had previously produced iPS cells by genetically manipulating mouse skin cells.

"Initial reports of the therapeutic efficacy of iPS cells in rodent models of sickle cell anemia and Parkinson's disease encouraged us to examine their use as a treatment for spinal cord injury," explains Tsuji.

The researchers successfully demonstrated the ability of mouse iPS cells to differentiate into functional neurons and two other important cell lineages, astrocytes and oligodendrocytes, using a method involving cell clusters called neurospheres. To evaluate the safety of the iPS cell-derived neurospheres, they injected them into a specific strain of immunodeficient mice to avoid tissue rejection. After establishing that the cells did not produce tumors and were therefore safe to use, the researchers tested their efficacy in a mouse model of spinal cord injury.






Sections of injured spinal cords of iPS-cell grafted mice. In mice grafted with 335D1 iPS-SNS cells (top) there was no evidence of tumors, however tumors were evident in the spinal cords of both 256H18 iPS-SNS (center), and 256H13 iPS-SNS (bottom) grafted mice.

They found that the transplanted 'safe' cells differentiated normally into neurons, astrocytes and oligodendrocytes without forming tumors, whereas other iPS cells (see image) re-evaluated as being 'unsafe' formed tumors as predicted.

They also showed that the safe iPS cells helped repair neural cells at the site of injury, facilitating the regrowth of vital nerve fibers. More importantly, they showed that mice receiving these transplants recovered a degree of locomotor function. In contrast, they found that mice treated with iPS cells pre-evaluated as being unsafe, in addition to developing tumors, gradually lost the transiently obtained functional recovery and died.

"Our findings suggest that pre-evaluated safe iPS-clone derived neural stem cells may be a promising cell source for transplantation therapy for human patients with spinal cord injuries," says Tsuji. ■

1. Tsuji, O., Miura, K., Okada, Y., Fujiyoshi, K., Mukaino, M., Nagoshi, N., Kitamura, K., Kumagai, G., Nishino, M., Tomisato, S., Higashi, H., Nagai, T., Katoh, H., Kohda, K., Matsuzaki, Y., Yuzaki, M., Ikeda, E., Toyama, Y., Nakamura. M., Yamanaka S. & Okano, H. Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. *Proceedings of the National Academy of Sciences USA* **107**, 12704–12709 (2010).


Keio University Graduate School of Medicine
35 Shinanomachi Shinjuku-ku Tokyo, 160-8582 Japan
www.gcoe-stemcell.keio.ac.jp

## Members of the Keio University Global COE Program "Education and Research Center for Stem Cell Medicine"

Hideyuki Okano (Program Leader)

**Tissue stem cell regulation and *in vivo* experimental medicine**
- Toshio Suda (Group Leader)
- Haruhiko Siomi
- Hideyuki Okano
- Michisuke Yuzaki

**Inflammation/immunological regulation and tissue regeneration**
- Shigeo Koyasu (Group Leader)
- Masayuki Amagai
- Yasunori Okada
- Akihiko Yoshimura

**Development of new cancer treatments targeting cancer stem cells and EMT**
- Yutaka Kawakami (Group Leader)
- Hideyuki Saya
- Naoto T. Ueno (MD Anderson Cancer Center, USA)
- Yuko Kitagawa
- Yasuyuki Ohnishi (Central Institute for Experimental Animals)

**Development of regenerative medicine for intractable diseases**
- Keiichi Fukuda (Group Leader)
- Hans Keirstead (University of California, Irvine, USA)
- Olle Lindvall (Lund University, Sweden)

**Practical implementation of feasible regenerative medicine**
- Yoshiaki Toyama (Group Leader)
- Kazuo Tsubota (Group Leader)
- Koichi Matsuo
- Akihiro Umezawa (National Research Institute for Child Health and Development)
- Hideaki Nakajima
- Masaya Nakamura

**Education and Research Center for Stem Cell Medicine**
- Yumi Matsuzaki (GCOE Flow Cytometry Core Facility)
- Shinji Makino (GCOE Keio University Small Fish Center)