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The Sky is the Limit

Simplicity for anyone at any time—The challenge of improving the CRISPR/Cas9 genome-editing technique

General Research Organization Project

Establishment of Methods to Generate Genome-Edited Animals and Their Application in Animal Model Development

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Genetic modification using genetic engineering methods is a technique that is indispensable in many areas of Life Sciences research, including medicine, biology and agriculture. The CRISPR/Cas9 genome editing method announced in 2012 involves simpler handling than previous methods and offers possibilities for many researchers to be able to attempt genetic modification of a variety of living species. On the other hand, there are still not many research facilities/laboratories that can take full advantage of the technique.

The research unit represented by Associate Professor Masato Ohtsuka of the Division of Basic Medical Science and Molecular Medicine, School of Medicine at Tokai University is aiming to lay the foundations for producing genome-edited organisms through improving and developing the CRISPR/Cas9 technique. We discussed this with Associate Professor Ohtsuka, Junior Associate Professors Mitsuhashi and Nakagawa, who are engaged in developing, applying, and verifying the technique.

How did genome editing technology change genetic engineering field? What is the genome editing technique called CRISPR/Cas9?

Ohtsuka: We are working on applying and improving genome editing techniques that use CRISPR/Cas9. CRISPR/Cas9 is a technique that is capable of cleaving genomic DNA at any position, and by using this method, it is possible to alter any gene of interest. This was first announced in 2012.

Previously, gene modifications were primarily conducted using mice in order to produce gene knockout mice or mice having exogenous genes in their genome. These have been used to analyze the functions of genes and to produce models of human diseases. However, gene modification was quite difficult at that time and was not possible without specialized technical skills. The recent development of CRISPR opened the door for any researchers to manipulate genes much easier than before.

The three of us have been pondering how CRISPR can be and should be used, and our intention is to establish and disseminate the basic technology for genome editing at Tokai University. I mainly use mice in my research, but Dr. Mitsuhashi uses a type of tropical fish called the zebrafish, and applications in other living organisms are also possible. The goal is to facilitate the production of more refined animal models of diseases and the future development of new gene therapy methodologies.

Mitsuhashi: Researches using CRISPR/Cas9 technologies are commonly performed in mice aiming at medical applications, but I am applying it to zebrafish. Zebrafish lay about 100 eggs at a time. It is a great advantage to produce a larger number of genetically modified animals at once compared with mice. Also, genome editing is generally performed on fertilized eggs, but the fertilized eggs of mice are very small and they are within the mother mouse's womb, making them difficult to handle. Zebrafish lay eggs as large as 1 mm outside the body that can be seen

Masato Ohtsuka

Associate Professor, Basic Medicine, Department of Medicine, School of Medicine, Tokai University
(Professor as of April 2019)

Principal investigator of the study "Establishment of methods to generate genome-edited animals and their application in animal model development." His goal is to establish the techniques for the production of genome-edited mice and apply these techniques to the development of techniques to regulate gene expression.

Hiroaki Mitsuhashi

Junior Associate Professor, Department of Applied Biochemistry, School of Engineering, Tokai University
(Associate Professor as of April 2019)

He specializes in molecular biology, molecular genetics, and musculoskeletal biology. He is involved in the establishment of techniques for the production of genome-edited small fish techniques applied to Zebrafish, as well as producing and analyzing fish disease models utilizing these techniques.

So Nakagawa

Junior Associate Professor, Basic Medicine, Department of Medicine, School of Medicine, Tokai University

Apart from genome science, his fields of expertise include molecular evolution and bioinformatics. He is involved in the analysis and evaluation of genes in genome-edited organisms using next-generation sequencing.

with an stereomicroscope, and so we can easily manipulate them for genome editing.

Zebrafish are also useful for education: in my laboratory, undergraduate students perform genome editing on zebrafish by themselves, resulting in a strong educational impact. It would be too difficult for the students to handle mouse fertilized eggs by themselves.

Nakagawa: My specialty differs from that of my two colleagues here in that I analyze the genetic information in the DNA that makes up a genome. DNA is formed from the four bases A, G, C, and T (adenine, guanine, cytosine, and thymine, respectively) and the combinations of these base codes form the genetic information necessary to produce an organism. For example, the human genome contains about three billion bases, and until just 10 years ago, it took an incredible amount of time and cost just to read that sequence. However, DNA sequencing technology has progressed in leaps and bounds, and currently, the human genome can be read for only about 100,000 yen.

In the era when decoding the genome was costly, it was impossible in a practical sense for individual researchers to read the genome of one of their own samples. Currently, cost reductions have resulted in actual sequence data being relatively easy to obtain, and the next challenge is to see how efficiently we can investigate whether there are any mutations in those sequences. Since genome data are huge, such a massive amount of information including mutations are studied by utilizing information processing technology with computers.

In the current project, I am in charge of whole genome sequencing of zebrafish to examine whether the desired modifications have been successfully implemented in the target region for genome editing using the CRISPR technique, and for confirming that unintended modifications have not arisen in areas outside of the target area.

The place where the researchers met one another, the "Research Exchange Meeting," and the General Research Organization Project

Ohtsuka: Our current study is being conducted by a three-person unit consisting of myself and Dr. Nakagawa in the School of Medicine, together with Dr. Mitsuhashi from the School of Engineering. The formation of this unit was sparked by the "Research Exchange Meeting" that began at Tokai University in 2015. The Research Exchange Meeting, as the name implies, is hosted by the Research Promotion Division as a meeting for exchanges between researchers, and as an opportunity for scholars at the school to present their research topics and issues that they are facing, as well as to have discussions.

In addition, Tokai University also has an internal research grant system called the "General Research Organization Project," which promotes collaborative studies that take advantage of the benefits of a university by bridging multiple departments, and supports research projects that seek external sources of funding, such as large-scale competitive funding. Our current unit, which was newly formed through the Research Exchange Meeting, applied to be a comprehensive research project and was selected, thus receiving a kick-off grant.

Mitsuhashi: I met Dr. Ohtsuka when I attended the Research Exchange Meeting in 2015 with the idea of possibly finding a research collaborator. Both Dr. Ohtsuka and I presented posters to introduce our own research and became interested in each other's work in the process.

Ohtsuka: My goal is to improve the CRISPR technique in order to allow more complex genetic manipulations to be performed more simply. My specialty is genetic engineering, so at the time of our meeting, I was already using mice to establish genome editing techniques. I heard that other people, including Dr. Mitsuhashi, wanted to begin the genetic manipulation of zebrafish.

Mitsuhashi: I originally studied muscular dystrophy, which is a disease of the muscles caused by a genetic abnormality. Muscular dystrophy affects fewer patients than cancer or diabetes does, so it is difficult to use samples from the patients directly, and it is a field that has not been making much progress. In our current study, we took the knowledge of genome editing techniques developed by Dr. Ohtsuka with mice and applied them to zebrafish, and we are conducting experiments to produce zebrafish with the same genetic abnormality that affects patients with muscular dystrophy. If we can observe the same symptoms in the zebrafish with the genetic abnormality, we can use them for drug testing.

Nakagawa: I am in the same academic department as Dr. Ohtsuka, but I didn't know about this project until we worked together as instructors for student training.

Ohtsuka: After manipulating the base sequence of a genome, it is necessary to know what changes have occurred, and in which part of the genomic DNA they have occurred in, as a result of the manipulation. Since Dr. Nakagawa's strength is in genome analysis, I thought that it would be advantageous to bring him on board.

Mitsuhashi: I am very grateful to be able to participate in this type of research project, thanks to the Research Exchange Meeting. I think it is a good system and so I very much hope it continues in the future.

The goals and dreams of genome editing, from improving editing efficiency to developing gene therapy

Ohtsuka: Without a doubt, genome editing techniques will progress to applications in

gene therapy for humans in the future. I would like to know what changes should be made to the genomic DNA and how much efficiency can be improved for future gene therapy in humans.

Mitsuhashi: Muscular dystrophy is currently untreatable. I am conducting research with a dream of finding a treatment for this disease. My still greater dream is for the research that began with a small tropical fish to lead to a treatment that will be useful to humans, so I would be very grateful if this could be realized.

Ohtsuka: When considering clinical applications, it is necessary to reduce to an absolute minimum the possibility of undesired mutations occurring. There are actually numerous similar base sequences within genomic DNA, and so there are cases when CRISPR/Cas9 ends up mistakenly cutting at a different location that has a base sequence very similar to the target sequence that is actually being targeted for cutting. This is why there are cases of undesired mutations occurring at locations other than the target sequence. Reducing and avoiding the occurrence of such nonspecific mutations is currently a hot topic that is being heavily studied.

Nakagawa: Genomes are not logically and efficiently designed to achieve their present sequence. Of course, the process of evolution led it to its current form, and so not everything is cleverly meshed together. The reality is that life carries on somehow, despite various issues. There are many places where the same sequence is repeated, and where the purpose of the sequence is not well understood. There are cases where such repeated sequences actually have very different and important functions, and where important genes are copied a number of times and are retained as a gene cluster.

Ohtsuka: DNA had been thought of as the blueprint for protein synthesis, but base sequences that code directly for proteins actually make up only 1.5–2% of the entire genomic DNA.

The regions that do not code for proteins are called noncoding regions. These are not always without purpose, as they have roles such as regulating the amount of protein synthesis, functioning as RNA, and the like, and so it is becoming increasingly clear that they can play certain roles in biological activities. The artificial modification of noncoding regions as well as of the locations that code for proteins may lead to treatments for diseases.

Nakagawa: It is now not enough to just obtain DNA sequences of given samples. There is currently real progress being made in using techniques, such as those developed by Dr. Ohtsuka, to modify genes by removing them or adding them in order to reveal the functions of each constituent element. I am very pleased to be working in an era when the roles of the genome and individual genes in our bodies are becoming understood in a dynamic way.

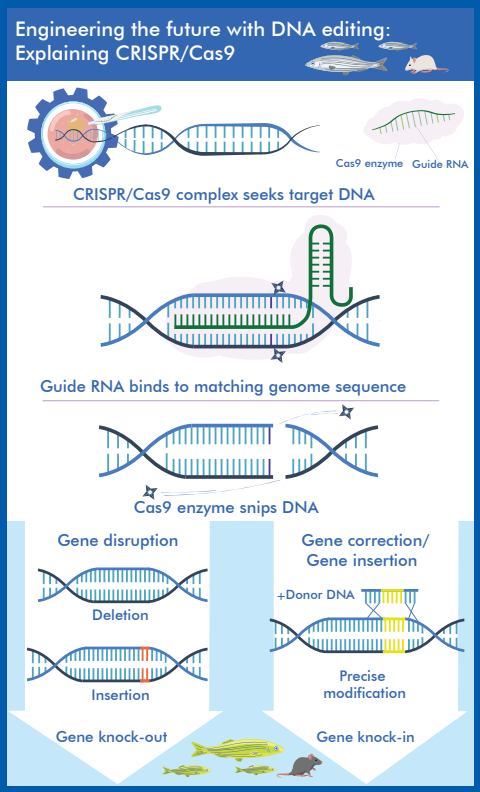
Ohtsuka: Our laboratories are individually small in scale, but my attitude is that I would like to use collaborative research as a research unit to develop groundbreaking techniques and model organisms that can have a worldwide impact.

I understand that research engaging in the development and improvement of new techniques does not tend to take place at for-profit corporations. Whereas companies take advantage of techniques that are already established, it is up to academia to use trial and error to wade through a sea of unfettered ideas and conceptualizations in order to develop techniques of unknown potential.

The research period as a comprehensive research project is over at the end of this fiscal year. Although we have already produced a certain level of results, since we have been fortunate enough to have this opportunity to engage in research together like this, I would like to continue to cooperate toward the creation and analysis of disease models.

Searching for a target sequence and cutting it with "scissors"—The basics of genome editing using CRISPR/Cas9

CRISPR/Cas9: This genome editing technique was developed using clues from the immune system found in bacteria, such as Escherichia coli. It is able to seek out specific base sequences in DNA and cut the double strands at that position.



When certain bacteria encounter an external threat, such as a phage (a virus that infects bacteria), they incorporate a portion of the enemy's DNA into their own base sequence in order to remember it. If an external threat invades again, RNA that has the base sequence corresponding to the stored DNA is synthesized, and this identifies whether the DNA of the new external threat contains the same base sequence as the memorized DNA. If that same base sequence is found, then that RNA guides the protein (enzyme) that cleaves the DNA to that location. Bacteria therefore inhibit phage infections by cleaving the phage DNA with such enzymes. (Note: The location in the bacterial base sequence where genetic information from phages is stored is called the CRISPR locus, and the enzyme that cleaves phage DNA is called the Cas9 protein.)

Using the CRISPR/Cas9 method, a solution set—consisting of the RNA (guide RNA) artificially designed to match a target DNA base sequence and the enzyme (Cas9) that cleaves the DNA—is introduced into the fertilized egg of an organism using a fine needle, electricity, or the like.

The cleavage of DNA in a cell is a phenomenon that also occurs in nature, and so the double strand break is repaired using the cell's repair function. However, it is also possible at this time for a repair error to occur (e.g., the loss or addition of some bases), or for a foreign DNA sequence to get inserted into the cleavage site, resulting in a modification of the genetic information in the DNA. The loss or addition of bases results in a gene knockout, whereas an artificial insertion of DNA fragment results in a gene knock-in.

The success rate of genome editing differs depending on the target species, cell type, intended operation, and the like. For knockouts, the success rate is about 50–100% in mice and about 50% in zebrafish.

NEWS

Tokai University Establishes First Social Partnership Chair "Kidney Disease Pathology Analysis Chair"

At Tokai University on December 1, Nephrology, Endocrinology and Metabolism Division of Medical Science Course, Faculty of Medicine, School of Medicine established the social partnership chair "Kidney Disease Pathology Analysis Chair." The school began a three-year, four-month-long joint research project with Kyowa Hakko Kirin Co., Ltd., which will last until March 2022. The social partnership chair, the first of its kind at Tokai, is an effort to promote the development of academics and society as well as improve and achieve Tokai's educational research initiatives through joint research funded by private companies.

From December 2015 to November 2018, the Medical Science Course, Nephrology, Endocrinology and Metabolism Division instituted an endowed chair under the same title with the financial support of Kyowa Hakko Kirin Co., Ltd.

Dr. Hirotaka Komaba

Centered around the work of university lecturer Dr. Hirotaka Komaba, the program aimed to explain the pathology of chronic kidney disease, particularly mineral and bone disorder. The social partnership chair will build upon the work accomplished under the endowed chair, moving forward with joint research that will lead to the development of new medicines and treatments.

Ranked 4th among private universities in Japan in the World University Rankings (by subject) *

The Times Higher Education's (THE) World University Rankings by subject attracts much attention every year. This year, Tokai University made the ranking in the five subject areas of Clinical, Pre-clinical & Health, Life Sciences, Physical Sciences, Computer Science, and Engineering & Technology, with an overall rank* of fourth place among private universities in Japan.

The THE evaluates universities around the world and ranks them in eleven academic fields including Arts & Humanities, Education, Business & Economics, Clinical, Pre-clinical & Health, Computer Science, and Life Sciences.

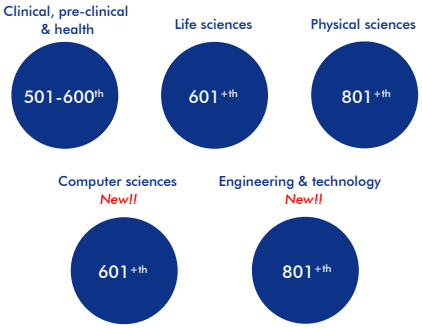
The evaluation indicators in this ranking were the same as those used for the overall THE world university rankings, which rigorously applied thirteen items in the five areas of teaching, research, citations, international outlook, and industry income and presented the results in the form of a ranked list.

*The overall rank, is calculated by Tokai University, refers to the number of academic fields in which each university made the ranking expressed as a ranked list.

■Times Higher Education Rankings 2019

(November 30, 2018)

Tokai University made the THE's World University Rankings (by subject) for five subjects: Clinical, Pre-clinical & Health, Life Sciences, Physical Sciences, Computer Science, and Engineering & Technology. The university had an overall rank of fourth among private universities in Japan.



Overall ranking - Private universities:

1st (10 subjects) Waseda University
2nd (7 subjects) Keio University
3rd (6 subjects) Ritsumeikan University
4th (5 subjects) Tokai University,
Tokyo University of Science
6th (4 subjects) Kinki University

EVENT REPORTS

Hosting the Agribusiness New Technology Presentation Meeting

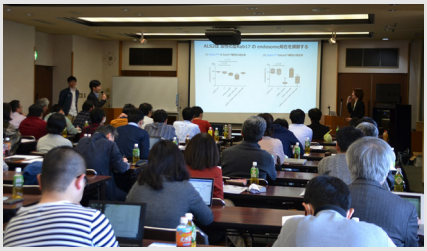
On Tuesday, November 20, 2018, Tokai University held an Agribusiness New Technology Presentation Meeting, at which Junior Associate Professor, Hideki Kinoshita, of the School of Agriculture, Department of Bioscience gave a lecture.

This event was part of a program of activities aiming to stimulate agribusiness through the cooperation of industry, government, and academia conducted by five private universities which have agricultural field in the Tokyo area: Tamagawa University, Tokyo University of Agriculture, Nihon University, Meiji University, and Tokai University. As Tokai University is currently chairing the group, the university managed the event as a forum for introducing seeds and presenting agricultural solutions from each university.

About 100 corporate representatives attended. After Junior Associate Professor Kinoshita's talk, they discussed possibilities for the future of cooperation between industry and academia.

The 14th Workshop held by Tokai University's Institute of Medical Sciences

On December 1 and 2, 2018, Tokai University's Institute of Medical Sciences held its 14th workshop at the Ikoinomura Ashigara (Kanagawa Prefecture). The institute is the base for cutting-edge research in the medical sciences at the university and promotes translational research that unifies basic and applied research in genomics, regenerative medicine, and drug development.



This workshop is held annually in order to promote the reporting of research results for both inside and outside the university, the training of young researchers, and cooperation with research institutions and various academic departments relevant to the medical sciences. For the past few years, it had been held as a one-day event at the Isehara campus. In response to a strong demand from the researchers, this year's workshop was held as a two-day, overnight event. General lectures and a keynote address were given.

About 70 faculty members and graduate students participated, not only from the School of Medicine, but also the School of Engineering and the School of Science. Ten individuals presented their research results in the general lectures held over both days. There were lively Q&A sessions and exchanges of opinions on research themes.

An exchange of opinions between a research support officer at the Technical University of Denmark and Tokai University

On November 21, 2018, a joint symposium was held by Tokai University with the Technical University of Denmark (DTU). In this symposium, an exchange of opinions took place between a research promotion officer at Tokai University and Ms. Mie Morthorst, an academic officer from DTU working at Research Support DTU, Research and Relations.

Ms. Morthorst introduced the research support system at DTU, measures enacted by Denmark and the EU for competitive research funds, and support for obtaining those funds. After an introduction of Tokai University's research promotion system, characteristic research, and examples of cooperation between industry and academia, there was an exchange of questions regarding the efforts made by both universities.