Please contact us »» uraoffice.adm@tmd.ac.jp

International collaboration reaps rewards in study of atrial fibrillation

Toshihiro Tanaka

Professor of Bioresource Research Center at TMDU

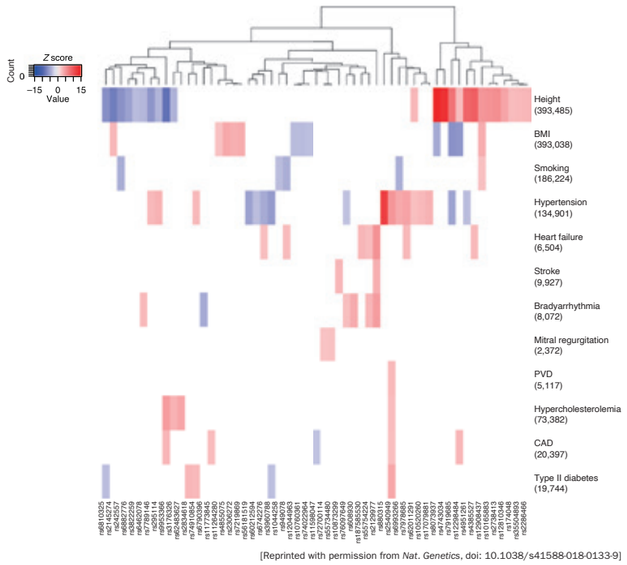
Q You have recently been involved in a large collaborative study of genetic loci associated with atrial fibrillation (AF). Please tell us about this work.

A: The study is the largest meta-analysis of genome-wide association studies of AF to date, and included more than half a million participants from 50 separate studies. The analysis identified 97 loci that were significantly associated with AF, 67 of which were novel. We were then able to link certain risk variants with candidate genes enriched within cardiac developmental, electrophysiological, contractile, and structural pathways, confirming their likely involvement with AF. Interestingly, many of the AF candidate genes encoded transcription factors, hinting at the complex, polygenic nature of AF.

Q You have previously carried out genome-wide association studies for AF, but the current study is a little different. Can you explain what sets it apart?

A: AF is a common heart rhythm disorder that significantly contributes to serious conditions such as heart failure and stroke. It is estimated that more than 33 million people are affected by AF worldwide. However, despite this global incidence, many association studies only focus on one, or perhaps several, ancestry groups. What sets the current study apart is the inclusion of patients from European, Japanese, African American, Brazilian, and Hispanic populations, and the fact that we performed both combined-ancestry and ancestry-specific meta-analyses. This approach is very important because while ancestry-specific loci were identified, we showed that the most common genetic susceptibility signals for AF were conserved across all populations, meaning that potential therapeutics need not be targeted to specific ancestry groups.

Cross-trait associations of AF risk variants with AF risk factors in the UK Biobank



[Reprinted with permission from *Nat. Genetics*, doi: 10.1038/s41588-018-0133-9]

Q The work also includes a phenome-wide association study. Can you tell us a little more about this?

A: Phenome-wide association studies allow us to assess the pleiotropic effects of a disease-associated variant — essentially, whether a particular variant is also significantly associated with any other phenotypes. Using data from the UK BioBank, we showed that distinct clusters of variants were associated with both AF and height, body mass index, or hypertension. These findings may help us to better understand the diverse mechanisms involved in AF.

Q The study involved many international collaborators. How does this align with TMDU's research objectives?

A: Our study involved collaborators from 21 countries across Europe, North and South America, Australia, and Asia. TMDU has a strong focus on international collaboration and our work helps strengthen TMDU's relationships with leading research institutions around the world.

Dr. Tanaka graduated from the University of Tokyo, School of Medicine where he received his MD and PhD. He conducted research as Assistant Professor at the University of Tokyo from 1997 to 1999, then moved to RIKEN in 2000, and took the post of Deputy Director of RIKEN Center for Genomic Medicine in 2009. Since 2013, he has been Professor of the Research Division at the Bioresource Research Center of TMDU and Professor of Human Genetics and Disease Diversity at Graduate School of TMDU.

Nat. Genetics, **doi: 10.1038/s41588-018-0133-9**

Circulating microRNAs as an early indicator of atrial fibrillation

Tetsuo Sasano

Professor of Cardiovascular Medicine at TMDU

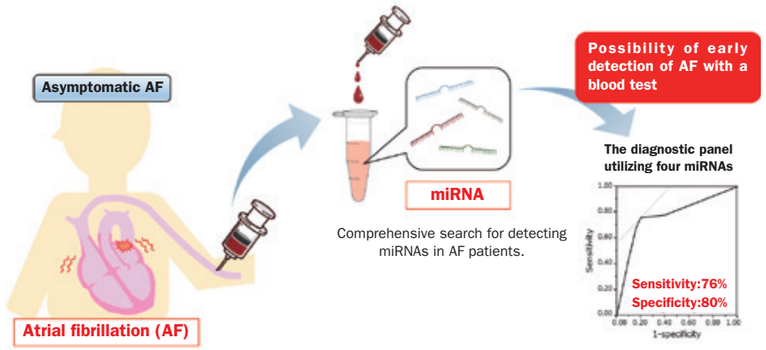
Q What made you focus on microRNAs (miRNAs) as biomarkers for atrial fibrillation (AF)?

A: These small, non-coding RNAs bind to target messenger RNAs and inhibit translation, thereby regulating gene expression. Recently, several studies have shown that miRNAs expressed in atrial tissue play a key role in the pathogenesis of AF. In addition, stable miRNAs have been detected in circulating blood. This led us to ask whether these miRNAs could be used as biomarkers for predicting AF.

Q Can you give us an overview of your latest findings?

A: We wanted to test our hypothesis that circulating miRNAs could be used as indicators of AF. To do this, we screened for 733 miRNAs in serum from patients with AF as well as 672 miRNAs in atrial tissues from mice with inducible atrial tachycardia. We compared the expression of the miRNAs in these patients and mice with that in suitable controls to identify miRNAs that were associated with disease. From this initial screening, we identified 11 miRNAs with significant changes in expression in both the patients with AF and the atrial tachycardia model mice. To confirm the association between the miRNAs and AF, we then quantified the expression of each of the candidate miRNAs in serum from 50 patients with AF and 50 healthy controls. This individual screening led us to identify four miRNAs that were upregulated in patients with AF. Encouragingly, the predictive accuracy of the four miRNAs as biomarkers for AF was very good, with a sensitivity of 76% and a specificity of 80%.

Prediction of AF with a blood test



Since AF strongly increases the risk of stroke, it is important to detect AF as early as possible, in order to start preventive therapy. However, about one-third of AF is asymptomatic, and it is sometimes difficult to detect AF in a case with paroxysmal AF. We screened circulating miRNAs in peripheral blood, and found four miRNAs associated with the presence of AF. The diagnostic panel utilizing these four miRNAs might be useful to predict an asymptomatic and/or undiagnosed AF with a blood test.

Q What are your next steps with this work?

A: Interestingly, only one of the miRNAs identified in our study has previously been directly linked to AF. While putative functions for the other three miRNAs have been suggested, the relationship between these miRNAs and AF is unclear. Therefore, we now hope to clarify the functional role of these miRNAs in relation to AF.

Q What are the clinical implications of your research?

A: AF is a risk factor for cardiogenic stroke, heart failure, and dementia but is often asymptomatic and can go undetected until the onset of serious illness. Therefore, the four miRNAs identified in our study could be developed into a diagnostic panel to predict asymptomatic AF via a simple blood test, allowing intervention before the onset of disease.

Dr. Sasano completed medical and graduate school at TMDU, where he received his MD and PhD. He became Associate Professor of Biofunctional Informatics at TMDU in 2011 and Professor of Cardiovascular Medicine there in 2019. His major research interests are arrhythmia, electrophysiology and gene therapy.

Circ J, **doi:10.1253/circj.CJ-17-1194**

Please contact us »» uraoffice.adm@tmd.ac.jp

Microhomology: key to high-throughput mouse gene cassette knock-in

Kohichi Tanaka

Professor of Molecular Neuroscience at TMDU

Q How has available technology hampered the generation of genetically engineered knock-in mice?

A: Traditional CRISPR/Cas relies upon homologous recombination (HR) and the generation of vectors containing large regions of homology. This approach is cumbersome, time-consuming, and reaches efficiencies of only 10% – 20%, thus hindering large-scale application. We previously reported that the cloning-free CRISPR/Cas system facilitates HR-mediated gene cassette knock-in with efficiency of up to 50%. This is still lower than the approximate 100% efficiency observed for non-homologous end joining (NHEJ)-mediated gene knockout. Therefore, we sought to improve gene cassette knock-in efficiency in mammalian cells and zygotes.

Q Your new gene cassette knock-in method uses microhomology-mediated end joining (MMEJ). How does this improve the efficiency of gene cassette knock-in?

A: In mammalian cells, double-stranded DNA breaks (DSBs) are usually repaired by NHEJ, so the efficiency of any HR-based technique, such as CRISPR/Cas, will be low. MMEJ is an alternative pathway for DSB repair. Importantly for us, microhomologies are frequently found in the majority of CRISPR/Cas DSB-repair sites in mice. We harnessed this to develop the highly efficient and convenient CRISPR/Cas-based precision insertion into the target chromosome (PITCh) system.

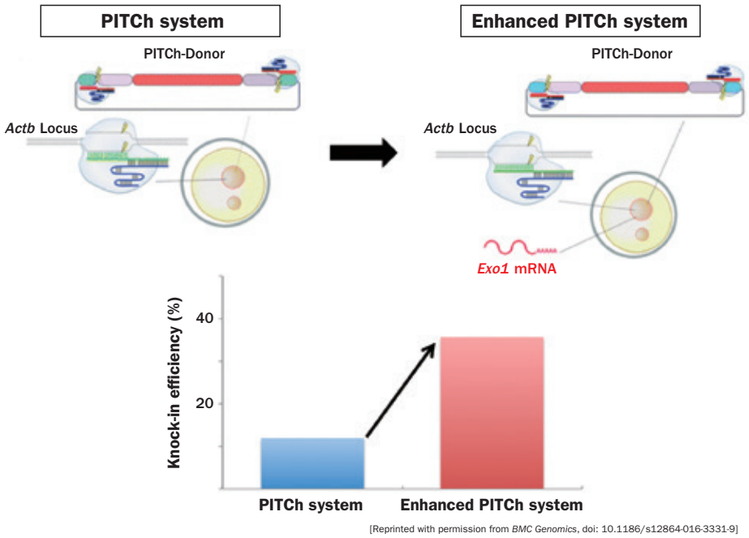
Q You were the first group to apply the PITCh system to mammalian cells. What does this mean for the generation of knock-in transgenic mice?

A: We performed a genetic screen to identify enhancers of MMEJ. Co-delivery of *Exo1*, involved in MMEJ, enhanced knock-in in human cells, and we applied this approach to generate knock-in mice. Using a combination of *Exo1*, PITCh, and the cloning-free CRISPR/Cas system increased the efficiency of knock-in mice to 30% from the approximate 10% achieved using PITCh and HR-based methods. The advantage of this approach over more traditional ones is the omission of laborious target vector construction.

Q What are the implications of this study for the field as a whole?

A: This approach requires a donor vector that can be generated using a single PCR, TA-cloning using primers conjugated with 40 bp of microhomology, and a genomic CRISPR RNA target sequence from any template plasmid containing marker or functional gene cassettes. This approach is exciting because it is scalable and can be used in a small research laboratory, or by a large consortium. This scalability, and the relative simplicity of the post-PCR TA-cloning, makes large-scale mouse knock-in projects feasible.

Generation of gene cassette knock-in mice by the enhanced PITCh system



[Reprinted with permission from *BMC Genomics*, doi: 10.1186/s12864-016-3331-9]

Dr. Tanaka received his MD and PhD from Niigata University. His postdoctoral research was performed with the Neural Network Team at RIKEN. He became a section chief of Neurodegenerative Diseases at the National Institute of Neuroscience in 1993 and assumed his present post at TMDU in 1998.

BMC Genomics, **doi: 10.1186/s12864-016-3331-9**

Novel oligonucleotide drug for gene therapy targeting neurological disease

Takanori Yokota

Professor of Neurology and Neurological Science at TMDU

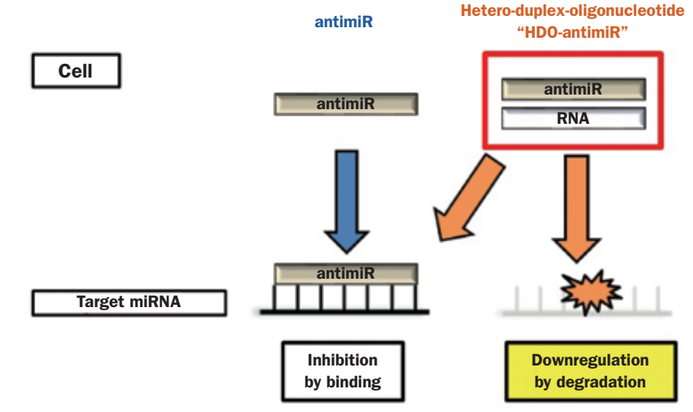
Q Dr. Yokota, you are Chairman of the Department of Neurology and Neurological Science at TMDU. Can you tell us about the key objectives and focus of the Department?

A: Given Japan's rapidly aging society, medical conditions that are associated with aging, such as stroke and Alzheimer's disease, have become a research focus. Innovations in molecular biology have enabled new possibilities for treating age-related neurological diseases. One of the main projects in our department is focused on the use of oligonucleotide drugs for gene therapy. Recently, we created a new class of oligonucleotide — DNA/RNA heteroduplex oligonucleotide (HDO) — which has great potential as a component of gene therapy.

Q What are the advantages of using HDO to treat neurological diseases?

A: The molecular structure and function of HDO are different from conventional genetic therapeutic approaches, and HDO has been found to be highly potent for molecular regulation. We were able to use HDO to develop a new form of a molecule that can inhibit specific types of microRNA (miRNA), which is implicated in various genetic diseases. This new type of anti-miRNA, or antimiR, may represent a new treatment approach for neurological disorders.

Intracellular mechanism of miRNA silencing by HDO-antimiR



This image illustrates the molecular mechanism for how double-stranded HDO-antimiR silences the targeted miRNA within cells in comparison to the original single-stranded antimiR.

Dr. Yokota received his MD from TMDU. His postdoctoral research was performed in several institutes including Tokyo Metropolitan Neurological Hospital and Sanford-Burnham Medical Research Institute in the United States. He returned to TMDU as Junior Associate Professor of Neurology in 2000, became Associate Professor of Neurology and Neurological Science in 2004, and assumed his present post in 2009.

Q What led you to focus on miRNA?

A: RNA plays an essential role in the regulation and expression of genes. As a type of RNA, miRNA is implicated in various cellular functions such as development, differentiation, growth, and metabolism. A number of human diseases may be caused by abnormal expression and organization of miRNA. Compounds that inhibit the activity of miRNA, or antimiRs, are being investigated for their potential utility in treating disease. Thus, we chose to focus on developing a new form of antimiR.

Q Can you tell us more about the new molecule that you developed?

A: We created HDO-antimiR, which has heightened efficacy against targeted miRNA and lower levels of toxicity in kidney than existing options. We found that HDO-antimiR was 12 times as efficient as conventional antimiR in terms of silencing targeted miRNA. Furthermore, HDO-antimiR has improved potency within cells. These improvements are due to the unique structure of HDO-antimiR, which has enabled it to behave differently from other types of miRNA inhibitors.

Q What are the clinical implications of your study?

A: Discovering new ways to silence malfunctioning miRNA could lead to new ways to treat diseases. In the future, HDO-antimiR technology may yield miRNA inhibitors for various diseases, including cancers, intractable neurological diseases such as Alzheimer's disease and Parkinson's disease, and cardiovascular disease, such as heart failure.

Nucleic Acids Res., **doi: 10.1093/nar/gkz492**

Please contact us »» uraoffice.adm@tmd.ac.jp

Using nanoparticles to deliver cancer-killing microRNA

Johji Inazawa

Professor of Molecular Cytogenetics at TMDU

Q Dr. Inazawa, your work focuses on microRNAs as possible anticancer therapies. What are microRNAs, and how can they be used in cancer medicine?

A: MicroRNAs are short RNA molecules that are produced naturally in cells. Unlike messenger RNAs, which are used to synthesize proteins and are typically thousands of nucleotides long, microRNAs are non-coding and on average only around 20 nucleotides in length. MicroRNAs bind to and silence messenger RNA, which allows them to fine-tune different cellular processes by regulating gene expression. It turns out that many of these microRNAs regulate genes involved in cancer, and drugs that mimic microRNA activity may allow us to silence cancer-causing pathways in tumor cells.

Q You recently published a study looking at *miR-634* and its potential role in treating pancreatic cancer. Why did you focus on this particular microRNA and type of cancer?

A: We previously discovered that *miR-634* can act as a potent inducer of cell death in several different kinds of cancer cells. In our prior research, we had examined *miR-634* in only a handful of cancer cell lines. In the current study, we explored on a much larger scale, in over 100 different types of cancer cells. We saw that the microRNA had a powerful effect on multiple cell lines derived from pancreatic cancer. This naturally led us to think that *miR-634* could be key to developing a novel therapeutic agent for this cancer type.

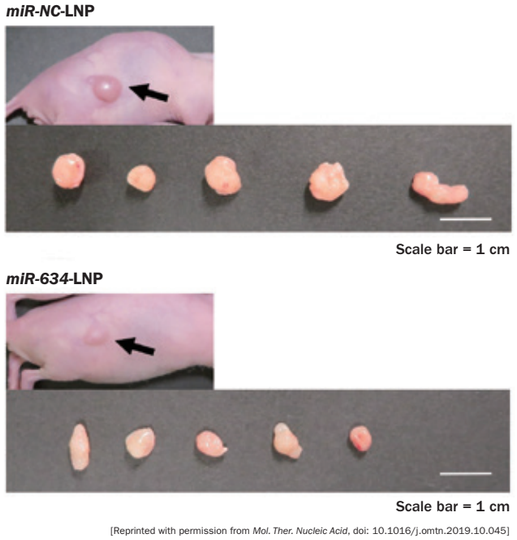
Q Can you describe the microRNA treatment and whether it was effective?

A: We used a lipid nanoparticle that allows us to deliver a mimic of *miR-634* through the bloodstream and into cancer cells. The mimic is structurally similar to *miR-634* and functions like the natural microRNA. We grafted pancreatic cancer cells into mice, then gave them microRNA treatment intravenously several times over a week. By day 21 we found that their tumors were, on average, less than half the size of those in mice given a control treatment.

Q What role do you think *miR-634* will play in the future of cancer therapy?

A: Therapies based on *miR-634* look to be quite feasible, not only for pancreatic cancer but for a variety of cancer types. Interestingly, *miR-634* exerts its effect on cell survival through the same pathways that confer chemoresistance, so it may even be possible to develop treatments for chemotherapy-resistant cancer. Our findings admittedly represent the earliest stages of drug development, but the initial results are very encouraging.

Representative images of pancreatic tumors removed from grafted mice on day 21 after treatment with a control microRNA (*miR-NC*-LNP) or therapeutic microRNA (*miR-634*-LNP)



[Reprinted with permission from *Mol. Ther. Nucleic Acid*, doi: 10.1016/j.omtn.2019.10.045]

Dr. Inazawa graduated from Kyoto Prefectural University of Medicine where he received his MD and PhD. He pursued postdoctoral research at Kyoto Prefectural University from 1982 to 1996, when he became Associate Professor at the University of Tokyo. He joined TMDU as Professor of Molecular Cytogenetics at the Medical Research Institute in 1998, and assumed the position of Director of the Bioresource Research Center in 2012.

Mol. Ther. Nucleic Acid,
doi: 10.1016/j.omtn.2019.10.045

Correlating circulating tumor DNA with patient prognosis in cancer

Sadakatsu Ikeda

Associate Professor of Precision Cancer Medicine, TMDU Medical Hospital

Q As Deputy Director of the TMDU Cancer Center, much of your work focuses on precision medicine. Can you explain what that means?

A: "Precision medicine" refers to personalizing medical treatment by using factors like genetics and molecular biomarkers in blood or tissue. It is sophisticated and, we hope, a more powerful way to treat disease. For example, cancer is traditionally diagnosed and treated based on where it originates in the body. But we're finding that the same molecular abnormality frequently underlies several cancer types, and that targeting abnormalities specific to each patient's cancer may be a more effective treatment approach.

Q One type of biomarker that has received attention lately is circulating tumor DNA (ctDNA). How can ctDNA personalize cancer care?

A: Circulating tumor DNA has enormous potential as a tool in cancer. As tumor cells die, they shed DNA, which enters the circulatory system and can theoretically be collected and analyzed using a simple, non-invasive blood draw. We are currently researching how ctDNA from these "liquid biopsies" can identify patients who are likely to have poorer outcomes.

Q Your team recently published an article describing this research. Can you elaborate on the study?

A: We looked at a gene called *MET,* which is commonly mutated or amplified in tumor cells. *MET* is an excellent biomarker candidate because it is associated with many types of cancer, and because there are treatments that target *MET*. We collected ctDNA from about 400 patients with different types of cancer, sequenced the DNA, and mapped it to a Real World Database, to identify *MET* abnormalities. We then reviewed the patients' medical charts to see if we could correlate their genetic findings to clinical findings.

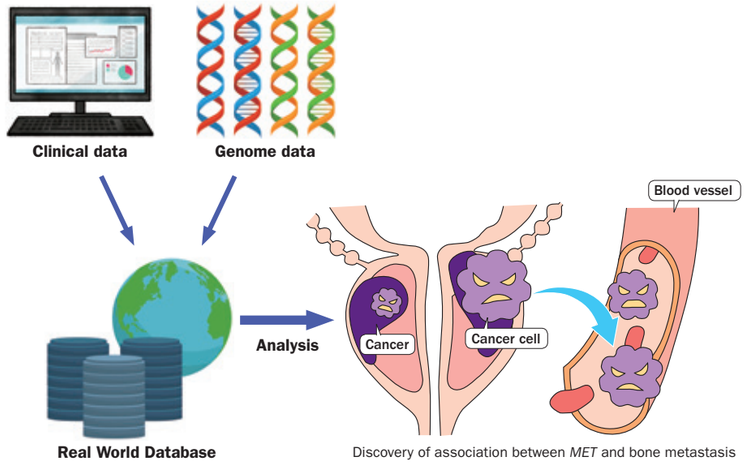
Q And what did you learn?

A: Our major finding was that patients with abnormal *MET* were more likely to have bone metastasis. Clinically, this nearly always means that the cancer has become incurable. In addition, these patients were more likely to have cancer-causing mutations in other genes, and a worse survival rate. We also found that the ctDNA analysis was sensitive — *MET* mutations were easier to detect with ctDNA than with DNA from a traditional tumor tissue biopsy.

Q What are the prospects for this method's use in precision medicine?

A: Our goal is to develop an assay to screen ctDNA with a blood draw, to help personalize the treatment plan for each patient. Reaching that goal is a long process, but our recent findings are an important step in that direction.

Utilizing patient data and Real World Database to predict patient prognosis



Discovery of association between *MET* and bone metastasis

Dr. Ikeda obtained his MD and PhD at Hokkaido University and performed his postdoctoral research at Harvard Medical School, Beth Israel Medical Center, the University of Michigan and The University of California, San Diego. He joined TMDU in 2016 and became Associate Professor of Precision Cancer Medicine at TMDU Medical Hospital.

Hematol. Oncol., **doi: 10.1186/s13045-018-0610-8**

Please contact us »» uraoffice.adm@tmd.ac.jp

Uncovering the duality of *Porphyromonas gingivalis*

Toshihiko Suzuki

Professor of Bacterial Pathogenesis, Infection and Host Response at TMDU

Q You study the molecular mechanisms of bacterial infection and the host immune response. Why is it important to study the *Porphyromonas gingivalis* infection process?

A: *P. gingivalis* is one of the main bacterial causes of periodontitis. Its colonization of the oral cavity and ensuing chronic inflammation have been linked to the progression of systemic diseases such as cardiovascular disease and cancer, as well as inflammatory diseases such as rheumatoid arthritis. By studying the mechanisms of *P. gingivalis* infection, we may be able to identify potential targets for therapeutics that can halt the infection process.

Q Can you tell us about your findings?

A: *P. gingivalis* produces a range of proteases called gingipains that allow it to utilize small peptides in the periodontal tissue. Intriguingly though, conflicting reports also suggest that gingipains can both suppress the host inflammatory response and trigger inflammasome activation. Using wild-type and gingipain mutant strains, we attempted to unravel the seemingly contradictory effects of *P. gingivalis* on the host immune response. Our results confirmed that *P. gingivalis* infection triggers NLRP3-mediated inflammasome activation, inducing an immune response; however, activation was equivalent for all strains. Interestingly, heat-inactivated bacteria still induced a reaction, with secreted factors seemingly responsible for inflammasome activation. These findings are significant because they show that gingipains are not responsible for the *P. gingivalis*-induced inflammatory response.

Q Did you find any evidence that gingipains suppress the host immune response?

A: Yes, our results suggest that gingipains dampen the host's inflammatory response. *P. gingivalis* infection induces host-cell production of inflammatory cytokines such as IL-1β, IL-18, and TNF-α. Immature pro-IL-1β and pro-IL-18 are converted to their mature forms through proteolytic cleavage by activated caspase-1. However, immunoblotting and ELISA-based analyses of *P. gingivalis*-infected human monocytes showed that while mature IL-1β, activated caspase-1, and TNF-α were present in the supernatant of gingipain mutant-treated cell cultures, these fragments were absent from the wild-type *P. gingivalis*-treated cell cultures. Further analyses confirmed that gingipains proteolytically degrade the secreted cytokines and activated caspase-1, presumably suppressing the host immune response.

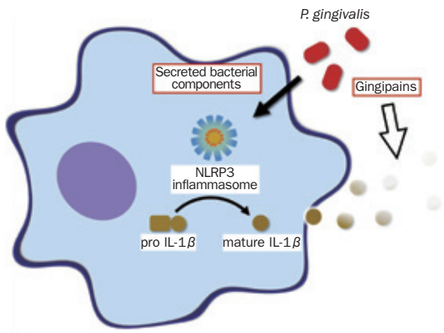
Q What are the future directions of your research?

A: The paradoxical effects of *P. gingivalis* in both triggering and suppressing the host immune response likely help it to stably colonize the periodontal tissues. However, we have yet to identify the secreted factors that trigger inflammasome activation. We now hope to characterize these factors at the molecular level to help us better understand the host-pathogen relationship.

Image of infected cells

Our image was selected as the cover art for the Dec. 4, 2018 issue of the *European Journal of Immunology*.

Paradoxical effects of *Porphyromonas gingivalis*



P. gingivalis triggers activation of NLRP3 inflammasome, resulting in the processing of IL-1β. On the other hand, it secretes gingipains that degrade IL-1β.

[Reprinted with permission from *Eur. J. Immunol.*, doi: 10.1002/eji.201847658]

Dr. Suzuki completed his graduate school at the University of Tokyo, where he received his Ph.D. He performed postdoctoral research at the Institute of Medical Science at the University of Tokyo. He became Professor at the University of the Ryukyus in 2006. He joined TMDU as Professor of Bacterial Pathogenesis, Infection and Host Response in 2015.

Eur. J. Immunol., **doi: 10.1002/eji.201847658**

Making multifunctional molecules

Takamitsu Hosoya

Professor of Chemical Bioscience at TMDU

Q Your research focus is on synthesizing multifunctional molecules. Please give us a brief overview of your latest publication.

A: Many biological analyses, including clinical tests, rely on multifunctional molecules to detect specific proteins, pathogens, or other target analytes. Usually, one end of the multifunctional molecule binds with the target, while the other attaches to a fluorescently tagged marker. There are advanced tests that require a "trifunctional" molecule with three binding sites in order to work correctly. However, adding a third functional group with conventional synthetic methods has proven to be very difficult, since the three groups need to be added sequentially, and usually require protection/deprotection reactions that are specific to that particular combination. Along with my TMDU colleagues, we were able to develop a new unified synthetic method for easily combining any three arbitrary functional groups in the same multifunctional molecule.

Q Describe this new method for creating trifunctional molecules.

A: We start by synthesizing a triazido platform molecule. Using three azido groups of different types — such as one sterically-hindered aromatic, one standard aromatic, and one aliphatic — we can easily perform orthogonal cycloaddition reactions to attach the three desired azidophilic ligands with high yields. Since this process is modular, we can even choose in which order to add the azidophiles. The final product contains the three chosen functional groups at the vertices of an essentially triangular-shaped multifunctional molecule. In the published research, we demonstrated our method by synthesizing a molecule containing a biotin linker, a HaloTag ligand, and a fluorescent BODIPY moiety.

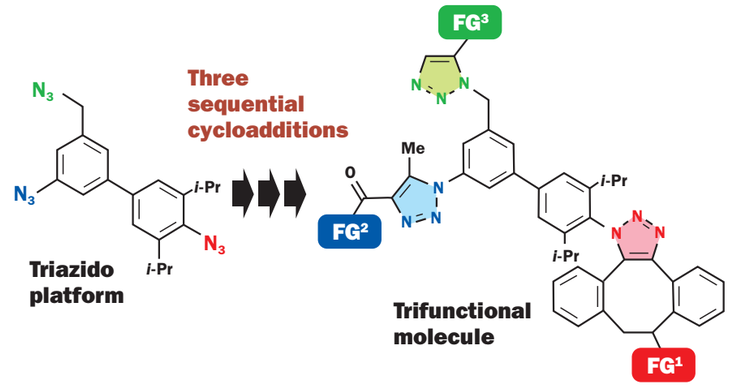
Q How does your research align with TMDU's focus areas?

A: We are very excited to contribute to TMDUs mission to provide new and better tools to clinicians and biologists to accelerate the delivery of rapid, accurate, and cost-effective health care diagnostic tests. We accomplish this by developing new synthetic methods to create a general platform for manufacturing multifunctional molecules. This advances TMDU's goal of enhancing public health and the quality of human life.

Q What are future directions for your research?

A: We hope to use this new method to prepare libraries of multifunctional molecules with various permutations of similar functional groups. Because we can now synthesize arbitrary combinations of groups with high yields, we can start applying them to the high-throughput screening of target analytes. This work may lead to new "point-of-care" rapid medical tests that can be performed cheaply with real-time results.

Combining three arbitrary functional groups



A facile strategy for the synthesis of trifunctional molecules involving three sequential selective triazole-forming reactions is proposed. This method exploits three kinds of mechanistically different azido-type-selective cycloadditions. Three different azidophiles could be efficiently connected to a triazido platform molecule with three types of azido groups in a consecutive manner, which rendered a practical trifunctional molecule readily available.

[Reprinted with permission from *Chem. Commun.*, doi: 10.1039/c8cc01195h]

Dr. Hosoya received his doctoral degree in Science at Keio University. From 1995 to 2005, he worked as Assistant Professor at Gifu University. He then worked as Associate Professor and Professor at Tokyo Institute of Technology. Since 2009, he has been Professor at TMDU. He also has been affiliated with RIKEN, and is now at the Center for Biosystems Dynamics Research there.

Chem. Commun., **doi: 10.1039/c8cc01195h**