Unraveling what it means to be alive

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Dive into BDR's intriguing research

Follow the heart

This time, we have Dr. Akane Sakaguchi from the Kimura Lab. I was surprised to learn that she makes decision based on her intuition, which was different from what I expected judging from her neat appearance. I guess we could call her an intuitive person. She is doing research on the heart, and the way she made the decision to do so was also very intuitive. I really enjoyed listening to her talk.

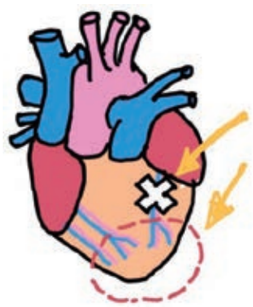
Yakushiji (HY) ▶ Where were you before coming to RIKEN?
Sakaguchi (AS) ▶ At the National Institute of Genetics (NIG). I received my Ph.D. in the lab of Dr. Yumiko Saga.
HY ▶ There are a lot of people at RIKEN BDR who used to work at NIG, aren't there?
AS ▶ That's right. At the time, if you wanted to do something working with mice, your options were limited.
HY ▶ Sure. By the way, where were you before that?
AS ▶ I was at the University of Hyogo.
HY ▶ Why there?
AS ▶ I found out that some universities made admissions decisions based only on the results of the National Center Test (for university admissions) after taking this test, and decided to submit my application to the University because I could.
HY ▶ That's a pretty muddled way to make life choices...
AS ▶ I'm often told that... (laughing).

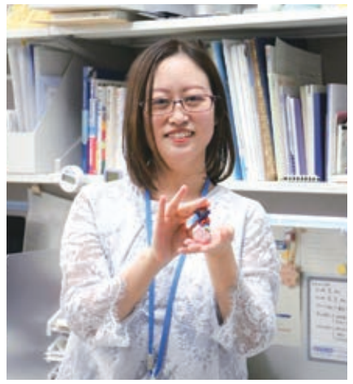
Conceiving ideas

HY ▶ I have the impression that you are more logical.
AS ▶ Sometimes people ask me, "Why did you decide to do that?" or tell me, "I've never thought of that."
HY ▶ Can you give me an example?
AS ▶ The goal of the laboratory I am working in now is heart regeneration, so we are looking for genes and substances that have some regenerative effects in the heart. I can't tell you all about it because I haven't been able to put it together in a paper, but there was one instance when I decided to try administering a certain substance on a whim...
HY ▶ Oh, I see.
AS ▶ Mammalian cardiomyocytes have the ability to proliferate before birth, and then the cells gradually lose this ability after birth. In humans, cardiomyocytes stop proliferating about one month after birth. It is thought that this occurs due to changes in the metabolic pathways. So, I tried treating the cardiac cells with a reagent that is commonly found in any laboratory. When I did this, I observed that the cardiomyocytes maintained their proliferative capacity, and this led to the start of one project.
HY ▶ What did your lab head, Dr. Kimura, say?
AS ▶ He just said, "I never thought of that."
HY ▶ Hahaha (laughing). I guess from Dr. Kimura's view, it was something he had never even thought to consider. But I think this kind of flexible mindset is important.
AS ▶ There was another time when we had a reagent that is known to regulate the cell cycle, and I decided to try administering this as well.
HY ▶ Yet another instance (laughing).
AS ▶ We have to generate myocardial infarction model mice by performing LAD ligation surgery. We had just received approval from the Institutional Animal Care and Use Committee for our project to practice the surgical procedure, and I thought it would be a shame not to do try anything afterward. I didn't need to do anything more than confirm that the procedure went well, but since I had the chance, I wondered what would happen if I tried administering the drug... There were several other candidate substances that I had submitted applications for, but we decided to try administering a well-stocked reagent in our lab. This led us to discover the regeneration of the injured heart, and this also led to another project that was later published as a paper.

Pulling out a heart from her pocket... !

AS ▶ This is the area that we do the surgery (suddenly revealing a plastic model of a heart).
HY ▶ I see... Wait. Where did that model come from?
AS ▶ I had it in my pocket. It's handy for explaining things.
HY ▶ I understand that, but you surprised me because it just appeared almost like magic.
AS ▶ The arteries are red and the veins are blue, and if you ligate this left anterior descending artery with a thread, areas beyond this point are not supplied with blood and oxygen and will become necrotic. All the cells in the ventricular wall beyond this point will die. After measuring cardiac function using echocardiography (or echo), we take a sample and make cross-sectional slices to prepare tissue sections. In the tissue section, you can see two holes, which are the left ventricle and the right ventricle. If you look at the muscle layer around those holes, because the cardiomyocytes cannot proliferate, the necrotic areas are thin and flimsy. The necrotic and flimsy areas have reduced cardiac function with weaker contractility, so we can measure cardiac function using echo waveforms.





Akane Sakaguchi

Research Scientist in the Lab for Heart Regeneration. After studying crystallized protein structures at the University of Hyogo and then heart development at the National Institute of Genetics, she joined RIKEN BDR in 2018 to carry out research on heart regeneration. She likes teas, perfumes, and cooking (but only side dishes that go well with alcoholic beverages).

Hideki Yakushiji

Business developer based in Kobe. He has a broad background in areas such as analytical chemistry, optics, biotechnology and IT. He is involved in a wide range of activities to assist in commercializing technologies and ideas born from academia, including setting up opportunities for idea sharing, finding investors, and strategic planning.

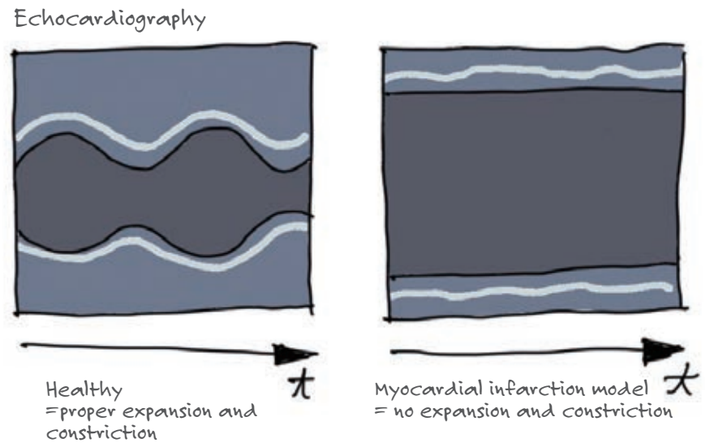
Confirmed using echo by the team leader

AS ▶ And the echo is done by the team leader, Dr. Kimura.
HY ▶ Really? By Dr. Kimura?
AS ▶ That's right. We perform echoes once a week, but it has to be a blind test. If the person performing the surgery or other experiments and the person performing the echoes are the same, they will know what kind of treatment has been performed on the mouse, so their hopes for a certain result may unknowingly come into play. If this happens, it is possible that the person will unconsciously take a picture of the area with the least amount of damage when conducting the echo. We need to avoid this.

For example, if you do an echo above the area where the surgery was performed, of course, the cardiomyocytes there are all healthy, so the waveform of the heart contraction is beautiful and the cardiac function comes out well. But if you know what the experiment (or hypothesis) is about, you will unconsciously want to take picture there, so you need to separate the person who did the experiment from the person who took the echo. It's absolutely essential that the person performing the echo does not know what experiment was done. In addition, Dr. Kimura was the only person with the right skills.
HY ▶ What do you mean?
AS ▶ The supplier of that echo instrument taught us how to use it and where to aim the ultrasound, but I was never able to master it...
HY ▶ Is it that difficult?
AS ▶ It is difficult to find the right angle to apply the echo device. When you do an operation, the heart is tied up with a thread, and this thread can adhere to the ribs and lungs, resulting in the heart being positioned in an abnormal direction. For example, if the heart positioning is tilted, you can't take a good picture of it at a normal angle. That's where experience comes into play, so I ask Dr. Kimura to help me.
HY ▶ I can see how that requires some skill.
AS ▶ Actually, having three people involved—one for the surgery, one for the echo and one for the analysis—would be ideal. And each person would not know what the others did. Even for the analysis, it's not impossible to extract the part of the analysis that produces the intended result.
HY ▶ How long do you carry out observations for?
AS ▶ It's usually anywhere from a few months to six months. I, myself, originally did experiments in embryology which spanned about seven days, so I've gotten much more patient (laughing).

Obtaining objective data

AS ▶ In a model of myocardial infarction, the necrotic area becomes thin and flimsy. However, if we administer a certain substance, this area will begin to regenerate (through the proliferation of cardiomyocytes), and the cardiac function will improve.
HY ▶ So you are observing this using the echo, right?
AS ▶ By measuring the thickness of the myocardium and the differences between diastole and systole, we can evaluate how well it is recovering. If the echo device is not positioned properly, then the echo image will not be taken correctly.
HY ▶ Wow, that makes a big difference.
AS ▶ Yes. The heart of a mouse is no more than two centimeters in size, so the data changes depending on subtle differences in how the echo device is applied. Therefore, it's very important to know where and at what angle to apply the echo device, and eliminate arbitrary factors. In addition, it is also very important to know what part of a particular echo image to quantify.
HY ▶ Really?
AS ▶ We take several datasets from the same individual, but the data changes depending on how it was taken, so it's the person doing the analysis who decides what part of the data will be used. Here, too, it is necessary to decide which points to analyze so that we are obtaining the data properly. Therefore, if you are influenced by your hypothesis about what results would be desirable, you will end up with skewed data.
HY ▶ I see. That's why it's best to have separate people doing the surgery, echo, and analysis.
AS ▶ That's right.



As her heart desires

HY ▶ So, why did you decide to study the heart in the first place?
AS ▶ I chose the heart mainly because it was beating. When you culture cardiac cells, you get clumps of cells called colonies forming and those colonies are beating. Among the colonies, some are small and beating independently, some are large colonies with several concentric areas beating in concert, and some are large colonies beating in separately toward each center. Seeing that was really interesting. I think we also cultured cells from the liver, which was attached to the heart when it was removed, and the thigh muscle, which was easy to remove because it was large, but I don't remember those results at all. I think the heart left a big impression on me.

So, I was looking for a laboratory in the Kanto region, which close to my parents' home, where I could do heart research. And this led me to Dr. Saga at the NIG. I met her for the first time at a graduate school information session at The University of Tokyo. After talking to her, I knew I liked her and contacted her about working in the lab without really looking into the details of her research.

As I am telling you this, I am realizing that it quite a muddled decision even for me (laughing).
HY ▶ Whatever it may be, I have a feeling that you're living your life following your heart (laughing).

Postscript

I really admired how she trusted her instincts to at least give something a try, where other people would over think things. It's not uncommon as adults to ponder this and that, and in the end not doing anything. The fact that she actually managed to collect data afterward is impressive.

Read other interviews ▶

BDR Research Highlights

Research highlights articles and press releases between July 2021 to October 2021.
Read these and other articles on the BDR website.

01

Basement membrane underpins tissue interactions in the skin

The extracellular matrix (ECM) is a complex scaffold composed of hundreds of regionally specialized proteins that performs multiple roles, including supporting and organizing cells in organs and conveying biochemical signals that control their growth and differentiation. Between the two main layers of skin—the outer epidermis and the inner dermis—lies a thin sheet of ECM called the basement membrane (BM), serving as a barrier and storehouse for growth factors but how it operates in interactions between tissues has remained unclear. By using gene-expression analyses and imaging techniques, Hironobu Fujiwara (Lab for Tissue Microenvironment) and his colleagues have systematically characterized the cellular origins, molecular identities and distribution patterns of molecules in the BM of mouse skin. They discovered that, within this tissue interface, there were a diverse series of regionally specialized niches, each impacted by the identity of nearby cells. A better understanding of these structures could also inform the development of tissue engineering strategies for treating hair loss and other skin conditions.

Tsutsui K, Machida H, Nakagawa A, et al. *Nat Commun* 12, 2577 (2021)

02

Genome editing meets marsupials

The opossum, which is thought to be the ancestor of all marsupials, has a variety of characteristics that are not found in other mammals. It develops without a functional placenta, and pups are born prematurely. Like humans, but not other non-marsupial mammals, it gets skin cancer simply by exposure to ultraviolet light. Also unlike other mammals, newborn opossum pups with spinal cord injuries have the ability to naturally heal themselves. Because of these unique characteristics, studying marsupial biology is gaining interest. Now, a research team led by Hiroshi Kiyonari (Lab for Animal Resources and Genetic Engineering) has taken advantage of new gene editing and reproductive technology to produce genetically modified opossum models for future analyses of the underlying genetics of unique marsupial characteristics.

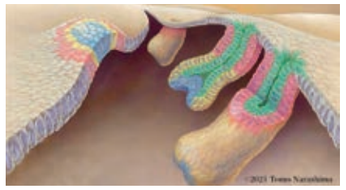
Kiyonari H, Kaneko M, Abe T, et al. *Curr Biol* 31, 3956-3963.e4 (2021)

03

A telescopic model of the development of hair follicles

Like other adult tissues in our bodies, hair follicles are regenerated from adult tissue stem cells. But the developmental origin of hair follicle stem cells has been largely unexplored because of the lack of markers for identifying and tracking each cell lineage. A team led by Hironobu Fujiwara (Lab for Tissue Microenvironment) has created time-lapse videos of hair follicle development over several days in cultured skin samples taken from mouse embryos. Playing these videos in reverse allowed them to trace the fate of cells in developed hair follicles back to their earliest origins. The analysis revealed that hair follicle stem cells originate from a specific zone in the outer ring of the early placode. They also showed that cell proliferation caused the two-dimensional concentric rings in the placode to transform into a series of cylindrical compartments arranged like the components of an extendable telescope—leading the researchers to dub this type of developmental process the telescope model.

Morita R, Sanzen N, Sasaki H, et al. *Nature* 594, 547-552 (2021)

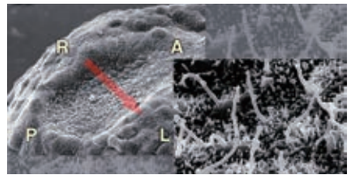


04

mRNA degradation induced by fluid flow breaks left–right symmetry in vertebrates

The first step in establishing left–right asymmetry in an embryo is the left–right breaking event. In fish, frog and mouse embryos, this begins with hair-like cilia generating fluid flow that runs leftwards. This fluid flow then downregulates *Dand5* mRNA on the left-hand side of the embryo. Now, Hiroshi Hamada and his team, together with other collaborators, have investigated the mechanism of *Dand5* mRNA suppression and revealed that there is an evolutionarily conserved 200-nucleotide region of the proximal 3'-untranslated region of *Dand5* mRNA. This conserved region is specifically recognized by the RNA-binding protein Bicc1, which recruits and binds to the mRNA-degrading enzyme Ccr4-Not deadenylase complex, resulting in *Dand5* mRNA degradation on the left side of the node.

Minegishi K, Rothé B, Komatsu KR, et al. *Nat Commun* 12, 4071 (2021)



05

Supercomputer simulations reveal how protein crowding in cells impacts interactions

The initial stages of drug development usually involve simulating the interactions between a molecule and its target protein. Although these simulations can suggest a drug is effective, the interaction can frequently fail to live up to its promise when tested on living cells. Yuji Sugita (Lab for Biomolecular Function Simulation) and his colleagues wanted to make simulations more accurate by accounting for cells' normally crowded environments. To do this, they developed a highly optimized software program called GENESIS for use with supercomputers and conducted microsecond-scale simulations of the interaction of an enzyme (c-Src kinase) with an inhibitor (PP1) in the presence of different concentrations of bovine serum albumin (BSA). They found that crowding by BSA reduced the amount of PP1 able to reach the enzyme by physically blocking its access and also by weakly and non-specifically interacting with it. Mikako Shirouzu (Lab for Protein Functional and Structural Biology) validated their results by performing laboratory tests using the actual proteins in similar conditions.

Kasahara K, Re S, Nawrocki G, et al. *Nat Commun* 12, 4099 (2021)

06

Persistent current-mode operation of NMR magnet with high-temperature superconducting joints

Superconducting nuclear magnetic resonance (NMR) magnets made from coils of wire that have been cooled to extremely low temperatures can sustain and maintain continuous current flow without being connected to an external power supply. NMR instruments makes use of the powerful magnetic field generated by NMR magnets to obtain information on structural and molecular dynamics of biological macromolecules or other materials. In 2018, Yoshinori Yanagisawa (Lab for Functional Ultra-High-Field Magnet Technology), Toshio Yamazaki (Lab for NMR Engineering and Structural Science) and their colleagues succeeded in developing the world's first NMR instrument with a magnet incorporating high-temperature superconducting (HTS) joints made from REBCO. They have now demonstrated for the first time that their NMR magnet with HTS joints can maintain and operate the instrument with a stable persistent current for an extended period (at least two years) with a magnetic field of 400 megahertz (MHz).

Yanagisawa Y, Piao R, Suetomi Y, et al. *Supercond. Sci. Technol.* 34, 115006 (2021)

Peek-a-LABoo

The RIKEN BDR-Otsuka Pharmaceutical Collaboration Center (RBOC) is one of the two collaboration centers at BDR and is a partnership with Otsuka Pharmaceutical Co. Ltd, a Japanese pharmaceutical and nutraceutical company. Launched in 2016, the RBOC is aspires to conceive novel and original results through collaborative research investigating disease mechanisms based on developmental, regenerative and systems biology approaches. In June 2020, the Neural Organogenesis Research Program headed by Hideya Sakaguchi was established under the RBOC to accelerate research specifically in areas of neurobiology and the understanding of neurodegenerative diseases. Originally trained as a neurologist, he developed an interest in generating three-dimensional brain tissues and joined the laboratory of the late Yoshiki Sasai at the former RIKEN Center for Developmental Biology (CDB) for his Ph.D. thesis research, where he gained experience generating brain organoids. We talked to Dr. Sakaguchi to find out more about the laboratory.

Neural Organogenesis Research Program, RIKEN BDR-Otsuka Pharmaceutical Collaboration Center

Q How many people are in the laboratory?

A There are currently three people. One research scientist, one person who fills the role as both lab assistant and technical staff, and me the research leader. We hope to welcome more researchers in the near future.

Q What are the main research themes of the laboratory?

A We are interested in shedding light on the mechanisms underlying the self-organizing development of the hippocampus using hippocampal organoids differentiated from pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). As we can also generate hippocampal neurons, we would like to also carry out applied research to explore the disease mechanisms of psychiatric diseases such as schizophrenia that are thought to be caused by abnormal function of hippocampal neurons.

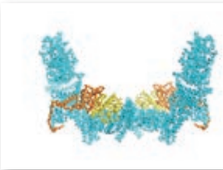
Q What are some strengths of the laboratory?

A A major strength of our lab is having honed the techniques for inducing differentiation of PSCs into hippocampal organoids as well as hippocampal neurons. While many groups have reported the successful generation of cerebral organoids from PSCs, very few groups, if any, have reported being successful in generating hippocampal organoids. We also have experience in inducing differentiation of cerebral, spinal cord and choroid plexus tissues from PSCs by recapitulating early developmental conditions in cell culture systems. We plan to apply these techniques to unveil fundamental developmental processes of the brain as well as neurological and psychiatric disease mechanisms.

Q Did the laboratory face any challenges trying to open during the early phase of the COVID-19 pandemic?

A Setting up the lab with the pandemic in full swing, we experienced delays in construction of the lab and also had difficulties obtaining necessary research equipment for the lab due to supply shortages, and as a result it took a while to get our lab in order. It was only around March 2021, that we were able to really get the ball rolling with our research projects.

On the cover!



Light blue hammock?

This is a graphic model of the three-dimensional structure of the DOCK-ELMO-Rac protein complex. DOCK (cyan) is involved in cell movement and maturation, while ELMO (orange) is known to regulate DOCK function. ELMO helps DOCK bind to the signaling protein Rac (yellow) to trigger downstream responses of DOCK activity. The biological activities in the body are maintained by the binding and unbinding of proteins to other proteins.

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