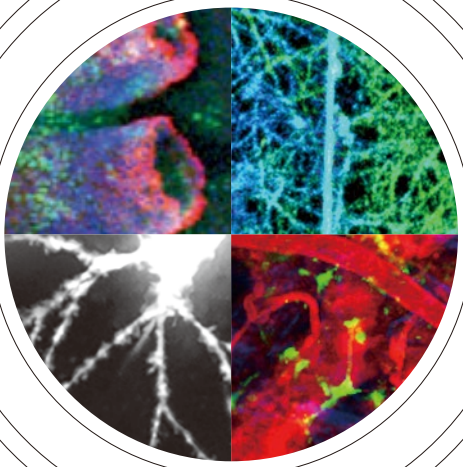
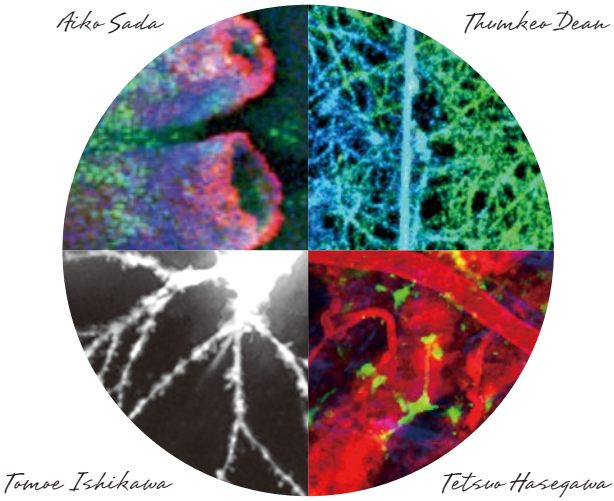


2020 NIKON JOICO AWARD

Through a microscope

Expand your world

Into the wide expanse



Microscopic images based on science have both artistic and academic qualities, and we believe that people who come into contact with these images will find new value and creativity. We want more people to experience the world as seen through a microscope, and it is with this in mind that we launched the NIKON JOICO AWARD in 2019.

The world seen through a microscope is not an imaginary world, but one based on science.

Even though this world differs through the perception of one researcher to another, we believe that the cutting-edge science hidden in microscope images—and above all, in the world of science—that researchers are working on with excitement, will stimulate viewers and lead them to a new world.

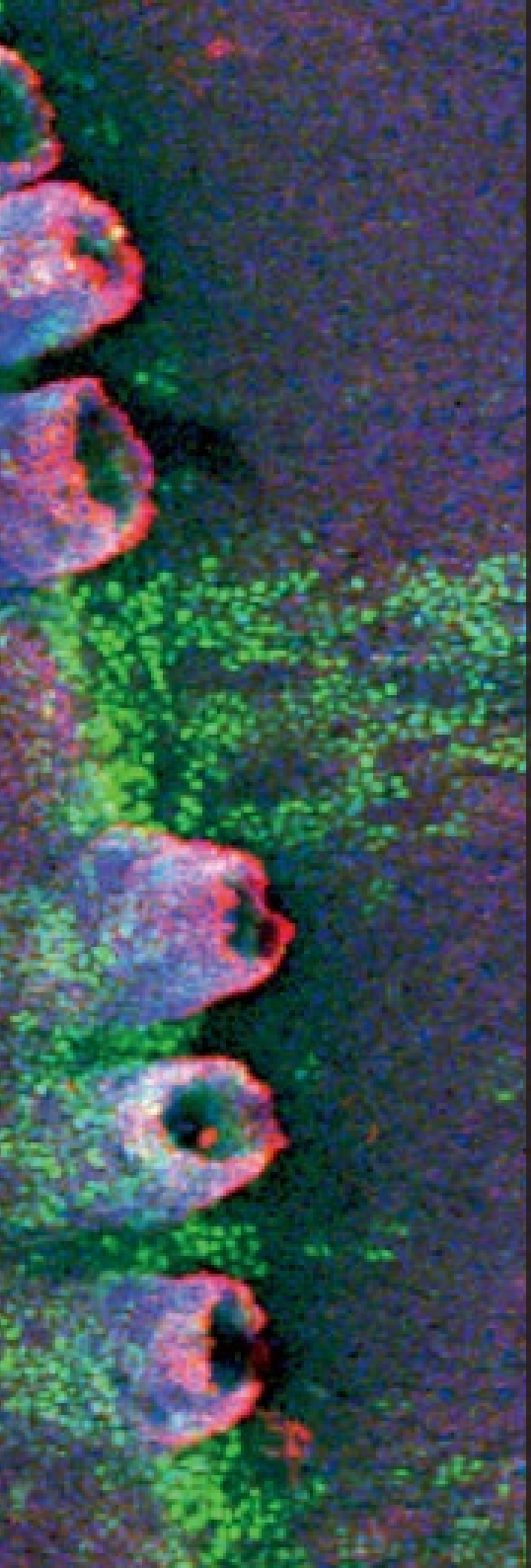
We will continue our activity,

"expanding the world through microscopy into the wide expanse"

as a place for researchers to communicate science and art through microscopic images and for visitors to the NIKON JOICO AWARD to experience the world as seen through microscopes.

Compartmentalized stem cell populations in the skin epidermis

The regular pattern formation and compartmentalization of heterogeneous stem cell populations in the skin was discovered.



First Prize JOICO Award

Aiko Sada

Associated Professor
Laboratory of Skin Regeneration and Aging International Research Center for Medical Sciences, Kumamoto University

Comments from the award recipient:

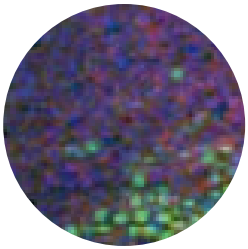
Thank you very much for choosing me for the first prize JOICO Award of the NIKON JOICO AWARD.

One of the most important things I do in my research is to look carefully under the microscope. I still remember a "wow" moment when I saw this picture for the first time. A "small notice" based on microscopic observation led to an unexpected discovery through imagination and patiently repeated experiments.

I would like to further explore these beautiful and mysterious skin stem cells.

Mouse skin

Detailed description	:	A tail skin derived from the H2B-GFP tet-off mouse Green: H2B-GFP (cells with a low division frequency) Red: K14 (an epidermal cell marker) Blue: Stained nuclei
Observation method	:	Confocal microscopy, inverted, fluorescence
Magnification	:	10x
Year	:	2014
Microscopic data	:	Z-stack image



Compartmentalized stem cell populations[1] in the skin epidermis

Brief overview of this research

The epidermis, the outermost layer of the skin, plays essential life-sustaining roles such as the prevention of water loss and the repair of damages, as well as functioning as a barrier against the external environment. In this study, we utilized genetic mouse tools and demonstrated that, in the mouse epidermis, actively-dividing cells that had been considered not to be stem cells—as well as slowly-dividing cells—act as stem cells. These two distinct epidermal stem cell populations[2] exhibited a compartmentalized localization pattern in the skin tissue. The epidermal stem cell compartment is likely involved in wound healing, cancer, and aging. This paper was selected for F1000Prime and received high commendation in the skin research field.

Paper

Sada A, Jacob F, Leung E, Wang S, White BS, Shalloway D and Tumbar T
Defining the cellular lineage hierarchy in the interfollicular epidermis of adult skin.
Nature Cell Biology. 2016, 18(6), doi: 10.1038/ncb3359

Introduction

Tissue stem cells are special types of cells capable of generating mature, differentiating cells while they maintaining themselves by self-renewal for the entire lifetime. They have attracted attention as an excellent cell source for regenerative medicine owing to their high self-renewal capacity. Abnormity of tissue stem cells leads to the impairment of functions of organs and tissues, which may cause disease development, tumorigenesis and aging. Thus, accurate identification of tissue stem cells to understand their properties and control mechanisms is an important first step toward the application of them for regenerative medicine and the treatment of cancers and aging.

Here, we would like to introduce our research relating to microscope images for this award, focusing on the mystery of stem cells. We successfully visualized the dynamics of stem cell divisions within skin tissues.

The development of regenerative medicine using epidermal stem cells and remaining questions

Skin epithelial tissue consists of the interfollicular epidermis and its appendages such as hair follicles.[3] The stem cells in the interfollicular epidermis and hair follicles play roles in homeostatic turnover and repair of damaged tissue (Figure 1). Epidermal and hair follicle stem cells function independently during homeostasis but have plasticity as well: They can respond to skin damage and contribute to each other's cell lineage by transforming the differentiation fate.

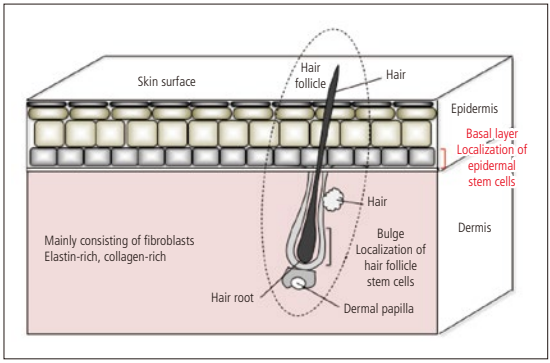


Figure 1. Skin structure and stem cells

Since the world's first successful burn treatment with the *in vitro* culture of human epidermal stem cells and autograft in the 1980s, skin regenerative medicine has been greatly developed. In 2017, it was reported that transplantation of epidermal sheets containing transgenic stem cells[5] to a patient with epidermolysis bullosa,[4] an intractable hereditary disease, led to a successful result where skin was regenerated over 80% of the whole body.

Please see Glossary on p.10 (each superscript number corresponds to the number for each term in it)

However, the already proved clinical usability so far is only for the regeneration of epidermis, the most superficial layer of the tissue, and it is still technically difficult to completely regenerate complicated skin structure including connective tissue.[6] In addition, **the location where epidermal stem cells localize in the skin tissue of living bodies and how they behave** remained unclear. It is vital to understand the basic characteristics of epidermal stem cells in order to achieve stable and succsessful regeneration of skin.

The discovery of compartmentalization of epidermal stem cells with distinct division frequencies

It had been considered that tissue stem cells prevent aging and tumorigenesis through the suppression of the division frequency to minimize the effects of DNA's damage and telomere[7] shortening. On the other hand, actively dividing cells had been regarded as progenitor cells[8] without the stem cell ability.

In this study, Sada et al. reported that slowly- and actively-dividing cells function as two distinct, independent stem cell populations in the mouse interfollicular epidermis (*Nat Cell Biol*, 2016). In the H2B-GFP tet-off system[11] that visualizes cells with various division frequencies, the transcription of the histone H2B-GFP takes place under the control of the epidermal cell-specific K5 promotor in the absence of doxycycline,[12] resulting in the accumulation of GFP proteins in the cells. When doxycycline is administered to mice, the transcription is suppressed and the amount of the GFP proteins decreases by half every cell division. Thus, actively dividing cells gradually lose the GFP signal, while infrequently dividing ones retain the high-level GFP signal for a prolonged period and are detected as label-

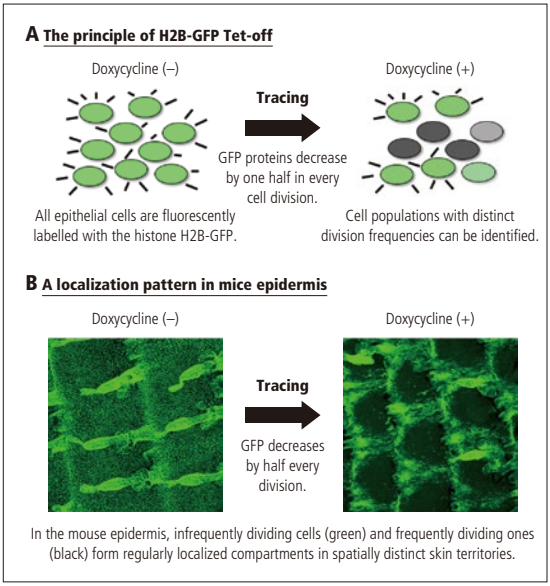


Figure 2. A system for visualizing epidermal cells with distinct division frequencies

retaining cells (LRC) (Figure 2). Interestingly, they found that the two distinct epidermal stem cells with distinct division frequencies exhibited a regularly localized, compartmentalized pattern in the skin tissue.

Originality and future perspective of this study

In the traditional stem cell models, it had been considered that tissue stem cells avoid stress and damage associated with cell division by lowering the division frequency. However, we found that cells which are actively dividing—as well as those with a low division frequency—act as stem cells in the mouse epidermis, and we discovered the compartmentalization of these stem cells in the skin tissue. It is suggested that the epithelial cell compartment may have a connection with **the skin's damage-repairing mechanism,** and with **skin diseases such as cancers, as well as the aging process**, but the importance of its existence is still unknown.

There is a need to understand stem cells and environmental factors comprehensively and to control them for functional skin regeneration. Our research will provide a basis for understanding and engineering the three-dimensional skin structure, just like those of living bodies (Figure 3).

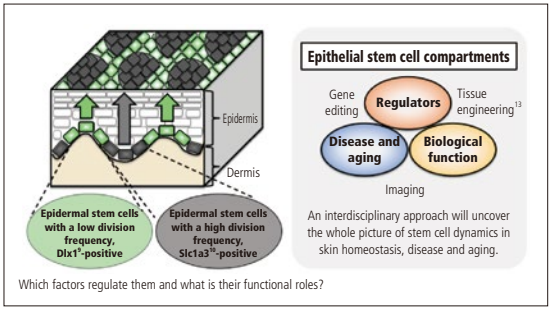


Figure 3. Future perspective: Elucidating the mechanisms of tissue regeneration and dysfunction by focusing on the three-dimensional skin-structure and heterogeneous stem cell populations.

Glossary

1. Epithelial stem cell

Epithelium is a layer of cells covering the surfaces of the body or tissue. Skin consists of epidermis (epithelium tissue) and dermis (connective tissue). Intestinal epithelium, corneal epithelium, and oral mucosal epithelium are all categorized as epitheliumal tissues. Stem cells existing in the epithelium tissue are collectively called epithelial stem cells.

2. Epidermal stem cell

Stem cells which generate skin epidermis. They play roles in epidermal turnover and damage-repairing. Epidermal stem cells localize at the basal layer and move to the upper layers with differentiation.

3. Hair follicle

A hair-surrounding organ. A hair follicle has a hair, an inner root sheath, a companion layer, and an outer root sheath structured in layers from its center outwards. Hair follicle stem cells (hair-generating stem cells) localize at the region called the bulge in the outer root sheath.

4. Epidermolysis bullosa

A skin disease in which epidermis and dermis dissociate. It generates blisters and erosions due to weak irritation. It is caused by genes defective in hemidesmosome-related proteins, which function for adhesion between epidermis and dermis.

5. Transgenic stem cell

It was reported by M De Luca et al. in *Nature* in 2017 that the treatment of epidermolysis bullosa was successful through the transplantation of cultured epidermal stem cells into which laminin genes were introduced by using genome-editing technique.

6. Connective tissue

A supporting tissue infilling the inter-tissue region. For example, skin dermis is a connective tissue, consisting of fibroblasts and an extracellular matrix such as collagen and elastin.

7. Telomere

A structure located at the end of chromosomes. It is known that its length shortens with cell divisions. The length of telomeres is considered important for the control of aging and tumorigenesis.

8. Progenitor cell

It is a relatively undifferentiated cell which is generated from stem cells but does not have the self-renewal ability like them. Progenitor cells divide only a limited number of times and are comitted to terminal differentiation.

9. Dlx1

A gene identified by the microarray analysis as a marker of epidermal stem cells with a low division frequency. It belongs to the Dlx family of transcriptional factor.

10. Slc1a3

A gene identified by the microarray analysis as a marker of epidermal stem cells with a high division frequency. It functions as a glutamate transporter and has important functions for the metabolic control of stem cells and cancer cells.

11. H2B-GFP tet-off system

Transgenic mice in which cell division frequencies can be visualized. Cell nuclei are stably labeled by the green fluorescent proteins (GFP) fused with histone H2B. The number of cell divisions taking place during the suppression of transcription by doxycycline can be detected as the GFP fluorescence intensity.

12. Doxycycline

A derivative of tetracycline and an agent to terminate the transcription in the tet-off system. It prevents a tetracycline-dependent transcription activation factor tTA from the binding to the promotor sequence TRE (tetracycline responsive element).

13. Regenerative engineering

A collaborative field of medicine and engineering aiming at regeneration of tissue damage using biomaterials such as cells and the extracellular matrix. It attracts attention as the next generation medicine.

Q & A

Q1 Why did you focus on the difference in the cell division frequency?

It had been known that cells with low division frequencies localize at the bulge region and behave as stem cells functioning for a long period in hair follicles in the skin. In addition, it had been suggested that cells with low division frequencies act as cells with special functions in other tissues. However, in the skin epidermis, there were many unclear points about the true nature of the tissue stem cells, and there were very few studies that focused on the difference in division frequency, so I thought that something interesting might be found.

Q2 What happens to the balance and compartmentalization of two types of epithelial stem cells with differing division frequencies upon skin damage repair and skin diseases such as cancers?

That is exactly our current research target. It is known that the two types of epidermal stem cells have potential to contribute to each other's cell lineage upon skin damage. Epidermal stem cells actively divide in the cases of cancers and skin inflammation, but the cell division ability decreases with aging. I think that change of the balance and compartments of the two distinct stem cells in various situations will lead to the understanding of the biological significance of the existence of two types of stem cell populations in the skin epidermis.

Q3 Are there any known mechanisms controlling the compartmentalization of epithelial stem cells?

It has been reported that signals from the dermis are one of the important regulators for the compartmentalization of epidermal stem cells. For example, the size of compartments changes depending on the Wnt signal in the dermis. Moreover, autonomous factors of epidermal stem cells, blood vessel patterns, the extracellular matrix, and the mechanical environment are considered to function for controlling the stem cell compartments and function, but detailed mechanisms are still unknown.

From award panel members

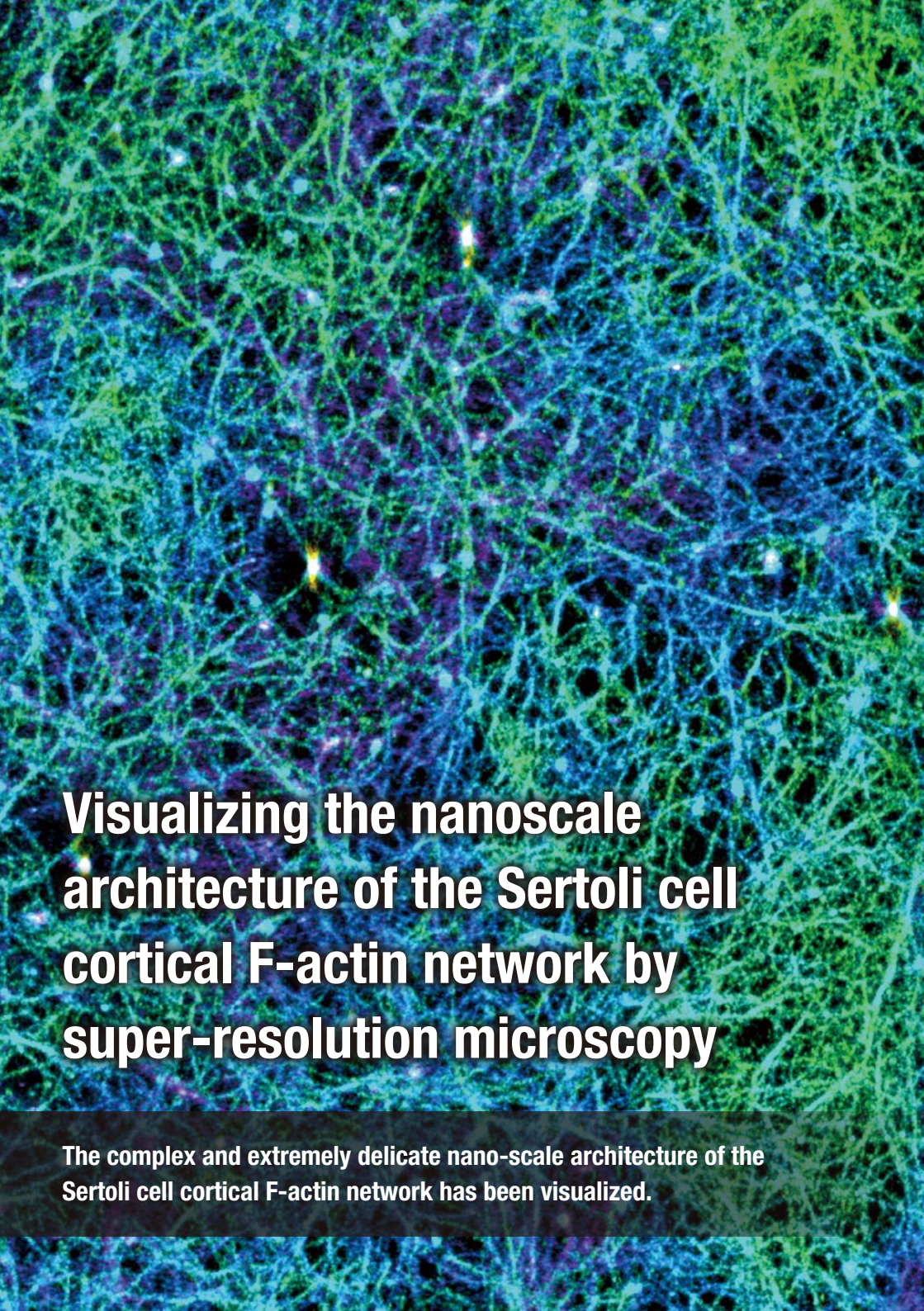
An amazing depiction of creepy structures that seem to be crawling out of the ground. It is of great academic value.

In addition to being beautiful, it is also valuable from an academic point of view.

The concept of visualizing the division frequency of stem cells by the fluorescence intensity of GFP deserves particular mention. The regular pattern of localization and compartmentalization of two types of stem cells with different division frequencies is not only scientifically valuable but also beautiful and artistic.

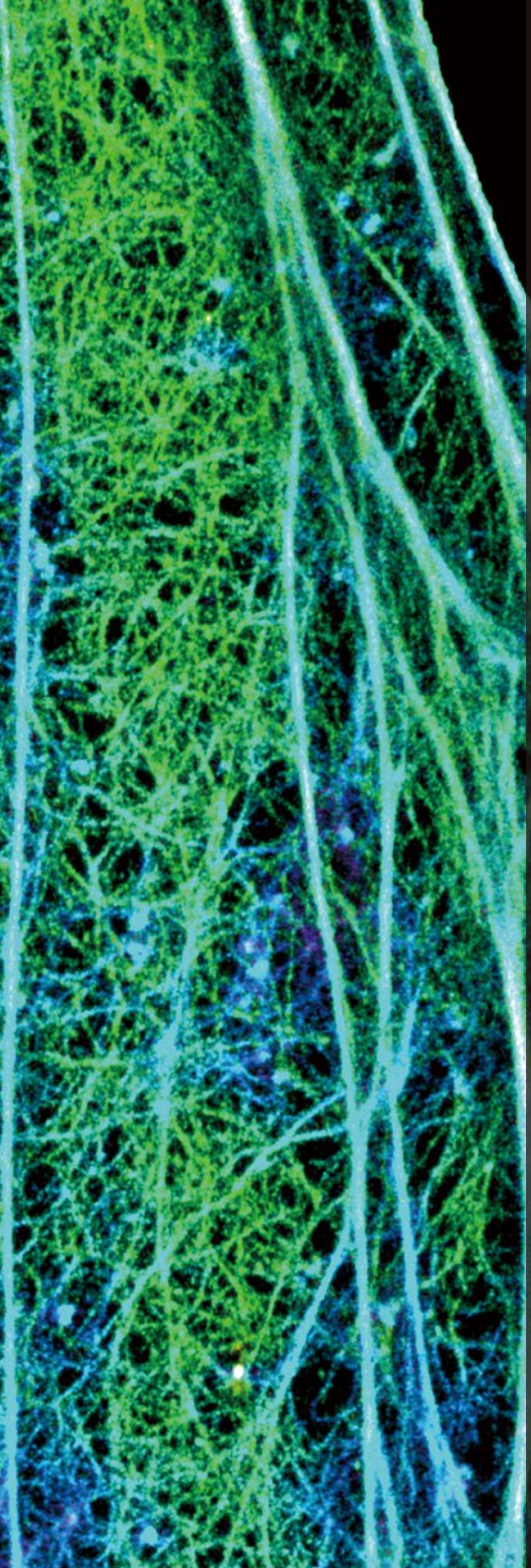
From an academic point of view, the use of GFP-labeled probes for histone proteins, which stably bind to chromatin, to elucidate the dynamics of stem cells in the skin—a fact that was previously unknown—is highly commendable.

It is a groundbreaking research that defies the existing theories and reveals the compartment of epithelial stem cells. The work is fantastic and evokes a sense of life.



Visualizing the nanoscale architecture of the Sertoli cell cortical F-actin network by super-resolution microscopy

The complex and extremely delicate nano-scale architecture of the Sertoli cell cortical F-actin network has been visualized.



/ Runner-up Prize /

Dean Thumkeo

Associate Professor
Department of Drug Discovery Medicine,
Graduate School of Medicine, Kyoto University

Comments from the award recipient:

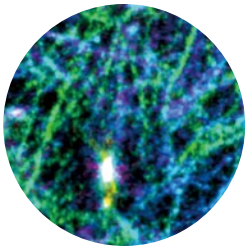
I feel very honored on this occasion to be presented with this runner-up prize of the NIKON JOICO AWARD. Thank you very much.

In the beginning of my research career, I used conventional optical microscopes to observe actin filaments in cell cultures. At that time, I was only able to see clearly just the thick bundles of actin, which are composed of multiple to several hundreds of actin filaments. However, by using a super-resolution microscope, it became recently possible, as shown in this work, to visualize individual actin filament and the mysterious three dimensional nano-scale architecture of actin filament.

I will continue conducting more imaging experiments and striving on to further elucidate unprecedented actin structures in the future.

Primary-cultured Sertoli cells derived from mouse testes

Detailed description	: Alexa647-Phalloidin staining Z-axis direction: Pseudo-color imaging (–300 to 300 nm)
Observation method	: Super-resolution microscopy, inverted, fluorescence
Magnification	: 100x
Year	: 2015
Microscopic data	: Still image



Visualizing the nanoscale architecture of the Sertoli cell[1] cortical F-actin network by super-resolution microscopy

Brief overview of this research

It is known that spermatozoa, which have a very unique morphology, are generated from the small spherical spermatids in the seminiferous tubules of testes. Previous studies have elucidated the close and critical interaction between spermatids and Sertoli cells, which are supportive cells existing in seminiferous tubules, in normal spermatogenesis. It is known that the cell-cell adhesion formed between spermatids and Sertoli cells is based on intercellular adhesion molecules[2] and the underlying[3] cortical cytoskeletal[4] actin. However, the actual structure of Sertoli cell cortical actin filament and how it is formed and maintained were almost unknown. In this study, we employed the super-resolution imaging STORM[5] and single-molecule live imaging technology. As the results, we found that mDia1/3 proteins[6] regulate normal spermatogenesis through actin polymerization and the generation of contractile actomyosin[7] continuous with the fine actin filaments network in Sertoli cells. These strucutures are indispensable for proper formation and maintenance of the adhesion between Sertoli cells and spermatids.

Paper

Sakamoto S*, Thumkeo D*#, Ohta H, Zhang Z, Huang S, Kanchanawong P, Fuu T, Watanabe S, Shimada K, Fujihara Y, Yoshida M, Ikawa M, Watanabe N, Saitou M, Narumiya S#

*Equal Contribution, #Corresponding Author

mDia1/3 generate cortical F-actin meshwork in Sertoli cells that is continuous with contractile F-actin bundles and indispensable for spermatogenesis and male fertility. *PLoS Biology* 2018, 16(9), doi: 10.1371/journal.pbio.2004874

It is known that spermatozoa, which have a unique shape, are produced from small, spherical spermatids in the seminiferous tubules of testes.It was previously shown that close interaction between spermatids and Sertoli cells, which are supportive cells existing in seminiferous tubules, is critical for normal spermatogenesis. The cell-cell adhesion formed between spermatids and Sertoli cells is based on intercellular adhesion molecules and the underlying cortical cytoskeletal actin. However, the details of the structure of the underlying cortical actin on the Sertoli cells side and how this structure is formed and maintained were largely unknown. Moreover, the physiological importance of such cortical actin was also unclear.

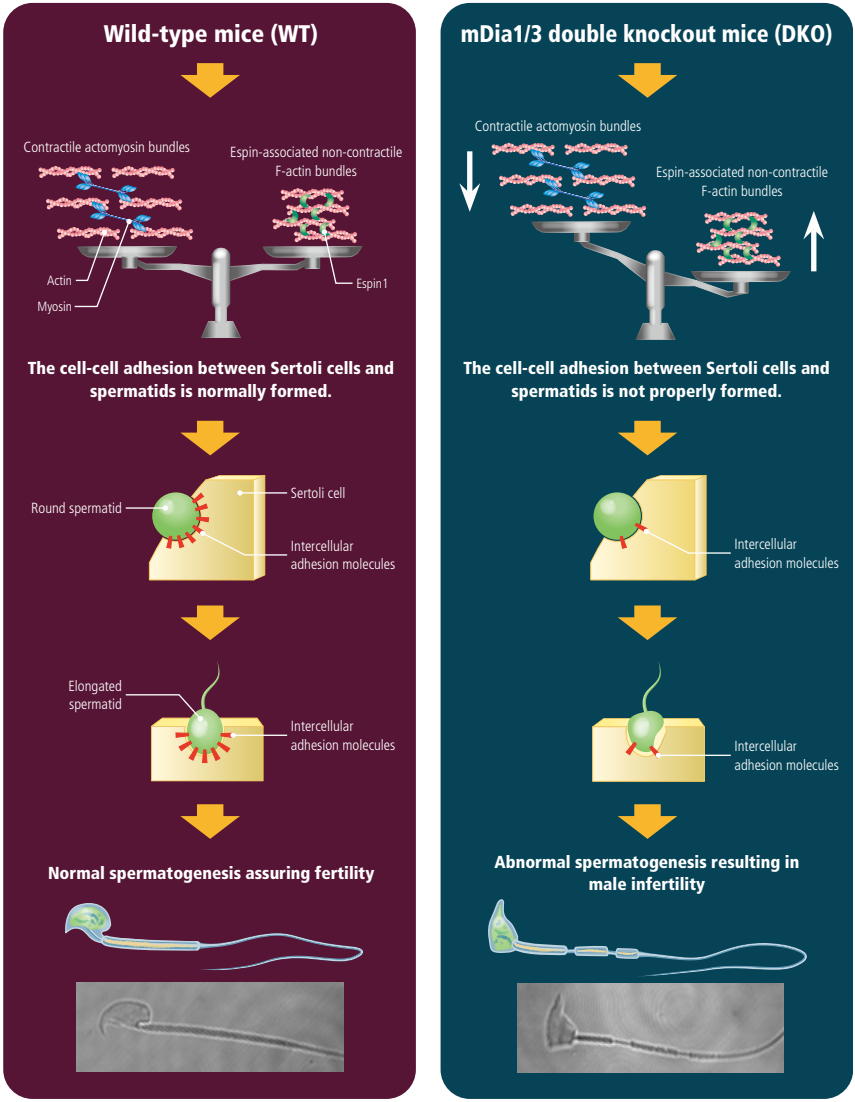
In this study, We've found that the mDia1/3 double knockout male mice were infertile. Further observation of spermatozoa and seminiferous tubules revealed that impaired spermatogenesis was the cuase of male infertility. mDia1 and mDia3 expression were both found in Sertoli cells,suggesting that the cause of male infertility of the mDia1/3 double knockout mice is due to abnormalities in Sertoli cells, but not spermatids. Subsequently, in order to clarify the functions of mDia1/3 in Sertoli cells, filamentous actin in primary-cultured Sertoli cells was observed using a super-resolution microscope based on stochastic optical reconstruction microscopy (STORM) at the XY resolution of ~20 nm and the Z resolution of ~50 nm. This revealed that filamentous actin form a meshwork structure with a mesh size of ~ 100 nm that exists beneath the Sertoli cell membrane and the density of this actin meshwork structure was significantly reduced in the double knockout of mDia1/3. Furthermore, to observe the dynamics of the actin meshwork, high-speed and high-resolution live imaging with spinning disk confocal microscopy for fluorescently labeled filamentous actin in primary-cultured Sertoli cells was conducted. As a result, two components were found to exist in the meshwork and they polymerized at the different rate (0.4 μm/s and 1.3 μm/s on average). Moreover, filamentous actin with the faster polymerization rate (1.3 μm/s) specifically impaired in the mDia1/3 double knockout cells. The higher polymerization rate was similar to the rate of actin polymerization[8] by mDia3, calculated from the single-molecule imaging for mDia3. These findings together indicate that mDia1/3 are involved in the formation and maintenance of meshwork actin structure.

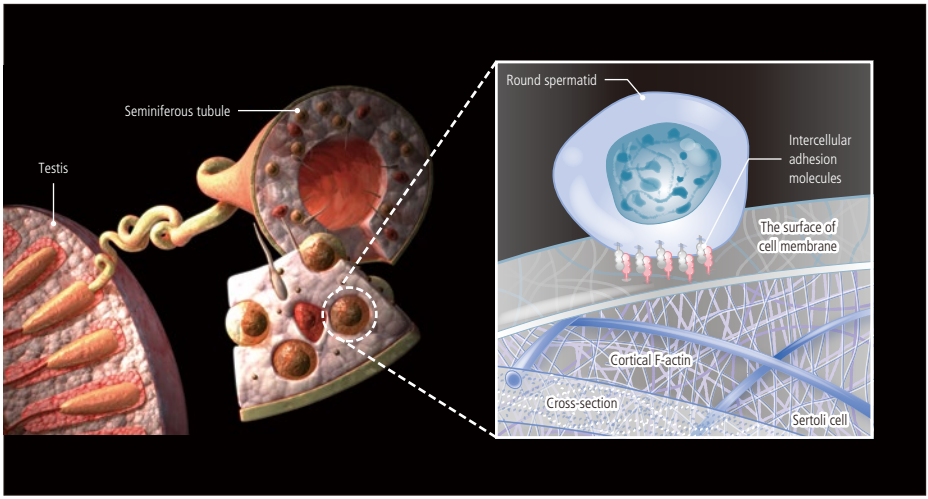
Please see Glossary on p.18 (each superscript number corresponds to the number for each term in it)

The functions of mDia1/3 unveiled in this study (working model)

The relationship between mDia and actin (actomyosin, Espin[9])

The formation of mDia1/3-dependent cortical F-actin meshwork in Sertoli cells





Schematic drawing: mDia1/3-generating cortical F-actin meshwork in Sertoli cells is required for the cell-cell adhesion between Sertoli cells and smaller round spermatids, and is indispensbale for spermatogenesis.

It has been shown previously using cultured cell lines that the contractile actomyosin bundle is critical for the formation and maintenance of the cell-cell adhesion. In this study, we performed super-resolution imaging with STORM microscope and revealed that mDia-dependent F-actin meshwork in primary-cultured Sertoli cells is in continuity with the contractile actomyosin bundles. In addition, we found that the number of the actomyosin bundles significantly decreased, but the non-contractile espin-containing F-actin bundles increased in the mDia1/3 double knockout Sertoli cells. These data indicate that the mDia1/3 double knockout causes the decrease of contractile actomyosin bundles that is continuous with the F-actin meshwork and the increase of noncontractile F-actin bundles. These together result in impaired formation and maintenance of the cell-cell adhesion between Sertoli cells and spermatids.

In summary, this study revealed that mDia1/3 are involved in the polymerization of actin meshwork and formation of contractile actomyosin that is continuous with the actin meshwork in Sertoli cells. Moreover, these structures are critical for the cell-cell adhesion between Sertoli cells and spermatids, and contribute to the normal spermatogenesis. These results together suggest that the abnormality of the actin cytoskeleton system in Sertoli cells might be one of the causes of male infertility. It is known that approximately half of the causes in spermatogenesis defects, which are the major cause of male infertility, are unknown. This study, which elucidated a part of the causes of male infertility, has the potential as a basis to the development of a novel therapy for some cases of male infertility in the future.

Glossary

1. Sertoli cell

A large somatic cell that interacts with spermatids in the seminiferous tubule to support the spermatogenesis. It is known that Sertoli cell supplies nutrients to spermatids and forms the blood-testis barrier. These 2 functions have been long considered as its major functions, but it is likely that other unknown functions are also exist.

2. Intercellular adhesion molecule

Molecules that physically connect two different cells. Claudin, cadherin, and nectin are known as representative molecules.

3. Underlying (cortical structure)

A structure consisting of thick actomyosin bundles and thin F-actin, which regulates the adhesion strength of intercellular adhesion molecules and is considered to connect with intercellular adhesion molecules through catenin.

4. Cytoskeleton

There are three kinds of cytoskeletons, that is, microtubules, intermediate filaments, and filamentous actin. They are involved in various cell functions such as cell migration and division as well as physically supporting the cell morphology.

5. STORM

Acronym for stochastic optical reconstruction microscopy. The process utilizes the photoswitchable property of fluorophores and activates only a portion of them to a fluorescent state to precisely determine the positional information of a particular molecule on the nm scale. By repeating this, eventually the positional information of almost all molecules is obtained, and a high-resolution image is constructed based on the information and calculation. Generally, it is said that an approximately 10-times-higher XY resolution image can be obtained with STORM than with conventional optical microscopes.

6. mDia1/3 proteins

Belonging to the formin family of proteins, mDia is a protein with actin polymerization activity. There are three isoforms in mammals, mDia1, mDia2, and mDia3. Previous studies have shown that mDia2 is related to the actin polymerization during cell division. On the other hand, the functions of mDia1 and mDia3 overlap, and it has been shown that they redundantly function in some cases such as cell migartion and intercellular adhesion regulation, but not for the actin polymerization during cell division.

7. Actomyosin

One of the actin bundle structures formed by the connection of myosin, a molecular motor, to filamentous actin. Hydrolysis of ATP by myosin causes the generation of the contractile force.

8. Actin polymerization rate

If we add actin monomers with appropriate amounts of ATP and $MgCl_2$ into a test tube, actin will spontaneously polymerize at a certain polymerization rate to form a filamentous structure. In the presence of profilin, one of the actin monomer-binding proteins, the polymerization rate drastically increases with the addition of proteins of the formin family such as mDia1.

9. Espin

One of the actin-binding proteins with the filamentous actin-bundling activity. Unlike actomyosin, the F-actin bundled by espin is not contractile.

Memo

Q & A

Q1 Do the mDia1/3 proteins investigated in this study also have important functions for spermatogonia and spermatocytes, precursors of spermatids?

In this study, we clarified that the functions of mDia1/3 in Sertoli cells are indispensable for differentiation of round spermatids to elongated spermatids and spermatozoa. But in addition, we also found that they are involved in the blood-testis barrier that is formed due to the intercellular adhesion between Sertoli cells. The blood-testis barrier is considered to be involved in the maintenance of the micro-environment appropriate for spermatogonia and spermatocytes. Thus, it is possible that mDia1/3 proteins also affect the function of these cells.

Q2 Why does the abnormal spermatogenesis happen due to the differences in actin polymerization rates and F-actin mesh sizes in the case of mDia1/3 double knockout?

The connection of spermatids and Sertoli cells is vital for spermatogenesis; the adhesion force regulators for intercellular adhesion molecules are intercellular adhesion molecule-underlying actomyosin bundles and the thin actin meshwork that is continuous with the actomyosin bundles. These actins repeat polymerization and depolymerization for homeostatic remodeling. However, in the case of mDia1/3 double knockout, the actin polymerization does not catch up with the depolymerization, resulting in a decrease in total mass of actin filaments, which subsequently weakens cortical actin and intercellular adhesion , and thus adhesion detachment.

Q3 The declining birthrate in Japan is a current social problem. How do you think the new findings about mDia1/3 obtained in this study will lead to diagnosis of male infertility and therapeutic development?

Almost half of the cuases in spermatogenesis defects, which are the major cause of male infertility, are unknown. This study indicates that abnormality of the actin cytoskeleton system in Sertoli cells may be one of the causes of male infertility. Therefore, mDia1/3 genetic variation might be useful for diagnosis. In addition, it was found in this study that at least part of the spermatogenesis defects are caused from the Sertoli cell side. Thus, we would like to suggest the possibility of therapeutic development for infertility by targeting the Sertoli cells (such as transgenesis or drug delivery).

From award panel members

In efforts to elucidate the functions of mDia, an actin polymerization regulator in the testis, the results of the localization analysis of actin filaments in Sertoli cells using localization microscopy are of high academic value. The quality of the images obtained is also extremely high.

The image is very beautiful.

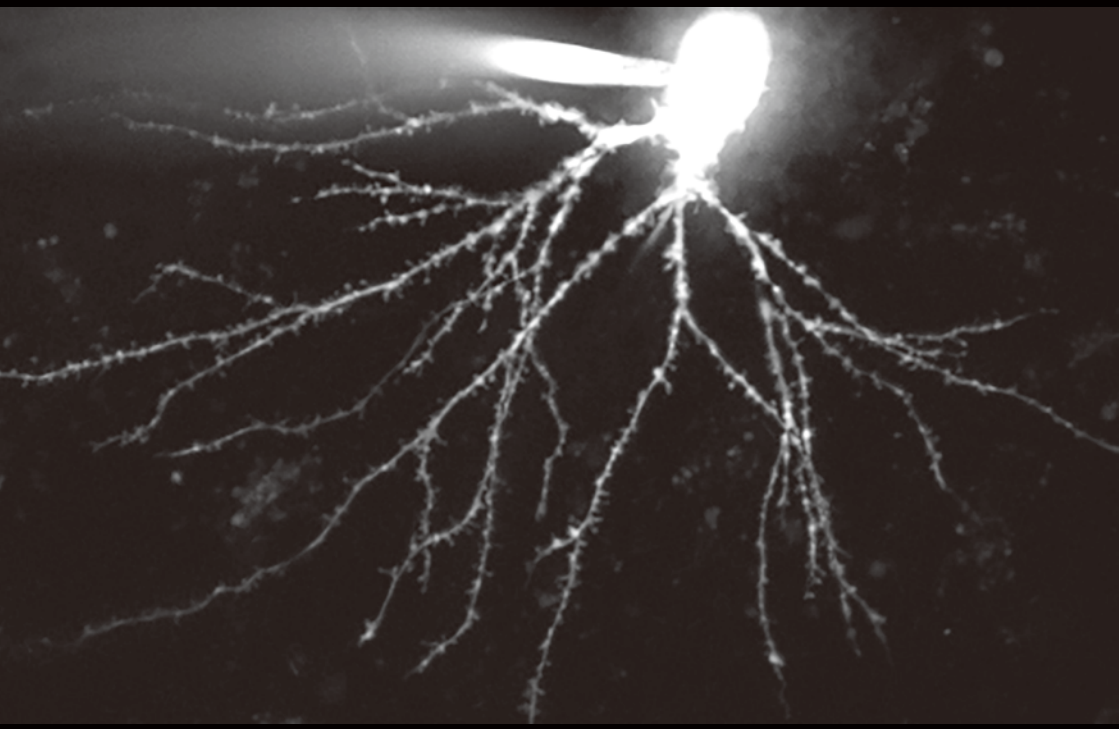
This is an impactful study that fully utilizes the potential of super-resolution microscopy. It gives us a sense of the fantastic nano-world.

The fine and beautiful structure of the actin cytoskeleton is beautifully captured using super-resolution microscopy.

The green and blue colors and the fine linear expression are beautiful, and the expression of delicacy is inspiring. The delicacy and dynamism of the structure are captured in a well-balanced manner.

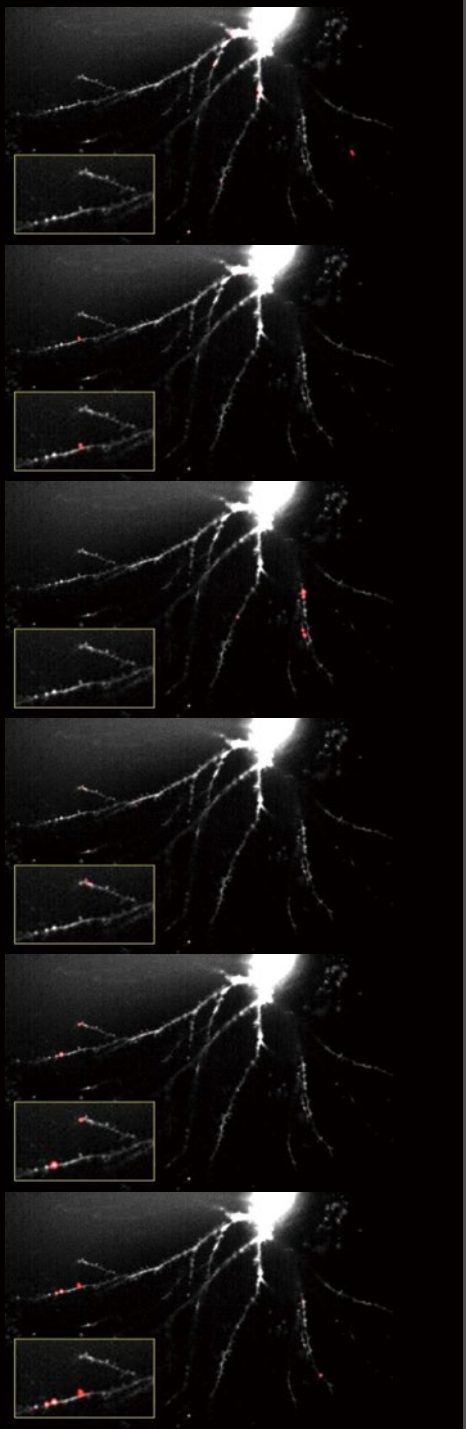
The beauty of the nano-structure of the actin cytoskeleton conveys the inspiration of the entrant.

The overlapping of the green and blue lines reminiscent of the Sea of Trees is beautiful. A unique color tone that is neither blue nor green has been created.



Sequential synaptic inputs during memory replay

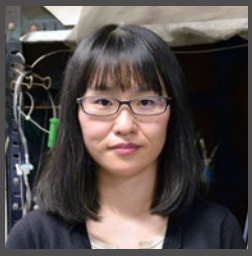
Large-scale, high-speed calcium imaging enabled to discover repeating sequential synaptic input onto specific pyramidal neurons during memory replay.



/ Special Prize /

Tomoe Ishikawa

Assistant Professor
Department of Pharmacology, School of Medicine
Keio University



Comments from the award recipient:

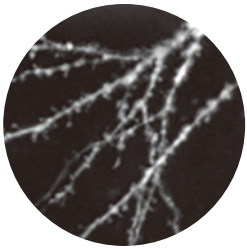
I am extremely honored to have been chosen to receive this special award.

It is an honor for me to receive the wonderful praise of my work as "sparklers shining in the dark". Calcium activity of synaptic inputs across dendritic arbor is so beutiful that I never tire of seeing them. Day after day, I am amazed to find that seemingly chaotic synaptic inputs are precisely governed to the order of a few micrometers. I will be giving my all to push forward to understanding the rules that govern the the brain function.

It would bring me great joy if this movie enables you to experience a glimpse into the brain's beauty and elaborate nature.

Rat brain (the hippocampal subfield CA1)

Detailed description : A fluorescent calcium indicator (Fluo-4)
Observation method : Confocal microscopy, upright, fluorescence
Magnification : 60x
Year : 2019
Microscopic data : Video



Sequential synaptic inputs[2] during memory replay[1]

Brief overview of this research

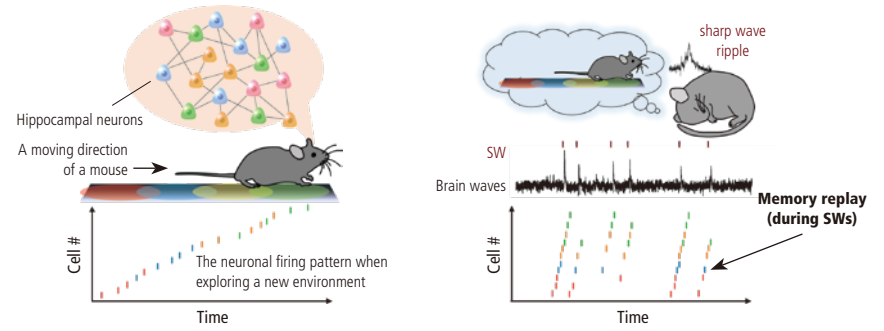
We discovered that neighboring spines[4] are activated serially along dendrites toward or away from cell bodies during specific brain waves, called sharp-wave ripples[3], which are frequently observed during memory reply. This result suggests that the neuronal firing patterns of upstream neuron[5] populations are converge on the adjacent dendritic spines of a portion of downstream neurons in precisely wired neuronal circuits. Squential synaptic inputs could be a novel algorithm in the brain[6] to explain the generation of a specific neuronal firing during memory replay.

Paper

Ishikawa, T., Ikegaya, Y.
Locally sequential synaptic reactivation during hippocampal ripples.
Science Advances. 2020, 6(7), doi: 10.1126/sciadv.aay1492

Ishikawa, T., Kobayashi, C., Takahashi, N., Ikegaya, Y.
Functional multiple-spine calcium imaging from brain slices.
STAR Protoc. 2020, doi: 10.1016/j.xpro.2020.10012

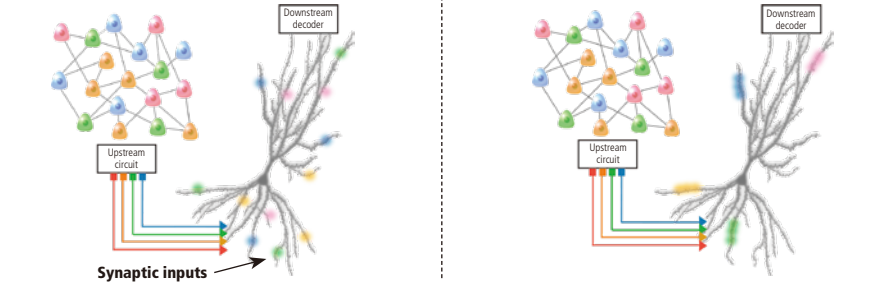
Background #1 Two stage model for the long-term memory



To understand the mechanism of long-term storage of a new memory, two stage (1) memory acquisition and 2) memory consolidation) model was proposed. However, **how neurons activated during memory acquisition spontaneously fire during memory consolidation, i.e., the mechanism of memory replay had remained unclear.**

Background #2 The spatiotemporal pattern of synaptic inputs[7] during memory replay (a hypothesis)

Neurons fires an action potential by receiving synaptic inputs from other (upstream) neurons.
The neuronal firing patterns depend on the spatiotemporal patterns in addition to the number of number of synaptic inputs.
Then, **what types of synaptic inputs do CA1 pyramidal neurons receive during SWs in which memory replay is frequently observed?**

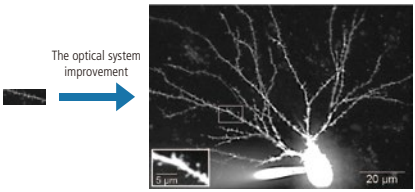


Our research purpose

To understand the mechanism of memory replay in subcellular level, we imaged the spatiotemporal patterns of synaptic inputs during SWs using the Nipkow-type confocal microscopy with the world's fastest scanning speed and largest number of recorded spines.

Please see Glossary on p.26 (each superscript number corresponds to the number for each term in it)

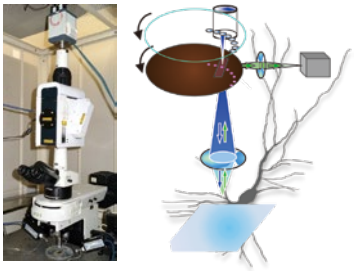
Development of a large-scale, high-speed functional spine imaging method[8] (Ishikawa, *Star protoc*, 2020)



A conventional field of view (–20 spines)

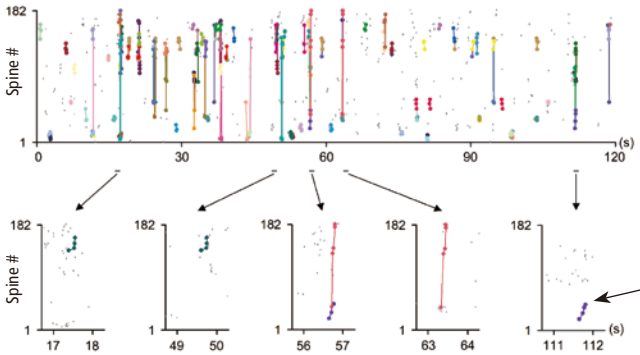
The field of view in this study (244 spines on average)

The high-speed imaging of synaptic inputs from several hundreds of spines at 100 Hz enabled to discover the sequential synaptic inputs.



Nipkow disk-type[9] confocal scanning unit was used in large-scale, high-speed functional multi-spine calcium imaging

Sequential synaptic inputs were discovered for the first time!

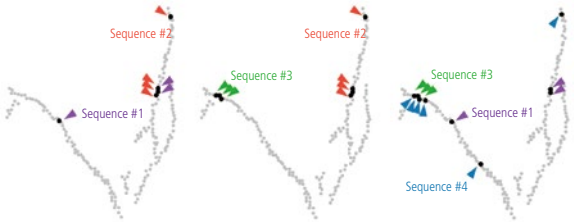


Through the complehensive analysis of big data exhibiting spatiotemporal synaptic input patterns, **we discovered neighboring spines repeatedly receive synaptic inputs serially in the same order; sequential syanptic inputs.**

Sequential synaptic inputs

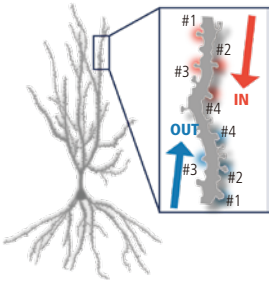
In the above rasterplot, we observed 216 events of 78 types of sequential inputs. Each color indicates a different type of sequential input.

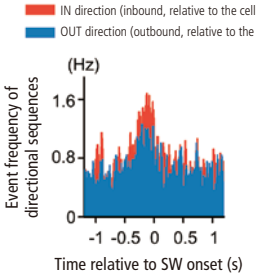
Sequential synaptic inputs, observed during SWs, are received by neighboring spines



A portion of neurons, which frequently fired during SWs, received the sequential synaptic inputs during SWs. In addition, the sequential synaptic inputs converge onto the neighboring spines within 10 μm.

Sequential synaptic inputs show directionality





Directional sequential inputs were observed more frequently during SWs. In terms of the comparison between the IN and OUT directions, the former occurred more frequently than the latter. The previous studies showed that inputs in the IN direction cause larger membrane potential fluctuations[10] than those in the OUT direction (Branco, *Science* 2010).

What happens if neighboring spines receive inputs?

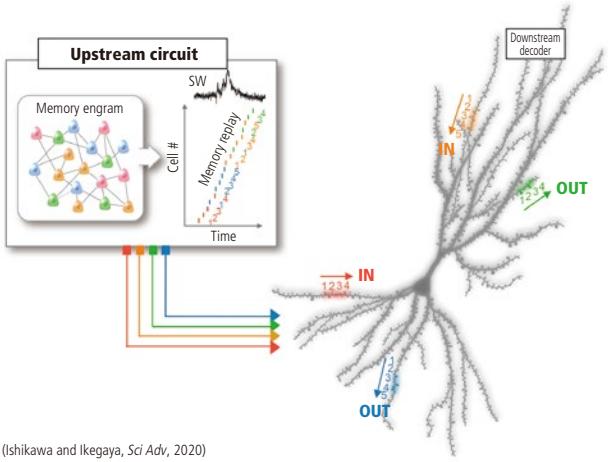
- Larger depolarization[11] are likely to be induced, compared with the case of the same number of dispersed inputs.
- Clustered synaptic inputs enable to induce of action potentials effectively.

What is the advantage of directional synaptic inputs?

- The inputs in the IN direction cause larger depolarization than those in the OUT direction.
- The subtle difference in timing of the neuronal firing of the same upstream neuron population results in a variation of the response of downstream neuron populations.
- The directionality of sequential inputs could enlarge computational capacity within a limited number of neurons.

Summary

In the hippocampal microcircuits, a part of neurons involved in SWs receive sequential synaptic inputs at adjacent spines.



(Ishikawa and Ikegaya, *Sci Adv*, 2020)

The importance of this study

- The existence of the sequential synaptic input was revealed.
- The sequential synaptic inputs are observed in a portion of pyramidal neurons during SWs, which implies a synaptic mechanisms of memory replay during SWs.
- Sequential inputs have not been incorporated into the current artificial intelligence (machine learning). It is possible to realize AI with more brain-like intelligence through the implementation of this phenomenon.

Glossary

1. Memory replay

The firing patterns observed during memory formation are likely to be repeated during sleep and resting state, which we call memory replay. Memory replay and sharp wave ripples[3] are considered to play an important role in the long-term memory preservation.

2. Sequential synaptic input

Specific spine assemblies repeatedly receive synaptic inputs serially in the same order. Our results suggest that memory replays of multineuronal spikes are distributed across dendritic spines of a postsynaptic neuron, with their spatiotemporal features preserved (sequential synaptic inputs).

3. Sharp wave (SW) ripple

Oscillatory patterns observed during sleeping or resting. It consists of the combination of SWs (2–30 Hz) and ripples (125–250 Hz). It is closely related to memory replay and considered to have important roles in long-term memory preservation.

4. Dendritic spine

A small membrane structure present on dendrites where majority of excitatory synaptic inputs are received. When neurotransmitters are emitted from the presynaptic region, they connect to the receptors on spines, leading to the inflow of sodium and calcium ions, which contributes to the downstream neuronal firing.

5. Upstream and downstream neurons

Neurons are connected with each other to organize neuronal circuits in the brain. Upstream neurons send information to down stream neurons in the neuronal circuits.

6. Algorithm in the brain

A rule of information processing among neurons. The sequential synaptic input discovered in this research is considered to activate cell bodies efficiently. Thus, for example, the incorporation of the algorithm into a neural network is possible to construct a more brain-like system.

7. Synaptic input

When an upstream neuron fired, neurotransmitters are emitted from the presynapses. The neurotransmitters bind to the receptors on the postsynpses of a downstream neuron and induce an ion influx. This is called the synaptic input. In this study, we observed calcium ion inflows caused by the excitatory transmission as fluorescence intensity fluctuations.

8. Large-scale, high-speed spine imaging method

A method to observe spatiotemporal pattern of synaptic inputs at a high speed and on a large scale through the combination of a Nipkow disk confocal unit and a CMOS camera. Two hundred or more spines on average can be imaged at 100 Hz—currently the world's largest number and fastest speed.

9. Nipkow disk-type

Spinning disk with a spiral of holes. The high-speed rotation of the disk can divide a laser, enabling simultaneous recording from multiple points. Nipkow disk-type confocal microscope enables high-speed, wide-field imaging with low photobleaching.

10. Membrane potential fluctuation

It is a fluctuation of somatic membrane potential caused by convergence of synaptic inputs from other neurons. A shift towards excitation is called depolarization and leads to neuronal firing.

11. Depolarization

In a normal state, a cell membrane is negatively charged. When exicitatory syanptic inputs are received, the membrane potential becomes less negative, which is called depolarization. When the degree of depolarization reaches a certain threshold, action potential occurs, which transmits an output to further downstream neurons.

Memo

Q & A

Q1 Could you describe the experimental system in this study?

In this study, we used hippocampal slice culture preparations (300 μm thick, days *in vitro* 10–20). First, cell-attached recording was performed using an electrode from a CA1 pyramidal cells. After that, negative pressure was applied to achieve whole-cell configuration to load the fluorescent calcium indicator molecules—which are originally contained in the electrodes—into the cells, enabling the measurement of calcium influx caused by synaptic inputs. Another electrode was placed to record local field potentials around the target neurons (approxinately 50 μm apart from the target neurons). After recording the synaptic inputs, regions of interest were set manually on the obtained image to calculate the fluorescence intensity change.

Q2 What is the mechanism of controlling the cell-selective sequential synaptic input discovered in this study?

This is an important question, but we do not have a good answer so far. Although it may be related to how upstream and downstream neurons connect with each other, many facets are still unclear in terms of the types of cells that form the synapses between CA3-CA1 circuits. In addition, neurons receive inhibitory inputs (working as a brake in the brain) as well as excitatory inputs (as an accelerator), and thus we need to consider the effect of inhibitory inputs.

Q3 Do you know anything about the relationship between the findings in this study and diseases such as memory disorders?

Since the sequential synaptic inputs were observed in only a portion of cells, we consider that the existence of the sequential inputs plays an important role in the formation of cell-selective firing patterns. Thus, if the sequential inputs are selectively inhibited, diseases such as memory disorders could be induced. But, it is challenging to inhibit specific sequential inputs. In the future, we would like to introduce new technologies to explore the functions of sequential synaptic inputs.

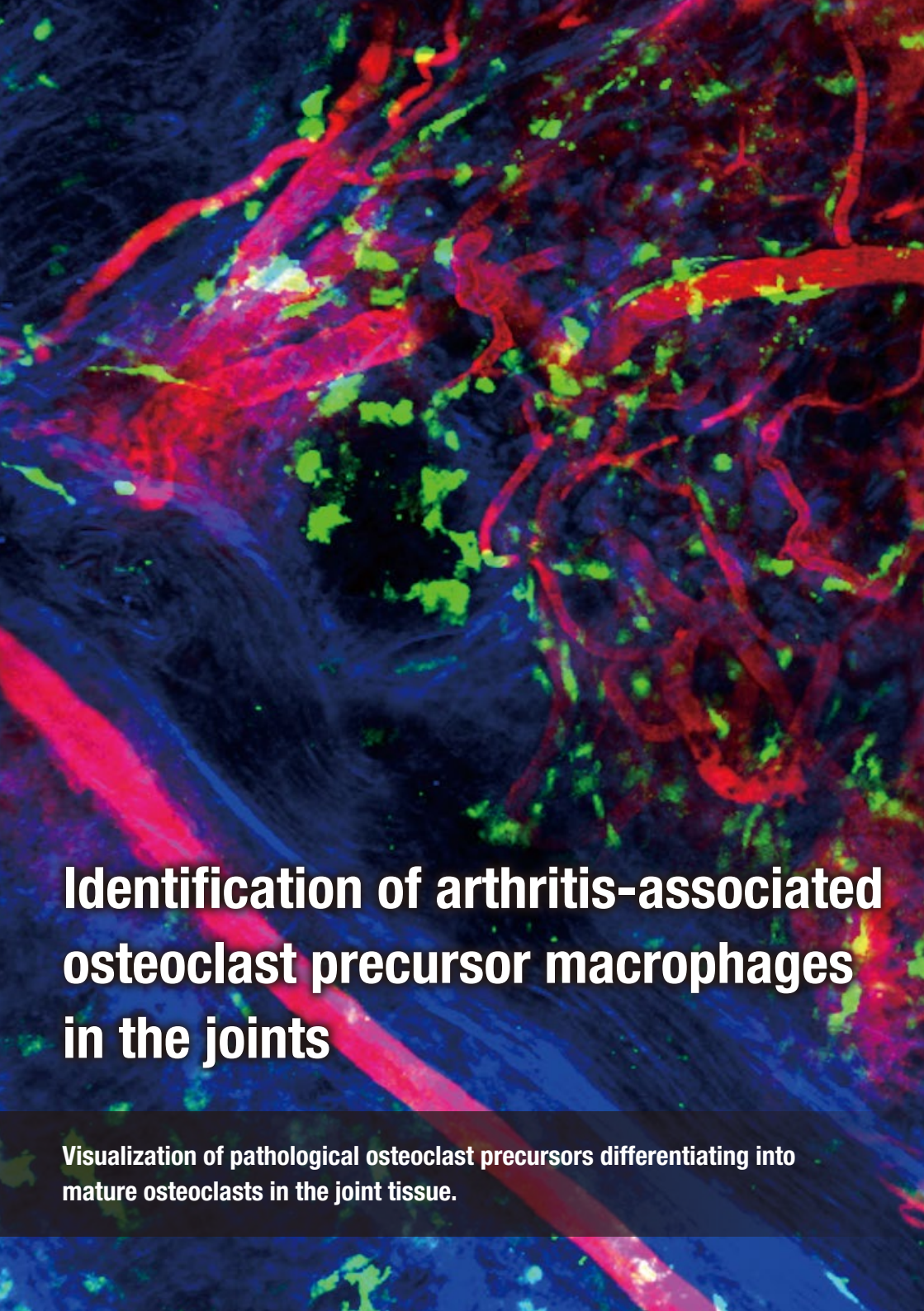
From award panel members

This work is scientifically valuable.

It has succeeded in capturing sequential synaptic inputs during memory replay at a high spatial and temporal resolution, and its scientific merit is very significant. Furthermore, the video capturing this phenomenon is reminiscent of sparklers in the dark, so it also has artistic merit.

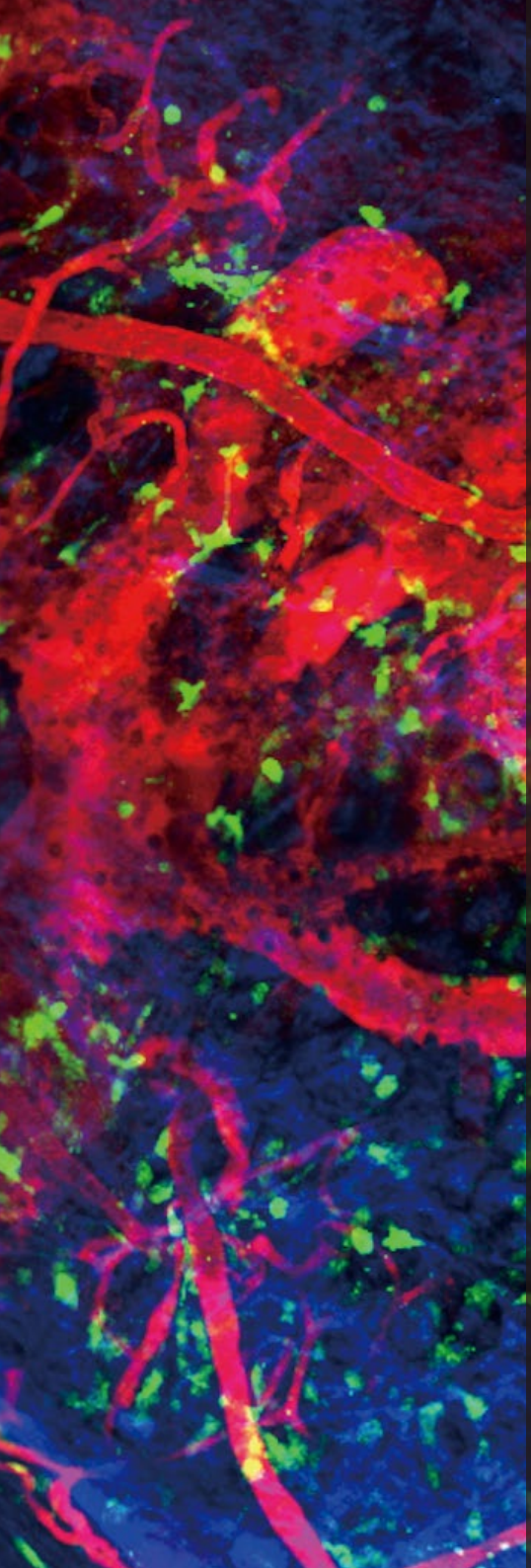
This is an important discovery which demonstrates the existence of sequential synaptic inputs in a slice preparation.

The images, which capture the work of the spine synapses (where neurons transmit information) in high temporal resolution, hold great value.



Identification of arthritis-associated osteoclast precursor macrophages in the joints

Visualization of pathological osteoclast precursors differentiating into mature osteoclasts in the joint tissue.



/ Special Prize /

Tetsuo Hasegawa

Assistant Professor, Division of Rheumatology,
Department of Internal Medicine, School of Medicine, Keio University
Vice Chief Physician, Department of Rheumatology,
Kawasaki Municipal Hospital

Masaru Ishii

Professor
Department of Immunology
and Cell Biology[1]
Graduate School of Medicine,
Osaka University

Jun-ichi Kikuta[1]

Associate Professor

Comments from the award recipient:

I am very honored to be selected for the prestigious NIKON JOICO AWARD Special Prize.

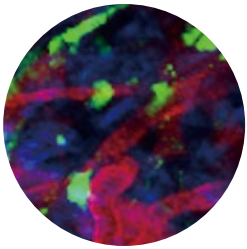
As in the saying "a picture is worth thousand words," imaging technology provides us an unbiased information, which is often more convincing than a lot of numerical data.

As a rheumatologist treating patients with rheumatoid arthritis, the surprise and excitement I feel when I successfully capture the moment of pathological osteoclast formation in the joint tissue leads to my motivation to further elucidate the pathogenesis of autoimmune diseases.

I would like to further explore the intravital imaging research to finally find a way to cure autoimmune diseases.

Mouse (DBA1/J) inflammatory joint

Detailed description	:	Second harmonic generation (fibrous tissue) Red: AF647 (blood vessel) Green: CX3CR1 (osteoclast precursor cell)
Observation method	:	Two-photon excitation microscopy, inverted, fluorescence
Magnification	:	25x
Year	:	2019
Microscopic data	:	Still image



Identification of arthritis-associated osteoclast precursor macrophages in the joints

Brief overview of this research

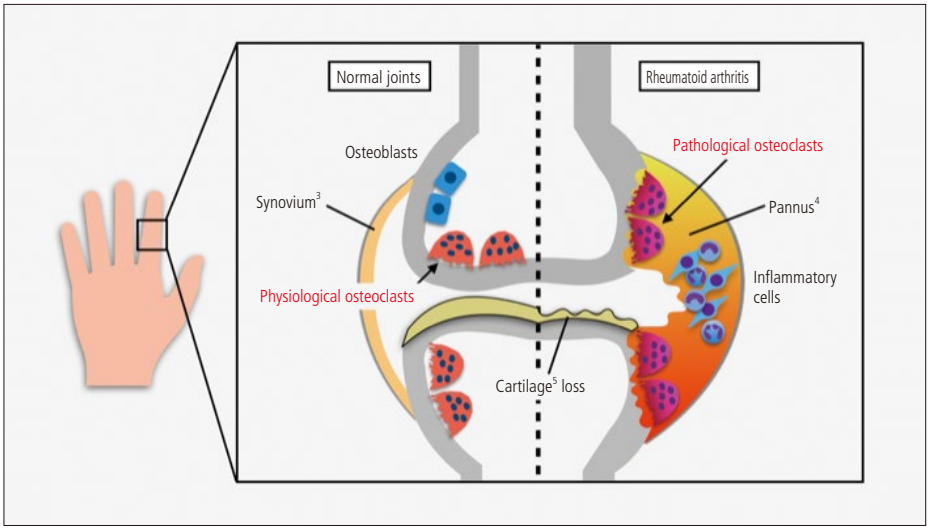
Osteoclasts[1] are multinucleated cells with its unique bone-resorbing capacity. They play key roles in homeostatic bone remodeling, while they are also involved in pathological bone destruction in rheumatoid arthritis (RA). This study is aimed to elucidate where and how the pathological osteoclasts are developed in the joint tissue through single-cell RNA sequencing analysis[2] and intravital imaging technologies.

Paper

1. Tetsuo Hasegawa, Junichi Kikuta, Takao Sudo, Yoshinobu Matsuura, Takahiro Matsui, Szandor Simmons, Kosuke Ebina, Makoto Hirao, Daisuke Okuzaki, Yuichi Yoshida, Atsushi Hirao, Vladimir V. Kalinichenko, Kunihiro Yamaoka, Tsutomu Takeuchi, Masaru Ishii
   Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1.
   Nature Immunology. 2019, 20(12), doi: 1038/s41590-019-0526-7

2. Tetsuo Hasegawa, Junichi Kikuta, Takao Sudo, Erika Yamashita, Shigeto Seno, Tsutomu Takeuchi, Masaru Ishii
   Development of an intravital imaging system for the synovial tissue reveals the dynamics of CTLA-4 Ig *in vivo*.
   Scientific Reports. 2020, 10(1), doi: 1038/s41598-020-70488-y

Where are the bone-absorbing osteoclasts?



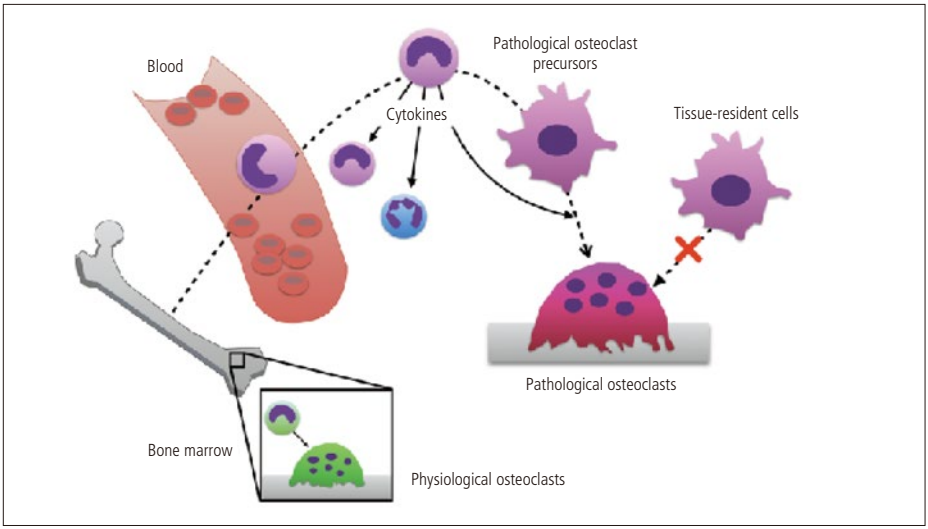
Osteoclasts are unique multinucleated cells with a bone-resorbing capacity, playing an important role in **the bone homeostasis (bone remodeling) through reciprocal interplays with osteoblasts, which form the bone inside the bone marrow.**

Rheumatoid arthritis causes inflammation of joint-enveloping membrane (synovium), leading to **the formation of pathological osteoclasts at the region where synovium contacts with the bone surface.** These "bad osteoclasts" destroy the articular bones, leading to increased mortality and morbidity in patients with RA.

Many of the previous studies had explored and analyzed cells in tissue such as bone marrow, the spleen, and blood. Nevertheless, **a precise analysis of osteoclast precursors (OPs) has not yet been performed in "inflamed synovium", the actual site of bone erosion in arthritis, mainly due to technical difficulties associated with isolating tiny synovial tissues. In addition, it remains unknown how the physiological bone remodeling and pahtological joint destruction differs.**

Please see Glossary on p.34 (each superscript number corresponds to the number for each term in it)

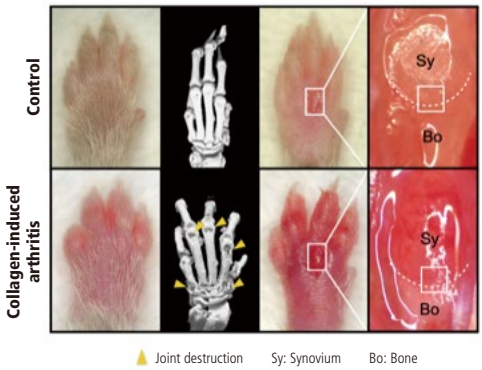
Where are the pathological osteoclasts in joints derived from?

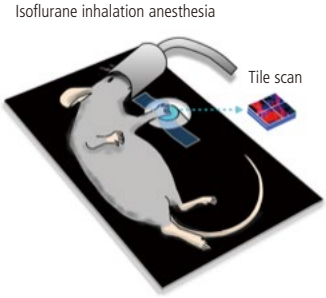


An analysis using bone marrow chimeric mice[6] revealed that **pathological osteoclasts are derived from bone marrow cells[8] through circulation and not from tissue-resident macrophages[7] in joints.** In addition, pathological osteoclast precursor cells express surface markers involved in antigen presentation, possess a capacity to efficiently differentiate into osteoclast with inflammatory cytokines, and only 10% of the precursors actually differentiate to mature osteoclasts within the joint tissue (described in the paper #1).

However, **there had been no experimental methods to verify if these pathological osteoclast precursor cells actually ingress into the synovial tissue and differentiate into mature osteoclasts.**

Visualization of joint tissue using intravital imaging technology





Cited from the paper #2, modified

We **developed a protocol to visualize the inflamed joint tissue of living mice using a multi-photon microscopy.**[9] Out of every joint in the entire body, we focused on the third metacarpophalangeal joint of the frontpaw because the interface between the synovium and bone is within 100 μm from the surface of the joint tissue. Exteriorizing the third metacarpophalangeal joint with micro-scissors under stereomicroscopic observation, we **identified the bare area where pathological osteoclasts cause devastating bone destruction,** and we performed the intravital imaging with an inverted multi-photon excitation microscope.

This enabled **real-time observation of pathological osteoclast precursors (CX3CR1,[10] EGFP positive cells, shown in green) and bone-destructing pathological osteoclasts.** We also visualized **the three-dimensional structure of newly formed blood vessels in the joint tissue.**

Previous studies have been mainly focusing on osteoclast-like cells developed *in vitro*. However, this research **enables a direct observation and analysis of inflamed synovium in the living animal, leading to clarify the pathogenesis of bone destruction in RA.**

Glossary

1. Osteoclast

Osteoclasts are multinucleated cell with a unique bone-resorbing ability. They are derived from monocyte/macrophage-lineage precursors. They reside inside of bone (bone marrow) and are involved in the bone remodeling under physiological condition, while they cause devastating joint destruction in patients with rheumatoid arthritis.

2. Single-cell gene expression analysis

This technology provides transcriptional profiling of individual cells and helps to understand what genes are expressed in what quantities at the single-cell level.

3. Synovium

Membrane existing inside the joint-enveloping articular capsule. It supports smooth movements of joints through synovial fluid production. In rheumatoid arthritis, this tissue is significantly inflamed and enlarged, leading to pathological bone destruction.

4. Pannus

Rheumatoid arthritis causes an inflammatory response in joints that leads to the formation of abnormal granulomatous tissue called pannus. This tissue invades the bone and cartilage, leading to articular bone erosion.

5. Cartilage

An elastic tissue covering the articular surface, which functions as a joint pad.

6. Bone marrow chimeric mouse

A mouse whose bone marrow cells are replaced by bone-marrow transplantation using those from another mouse after radiation and chemotherapy. It is utilized to evaluate whether specific cells or pathological conditions are derived from or induced by bone marrow cells.

7. Macrophage

One type of leukocytes that widely distributes throughout the whole body. They are involved mainly in phagocytosis, digestion of dead cells, and invading bacteria in the body. It acquires various functions depending on the surrounding microenvironment. They have different names according to the tissue they reside in, such as Kupffer cells in the liver, microglias in the brain, and osteoclasts in the bone marrow.

8. Bone marrow cell

Cells reside within the bone marrow cavity. It includes bloods cells originating from hematopoietic stem cells, and mesenchymal cells.

9. Multi-photon excitation microscopy

Two-photon microscopy is a fluorescence imaging technique that allows imaging of living tissue. Unlike traditional fluorescence microscopy, two-photon excitation requires simultaneous excitation by two photons with longer wavelength than photons used in traditional fluorescence microscopy. Two-photon excitation is useful for intravital imaging due to its deeper tissue penetration, efficient light detection, and reduced photobleaching.

10. CX3CR1

A receptor of a chemokine, CX3CL1. It is utilized as a surface marker of monocyte/macrophage-lineage cells and of some types of lymphocytes.

Memo

Q & A

Q1 What determines the capacity to differentiate into mature osteoclasts?

Receptor activator of nuclear factor-kappa B ligand (RANKL) and macrophage colony stimulating factor (M-CSF) are the two essential cytokines involved in osteoclastogenesis. Although pathological osteolast precursor macrophages express receptors for both of the cytokines, synovium-resident macrophages are reported to not express the receptor for M-CSF. Therefore, the difference of expression pattern for the receptor of key cytokines involved in osteoclastogenesis may be involved in determining the capacity to differentiate into osteoclasts in the synovium.

Q2 What aspects did you have the hardest time in bio-imaging?

Visualization of pathological osteoclasts in the joint tissue has been difficult for several reasons. First, the abundant red blood cells in the inflamed synovial tissue scatter light and impede the deep tissue imaging. Second, the inflammatory synovium is composed of multiple layers with various cell types, limiting the observation depth. Third, the skeletal system is directly connected throughout the body and respiratory movement causes the visual field of the joint tissue to drift. Hence, it was hard to establish a protocol to overcome these obstacles.

Q3 How do you expect to utilize the research outcome of this study in the development of a new therapy of rheumatoid arthritis?

Future applications of novel technologies, including new fluorescent probes and optogenetic techniques, will further reveal the intravital behaviour and function of immune cells in the synovium, leading to the development of novel therapeutic approaches to rheumatoid arthritis.

From award panel members

In relation to the synovial membrane, which is the site of major lesions in rheumatoid arthritis, the development of a technique to visualize the dynamics of osteoclast precursors and the process of synovial tissue reorganization at the individual level is highly commendable from an academic perspective.

The image is beautiful with high resolution.

It is powerful, dynamic, and has a strong impact.

The dynamism and the pressure of destruction are exquisitely expressed, and the excitement of the researchers can be felt.

It just feels powerful.

The description is beautiful, like green sparks of fire scattered among the burning red flames. It is also appropriate for imaging joint inflammation.

Award panel members

Masaru Ishii

Professor
Department of Immunology
and Cell Biology
Graduate School of Medicine,
Osaka University

Shigeo Okabe

Professor
Department of Cellular Neurobiology
Graduate School of Medicine
and Faculty of Medicine,
The University of Tokyo

Keigo Kohara

Principal Investigator, Assistant Professor
Department of Cellular
and Functional Biology
Institute of Biomedical Science,
Kansai Medical University

Tomomi Nemoto

Professor
Biophotonics Research Group, Exploratory
Research Center on Life and Living Systems,
National Institutes of Natural Sciences
Division of Biophotonics,
National Institute for Physiological Science

Hirokazu Hirai

Professor
Department of Neurophysiology &
Neural Repair, Graduate School of Medicine,
Gunma University

Design Center, Nikon Corporation

Nobuo Hashimoto

Center Manager

Nobuhiko Iwamura

Manager of Life Imaging Lab

Jun Konno

Group Manager
Experience Design Group

Kenji Baba

Group Manager
Industrial Design Group

Akiya Maekawa

Group Manager
UI & Interaction Design Group

Kumiko Saito

Group Manager
Corporate Branding Group

First Prize JOICO Award

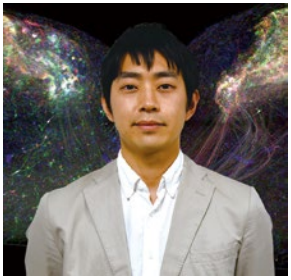
Long-Distance, Rapid Calcium Signaling in Plants

Masatsugu Toyota
Associate Professor
Graduate School of Science & Engineering
Saitama University

Runner-up Prize

A Secret Inside Flowers

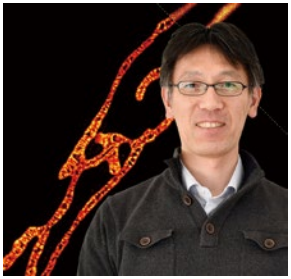
Yoko Mizuta
Designated Assistant Professor
Institute for Advanced Research/Institute for Transformative Bio-Molecules
Nagoya University



Special Prize

Auditory brainstem circuits detecting interaural time difference

Ryo Egawa
Designated Assistant Professor
Laboratory of Cell Physiology, Graduate School of Medicine
Nagoya University



Special Prize

A super-resolution image of the inner mitochondrial membrane developed in starved cells

Masayasu Taki
Designated Associate Professor
Institute of Transformative Bio-Molecules
Nagoya University

NIKON JOICO AWARD

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1-6-3 Nishioi, Shinagawa-ku, Tokyo 140-0015, Japan
TEL: +81-3-3773-8138
E-mail: Nsl-bio.Marketing@nikon.com
Website: https://www.healthcare.nikon.com/ja/ss/joicoaward/

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