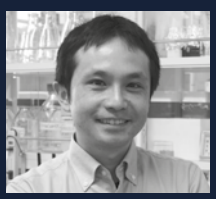
Impact Objective

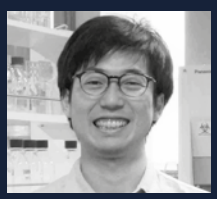
- Uncover the signalling pathways behind choices cells make to build fundamental understanding of DNA repair and to its potential use in tackling cancer

DNA repair and cancer research

Dr Atsushi Shibata and Dr Takaaki Yasuhara talk about their work examining the way a cell repairs breaks in its DNA that could be fatal if handled incorrectly



Dr Atsushi Shibata



Dr Takaaki Yasuhara

What are some of the obstacles to building on our existing knowledge in this field?

AS: DNA repair is the most important cellular system to maintain genome stability. Therefore, the lack of DNA repair causes multiple diseases, including cancer tumorigenesis. Thus, elucidating the mechanisms underlying genome stability after DNA damage will contribute to propose an approach for cancer prevention. Equally, I am interested in DNA repair and its signalling in cancer therapy. In fact, most cancer therapies (radiotherapy and most chemotherapy) introduce DNA double strand breaks (DSBs) in cancer cell DNA, causing a cancer cell killing effect. However, the capability of DNA repair and signalling in cancer cells has not been well considered prior to this. Based on this background, I would like to know the precise mechanism of DSB repair and signalling at a molecular level. Additionally, I would like to exploit our cutting-edge knowledge to improve the strategy of cancer therapy.

Have you seen any results from this work that you are particularly pleased with?

TY: We found that DNA/RNA hybrids are formed following DSB induction at sites of high transcriptional activity. But with time, the DNA/RNA hybrids have to be resolved for the progression of homologous recombination repair (HRR). However, the factor and mechanism were unknown. Here, we found that Rad52 and XPG are required for resolution of DNA/RNA hybrids to progress HRR. From data published in previous literatures (such as Rad52 is DNA/RNA hybrid binding protein and XPG endonuclease activity has a role to resolve DNA/RNA hybrid structure) we speculated these are involved in the resolution of DNA/RNA hybrids at DSBs. Then, our speculation proved correct. I think we were excited when we knew our hypothesis was right! This work has been published in Cell (2018.175(2):558-570).

Have you faced any challenges in your research? How have you overcome them?

TY: The visualisation of DNA/RNA hybrids, Rad52 and XPG was difficult and our biggest challenge during this work. In order to study this, we made use of a near infrared laser (730 nm). This laser is able to introduce hundreds to thousands of DSBs at the irradiated regions. Because so many DSBs can be introduced at selected regions, the formation of DNA/RNA hybrids and Rad52 recruitment is visualised even if the signal of a single DSB is not visualised under normal microscopy. This allowed us to see the formation of DNA/RNA hybrid and Rad52 recruitment at DSB sites, a key revelation in the understanding of repair pathway choice in cells.

What does the future hold for your studies?

AS: As my ultimate goal, I would like to perfectly elucidate the mechanism of DSB repair and understand the orchestrating of multiple repair networks over time. I would like to understand the spatiotemporal regulation of DNA repair. For this purpose, we are now using super-resolution microscopy to visualise precise DNA damage structure and its dynamic DNA repair response in 3D. Time-lapse analysis will be introduced into the work to include the concept of 4D. In terms of transcription-associated DSB repair, we are now investigating the mechanism of repair in G0/G1 cells, because most cells in human body , even in cancer cells, are arrested in G0/G1 phase. Since G0/G1 cells are not able to use HRR pathway, the repair pathway for TA-DSBs in G0/G1 cells must be distinct to that in S/G2. On the other hand, I would like to contribute to cancer therapy. In 2017 we published a paper in Nature Communications demonstrating the involvement of DNA damage response in anti-PD-1 immunotherapy and I'd like to build on that. I am collaborating with Dr Takaaki Yasuhara on both these projects.

Choosing the right path

Researchers are investigating how the cell decides which DNA repair pathway to use in order to fix double-strand breaks

The ability to repair breaks and mutations in DNA is essential for a cell's – and therefore an organism's – survival. Breaks and other mutations arise naturally over time and are dealt with by the cell using several different pathways. Incorrect or non-existent DNA repair can lead directly to either cell death or the development of a cancerous cell. A break in double strand in the DNA is particularly serious. Dr Atsushi Shibata, a Principle Investigator and Senior Lecturer at Gunma University in Japan explains that the cell has two key ways to deal with this sort of break. 'These are homologous recombination repair (HRR) and non-homologous end-joining (NHEJ),' he says. 'HRR is the more accurate pathway, while NHEJ provides a quicker fix, but it has a risk of error under some circumstance.' This makes HRR perfectly suited to repairing important parts of the genome whilst NHEJ is better suited to less key regions.

The molecular mechanisms behind these repair pathways have been extensively studied. However, the question of how a particular pathway is selected over another in different situations is less well characterised, particularly in human normal cells as well as cancer cells. Uncovering the signalling pathways behind this choice the cell makes is both very important to our fundamental understanding of DNA repair and to its potential use in tackling cancer. Shibata is collaborating with Dr Takaaki Yasuhara from the University of Tokyo to investigate these pathways. They have revealed some of the inner workings behind the choice of repair pathway.

Previously, Shibata has shown that the NHEJ was the default choice for repairing DSBs, but that HRR is then selected if the re-joining does not occur properly. He has also shown a fundamental role of MRE11 nuclease in determining the type of repair that occurs at a DSB. Additionally, Shibata has shown the crucial role BRCA1 has in directing the repair of DSB towards the HRR pathway. In short, his work in the field is internationally recognised. 'These studies have contributed to the establishment of research basis for the recent discovery in the study of transcription-associated HRR,' observes Shibata.

PREVENTING CANCER

Shibata's most recent focus is on the mechanisms behind DSB repair signalling in highly active genes. These are genes that are extremely important to the cell and are therefore constantly being transcribed. 'Given the importance of the genes, it is imperative for the cell that they undergo the accurate HRR rather than NHEJ,' highlights Shibata. 'The question that arose from this, therefore, concerned how the cell is able to consistently recruit the HRR pathway over the NHEJ pathway at highly active genes.' Answering this is crucial to understanding how cells are prevented from becoming cancerous. This is where his collaboration with Yasuhara was crucial to solving the problem.

Yasuhara uses a near-infrared laser to create thousands of DSBs at the site of highly active genes. This allows them to ensure that they are able to monitor the type of repair that is being undertaken at that site, even if the signal from one break is not visualised. The DNA/RNA hybrid that is found during transcription forms a particular structure known as an R-loop. 'This R-loop is recognised by two proteins: Rad52 and XPG,' outlines Yasuhara. 'These proteins are essential to promoting the use of HRR to fix the DSBs.' They found that deficiency in either of those proteins was sufficient to lead to the DSB being repaired by NHEJ, something which is highly undesirable in biologically significant active genes.

These results are ground-breaking by themselves; however, they also offer another potential strategy for cancer prevention as well as therapy. This is something the two will continue working on together alongside further foundational science projects that continue to probe the repair of DSBs at highly active genes by looking at them in three dimensions and over time which would be a first in the field.

Project Insights

FUNDING

- JSPS KAKENHI JP26701005, JP17H04713 (Dr Atsushi Shibata)
- The Takeda Science Foundation (Dr Atsushi Shibata)
- The Uehara Memorial Foundation (Dr Atsushi Shibata)
- JSPS KAKENHI JP18K18191 (Dr Takaaki Yasuhara)

CONTACT

Dr Atsushi Shibata

T: +81 27 220 7977
E: shibata.at@gunma-u.ac.jp
W: http://shibatalab.com/english/

BIOS

Dr Atsushi Shibata is a Senior Lecturer (Principal Investigator) at Gunma University in Japan. He graduated the Kyoritsu College of Pharmacy, Japan. Shibata completed his PhD at the Tokyo Medical and Dental University, Japan and has previously worked in Professor Penny Jeggo's laboratory at the University of Sussex, UK.

Dr Takaaki Yasuhara gained his PhD at the University of Tokyo, where he currently works as an Assistant Professor.