© 2023. Published by The Company of Biologists Ltd | Journal of Cell Science (2023) 136, jcs261398. doi:10.1242/jcs.261398

FIRST PERSON

First person – Tatsuki Tsuruoka

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping researchers promote themselves alongside their papers. Tatsuki Tsuruoka is first author on 'Development of a fluorescence reporter system to quantify transcriptional activity of endogenous p53 in living cells', published in JCS. Tatsuki conducted the research described in this article while a Master's course student in Kazuyasu Sakaguchi's lab at Hokkaido University, Sapporo, Japan. He is now a PhD student in the lab of Kazuhiro Aoki at Quantitative Biology Research Group, Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, Aichi, Japan, investigating the regulatory mechanisms of cellular responses mediated by the p53 signaling pathway.

Tatsuki Tsuruoka

How would you explain the main findings of your paper in lay terms?

p53 is a tumor suppressor whose function is lost in many tumors due to gene mutations. p53 functions as a transcription factor, integrating a variety of upstream signals and simultaneously regulating the transcription of multiple downstream genes. In this way, p53 suppresses tumor formation by inducing various cellular responses such as temporarily halting cell division and irreversibly promoting cell death. To better understand the mechanism of p53-mediated regulation of these responses, we have developed a highly sensitive reporter system that allows us to monitor temporal changes in p53 transcriptional activity at the single-cell level. Using this p53 reporter system, we show that the transcriptional activation of p53 can strongly vary from cell to cell. Such heterogeneity might be a source of a phenomenon called fractional killing in which chemotherapy kills only a fraction of tumor cells, which is one of the factors that can prevent efficient cancer treatment. We also explored the factors that produce heterogeneity in p53 transcriptional activity under etoposide (an anticancer drug) and ultraviolet light (UV) exposure. Despite similar heterogeneity in both conditions, the heterogeneity of p53 transcriptional activity with etoposide treatment can be largely explained by the cell cycle phase, while with UV treatment there is little correlation with the cell cycle. This suggests that the source of heterogeneity in tumor cells differs depending on the type of stress they are exposed to. Our p53 reporter system and the findings obtained using this system will contribute extensively to the study of the p53 signaling pathway.

Were there any specific challenges associated with this project? If so, how did you overcome them?

Since I started this research when I was an undergraduate student, the first obstacle was learning basic biological experimental techniques and acquiring a lot of knowledge from reading related papers. In addition, no one in my laboratory had handled time-lapse fluorescence imaging and single-cell tracking by image analysis before, so I had to go through a trial-and-error process to construct and improve the experimental system and image analysis process. Furthermore, once some correlation between p53 transcriptional activity and cell cycle was observed, I needed to more directly demonstrate these causal relationships. Finally, I was able to obtain nice data by using a simple cell cycle synchronization technique.

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

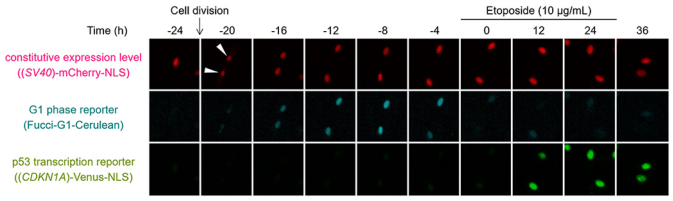
I experienced an 'eureka' moment when I quantified p53 expression levels and phosphorylation of serine at position 15, a typical post-translational modification of p53, by immunofluorescence staining. Initially, I thought that the cell cycle-dependent variation in p53 transcriptional activity during etoposide treatment could be largely explained by the variation in p53 expression levels. However, the experimental results revealed that the expression level of p53 did not fluctuate significantly, whereas the post-translational modification signal of p53 showed large fluctuations. I believe that this result is both surprising and important, as it suggests which step in the p53 signaling pathway is the source of the heterogeneity.

Why did you choose Journal of Cell Science for your paper?

Journal of Cell Science publishes 'Tools and Resources' articles, which report on practical and novel tools aimed at researchers in cell biology. We decided to submit our manuscript to JCS in order to make our p53 reporter system known to many researchers, including the p53 research community. We hope that our reporter system and its design framework will be useful to the wider community.

Tatsuki Tsuruoka's contact details: Quantitative Biology Research Group, Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan.
E-mail: t-tsuruo@nibb.ac.jp

Journal of Cell Science (2023) 136, jcs261398. doi:10.1242/jcs.261398



Time-lapse imaging of endogenous p53 activity in living cells. Simultaneous time-lapse imaging of the constitutive protein expression level [(*SV40*)-mCherry-NLS], cell cycle phase (Fucci-G1-Cerulean), and transcriptional activity of endogenous p53 [(*CDKN1A*)-Venus-NLS] under etoposide treatment.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

I could not have completed this research without the support of the laboratory staff. Prof. Sakaguchi taught me not only through his direction of the research and discussions about the data, but also through his attitude toward research and the importance of viewing things from different perspectives. Dr Imagawa gave me direct instructions on countless occasions, from teaching me experimental techniques, to developing data analysis methods, to interpreting results. He was kind and sometimes strict with me as a student, based on his belief that 'students and faculty should always be on an equal footing in research'. He always took the time to carefully respond to my questions and help improve my comprehension. Dr Kamada gave me very insightful remarks and valuable advice on how to improve my presentation material in various situations, such as in seminars for the laboratory.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I originally enjoyed science in a very broad sense, and enjoyed reading technical books and papers in a variety of fields. I also found scientific work fascinating, as I would be the first to experience interesting discoveries in the process. I chose biology as my research field for two reasons. First, life is very complex and there is still much that is unknown. Second, new concepts and research methods are being developed at a tremendous pace by many researchers. I am currently focusing on the p53 signaling pathway to understand the regulatory mechanisms of cellular functions involving this pathway. In order to achieve this, after completing my Master's degree, I entered my current laboratory as a PhD student. Although I was anxious about changing laboratories, I feel that I have grown as a researcher by working in a different environments and learning experimental methods and concepts in a new field.

What's next for you?

I am currently a PhD student in the Department of Quantitative Biology (Prof. Kazuhiro Aoki) at the National Institute for Basic Biology in Japan. I plan to continue to study the p53 signaling pathway, and my research is focused on the development of optogenetic tools that can manipulate p53 activity and its applications for controlling cellular functions.

Tell us something interesting about yourself that wouldn't be on your CV

I like outdoor activities, especially mountain trekking. I like to go hiking as a day trip, but I prefer to traverse the mountains for days at a time carrying a tent. My favorite mountain areas are Daisetsuzan (in Hokkaido) and the Japanese Southern Alps. I also love taking an Onsen (hot spring) bath after descending a mountain.

Reference

Tsuruoka, T., Nakayama, E., Endo, T., Harashima, S., Kamada, R., Sakaguchi, K. and Imagawa, T. (2023). Development of a fluorescence reporter system to quantify transcriptional activity of endogenous p53 in living cells. *J. Cell Sci.* **136**, jcs260918. doi:10.1242/jcs.260918