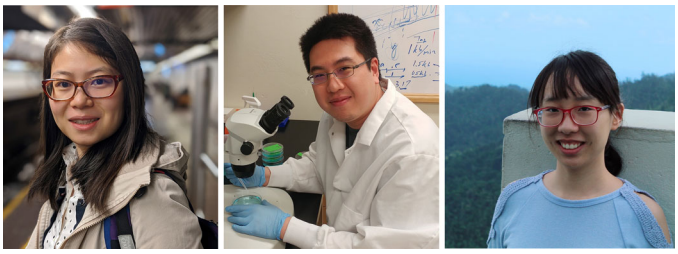
© 2020. Published by The Company of Biologists Ltd | Journal of Cell Science (2020) 133, jcs253070. doi:10.1242/jcs.253070

FIRST PERSON

First person – Wenqing Zhou, Alan Hsu and Yueyang Wang

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Wenqing Zhou, Alan Hsu and Yueyang Wang are first authors on 'Mitofusin 2 regulates neutrophil adhesive migration and the actin cytoskeleton', published in JCS. Wenqing, Alan and Yueyang conducted the research described in this article while PhD students in Qing Deng's laboratory at Purdue University, West Lafayette, IN, where Yueyang is currently studying mechanisms of neutrophil migration and activation, including the response of neutrophils to tissue injury and infection. Wenqing is now a postdoctoral associate in the lab of Gregory Sonnenberg at Weill Cornell Medicine, New York, NY, investigating the mechanisms regulating immune homeostasis and response in the gastrointestinal tract. Alan is now a postdoctoral fellow in the lab of Dr Hongbo Luo at Harvard Medical School and Boston Children's Hospital, Boston, MA, where he is investigating the factors that govern neutrophil function and how they impact immune responses to pathogen challenges.



Wenqing Zhou (left), Alan Hsu (middle) and Yueyang Wang (right)

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

W.Z.: Neutrophils are our first line of immune defense against infection. When infection or injury occurs, they are usually the first responders that migrate to inflammation sites. Mitochondria are the powerhouses of cells and provide energy. However, neutrophils do not rely on mitochondria for energy production. The functions of mitochondria in neutrophil biology still remain elusive. We found that the mitochondrial dynamics gene mitofusin 2 (*MFN2*) is important for neutrophil migration. When the *mfn2* gene is depleted in neutrophils in zebrafish, the neutrophils are surprisingly trapped in the vasculature and fail to respond to inflammation. In addition to zebrafish, we further confirmed a defect in neutrophil migration towards stimulation in other model systems, including human neutrophil-like cells and a mouse model. Finally, we identified that mitofusin 2 mediates the interaction between mitochondria and the ER membrane, which maintains appropriate intracellular signaling to control the cell migration machinery. We have characterized a previously unappreciated role in cell migration for a gene regulating mitochondrial dynamics.

A.H.: Neutrophils are cells in our immune system that are the first responders to a threat or infection. In order to reach, or 'migrate' to, the inflamed site, neutrophils need to undergo a complex process of sensing where to go and clinging to surfaces in order to move in an orderly fashion (sort of like rock climbing). In this story we discovered that *MFN2*, a gene better known for its function in regulating the mitochondria (the power plant of the cell), has an alternative role in helping neutrophils migrate. When lost, neutrophils cannot cling on to the 'wall' or surface, thus cannot move as efficiently. To understand this, we gave our cells a prosthetic linker that 'grabs' the ER and mitochondria together, and BOOM, the migration was restored. In summary, our research opens up new possibilities to regulate neutrophil migration and presents a new function for MFN2, highlighting the beauty of our cells and evolution.

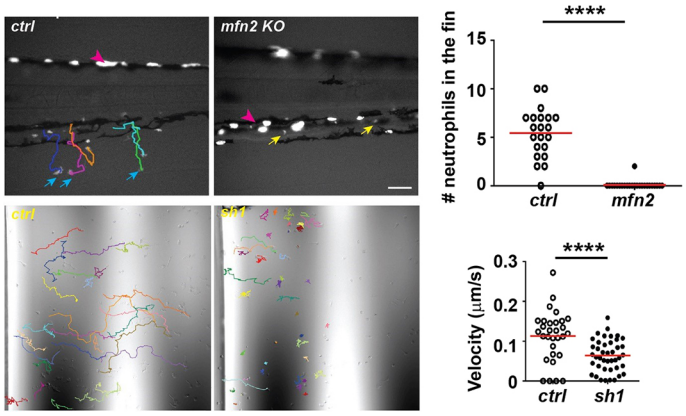
Y.W.: We found an unexpected role of the mitochondrial protein MFN2. It functions as a bridge between mitochondria and the ER in neutrophils. Ca^{2+} in the ER can cross the bridge and give signals to mitochondria. Without this bridge, mitochondria cannot receive the right signals, so neutrophils cannot move properly.

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

W.Z.: The first challenge was the tissue-specific knockout of *mfn2* in neutrophils in zebrafish. The neutrophil-specific knockout approach was not available when we started this project. We adapted the CRISPR/Cas9 system, and optimized and established it in zebrafish neutrophils. Although our system worked effectively in zebrafish, the fluorescent protein co-expressed for screening was dim, especially in the *mfn2* transgenic line, where all neutrophils circulate in the bloodstream and flow very fast. It took lots of optimization of live imaging and patience to catch these cells. The second challenge was to switch to cell culture to investigate the molecular mechanisms. We are a zebrafish lab, and cell culture was a new field to us. We started all the cell-line-based assays, including purchasing incubators and other essential equipment. All beginnings are hard. It took us a lot of time and effort to finally become familiar with the cell lines and to establish cell-based assays in the lab. We would like to thank Dr Orion Weiner for sharing the human neutrophil-like cell line, and Dr Anna Huttenlocher and Dr Carole Parent for sharing protocols with us. The mouse work was a challenge for us as well. Our lab had no experience with mouse models before. In order to expand our findings, we initiated the mouse work with tremendous help from our neighboring labs. Another challenge was to build the *in vitro* flow-adhesion assay. In zebrafish, we observed that *mfn2*-deficient neutrophils circulate in the vasculature and fail to extravasate. To mimic the blood flow *in vivo*, we established an *in vitro* flow-adhesion chamber and we tried very hard to control the cell flow rate, avoid bubbles in the tube, and optimize the live imaging. Besides these, personally, how to balance work and life is a big challenge for me. I had a

Wenqing Zhou's contact details: Weill Cornell Medicine, 413 East 69th St, New York, NY 10021, USA.
E-mail: wez4003@med.cornell.edu
Alan Hsu's contact details: Harvard Medical School and Boston Children's Hospital, 814 Enders Building, 320 Longwood Ave., Boston, MA 02115, USA.
E-mail: alan.hsu@childrens.harvard.edu
Yueyang Wang's contact details: Department of Biological Sciences, Purdue University, 915 W State St, Lilly Hall, Room G206, West Lafayette, IN 47906, USA.
E-mail: wang3654@purdue.edu

Journal of Cell Science (2020) 133, jcs253070. doi:10.1242/jcs.253070



MFN2-deficient neutrophils fail to perform chemotaxis.

several-month-old baby to take care of when I was working on this project. I really appreciate my highly supportive family, whose support meant I could focus on the project when I was in the lab.

A.H.: For me personally, to decipher the role of MFN2 in regulating neutrophil migration, either through physical tethering or mitochondrial dynamics itself, was tricky because genetic ablation would impair both. We tackled this by expressing an artificial tether in our MFN2-knockdown cells to link the mitochondria and ER back together. It was difficult to hit a sweet spot as this 'forced' interaction, if not delicately done, would lead to cell death. Thus through multiple rounds of construct design and cloning, we were able to show that a 'prosthetic' linker could rescue neutrophil migration, reduce Rac activation levels, and restore mitochondria localization.

Y.W.: The half-life of neutrophil cells is too short to culture *in vivo*. We used a neutrophil-like cell line, dHL-60 cells. Still, these are pretty fragile and can be activated after exposure to many types of stimuli. It took us some time to complete the gene modifications and verifications in this cell line.

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

W.Z.: Actually, I think I had lots of 'eureka' moments when I was doing this project. I remember the first time I saw the majority of *mfn2*-deficient neutrophils circulating in vasculature of zebrafish embryos. I could not believe my eyes, because circulating neutrophils had never been reported in zebrafish embryos before. I ran to grab my supervisor from her office to look at those embryos, and we both were so astonished and excited. The first time I saw MFN2-knockdown neutrophils barely moving in the chemotaxis assay, the first time that the *in vitro* flow-adhesion assay worked, the first time I saw disrupted mitochondrial structure, the first time I saw a hyperactivation of Rac and the first time I saw rescued chemotaxis with Rac inhibitors were are all eureka moments for me.

A.H.: Yes, two, in fact. The first one was after my colleague characterized the phenotype of migration in zebrafish and HL-60 cells, and I performed the *in vivo* recruitment assay in a mice peritonitis model. Since neutrophil recruitment is a complex multi-layered process, I jumped off of my seat when I saw that there was significant impairment in neutrophils recruited to the peritoneal cavity in our conditional knockout mice. The second was the experiment I mentioned above, where we could use artificial bridging to rescue an *MFN2* genetic abrogation, thus restoring the migration phenotype.

Y.W.: The phospho-western blot assay for neutrophil samples was a struggle for us due to the high activity of proteases and phosphatases in neutrophils. We tried several different protocols and finally found a good way to perform this experiment. It amazed me how the Rac inhibitors bring down the heightened Rac activation in the *MFN2*-deficient cells without affecting that in the control cells as much.

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

W.Z.: We chose Journal of Cell Science because it has a broad audience of cell biologists. According to the description of the journal, we believe it is appropriate to report our findings involving mitochondria, immune cell chemotaxis and intracellular signaling here.

A.H.: We felt that JCS provides a good group of readers and a broad scope of audience to share our work with. I personally feel that our work, though cool and exciting, raises more possibilities and work to do. But we needed to wrap up somewhere to deliver a straight and strong point and keep our story concise. The way the JCS editorial board and journal are laid out was perfect for what we wanted to share.

Y.W.: I am very happy that our paper was accepted by Journal of Cell Science.

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

W.Z.: Dr Qing Deng, my PhD supervisor, has been highly supportive in my research and life. Besides providing insightful advice on projects, she often shares her experience with me about work–life balance as a female scientist. She always encourages me to pursue what I want to do in research and also in life. I am very grateful for her trust and confidence in me.

A.H.: Dr Stan Gelvin at Purdue University, to whom I owe deep gratitude for filling me in, because at the time I was lacking background in molecular biology and current biological methods. Furthermore, Dr Gelvin would often speak to me about the pros and cons of academia and steer me toward a correct mindset to approach science – with rigor and unrelenting passion.

Y.W.: I want to thank my partner Alan Hsu. Wenqing started the project, and Alan and I had a good time working together to finish the project after Wenqing's graduation. He inspired me and gave me new perspectives about immunology, neutrophil biology and scientific careers.

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?

W.Z.: The exciting moments when assays are worked out, the thrilling moments when a hypothesis being tested is correct, and the proud moments when my work is reported motivate and intrigue me to pursue a career in science. At the time when I read the email about acceptance of my first manuscript six years ago, I was so thrilled and I called my best friend immediately to tell her my exciting news, then I realized that this is what I want to do in my life.

A.H.: My first curious question came from my time in clinic, when I wondered how dengue virus causes bone marrow suppression. Can dengue virus directly infect bone marrow cells? If so, which lineage? After getting a feel for testing hypotheses and designing experiments to address my curiosity during my early years in

Journal of Cell Science (2020) 133, jcs253070. doi:10.1242/jcs.253070

science, I decided to further my training by pursuing a PhD. The next big moment came when I discovered that terminally differentiated neutrophils required cell cycle proteins for their function, which seemed counterintuitive at the time, but the pleasure and joy of setting out to prove this and to find it is true was unparalleled. I carry the same curiosity to this day, and seek to understand biology and immunology. I look forward hopefully to my next chapter and 'a-ha' moment, as they are what fuels me.

Who are your role models in science? Why?

W.Z.: My PhD supervisor Dr Qing Deng and my current mentor Dr Gregory Sonnenberg are my role models in science, as both of them are intelligent, diligent and passionate about science. Importantly, they are highly supportive, training their trainees as independent scientists, and caring about not only their research projects but also their careers and lives.

A.H.: Trained as an infectious disease specialist, then moving on to study neutrophils in the context of cell migration and now shifting to neutrophil immunology, I especially look up to Dr Michael Diamond, as his interdisciplinary training and his scientific reflex and thinking are all I hope to be, and more. But on a more heartfelt note, I would say I look up to my past and current mentors Dr Guey-Chuen Perng, Dr Qing Deng, and Dr Hongbo Luo, as they all share qualities of diligence, intelligence, scientific logic, and most of all patience and passion to mentor a young and junior scientist-to-be such as myself.

What's next for you?

W.Z.: Currently, I am a postdoctoral associate at Weill Cornell Medicine, pursuing my interests in immune homeostasis and regulation at mucosal sites. I hope to pursue a career in academia as an independent investigator.

A.H.: I have recently started my postdoc training in Dr Hongbo Luo's lab at Harvard Medical School, where I am continuing to learn about the more immunological and developmental aspects of neutrophils. After my training, I hope to continue my passion and pursue a career in academia and hopefully pass on the torch, as so many have done for me.

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

W.Z.: I am a big fan of suspense movies. I enjoy thinking and figuring out puzzles and mysteries as the movie goes, just like I really enjoy asking questions, developing a hypothesis, testing the hypothesis and addressing questions during research.

A.H.: Besides trying my best and being as detailed and rigorous I can be in science, I carry the same attitude to other aspects in life, including a long-time leisure activity of mine – gaming. So much so that I achieved number one in the world rankings for a game I play – Starcraft. Playing video games not only helps me to relax but also to learn collaboration and teamwork. And just like in my science, I could not do it without the help of great mentoring, great coaching and an exceptional team!

Reference

Zhou, W., Hsu, A. Y., Wang, Y., Syahirah, R., Wang, T., Jeffries, J., Wang, X., Mohammad, H., Seleem, M. N., Umulis, D. et al. (2020). Mitofusin 2 regulates neutrophil adhesive migration and the actin cytoskeleton. *J. Cell Sci.* **133**, jcs248880. doi:10.1242/jcs.248880