

Live-animal imaging of native haematopoietic stem and progenitor cells: An interview with Constantina Christodoulou, Joel Spencer and Shu-Chi (Allison) Yeh

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Co-first authors (Left to Right): Dr. Constantina Christodoulou, Dr. Joel Spencer and Dr. Shu-Chi (Allison) Yeh

Over the last few decades, hematopoietic stem cells (HSCs) have been prospectively identified and studied based on their ability to functionally regenerate and maintain the entire blood system through serial transplantation. While many important determinants of HSC activity have been elucidated through these foundational assays, less is known as to how HSCs function in unperturbed states within their native microenvironments. Advancements in genetic tools have recently begun to open up opportunities for researchers to address

these gaps in our understanding of HSC biology. Here, I interviewed co-first authors Dr. Constantina Christodoulou, Dr. Joel Spencer and Dr. Shu-Chi (Allison) Yeh, who provided some further commentary on their recent successful efforts to image HSCs in live animals with the support of collaborative teams led by Dr. Fernando Camargo and Dr. Charles Lin (Christodoulou et al., 2020).

1. First off, congratulations on your monumental achievement of imaging HSCs in the native live-animal environment. For all readers of your work, what do you think are the most important take-home messages that should be gleaned from your work?

C: Thank you for this opportunity! For myself, I think the results of our work suggest that while activation of HSCs is correlated with stress or stimuli, they may not be as active as many of us have originally thought within their native conditions. For the first time, we are moving into a period where we can dissect what these cells are doing in their natural environment. We now have a tool to test a lot of previously held assumptions about HSCs in their native microenvironments and have importantly identified different restricted bone marrow regions important to their cellular behaviours.

J: Thank you! It is definitely exciting that we now have a tool to visually study native mammalian HSC biology *in vivo*. We are now able to visually determine how HSCs respond to various stressors and have tied their behaviours to bone remodeling processes. We have also been able to directly measure local oxygen tension surrounding these cells which has been a long-term goal of mine.

A: The degree of heterogeneity revealed in the bone marrow environment and dynamic changes that occur with bone remodelling also brings up the possibility that it may be a bit insufficient to classify the HSC niche as a static compartment. With this model, we can now further characterize HSC niche components downstream of bone remodeling, such as calcium gradients and the surrounding vascular architecture.

2. What were some of the technical barriers encountered or challenges faced that needed to be overcome in this work?

J: First off, all of this imaging work built on the foundation of important validation data brought forward by Constantina to verify that we were indeed imaging functionally tested HSCs. We had to learn what combination of reporters to use (or not to use) given the purity and rarity of cells we needed to visually identify. Once we figured out the correct combination, it was still very challenging to quickly scan the whole calvarial bone marrow in a live mouse to find such rare and relatively dim GFP cells on a backdrop of thousands of autofluorescent cells. I had to train my eyes to quickly and accurately find these cells so we

could study them. We also had to optimize imaging parameters to account for prioritized volumetric versus temporal data acquisition, realigning imaging drift, and all the nuances that come with live animal imaging.

A: I too had to train my eyes when we first imaged the mice to distinguish between true signal over autofluorescence. In multicolor imaging, especially when working with dyes with overlapped excitation or emission spectra, it was important to make sure the fluorescent signals in different acquisition channels did not crosstalk and that our chosen staining dyes for the bone remodeling experiments would not interfere with each other. We also had to pay attention to the anesthesia depth and hydration status of each animal during long hours of imaging acquisition in order to capture dynamics at the cellular level.

C: There was definitely some transitioning of expectations from linking the GFP signal seen beautifully on flow cytometry with what we were hoping to measure live and *in situ*. With the animal imaging work, we had to optimize the type and amount of anesthesia used in each animal and ensure proper hydration; this variable affected the breathing patterns, which would alter the focus of imaging as well as the assignment of imaging coordinates at times. These experiments involved a lot of fine-tuning for sure!

3. What are some of the most interesting questions and/or next steps this work raises for future studies?

C: I think it would be really interesting to now combine this model with models of leukemogenesis to understand how the bone marrow environment changes, what happens to HSCs during their conversion into leukemia stem cells (LSCs) and the cellular dynamics that occur during and throughout this process. A second important question would be to understand how these particular regenerative regions in the bone marrow around HSCs enable their ability to proliferate, expand or differentiate.

A: I would love to dig deeper on the downstream effects of bone marrow remodeling, such as the coupling between bone remodeling with the vascular niche, calcium gradients, composition of resident immune cells, and whether the different cavity types are supportive of other hematological malignancies.

J: I agree – we can now closely study how HSCs respond to various stressors, degree of hypoxia and so much more.

4. What do you think were important ingredients that enabled a successful collaboration from your experience in this project?

C: I believe that open communication between us three was key to this project, as I recall having numerous conversations touching on whether we could broach this technical possibility for such a complex project aim to define the HSC niche. We also brought a lot of

respect for each others' skills and expertise, which really made all of this possible.

J: There were a lot of conversations, not only about science, but also getting to know each other. Science is difficult and there is often a lot of failure. A lot of our conversations brought clarity towards knowing which paths to take and centred on building trust and encouraging each other. Along with the support of our principal investigators, we could continue being determined and push forward to the finish line. This trust and teamwork were especially important as I transitioned to UC Merced in Fall of 2017 to start my lab and continue this work.

A: I also really enjoyed being part of discussions as our various backgrounds stimulated us to think more and to address important questions that spanned our disciplines. This project was multidisciplinary in nature, so we needed to truly work collaboratively in order to attain a successful outcome.

5. What advice would you offer to trainees interested in establishing a successful research career?

C: Hard work always pays off even in very difficult fields. Don't be discouraged and be true to your beliefs. Your work will be reciprocated at some point as it is seen by scientists in the community. Surround yourself with good mentors – ones who provide you freedom to explore what may be controversial or scary (as these are what makes science substantially move forward) and who can also bring out your curiosity in carrying out experiments to test interesting ideas that could very well lead to some amazing outcomes. These are the type of leaders you should see as exemplar role models to emulate in how you should contribute to the research world.

J: Work with people who care about each other and the work. Find a support system where you can talk to peers, reminding us that we are not alone, and that there are ways to overcome many challenges in life (e.g. financial difficulties, starting a family). It's also important to find a good mentor or mentors. A good mentor provides opportunities for people whose potential they can see and tease out (sometimes through previous failures) and in some cases, takes risks on individuals based on such insight and not just those who look good on paper. I was fortunate to have a phenomenal mentor in Dr. Charles Lin and I hope to continue his example in my lab.

A: A good mentor also identifies areas where a mentee may lack and responds by providing training opportunities to get them to a fuller level of independence, so that they are able to build up a level of confidence to talk about their work, see themselves as qualified candidates for various jobs. Always try to seek for opportunities and don't be afraid of failures (e.g. getting reject letters from grant competitions). All of these practices will be valuable experiences and training while establishing one's research career.

Reference:

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