See-through zebrafish, new imaging method put blood stem cells in high-resolution spotlight

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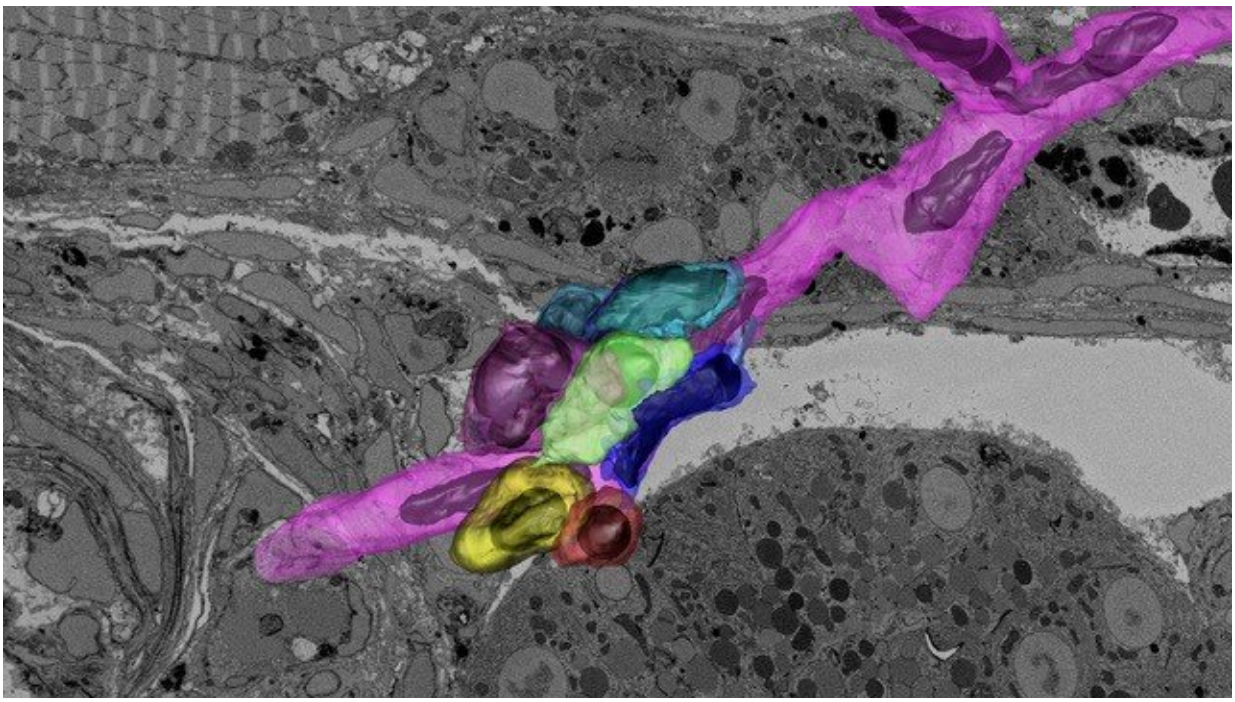

Tracing features in a large 3D electron microscopy dataset reveals a zebrafish blood stem cell (in green) and its surrounding niche support cells, a group photo method that will help researchers understand factors that contribute to blood stem cell health—which could in turn help develop therapies for blood diseases and cancers. Credit: Keunyoung Kim

For the first time, researchers can get a high-resolution view of single

blood stem cells thanks to a little help from microscopy and zebrafish.

Researchers at the University of Wisconsin–Madison and the University of California San Diego have developed a method for scientists to track a single blood stem cell in a live organism and then describe the ultrastructure, or architecture, of that same cell using electron microscopy. This new technique will aid researchers as they develop therapies for blood diseases and cancers.

"Currently, we look at stem cells in tissues with a limited number of markers and at low resolution, but we are missing so much information," says Owen Tamplin, an assistant professor in UW–Madison's Department of Cell & Regenerative Biology, a member of the Stem Cell & Regenerative Medicine Center, and a co-author on the new study, which was published Aug. 9 in *eLife*. "Using our new techniques, we can now see not only the stem cell, but also all the surrounding niche cells that are in contact."

The niche is a microenvironment found within tissues like the bone marrow that contain the blood stem cells that support the blood system. The niche is where specialized interactions between blood stem cells and their neighboring cells occur every second, but these interactions are hard to track and not clearly understood.

As a part of the new study, Tamplin and his co-lead author, Mark Ellisman, a professor of neuroscience at UC San Diego, identified a way to integrate multiple types of microscopic imaging to investigate a cell's niche. With the newly developed technique that uses confocal microscopy, X-ray microscopy, and serial block-face scanning electron microscopy, researchers will now be able to track the once elusive cell-cell interactions occurring in this space.

"This has allowed us to identify cell types in the microenvironment that

we didn't even know interacted with stem cells, which is opening new research directions," Tamplin says.

As a part of this study, Tamplin, and his colleagues, including co-first authors Sobhika Agarwala and Keunyoung Kim, identified dopamine beta-hydroxylase positive ganglia cells, which were previously an uncharacterized cell type in the blood stem cell niche. This is crucial, as understanding the role of neurotransmitters like dopamine in regulating blood stem cells could lead to improved therapeutics.

"Transplanted blood stem cells are used as a curative therapy for many blood diseases and cancers, but blood stem cells are very rare and difficult to locate in a living organism," Tamplin says. "That makes it very challenging to characterize them and understand how they interact and connect with neighboring cells."

While blood stem cells are difficult to locate in most living organisms, the zebrafish larva, which is transparent, offers researchers a unique opportunity to view the inner workings of the blood stem cell niche more easily.

"That's the really nice thing about the zebrafish and being able to image the cells," Tamplin says of animal's transparent quality. "In mammals, blood stem cells develop in utero in the bone marrow, which makes it basically impossible to see those events happening in real time. But, with zebrafish you can actually watch the stem cell arrive through circulation, find the niche, attach to it, and then go in and lodge there."

While the zebrafish larva makes it easier to see blood stem cell development, specialized imaging is needed to find such small cells and then detail their ultrastructure. Tamplin and his colleagues spent over six years perfecting these imaging techniques. This allowed them to see and track the real-time development of a blood stem cell in the

microenvironment of a live organism, then zoom in even further on the same cell using electron microscopy.

"First, we identified single fluorescently labeled stem cells by light sheet or confocal microscopy," Tamplin says. "Next, we processed the same sample for serial block-face scanning electron microscopy. We then aligned the 3D light and electron microscopy datasets. By intersecting these different imaging techniques, we could see the ultrastructure of single rare cells deep inside a tissue. This also allowed us to find all the surrounding niche cells that contact a blood stem cell. We believe our approach will be broadly applicable for correlative light and electron microscopy in many systems."

Tamplin hopes that this approach can be used for many other types of stem cells, such as those in the gut, lung, and the tumor microenvironment, where rare cells need to be characterized at nanometer resolution. But, as a developmental biologist, Tamplin is especially excited to see how this work can improve researchers' understanding of how the blood stem cell microenvironment forms.

"I think this is really exciting because we generate all of our blood stem cells during embryonic development, and depending on what organism you are, a few hundred or maybe a few thousand of these stem cells will end up producing hundreds of billions of new blood cells every day throughout your life," Tamplin says.

"But we really don't know much about how stem cells first find their home in the niche where they're going to be for the rest of the life of the organism. This research will really help us to understand how stem cells behave and function. A better understanding of stem cell behavior, and regulation by surrounding niche cells, could lead to improved stem cell-based therapies."

More information: Sobhika Agarwala et al, Defining the ultrastructure of the hematopoietic stem cell niche by correlative light and electron microscopy, *eLife* (2022). DOI: 10.7554/eLife.64835

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