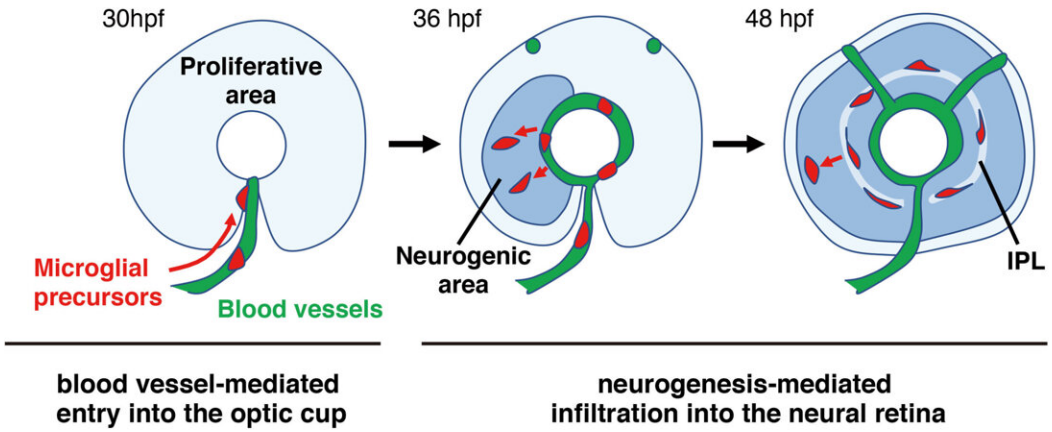
Study reveals journey of immune cells in developing zebrafish

December 7 2021



A model showing how microglial precursors colonize the developing zebrafish retina. As can be seen, the microglial precursors use the blood vessels to enter the optic cup and then disperse to areas undergoing neurogenesis. Credit: OIST

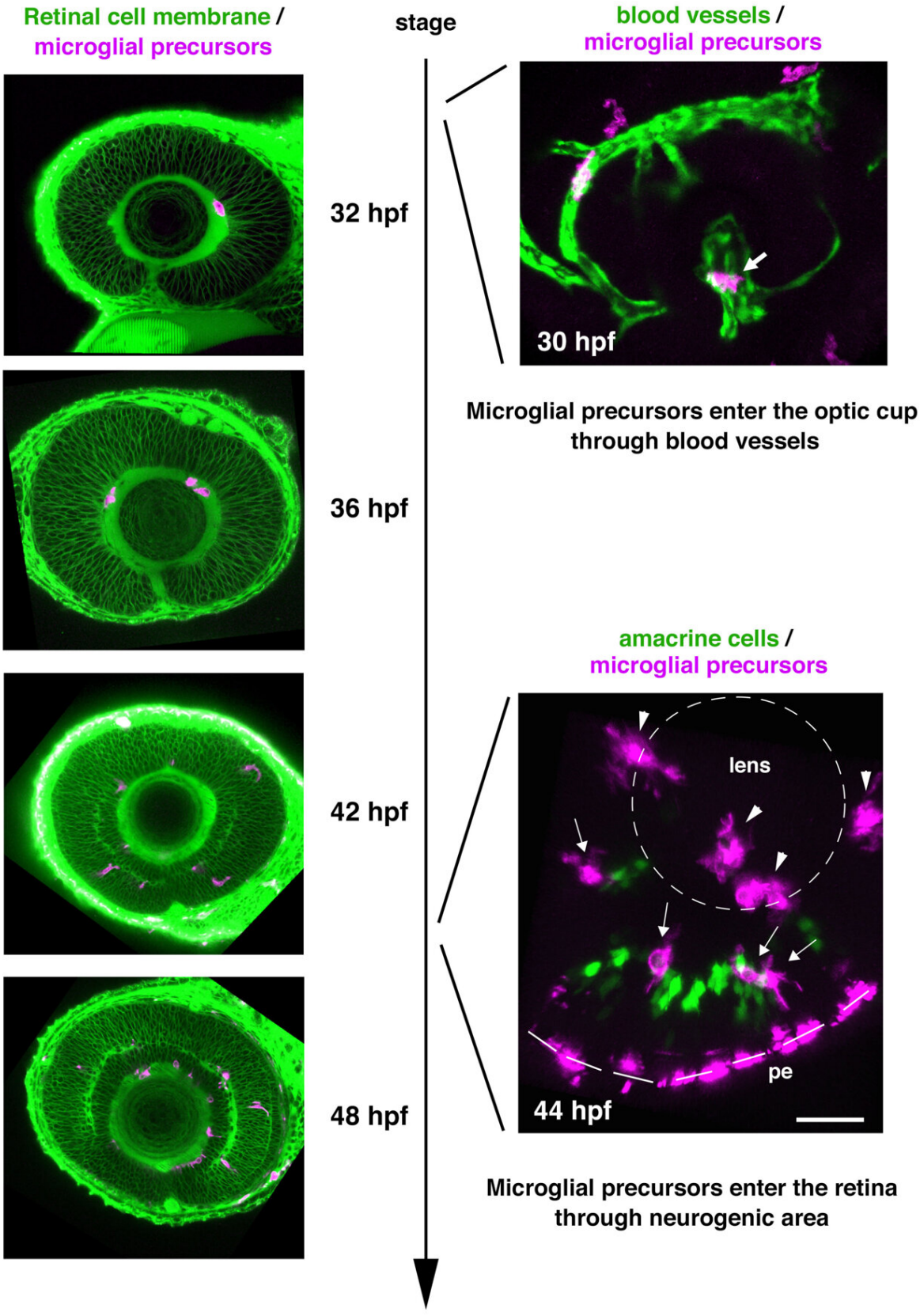
Microglia, the immune cells of the brain, form the first line of defense against neurodegenerative diseases and traumatic brain injuries. They maintain brain homeostasis, the stable condition necessary for survival, by acting like tiny vacuum cleaners—congregating at damaged areas, removing dead, infected, or injured brain cells and tidying up unnecessary synapses. However, microglia don't originate in the brain,

rather their precursor form travels there during development from their place of origin, another section of the embryo called the peripheral mesoderm. Many questions around this process, such as how the microglial precursors find their way, are currently unanswered. And, given how important these cells are for brain homeostasis in all animals, including humans, answering these questions could have a myriad of health benefits.

"Microglia have been implicated in several neurobiological diseases such as Alzheimer's. Revealing how they work could shed light on these diseases," stated Prof. Ichiro Masai who leads the Developmental Neurobiology Unit at the Okinawa Institute of Science and Technology Graduate University (OIST). "Microglia are found throughout the brain, but our most recent paper looks at how they colonize the retina—the neural tissue of the eye, which is one of the first brain regions to accommodate these cells."

Reporting in *eLife*, Prof. Masai and former OIST Ph.D. student Dr. Nishtha Ranawat revealed that, to successfully colonize the retina, microglia require two processes to have occurred—the blood vessels must have formed inside the eye, and neurogenesis, the process of neuron formation, must have begun within the retina.

To uncover these requirements, the researchers imaged transparent embryonic zebrafish from 24 hours after fertilization to 60 hours after fertilization. They tagged the microglia precursors with fluorescence, which allowed them to be tracked. From their original place, these precursors first traveled to the yolk, and started migrating towards the various brain regions from there.


Retinal cell membrane /
microglial precursors
stage
blood vessels /
microglial precursors
32 hpf
30 hpf
Microglial precursors enter the optic cup
through blood vessels
36 hpf
amacrine cells /
microglial precursors
42 hpf
lens
pe
44 hpf
48 hpf
Microglial precursors enter the retina
through neurogenic area

Images of a zebrafish retina from 30 hours after fertilization (hpf) to 48 hpf. The microglial precursors are tagged with purple fluorescence, whilst the developing zebrafish retina is green. The right upper image shows, at around 30 hpf, the first microglial precursors entering the eye via the blood vessels. The right lower image shows, at between 42 and 48 hpf, the microglial precursors colonizing the rest of the retina through areas undergoing neurogenesis. Credit: OIST

"The developing brain is very packed tissue," explained Prof. Masai. "It's hard to imagine how the microglia enter it and move around inside. But we found that they use the blood vessels as pathways to enter the eye."

However, the journey doesn't end there. Once the microglia arrive at the entrance of the retina, they remain associated with the blood vessels between the retina and the lens until another process begins—neurogenesis. Neurogenesis is the process of neuron formation. The researchers found that microglia could only infiltrate areas of the retina where neurogenesis was occurring. At around 60 hours after fertilization, the microglia were in place, spread throughout the retina.

To further determine that the blood vessels and neurogenesis were important components for microglia migration, the researchers hindered, in one experiment, the blood vessels from forming and, in another experiment, neurogenesis from occurring. In both cases, microglia were unable to enter the retina.

"This study highlights the microglia's dependence on the developing retina blood vessels and their preference for differentiating neurons," said Dr. Ranawat, who is the lead author of this study and now a

Postdoctoral Scholar at Burke Neurological Institute in the U.S.. "In the future, this knowledge could lead to targeted microglia stem cell therapies for neurodegenerative diseases. The next step is to find the molecule dictating the interaction between the microglia and the blood vessels and neurons."

More information: Nishtha Ranawat, Ichiro Masai, Mechanisms underlying microglial colonization of developing neural retina in zebrafish, *eLife* (2021). DOI: 10.7554/eLife.70550

Provided by Okinawa Institute of Science and Technology