Possible new plan of attack for opening and closing the blood-brain barrier

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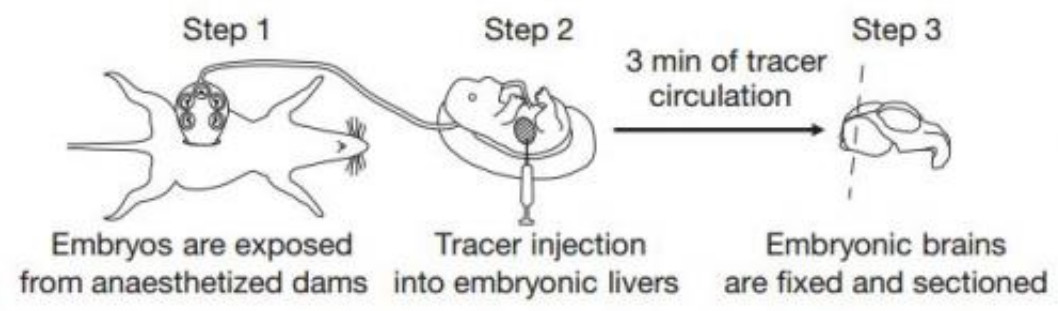

, In utero embryonic liver tracer injection method; fenestrated liver vasculature enabled rapid tracer uptake into the embryonic circulation. Credit: Nature, doi: 10.1038/nature13324

Like a bouncer at an exclusive nightclub, the blood-brain barrier allows only select molecules to pass from the bloodstream into the fluid that bathes the brain. Vital nutrients get in; toxins and pathogens are blocked. The barrier also ensures that waste products are filtered out of the brain and whisked away.

The blood-brain barrier helps maintain the delicate environment that allows the human brain to thrive. There's just one problem: The barrier is so discerning, it won't let medicines pass through. Researchers haven't been able to coax it to open up because they don't know enough about how the barrier forms or functions.

Now, a team from Harvard Medical School has identified a gene in mice, Mfsd2a, that may beresponsible for limiting the barrier's permeability—and the molecule it produces, Mfsd2a, works in a way few researchers expected.

"Right now, 98 percent of small-molecule drugs and 100 percent of large-molecule drugs and antibodies can't get through the blood-brain barrier," said Chenghua Gu, associate professor of neurobiology at HMS and senior author of the study. "Less than 1 percent of pharmaceuticals even try to target the barrier, because we don't know what the targets are. Mfsd2a could be one."

Most attempts to understand and manipulate blood-brain barrier function have focused on tight junctions, seals that prevent all but a few substances from squeezing between barrier cells. Gu and her team discovered that Mfsd2a appears to instead affect a second barrier-crossing mechanism that has received much less attention, transcytosis, a process in which substances are transported through the barrier cells in bubbles called vesicles. Transcytosis occurs frequently at other sites in the body but is normally suppressed at the blood-brain barrier. Mfsd2a may be one of the suppressors.

"It's exciting because this is the first molecule identified that inhibits transcytosis," said Gu. "It opens up a new way of thinking about how to design strategies to deliver drugs to the central nervous system."

Because Mfsd2a has a human equivalent, blocking its activity in people could allow doctors to open the blood-brain barrier briefly and selectively to let in drugs to treat life-threatening conditions such as brain tumors and infections.

Conversely, because researchers have begun to link blood-brain barrier degradation to several brain diseases, boosting Mfsd2a or Mfsd2a could

allow doctors to strengthen the barrier and perhaps alleviate diseases such as Alzheimer's, amyotrophic lateral sclerosis (ALS) and multiple sclerosis. The findings may also have implications for other areas of the body that rely on transcytosis, such as the retina and kidney.

The study was published May 14 in *Nature*.

Back to the beginning

As developmental biologists, Gu and her colleagues believed watching the barrier develop in young organisms would reveal molecules important for its formation and function.

The team introduced a small amount of dye into the blood of embryonic mice at different stages of development and watched whether it leaked through the walls of the tiny capillaries of the mice's brains, suggesting that the blood-brain barrier hadn't formed yet, or stayed contained within the capillaries, indicating that the barrier was doing its job. This allowed them to define a time window during which the barrier was being built.

The team was able to do this by devising a new dye injection technique. Researchers studying blood-brain barrier leakage in adult organisms can inject dye directly into blood vessels, but the capillaries of embryos are too small and delicate. Instead, researchers typically inject dye into the heart. However, according to Gu, this can raise blood pressure and burst brain capillaries, making it difficult to tell whether leakage is due to blood-brain barrier immaturity or the dye procedure itself. She and her team used theirvascular biology expertise to identify an alternate injection site that would avoid such artifacts: the liver.

"This allowed us to provide definitive evidence that the blood-brain barrier comes into play during embryonic development," said Ayal Ben-Zvi, a postdoctoral researcher in the Gu lab and first author of the study.

"That changes our understanding of the development of the brain itself."

Telltale pattern

Now that they knew when the barrier formed in the mice, the team compared endothelial cells—the cells that line blood vessel walls and help form the blood-brain barrier—from peripheral blood vessels and cortical (brain) vessels and looked for differences in gene expression. They made a list of genes that were expressed only in the cortical endothelial cells. From thatlist, they validated about a dozen invivo.

The team could have studied any of the genes first, but they were most intrigued by Mfsd2a because of its expression pattern. In addition to being switched on in brain vessels, it was active in the placenta and testis, two other organs that have barrier-type functions. Also, the gene is shared across vertebrate organisms that have blood-brain barriers, including humans.

Gu and the team then conducted experiments in mice that lacked the Mfsd2a gene. They found that without Mfsd2a, the blood-brain barrier leaked (although it didn't prevent the blood vessels themselves from forming in the first place). The next question was why.

"We focused on two basic characteristics: tight junctions between cells, which prohibit passage of water-soluble molecules, and transcytosis, which happens all the time in peripheral vessels but very little in the cortical vessels," said Gu. "We found the surprising result that Mfsd2a regulates transcytosis without affecting tight junctions. This is exciting because conceptually it says this previously unappreciated feature may be even more important than tight junctions."

"At first we were looking at tight junctions, because we were also biased by the field," said Ben-Zvi, who will be starting his own lab later this

year at The Hebrew University of Jerusalem. "We weren't finding anything on the electron micrographs even though we knew the vessels leaked. Then we noticed there were tons of vesicles.

"It really shows that if you do systematic science and see something strange, you shouldn't dismiss it, because maybe that's what you're looking for."

Next steps

The team also began to study the relationship between the cortical endothelial cells and another contributor to the blood-brain barrier, cells called pericytes. So far, they have found that pericytes regulate Mfsd2a. Next, they want to learn what exactly the pericytes are telling the endothelial cells to do.

Other future work in the Gu lab includes testing the dozen other potential molecular players and trying to piece together the entire network that regulates transcytosis in the blood-brain barrier.

"In addition to Mfsd2a, there may be several other molecules on the list that will be good drug targets," said Gu. "The key here is we are gaining tools to manipulate transcytosis either way: opening or tightening."

As important as the molecules themselves, she added, is the concept.

"I personally hope people in the blood-brain barrier field will consider the mind-shifting paradigm that transcytosis could be targeted or modulated," said Ben-Zvi.

Better understanding—and potentially being able to manipulate—the molecular underpinnings of transcytosis could aid in the study and treatment of diseases in tissues beyond the brain, from the intestines

absorbing nutrients to the kidneys filtering waste.

Being able to open and close the blood-brain barrier also promises to benefit basic research, enabling scientists to investigate how abnormal barrier formation affects brain development and what the relationship may be between barrier deterioration and disease.

More information: Paper: Mfsd2a is critical for the formation and function of the blood–brain barrier, dx.doi.org/10.1038/nature13324

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