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PHYSIOLOGY

Double function at the blood–brain barrier

Two aspects of the blood–brain barrier — the transport of lipids to the brain and the transport of molecules across cells lining blood vessels — have been shown to be regulated by the same protein, Mfsd2a.

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The blood–brain barrier (BBB) is a double-edged sword. On the one hand, this cellular interface helps to maintain a constant, optimal environment for neuronal function through a combination of barriers and selective transport systems that regulate the passage of wanted and unwanted molecules. But on the other hand, it presents a formidable challenge to medicine because it stops most drugs from passing from the bloodstream to the brain. Two papers published on *Nature*'s website today add considerably to our rudimentary understanding of the BBB: Nguyen *et al.*[1] unravel how an essential omega-3 fatty acid is transported across it, and Ben-Zvi *et al.*[2] identify a mechanism involved in suppressing the vesicle-mediated transfer of blood-plasma constituents to the brain.

Astonishingly, these two seemingly unrelated processes depend on the same gene, *Mfsd2a*, which encodes a transmembrane protein that is specific to the endothelial cells that line blood vessels in the brain. Two birds killed with one stone, it seems. But the discoveries also illustrate the contradictory properties of the BBB: Mfsd2a transports lipids that are essential for brain growth and function, while at the same time suppressing a transport route across the BBB that might be ideal for the delivery of complex drugs, such as antibodies, into the brain.

Mfsd2a is a member of the major facilitator superfamily (MFS) of secondary-active transporters[3] — proteins that use the electrochemical potential of solutes to shuttle specific substrates across lipid membranes. Known examples are the glucose transporters (GLUT1–4), but many MFS transporters do not yet have described substrates and functions[4]. Previous work has shown that *Mfsd2a* expression is induced by factors in the liver during fasting[5], and the protein has also been implicated in antibiotic transport[6] and in cell fusion in the human placenta[7,8]. However, Mfsd2a is strongly evolutionarily conserved, which seems inconsistent with a human-specific role in the placenta, and instead suggests that its primary function lies elsewhere. Moreover, Mfsd2a is found at only low levels in the liver, but it is highly expressed throughout the brains of several species, and mice lacking Mfsd2a have normal liver metabolism but develop neurological disorders[9]. Now, both Nguyen *et al.* and Ben-Zvi *et al.* determine that Mfsd2a is specifically and constitutively expressed by brain endothelial cells, suggesting that it has a role in the BBB.

Nguyen *et al.* extended the anatomical and behavioural characterization of mice lacking *Mfsd2a*, finding that these animals have small brains and a range of motor and cognitive defects, and have reduced numbers of certain neuron types. Noting that these defects were reminiscent of omega-3 fatty-acid deficiency, the authors compared the lipid composition of brains from *Mfsd2a*-knockout and control mice and found reduced levels of a certain omega-3 fatty acid, docosahexaenoic acid (DHA), in the mutant animals. *In vitro* analysis revealed that Mfsd2a transports DHA only when this fatty acid is attached to the lipid lysophosphatidylcholine (LPC). *In vivo* experiments confirmed that the brain's uptake of DHA occurs mainly through Mfsd2a-dependent transport of LPC–DHA (Fig. 1). Although the importance of DHA for normal brain growth and function was already known[10], the role of LPC as its carrier across the BBB, by means of Mfsd2a, represents a breakthrough in our understanding of how essential fatty acids enter the brain.

Ben-Zvi and colleagues approached Mfsd2a from a different angle. They searched for gene transcripts whose expression by vascular cells correlated with the development of the BBB. Previous work had indicated that, in mammals,

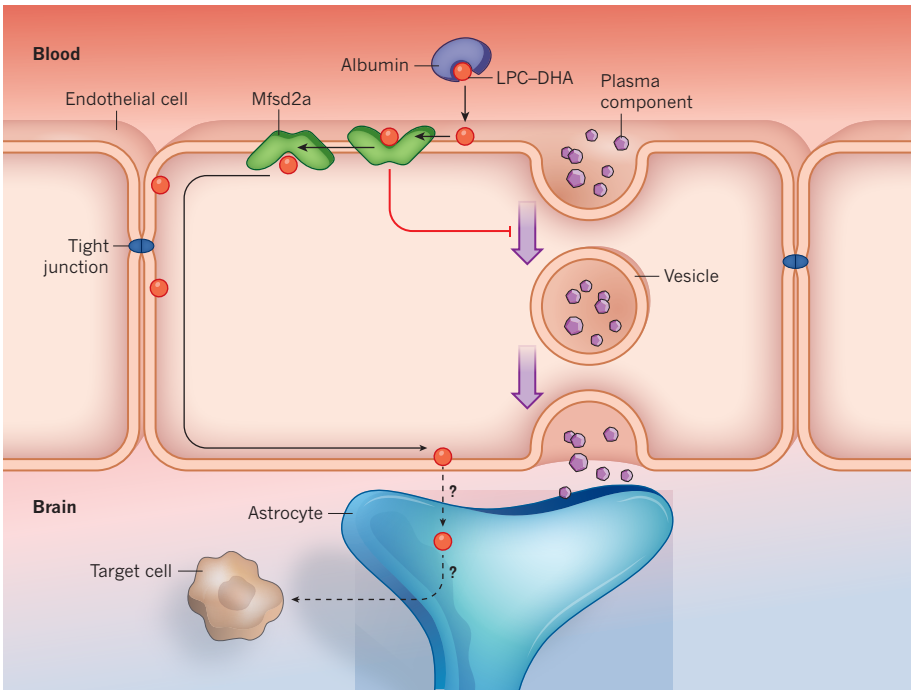

Figure 1 | Dual role for Mfsd2a. Lysophosphatidylcholine–docosahexaenoic acid (LPC–DHA) is bound to the protein albumin during transport in the blood. When it reaches the blood–brain barrier, LPC–DHA detaches from albumin and is adsorbed to the outer lipid leaflet of the cell membrane of endothelial cells. Nguyen *et al.*[1] show that Mfsd2a binds LPC–DHA and transfers it to the inner lipid leaflet, potentially allowing it to bypass the tight junctions between endothelial cells and reach the brain-facing side of the cell. The targets of DHA in the brain are unknown, as is the method by which it passes through the 'end-feet' structures of astrocyte cells, which completely cover the brain's blood vessels. Ben-Zvi *et al.*[2] also show that Mfsd2a suppresses transcytosis in endothelial cells, a transport mechanism by which components of the blood plasma, including proteins, are transferred across the cell in vesicles.

the BBB forms, at least in part, before birth, but it was not clear exactly when this occurs and whether there are regional differences. To observe the development of the BBB, the authors injected fluorescent tracer dyes into the livers of fetal mice, from which the dyes entered the systemic circulation. The authors were then able to map the maturation of the embryonic BBB with high spatio-temporal precision and thus to select a suitable development stage for transcript profiling. They found that Mfsd2a was prominently overexpressed in the brain-cortex endothelium compared with the lung vascular endothelium at embryonic day 13.5, and that the BBB in *Mfsd2a*-knockout mice was impaired from embryonic day 15.5 to adulthood.

The authors also found that, compared to control mice, the brain endothelial cells of *Mfsd2a*-knockout mice displayed increased transcytosis — the transfer of molecules from one side of a cell to another in membrane-bound vesicles (Fig. 1). Increased transcytosis has also been reported for mice with a reduced density of pericytes (cells that wrap around the endothelial cells of small blood vessels) in the brain[11,12], which led Ben-Zvi *et al.* to the finding that the expression of Mfsd2a in brain endothelial cells depends on the presence of pericytes. This transcytotic route is particularly attractive from a drug-delivery perspective because there is no evidence for restrictions on the molecular mass or physico-chemical properties of the cargo transported by this process[11]. Targeting pericytes as a way of opening the BBB for drug passage would be illogical (because these cells lie on the brain side rather than the blood side of the barrier), but targeting Mfsd2a in the endothelial-cell luminal membrane (which contacts the blood) could be feasible.

These two studies provide the first molecular handle on lipid and membrane-vesicle transport across the brain endothelium, but there are still several details to be explored. How does Mfsd2a regulate endothelial transcytosis? Is it a direct mechanism, or does it act indirectly through deficient lipid transport to the brain? Other causes of lipid deficiency, such as a dietary lack of DHA, might shed light on this question.

Regarding the transport of DHA across the BBB, only the first step has been elucidated so far, and it will be interesting to explore how the lipid (or lipids) transported by Mfsd2a completes its passage across the multicellular BBB and into the brain. Nguyen and colleagues' findings suggest that Mfsd2a translocates LPC–DHA from the outer leaflet of the endothelial-cell luminal membrane to the inner leaflet. Lipids in the inner leaflet, but not the outer leaflet[13,14], can bypass the tight junctions between cells, which may allow diffusion of LPC–DHA from the luminal to the abluminal membrane, which faces the brain, but how further transport of DHA in the brain occurs is unclear. The functions of DHA in the brain are also not known, although possibilities include structural roles in membranes or signalling roles in regulating cell behaviour. Further studies of *Msfd2a*-knockout mice should allow these and other questions to be addressed. ■

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