Perspective

The blood-brain barrier models to study apolipoprotein E genotypes in Alzheimer's disease

Irundika HK Dias*, Rachelle Taiwo, Dan Ma

Alzheimer's disease (AD) is a neurodegenerative disease that is characterized by an age-dependent progressive decline of memory, impairment of cognitive functions and changes in personality and behavior. Despite the improvement in understanding of the mechanisms underlying the disease, AD remains an incurable complex disorder with multifaceted pathophysiology to date. Apolipoprotein E (ApoE) is the main cholesterol carrier in the brain that supports lipid transport between brain cells. The individuals carrying the APOE4 allele are known to be at increased risk of developing AD compared with those carrying the more common APOE3 allele. Many researches have been undertaken to understand the role of APOE4 on brain cells and in AD (Shi et al., 2017). Despite the APOE allele being identified as an important genetic risk factor for cardiovascular disease and formation of blood vessels, there is comparatively less research focused on the blood-brain barrier functions in AD. The complex nature of the blood-brain barrier (BBB) and species differences hindered the development in this field. Recent advancement to induced pluripotent stem cell (iPSC) technologies provides an ideal platform to fine-tune BBB models and the possibilities to develop isogeneic models now allow us to improve our knowledge of the BBB and to model more disease relevant models.

In addition to AD-related Aβ and tau pathologies, cerebrovascular dysfunction and vascular pathology is a major contributor in cognitive decline and neuronal loss in AD. Cerebral capillaries account for approximately 85% of cerebral vessel length (Zlokovic, 2011) and serve as important sites of exchanging molecules, metabolites and nutrients between the brain and systemic circulation. These capillaries are lined by microvascular endothelial cells from the blood-brain barrier which results in a tight barrier with high transendothelial electrical resistance and restricted movement of solutes by paracellular and transcellular pathways (Zlokovic, 2011; Profaci et al., 2020).

Endothelial cells that line the BBB are adjoined by specific protein tight junctions (e.g., occludins, ZO-1, ZO-2, ZO-3, claudins and cingulins) with their basement membrane, and supported by underlying brain pericytes, astroglial foot processes and other brain cells (**Figure 1**). As the most abundant cells in the brain, astrocytes provide a supporting environment not only for neuronal survival and function but also for maintaining healthy BBB. Evidences support a synergistic role for the regulation of structure and function of BBB by cell types other than astrocytes and highlight the importance of cellular communications. In healthy brains th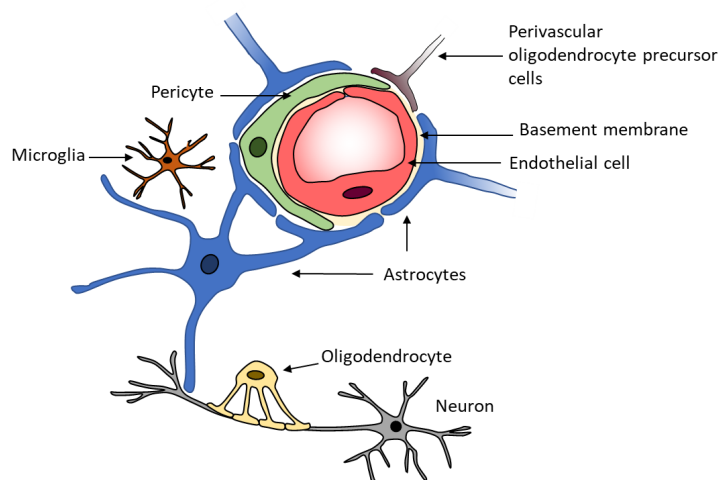ese layers display specific and tightly regulated transport mechanisms between the blood and ventricular cerebrospinal fluid (Zlokovic, 2011). Therefore, as the gatekeeper for peripheral circulation and the brain, the BBB plays an important role in pathophysiology of neurodegenerative diseases.

Influence of ApoE on the BBB: ApoE in the brain is predominantly expressed by glial cells, astrocytes, microglia and to a lesser extent by neurons. ApoE lipoproteins accumulate lipids from cellular efflux mechanisms with the aid of the ATP-binding cassette transporters such as ABCA1 and shuttle lipids between brain cells. Mice lacking the lipid transporter ABCA1 were found to have markedly decreased ApoE levels and lipidation of ApoE in the central nervous system and increased brain deposition of Aβ. Among three human isoforms of APOE (APOE2, APOE3 and APOE4), APOE4 allele is the strongest risk factor for late-onset and early-onset forms of AD. The ability of ApoE isoforms to induce lipid efflux from brain cells are different, with ApoE2 presenting the greatest efficiency and ApoE4 being the least efficient.

Previous observational studies indicate an association between elevated cholesterol, the ApoE4 allele, and AD. We have shown that cholesterol oxidation products are higher in AD patients with vascular comorbidities and these can increase endothelial barrier permeability and release of inflammatory cytokines (Dias et al., 2018). In addition to its primary function of cholesterol transport, ApoE has been shown to have several additional functions, including the processing, aggregation, deposition, and clearance of Aβ (amyloid-β)-the primary component of amyloid plaques in AD and cerebral blood vessel deposits in cerebral amyloid angiopathy. Both APOE4 allele carrying older adults and individuals with APOE4-associated disorders has shown neurovascular dysfunction and declined cerebral blood flow. To explore this, further research has undertaken in animal models and observed that expression of APOE4, but not APOE2 and APOE3, can lead to BBB breakdown and susceptibility to injury in mice (Hafezi-Moghadam et al., 2007). Disruption to BBB led to neuronal uptake of multiple blood-derived neurotoxic proteins in these animals subsequently reducing microvascular and cerebral blood flow. However, to date, much of the research into ApoE-mediated cellular dysfunction in the brain has largely been focused on ApoE4 expression in neurons and astrocytes rather than in vascular endothelial cells (ECs). Therefore, mechanisms of ApoE4-mediated dysfunction within ECs expressing ApoE4 are not clear and, to date, only few models exist in which to investigate the role of ECs in AD.

BBB model systems: The development of advanced BBB models for research on neurodegeneration is essential to discover new therapeutic strategies and to explore the pathogenesis of these diseases. From a translational and pharmacological point, BBB models act as tools to facilitate drug permeability screening and development of new therapeutics or to find solutions to bypass the intrinsic resistance of the BBB to the central nervous system. It is reported that over 98% of compounds intended for therapeutic use in the brain never reach the market because of their

Figure 1 | **Blood-brain barrier is supported by multiple cell types in the brain.**
The endothelial cells that surround brain micro vessels consist of specialized tight junctions to allow restricted access to between blood and brain compartments. At the blood-brain barrier, astrocytes provide maintenance and repair through the release of several effector molecules and chemical factors such as vascular growth factors. Neuronal interactions with endothelial cells increase brain endothelial barrier resistance by aiding in tight junction protein synthesis and localization. As the resident immune cells of the brain, microglia could polarize into distinct proinflammatory or anti-inflammatory phenotypes and provide a protective role at the blood-brain barrier. Pericytes help to maintain and stabilize the monolayer of brain endothelial cells by inducing the formation of tight junctions, regulating angiogenesis and depositing extracellular matrix. Perivascular oligodendrocyte precursor cells wrap the micro vessels with their fine processes and integrate dynamic interactions with neuronal, glial and vascular cells.

Perspective

inherent inability to reach the brain (Mensch et al., 2009). The development of a functional BBB model will be of interest not only for screening potential therapeutics to better predict their entry into to the brain, but also to understand whether mutations linked to neurodegenerative disease predispose to BBB dysfunction.

In recent years, with the recognition of more diseases that are thought to be involved in the BBB and unavailability of suitable *in vitro* model systems encouraged the use of the ever-growing number of animal models. There are a number of APOE4 knock-in mice models have been developed to express a humanized APOE4 allele. *In vivo* methods including the single carotid injection technique, *in situ* perfusion techniques, intravenous injection technique, brain efflux, or intracerebral micro dialysis are considered as some of the most accurate ways of determining BBB penetration. However, these techniques are invasive, time-consuming and have a low throughput compared to cellular models. To circumvent these drawbacks, a wide range of cellular models, including primary cells and immortalized cell lines of human origin, have been used across labs. However, a major weakness of immortalized cell lines with a cancerous origin is that that they are highly proliferative and are not able to form tight barriers questioning their usability as a representative BBB model. A previous study found that primary and immortalized human cell lines show substantial variation in their APOE genotype status (Schaffer et al., 2014). In addition, both *in vivo* and animal cell-derived *in vitro* models show inevitable species differences that hinder their translation to clinical neuroscience and drug development. A study by Shin and colleagues successfully developed a 3D human neural cell culture model of AD using human-origin neural progenitor cells expressing amyloid precursor protein or amyloid precursor protein/presenilin 1 with familial AD mutations (Shin et al., 2019). These studies show the possibility of developing simplified *in vitro* models to mimic barrier impairment observed in AD and it would be valuable to investigate the influence of APOE genotype in such models.

Recent advancement in iPSC-derived models provide a feasible option for overcoming many of these limitations by creating a BBB model with a human origin and provide the possibility for generating an isogenic model with all cell types originating from the same human individual. Differentiated iPSCs display many key characteristics of human brain microvascular endothelial cells (hBMECs), including an increased expression of tight junctions, low permeability to solutes and appropriate expression of nutrient and efflux transporters, making hBMECs a substantially advanced *in vitro* model of the human BBB. It is evident that co-culturing other brain cells associated with BBB, such as astrocytes and pericytes, to BBB models exhibit greater barrier strength, represented by higher transendothelial electrical resistance and lower permeability than single cell models. Previous studies showed that monolayers of iPSC-derived hBMECs can reach sub-physiological barrier tightness and attained physiological transendothelial electrical resistance values (1500–8000 Ω cm^2) when co-cultured with astrocytes and/or pericytes (Wang

et al., 2017). Taken together, these results suggest that hiPSC-derived isogenic models can maintain physiological barrier function and has a promising future application. There are only few studies have investigated the effects of APOE4 genotype on iPSC-derived BBB models (Rieker et al., 2019). Compared to previous *in vivo* and *in vitro* models, iPSC-derived BBB models will resemble the human BBB to study molecular mechanisms underlying ApoE-mediated vascular dysfunction and to test potential therapies to prevent cellular changes. A previous study by Lin et al. (2018) used CRISPR/Cas9 technology to create isogenic APOE4 iPSC lines from unaffected parental APOE3 cells. These APOE4 iPSC-derived neurons, astrocytes and microglia-like cells recapitulated phenotypes associated with AD that demonstrated the feasibility of establishing *in vitro* human-like BBB models.

To date, cultured cells including iPSC cells are used in a number of BBB model systems ranging from simple monolayers to more complex spheroid and microfluidic models. While early BBB models were based on two dimensional transwell systems that allowed easy access to both apical and basolateral compartments and co-culture of other BBB cell types, more recent advancements incorporate critical components of the flow and sheer stress that hBMECs are constantly being exposed to in the brain microenvironment (Vatine et al., 2019). Compared to static transwell methods, development of 3D model systems to mimic BBB in a microfluidic platform enables researchers to quantitative evaluation of permeability, enhances imaging capabilities, and allows integration of multiple experimental steps and cost-effective reagent and sample size for high throughput screening (Delsing et al., 2020).

Whilst there is emerging interest, there are still more challenges to overcome. For example, cost efficiency of iPSC cell cultures, limited availability of donor cells and the maintenance of efficient long-term barrier properties hinder fast advancement of this field (Delsing et al., 2020). The emerging new protocols to speed up iPSC differentiation, increase the yield, purity, and maturity of various brain cell types that are vital for establishing human-like BBB models. Therefore, future research is promising and has the potential for testing new drug targets, investigating disease mechanisms and personalized *in vitro* modeling of the BBB.

Irundika HK Dias[*], Rachelle Taiwo, Dan Ma

Aston Medical School, College of Health and Life Sciences, Aston University, Birmingham, UK
*Correspondence to: Irundika HK Dias, PhD, diashki1@aston.ac.uk.
https://orcid.org/0000-0002-6620-8221 (Irundika HK Dias)

References

Delsing L, Herland A, Falk A, Hicks R, Synnergren J, Zetterberg H (2020) Models of the blood-brain barrier using iPSC-derived cells. Mol Cell Neurosci 107:103533.

Dias IHK, Brown CL, Shabir K, Polidori MC, Griffiths HR (2018) miRNA 933 Expression by endothelial cells is increased by 27-hydroxycholesterol and is more prevalent in plasma from dementia patients. J Alzheimers Dis 64:1009-1017.

Hafezi-Moghadam A, Thomas KL, Wagner DD (2007) ApoE deficiency leads to a progressive age-dependent blood-brain barrier leakage. Am J Physiol Cell Physiol 292:C1256-1262.

Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, Cam HP, Gjoneska E, Raja WK, Cheng J, Rueda R, Kritskiy O, Abdurrob F, Peng Z, Milo B, Yu CJ, Elmsaouri S, Dey D, Ko T, Yankner BA, Tsai LH (2018) APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. Neuron 98:1141-1154.

Mensch J, Oyarzabal J, Mackie C, Augustijns P (2009) In vivo, in vitro and in silico methods for small molecule transfer across the BBB. J Pharm Sci 98:4429-4468.

Profaci CP, Munji RN, Pulido RS, Daneman R (2020) The blood-brain barrier in health and disease: Important unanswered questions. J Exp Med 217:e20190062.

Rieker C, Migliavacca E, Vaucher A, Mayer FC, Baud G, Marquis J, Charpagne A, Hegde N, Guignard L, McLachlan M, Pooler AM (2019) Apolipoprotein E4 expression causes gain of toxic function in isogenic human induced pluripotent stem cell-derived endothelial cells. Arterioscler Thromb Vasc Biol 39:e195-207.

Schaffer S, Lam VYM, Ernst IMA, Huebbe P, Rimbach G, Halliwell B (2014) Variability in APOE genotype status in human-derived cell lines: a cause for concern in cell culture studies? Genes Nutr 9:364-364.

Shi Y, Yamada K, Liddelow SA, Smith ST, Zhao L, Luo W, Tsai RM, Spina S, Grinberg LT, Rojas JC, Gallardo G, Wang K, Roh J, Robinson G, Finn MB, Jiang H, Sullivan PM, Baufeld C, Wood MW, Sutphen C, et al. (2017) ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature 549:523-527.

Shin Y, Choi SH, Kim E, Bylykbashi E, Kim JA, Chung S, Kim DY, Kamm RD, Tanzi RE (2019) Blood-brain barrier dysfunction in a 3D in vitro model of Alzheimer's disease. Adv Sci 6:1900962.

Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, Rahnama M, Barthakur S, Kasendra M, Lucchesi C, Kerns J, Wen N, Spivia WR, Chen Z, Van Eyk J, Svendsen CN (2019) Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications. Cell Stem Cell 24:995-1005.

Wang YI, Abaci HE, Shuler ML (2017) Microfluidic blood-brain barrier model provides in vivo-like barrier properties for drug permeability screening. Biotechnol Bioeng 114:184-194.

Zlokovic BV (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 12:723-738.