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Blood-Brain Barrier Models Derived from Individual Patients: A New Frontier

[SUBTITLE] An Editorial Highlight on "An Isogenic Blood-Brain Barrier Model Comprising Brain Endothelial Cells, Astrocytes and Neurons Derived from Human Induced Pluripotent Stem Cells"

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Abstract

This Editorial highlights a study by Canfield and coworkers in the current issue of the Journal of Neurochemistry in which the authors derived endothelial cells, astrocytes and neurons from human induced pluripotent stem cells (iPSCs). Their model of the human blood-brain barrier (BBB) shows excellent permeability characteristics, with transendothelial electrical resistances (TEERs) and passive permeabilities close to those found *in vivo*. Importantly, this model could be created from a single patient raising potential new frontiers for BBB research.

Abbreviations:

ABC: ATP binding cassette
BBB: blood-brain barrier
iPSCs: induced pluripotent stem cells
NVU: neurovascular unit
TEER: transendothelial electrical resistance

[Main text]

In this issue of Journal of Neurochemistry, Canfield et al. used human induced pluripotent stem cells (iPSCs) to derive endothelial cells, astrocytes and neurons (Canfield et al., 2016). Co-culture of those cells resulted in a human blood-brain barrier (BBB) model with excellent permeability characteristics, with transendothelial electrical resistances (TEERs) and passive permeabilities close to those found *in vivo*. This study continues the groundbreaking work from the laboratories of Eric Shusta and Sean Palecek on using iPSCs to model the BBB (Lippmann et al., 2014, Lippmann et al., 2012, Lippmann et al., 2011). Very importantly, in the current study, the authors also showed that such models can be derived from a single patient. Thus, provided the model is reproducible across patients, this approach raises the possibility of examining how genetic mutations in a patient change

BBB and neurovascular unit (endothelial and perivascular cells; NVU) function both in normal and stressed, disease-like, conditions. Alterations in BBB/NVU function may impact the course of neurological diseases and the delivery of therapeutics. In addition, studies of cells derived from patients with genetic mutations may provide insight into the mechanisms that normally regulate the human BBB/NVU.

With a few exceptions (e.g. the effects of glucose transporter type 1 deficiency syndrome in humans (Leen et al., 2014)), our knowledge of the effects of mutations on BBB function have been derived from targeted mutations, usually gene deletions, in mice. Thus, for example, mouse knockout studies showed the importance of p-glycoprotein as an efflux transporter at the BBB (Schinkel et al., 1994) and claudin-5 in regulating BBB permeability (Nitta et al., 2003). Such studies have greatly advanced the field, but there are some caveats. First, much less is known about the BBB implications of the gene variants that naturally occur in humans. An exception is the p-glycoprotein polymorphisms in relation to antidepressant usage (Breitenstein et al., 2015). A second, broader, issue relates to whether there are species differences in BBB function. Thus, for example, differences have been reported between ATP binding cassette (ABC) transporter activity and expression at the BBB between species (Uchida et al., 2011). Species differences at the BBB/NVU are an understudied area, and they may be one underlying difficulty in modeling human neurological diseases in rodents.

The human BBB/NVU model developed by Canfield et al. (Canfield et al., 2016) is, therefore, an important resource. Currently, human BBB/NVU studies rely on limited amounts of tissue for primary culture (e.g. epilepsy resections), commercial cells with sparse documentation as to source, or human immortalized cell lines (Helms et al., 2016). The mostly commonly used cell line, hCMEC3/D3, while useful for many studies, has a TEER an order of magnitude less than the model presented by Canfield et al. (Canfield et al., 2016, Helms et al., 2016). Even primary cultures with human brain endothelial cells generally have much lower TEER values. The use of iPSCs also has an advantage in scaling; i.e. these cells can be grown in much greater quantities compared to primary cultures. Combining the iPSC approach with high efficiency gene editing (CRISPR/CAS9) would also, in principle, permit study of a large array of human variants even if samples are not

available from patients. The same approaches could also permit the generation of isogenic controls for patient derived iPSCs.

One important element that is absent from the BBB model developed by Canfield et al. (Canfield et al., 2016) is the pericyte. These cells have an important impact on BBB function (Armulik et al., 2010) and Shusta and his collaborators have previously described a BBB model using primary pericytes that had very high TEERs (Lippmann et al., 2014). The use of human iPSCs to produce brain pericytes would be a major addition to the model. Also, while the model has excellent passive permeability properties, the BBB has important additional properties. How well this iPSC-derived model recapitulates the in *vivo* BBB with respect to transporters, transcytosis and the metabolic barrier remains to be fully elucidated. The addition of pericytes and physiological shear stress may have an impact on those barrier properties.

In conclusion, great strides are being made in the development of human iPSC-derived BBB/NVU models. Such models will help in our understanding of the human BBB/NVU, the impact of genetic mutations and disease states and whether there are species differences in BBB/NVU function knowledge. Such modeling may also allow an understanding of how to tailor therapeutic dosing to an individual patient.

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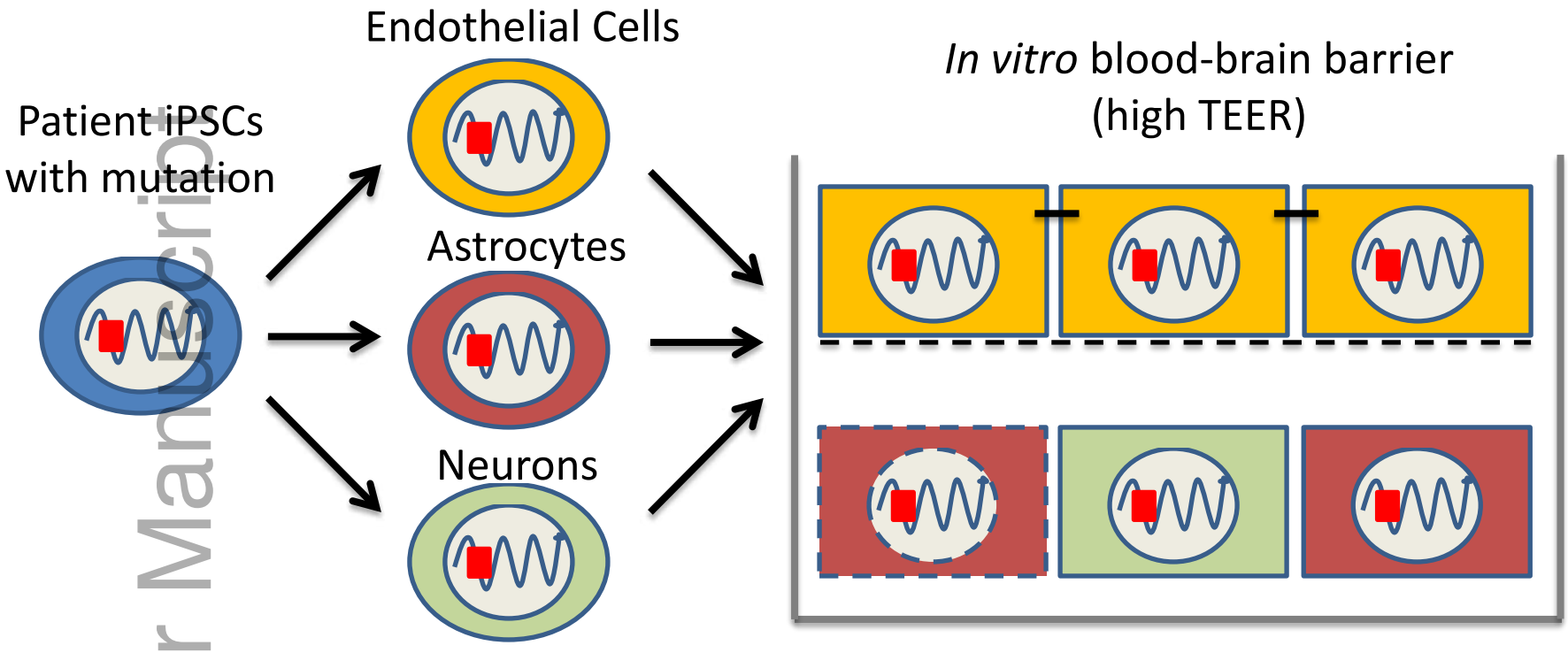

Figure. iPSCs from a single patient can be used to derive endothelial cells, astrocytes and neurons. These in turn can be used to create an in vitro blood-brain barrier model with an excellent transendothelial electrical resistance (TEER).