Biochimica et Biophysica Acta 1862 (2016) 381–394

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Genetic manipulation of brain endothelial cells in vivo☆

Julian C. Assmann [a], Jakob Körbelin [b], Markus Schwaninger [a,*]

[a] Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany
[b] University Medical Center Hamburg-Eppendorf, Hubertus Wald Cancer Center, Department of Oncology and Hematology, Martinistr. 52, 20246 Hamburg, Germany

ARTICLE INFO

Article history:
Received 16 July 2015
Received in revised form 30 September 2015
Accepted 5 October 2015
Available online 8 October 2015

Keywords:
Cre
Tet-on
Tet-off
Adeno-associated virus
Slco1c1
Liposomal transfection

ABSTRACT

Brain endothelial cells take center stage of the blood–brain barrier. They maintain homeostasis in the central nervous system (CNS) and are involved in the pathophysiology of many neurological diseases. So far investigations of their function have largely depended on in vitro models that lack the important impact of other cells and compartments in the mammalian CNS. A full evaluation of their role in a systemic context requires in vivo experiments. Here, we review recent innovative tools by which brain endothelial cells can be genetically manipulated in living organisms. We focus on conditional techniques for cell-specific deletion or overexpression of genes in mice. In a translational perspective, we summarize previous attempts to transduce brain endothelial cells in vivo using viral vectors or to transfect them with diverse methods. Available techniques provide the experimental basis for achieving a more refined picture of brain endothelial function in health and disease and to target this cell population for gene therapy. This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

1. Morphology of the blood–brain barrier

The blood–brain barrier (BBB) was discovered more than 100 years ago when Paul Ehrlich noted that administering some dyes into the blood stream stained all tissues except for the CNS [1]. In the following years, the concept of a BBB was formed by several researchers (Biedl and Kraus, Roux and Borrel, as well as Lewandowsky) based on similar observations (reviewed in detail in [2]). A crucial experiment pointing to the existence of the barrier was carried out by Ehrlich's student Edwin Goldmann [3]. He expanded Ehrlich's work by injecting dyes directly into the CNS, which left the periphery unstained, supporting the idea that there is indeed a barrier that restricts diffusion of dyes in both directions. It took several decades and the advent of electron microscopy until Reese and Karnovsky identified tight junctions between brain endothelial cells as the anatomical basis of the BBB [4]. Tight junctions seal endothelial cells and block the passage of hydrophilic or large molecules that are not able to diffuse through the endothelial plasma membrane. As a consequence, the BBB has an exceptionally high transendothelial electrical resistance compared to the peripheral vasculature [5].

On a molecular level, tight junctions are composed of proteins that form cell–cell contacts and are connected to the cytoskeleton. Tight junctions are mainly formed by members of the claudin family including more than 20 members. Claudin-3, -5, and -12 are characteristic for the BBB and important for tight junction integrity. They restrict diffusion of molecules through homophilic interactions between cells, only allowing small gaseous (e.g., O_2, CO_2) or lipophilic substances to pass. In addition, tight junctions contain other transmembrane proteins such as occludin or junctional adhesion molecules (JAM). Besides tight junctions, cell–cell contacts are strengthened by adherens junctions formed by VE-cadherin and PECAM-1.

To accomplish the specific properties of the BBB, brain endothelial cells need the helping hand of their neighbors. Cerebral vessels are almost completely ensheathed by astrocyte endfeet, which together with endothelial cells, produce the basement membrane [6]. By releasing soluble mediators, such as sonic hedgehog, astrocytes induce BBB properties in endothelial cells [7]. Tightness of the BBB is further supported by pericytes. Surrounded by the basement membrane, pericytes are located on the parenchymal side of endothelial cells and cover about 70% of capillaries [8]. Similar to astrocytes, pericytes can induce barrier properties in endothelial cells and are important for initial guidance and maturation of the developing BBB. A reduction of pericyte number increases transcytotic transport through the BBB [8].

Apart from astrocytes and pericytes, nerve fibers are found in close vicinity of brain endothelial cells [9]. The changing activity of neurons requires a flexible adaption of energy supply. To match the neurons'

Abbreviations: AAV, Adeno-associated virus; ABC, ATP-binding cassette; AD, Alzheimer's disease; BAC, Bacterial artificial chromosome; BBB, Blood–brain barrier; CNS, Central nervous system; EAE, Experimental autoimmune encephalomyelitis; IHC, Immunohistochemistry; MS, Multiple sclerosis; OAT, Organic anion transporter; OATP, Organic anion transporting polypeptide; PAC, Phage artificial chromosome; rtTA, Reverse tetracycline transactivator; TRE, tTA responsive element; tTA, Tetracycline transactivator; β-gal, β-Galactosidase.

☆ This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

* Corresponding author at: Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany.
E-mail address: markus.schwaninger@pharma.uni-luebeck.de (M. Schwaninger).

http://dx.doi.org/10.1016/j.bbadis.2015.10.006
0925-4439/

variable energy demand, blood flow increases in regions of neuronal activity providing a functional link between neurons and brain endothelial cells. How this neurovascular coupling works on a molecular level and which cells are directly involved is still a matter of debate. While astrocytes and pericytes have received a lot of attention, the contribution of endothelial cells to neurovascular coupling has not been studied in-depth.

The set of cells that form the BBB and control perfusion is often summarized as the neurovascular unit. Some authors also include microglia, the main resident immune cell in the brain. Their activation in pathological settings can lead to secretion of numerous cytokines that influence BBB permeability. In the last few years, the effect of microglia during BBB formation has been investigated in several studies, pointing to a role in embryonic brain angiogenesis [10].

The physical barrier maintained by the cells of the neurovascular unit with brain endothelial cells as the key player facilitates the tight control of the neural environment. Aside from protecting the CNS from toxic compounds, it maintains brain homeostasis and prevents uncontrolled influx of metabolites and potential neurotransmitters, the concentration of which considerably varies in blood (i.e. glutamate due to food intake). Furthermore, brain endothelial cells and the BBB restrict immune surveillance of the CNS, which is why this tissue is usually considered as immune-privileged, although recent studies also showed regular T cell patrolling and dendritic cell activity in the brain [11].

2. Relevance of brain endothelial cells and the BBB for pharmacotherapy

In addition to forming a passive boundary, brain endothelial cells express the machinery to actively extrude lipophilic and other compounds breaching the barrier and accumulating within the CNS. Compared to endothelial cells in other tissues, brain endothelial cells show a low rate of pinocytotic uptake and express a comprehensive set of transporter proteins that mediate this extrusion. Well-known transporters in the BBB belong to the ATP-binding cassette (ABC) transporter class. The most prominent example is P-glycoprotein (PGP, ABCB1), which is responsible for exporting a wide range of lipophilic, organic substances including many prescribed drugs [12,13]. Other transporters include the breast cancer resistant protein (BRCP, ABCG2) and MRP4 (ABCC4). Together, they restrict unspecific passage of compounds that are able to penetrate brain endothelial cells and therefore pose a major challenge for the treatment of neurological diseases.

For this reason, other strategies to cross the BBB have been explored. They can be divided into a) circumventing the BBB by intrathecal or intracerebroventricular application of drugs; b) increasing BBB permeability to facilitate passive entrance by diffusion; or c) capitalizing on existing transport mechanisms of brain endothelial cells. By directly injecting drugs into the cerebrospinal fluid (CSF) or into the brain, the BBB is bypassed. However, since diffusion in the extracellular space of the CNS is slow, the exposure of tissue to significant drug concentrations is locally restricted. Considering the risks of surgery or anesthesia that may be required, this route of drug administration is rarely used.

Transient opening of the BBB before drug administration to enhance paracellular transport is another option. Inducing an osmotic shock by administration of mannitol has been shown to transiently, albeit unspecifically, open the BBB and is used to improve effectiveness of chemotherapeutics in malignant brain tumors. To increase specificity, the use of focused ultrasound has gained some attention in recent years as it showed targeted opening of the BBB for only a limited time frame. In combination with the injection of microbubbles, the energy required to induce BBB opening does not seem to inflict tissue damage and animal studies show no adverse long-term side effects. This method, besides its use in models of brain tumors, has also improved cognitive function in a mouse model of Alzheimer's disease (AD) [14].

The third pathway used to overcome the BBB takes advantage of brain endothelial cell transport mechanisms via carriers or receptors (see Section 3 "Brain endothelial cells connect blood and brain"). Coupling of drugs to molecules that are cognate substrates of transporters in brain endothelial cells can increase brain uptake.

Apart from active transport, the formulation of liposomes, nanoparticles or viruses has been tested as a vehicle to deliver drugs by adsorptive endocytosis, examples of which are discussed below as a method to modulate gene expression in brain endothelial cells.

3. Brain endothelial cells connect blood and brain

Due to its high metabolic demands, the CNS critically depends on the supply of energy substrates and other nutrients. Endothelial cells are in a strategic position to feed the CNS. They enable the transport of selected nutrients required for brain function, which accounts for about 20% of the body's glucose and oxygen consumption [15]. To accomplish this task, they express specialized receptors and transporters. In order to cover the high demand of glucose, brain endothelial cells express the insulin-independent glucose transporter GLUT-1 that mediates a constant influx of glucose as energy substrate. Additional carriers facilitate the influx of hormones and nutrients like amino acids (e.g., large amino acid transporter, Lat1), monocarboxylic acids (e.g., monocarboxylate transporter 1, MCT1), vitamins (e.g., SVCT2 for vitamin C), and thyroxin (solute carrier organic anion transporter 1c1, Slco1c1). Transport of larger molecules is often mediated by specific receptors as described for insulin, leptin, transferrin, lipoproteins and others [16].

Because of its position at the blood–CNS interface, the brain endothelium has the potential to serve as a signaling platform in both directions. Inflammatory stimuli secreted in the parenchyma by microglia and astrocytes induce the expression of adhesion molecules on the luminal side which allows for cross-talk with circulating immune cells [10]. Conversely, cytokines and other factors circulating in the blood due to peripheral inflammation stimulate brain endothelial cells to release secondary mediators that subsequently mount a neural response, including fever and sickness behavior, to the peripheral insult [17,18].

These examples demonstrate that brain endothelial cells not only form a barrier but they bridge periphery and CNS. However, much remains to be learnt about their precise role in physiology.

4. Disturbance of brain endothelial cells in neurological diseases

The prominent position of brain endothelial cells and the BBB between blood and CNS makes them susceptible to involvement in a variety of neurological diseases. This involvement can either appear as simple physical disruption of the barrier as seen in stroke and traumatic brain injury or as more complex changes of the brain endothelial cell phenotype modulated by the disease context. In many cases, it is not known whether disturbance of the BBB and brain endothelial cells is a primary cause or a consequence of the disease.

A number of reviews have focused on the role of the BBB in diseases [19–21] and we will only provide some illustrative examples of its multifaceted role in disease induction and progression. During acute damage to the brain as found in stroke or traumatic brain injury, inflammatory cytokines activate matrix metalloproteases (MMP) that degrade parts of the extracellular matrix. They are also capable to disrupt tight junctions as already shown for claudin-5 and occludin [22]. This process facilitates opening of the BBB and development of vasogenic edema.

Apart from these acute disorders, many chronic neuroinflammatory or neurodegenerative diseases involve brain endothelial cells. Multiple sclerosis (MS), an autoimmune disease that causes demyelination in the brain and spinal cord, is a well-known example in which the usually immune-privileged CNS has to face massive immune cell infiltration. Although the initial route of entry for autoreactive $CD4^+$ T cells is thought to be the subarachnoidal space or choroid plexus, brain endothelial cells in postcapillary venules are a major site of immune cell migration during disease development [23]. This results in a disruption of BBB components, caused by the release of proinflammatory cytokines that are able