Biochimica et Biophysica Acta 1862 (2016) 452–460

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Molecular alterations of the blood–brain barrier under inflammatory conditions: The role of endothelial to mesenchymal transition☆

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ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 9 October 2015
Accepted 14 October 2015
Available online 19 October 2015

Keywords:
Blood–brain barrier
Multiple sclerosis
Endothelial to mesenchymal transition
Epithelial to mesenchymal transition
Neuroinflammation
Snail

ABSTRACT

Impairment of the protective properties of the blood–brain barrier (BBB) is a key event during numerous neurological diseases, including multiple sclerosis (MS). Under these pathological conditions, the specialized brain endothelial cells (BECs) lose their protective function leading to neuroinflammation and neurodegeneration. To date, underlying mechanisms for this loss of function remain unclear. Endothelial to mesenchymal transition (EndoMT) is a dynamic process by which endothelial cells (ECs) dedifferentiate into mesenchymal cells and as a result lose their specific phenotype and function. As yet, little is known about the involvement of this process in the impaired function of the BECs under pathological conditions such as MS. Interestingly, several signaling pathways that can induce EndoMT are also involved in different central nervous system (CNS) pathologies associated with BBB dysfunction. In this review, we first discuss the structure and function of the BBB highlighting the changes that occur during MS. Next, we will summarize recent findings on the pathways underlying EndoMT, and finally, we will discuss the potential role of EndoMT during BBB dysfunction in neurological disorders. This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

1. The blood–brain barrier

The BBB is a selective barrier composed by specialized BECs tightly connected through specific proteins present in the tight junction (TJ) and adherens junction (AJ) structures (Fig. 1). This close interconnection between the BECs provides a 'physical barrier' to solutes from the blood to the brain [1–3]. The BBB has a major role in maintaining brain homeostasis: it supplies nutrients to and excludes waste products from the brain; it limits both transcellular and paracellular passage of cells and molecules from the systemic circulation into the CNS and vice versa, thereby controlling the critical microenvironment necessary for proper neuronal function. Transcellular diffusion of hydrophilic molecules is limited due to a low rate of transcytotic vesicles, an extremely low pinocytotic activity and expression of active efflux membrane pumps of the ATP-binding cassette family such as P-glycoprotein, which drive cellular exclusion of more lipohilic compounds. In order to regulate the crucial influx of components needed within the CNS, BECs possess specific transporters that actively transport nutrients into the CNS, for instance glucose transporters (GLUT 1-3) [4].

Paracellular diffusion of hydrophilic molecules and immune cells trafficking is restricted by a complex network of TJ proteins which seal the inter-endothelial space [1,3]. The junction complexes that provide the characteristic phenotype of the BBB are not static but are dynamic structures that respond to the local microenvironment of the brain endothelium [5]. TJ complexes in itself can activate intracellular signaling pathways directly by engaging signaling proteins or growth receptors, or indirectly by capturing transcription factors at the plasma membrane [6]. Moreover, the transmembrane junctional proteins are connected to the cytoskeleton through the interaction with intracellular adaptor proteins [1,5,7]. Important proteins regulating TJ complex formation include occludin, which was the first of the TJ proteins to be discovered,

Abbreviations: α-SMA, Alpha smooth muscle actin; AD, Alzheimer's disease; AJ, Adherent junction; BBB, Blood–brain barrier; BECs, Brain endothelial cells; CCL2,-5, Chemokines (C-C motif) ligand 2, -5; CCM, Cerebral cavernous malformation; CXCL8,-10, Chemokines (C-X-C motif) ligand 8, -10; CNS, Central nervous system; DLL1-3-4, Delta-like ligand 1, -3, -4; ECs, Endothelial cells; EMT, Epithelial to mesenchymal transition; EndoMT, Endothelial to mesenchymal transition; ECM, Extra cellular matrix; EAE, Experimental autoimmune encephalomyelitis; FSP-1, Fibroblast specific protein 1; GBS, Group B Streptococcus; GM, Gray matter; GLUT 1-3, Glucose transporters; Gd-DTPA, Gadopentate dimeglumine; GSK3β, Glycogen synthase kinase 3 beta; Hh, Hedgehog; ICAM-1, Intracellular adhesion molecule 1; IL-8, -17, -22, Interleukin 8, -17, -22; IL-1β, Interleukin 1 beta; IFN-β, Interferon beta; IFNγ, Interferon gamma; JAMs, Junctional adhesion molecules; MMPs, Matrix metalloproteinases; MRI, Magnetic resonance imaging; MS, Multiple sclerosis; NAWM, Normal appearing white matter; NICD, Notch intracellular domain; NVU, Neurovascular unit; PECAM-1, Platelet endothelial cell adhesion molecule 1; PP-MS, Primary progressive MS; PR-MS, Progressive-relapsing MS; RR-MS, Relapsing-remitting MS; Shh, Sonic hedgehog; SP-MS, Secondary progressive MS; TJ, Tight junction; TGFb, Transforming growth factor beta; TNFα, Tumor necrosis factor alpha; VCAM-1, Vascular cell adhesion molecule 1; VE-cadherin, Vascular endothelial cadherin; WM, White matter; Wnt, Wingless-type murine-mammary-tumor virus integrations site family; ZO 1, -2, -3, Zonula occludens 1, -2, -3.

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

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http://dx.doi.org/10.1016/j.bbadis.2015.10.010
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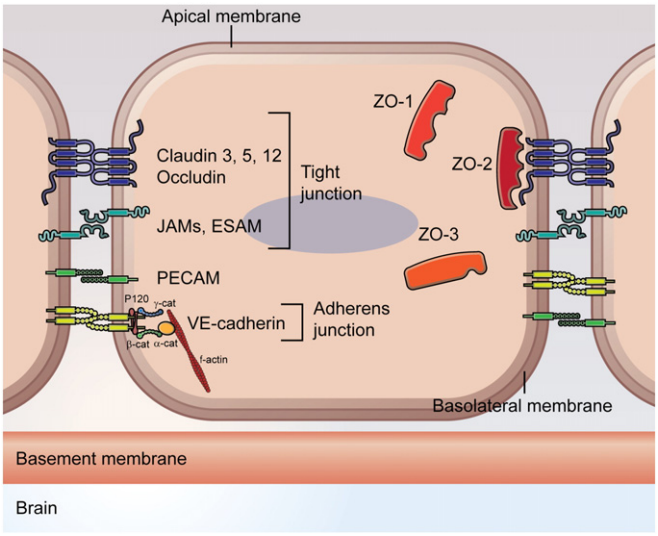

Fig. 1. Simplified scheme showing the molecular composition of endothelial TJs and AJs. Occludin and the claudins proteins are the most important membranous components of the TJs. ZO-1, -2, and -3 are scaffolding proteins and provide structural basis for the assembly of the TJs. JAMs and endothelial selective adhesion molecule (ESAM) are members of the immunoglobulin superfamily involved in the formation and maintenance of TJs. VE-cadherin is the most important molecule of endothelial AJs. In addition to VE-cadherin, PECAM-1 mediates homophilic adhesion.

claudins (in particular claudin-1, -3, -5, and -12) and junctional adhesion molecules (JAM-A, JAM-B, and JAM-C) [1,5,8,9]. The most important adaptors include proteins from the zonula occludens family (ZO-1, ZO-2, and ZO-3) which are scaffolding proteins that bind several effector proteins [4,13].

Vascular endothelial cadherin (VE-cadherin) plays a major role in cell–cell contacts and function of the AJ by binding binds to β-catenin and γ-catenin which anchor the complex to actin cytoskeleton [7,10]. In addition to VE-cadherin, platelet endothelial cell adhesion molecule 1 (PECAM-1) mediates homophilic adhesion. It is currently accepted that the functionality of the BBB is only possible through close contact between the brain endothelial cells and other cell types like astrocytes, pericytes, and neighboring CNS cells, such as microglia and neurons, creating a dynamic structure named the neurovascular unit (NVU) [11–13].

1.1. Cellular composition of the neurovascular unit

One of the most important cell type for the function of the NVU are the astrocytes, star-shaped glial cells, that provide support to other cell types, often through uptake and dispense of biochemical compounds. Astrocytes have been well known for their regulatory functions in maintaining neurotransmitter and ionic homeostasis in neuronal signaling, as well as providing feedback to neurons and to the vasculature of the CNS [14,15]. An additional role of astrocytes has been identified at the BBB, where astrocytic processes, called end-feet, enclose the BECs of the brain capillaries and closely regulate ion flow, blood volume, and cerebral blood flow [16]. Within the CNS, astrocytes consist of a heterogenic population of cells, including fibrous astrocytes and protoplasmic astrocytes. Fibrous astrocytes are predominantly found in the white matter (WM) of the CNS and posses a “star-like” appearance. Their main functions are to promote myelination of axons and maintenance of myelin as well as remyelination after myelin damage [17]. Protoplasmic astrocytes are found in the gray matter (GM) of the CNS and show more irregular processes which contact both synapses and blood vessels. Unlike the fibrous astrocytes, the main role of the protoplasmic astrocytes is to contribute to proper BBB function [18–20].

Another cell type in the NVU involved in the induction and maintenance of barrier properties are the pericytes, perivascular cells that wrap around small blood vessels and thereby communicate with BECs through direct physical contacts [21]. Pericytes coordinate the impermeability of the BBB and regulate cerebral blood flow [22,23]. The crucial importance of the pericytes in the NVU has recently been demonstrated in vivo using pericytes deficient mice [22].

The third cell type of the NVU is the neurons which are strictly dependent on both influx and efflux of molecules across the BBB. In order to exert their function, neurons require high amounts of glucose, which is used as energy source. Neuronal signaling is a process which is high in demand of energy consumption, but it is far from being homogeneous throughout the different brain regions and it varies over time. Feedback mechanisms between neuronal tissues and the NVU are crucial to meet this fluctuating demand. Whether neurons are critical in the development of the BBB phenotype is still unclear, few reports suggest that they can modulate the barrier phenotype by secreting factors that influence BBB maintenance [1,13,24].

In addition to astrocytes, pericytes, and neurons, the extracellular matrix (ECM) also interacts with the cerebral microvascular endothelium. The ECM is a dynamic, physiologically active component of all living tissues and it is made up of water, proteoglycans, minerals, and fibrous proteins. The main function of the ECM is to provide structural and biochemical support to the BBB [11]. Furthermore, the ECM has also been implied to contribute to the functionality of the BBB since the expression of matrix proteins can influence the expression of endothelial TJs [13,25]. Since the disruption of the ECM is strongly associated with increased BBB permeability in pathological states [13], an intact structure of the ECM is required for the correct functioning of the BBB [13,26].

Perivascular macrophages are also part of the NVU [27]. They arise from monocyte-derived macrophages that reside in the proximity of blood vessels and are regularly replaced [28]. It has been shown that perivascular macrophages are able to alter the function of the BBB by decreasing its paracellular permeability in vitro [27].

The last components of the NVU are the microglia cells, a type of mononuclear phagocytes that are located within the parenchyma of the CNS. Unlike macrophages, which are either monocyte or yolk-sac derived [29,30], microglia are formed from differentiated yolk-sac derived macrophages [31]. Microglia resides on the nervous part of the BBB and thus forms one of the first defense mechanisms against pathogens and harmful compounds that cross the BBB. Additionally, several studies also imply that microglia, once activated, can directly alter BBB function through secretion of matrix metalloproteinases (MMPs), enzymes that may degrade the ECM and thereby increase BBB permeability [32–34].

Overall, the function of the BBB is dynamically regulated by the concerted action of the different cell types and proteins to maintain the proper functioning of the BBB to ensure optimal neuronal function.

2. The blood–brain barrier under inflammatory conditions

2.1. Multiple sclerosis

A neurological disorder which is marked by a dysfunctional BBB is MS. MS is a progressive neurodegenerative disease of the CNS with an autoimmune component in which reactive lymphocytes recognize and attack myelin antigens. It has been estimated that approximately 2.5 million people suffer from MS worldwide with a typical age of onset between 20 and 50 years with a female:male ratio of 3:1 [35,36]. MS is characterized by inflammation, immune cell infiltration into the CNS, demyelination, and ultimately axonal loss [37,38]. In MS, lesion formation is a local phenomenon that occurs predominantly in the WM of the CNS, mostly in the spinal cord, brain stem, optic nerve, and periventricular areas [39]. Because the neurological symptoms are highly dependent on the location of the lesions within the CNS, MS is seen as a heterogeneous disease.

Based on the course of the disease, MS can be divided into four major categories: Relapsing-remitting MS (RR-MS), secondary progressive MS (SP-MS), primary progressive MS (PP-MS), and progressive-relapsing MS (PR-MS) [40]. RR-MS is the predominate form of MS and affects approximately 85% of MS patients. It is characterized by acute