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

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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.

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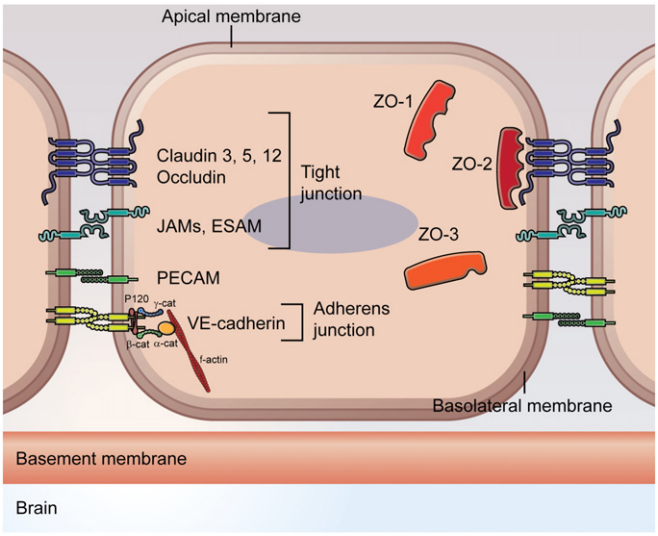

Fig. 1. Simplified scheme showing the molecular composition of endothelial TJs and AJs. Occludin and the claudins proteins are the most important membranous components of the TJs. ZO-1, -2, and -3 are scaffolding proteins and provide structural basis for the assembly of the TJs. JAMs and endothelial 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

attacks (relapses) that last for days to weeks, followed by a period of partial or full recovery (remission) of the clinical symptoms. A great majority of RR-MS patients (around 60%) will develop SP-MS which is characterized by initial relapses followed by a more progressive phase with gradual deterioration of neurological function. PP-MS patients experience a steady functional decline from the onset of the disease, without suffering from attacks. Similar to PP-MS, PR-MS is characterized by steady functional decline from the onset of the disease. However, PR-MS patients suffer from acute attacks, particularly in the late stage of the disease progression.

Due to its heterogeneous nature, there are no single tests or specific clinical diagnostic tools for MS. MS is often clinically diagnosed by neurological and cognitive evaluation, clinical history of the patients combined with magnetic resonance imaging (MRI) scans to visualize the lesions in the CNS [41]. Because MS is considered to be an autoimmune disease, the majority of currently developed therapies consist of corticosteroids administration and anti-inflammatory agent, such as the interferon beta (IFN-β), in combination with physical therapy [37,40]. Despite the benefit of immunosuppressing strategies in reducing the number of attacks, this therapeutic approach does not halt disease progression, nor can it offer relief in progressive forms of MS. Moreover, immunosuppressing therapies may lead to serious side effects, and therefore, there is a strong need to develop more specific drugs that can modulate disease's progression.

As the breakdown of the BBB is a key early event occurring during MS pathogenesis, disease-modifying therapies that prevent BBB dysfunction or accelerate its recovery may well provide a novel way of treating MS patients [42].

2.2. Dysfunction of the blood–brain barrier during multiple sclerosis

In MS, the BBB is inflamed and loses its protective function both during the relapsing-remitting and the progressive phases of the disease. Due to the BBB disruption and inflammation, numerous blood-derived lymphocytes and monocytes enter the brain by crossing the damaged BBB [43,44]. It is known that activated leukocytes and CNS-resident cells secrete pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), tumor necrosis factor alfa (TNFα), and interferon gamma (IFNγ) which can alter the structural architecture of the junctions of the BBB thereby increase BBB permeability [43].

A large number of imaging studies indicate the enhanced permeability of the BBB already in early stages of diseases [45]. Leakage of the BBB has also been characterized using the MRI enhancing contrast agent gadopentate dimeglumine (Gd-DTPA) in mice with experimental autoimmune encephalomyelitis (EAE), the experimental animal model for MS [46,47]. In addition to Gd-DTPA, other studies have used very small super-paramagnetic iron oxide particles or ultra small particles of iron oxide to detect monocyte infiltration in the CNS [48,49].

As it seems likely that alterations in BECs junctions contribute to permeability enhancement of the BBB as seen in MS, several studies have tried to understand the changes in junction proteins. One study examined the role of ZO-1 by measuring leakage of the blood endogenous fibrinogen protein [50]. Altered localization of ZO-1 expression in blood vessels was found in active MS lesions in postmortem material of patients, and to a lesser extent in normal appearing white matter (NAWM). These defects in correct localization of ZO-1 to the TJ complexes were thought to be correlated with BBB leakage. Moreover, Kirk et al. have also described that irregularities in the TJ protein ZO-1 and BBB leakage persisted also in inactive lesions, suggesting that increased BBB permeability can persist even when the inflammation is absent [50]. Furthermore, in mice suffering from EAE, cell-specific ectopic expression of claudin-1 in ECs reduces BBB leakiness and ameliorates the chronic phase of EAE without affecting leukocyte migration across the BBB [51]. This could be explained by the fact the transendothelial migration of leukocytes may occur via two pathways: paracellular, in which the leukocyte crosses the BBB by passing between adjacent endothelial cells, or transcellular, in which the leukocyte travels through the BECs, leaving the junctions intact [52]. Using a murine model of MS, which makes use of a virus to cause inflammation in the CNS [53], it was found that $CD8^+$ T-cells may activate astrocytes. This activation of astrocytes in turn leads to a decreased level of claudin-5 and occludin of ECs, thereby aggravating disease. In addition, cytokines like interleukin-17 (IL-17) and interleukin-22 (IL-22) produced by T helper 17 cells have also been shown to down-regulate the expression of ZO-1 and occludin in BECs [54].

Importantly, the effect of inflammation is not limited to changes in the junctional proteins, but it also promotes an activated inflammatory phenotype of ECs of the BBB. Inflamed BECs show increased expression of adhesion molecules, like intracellular adhesion molecule 1(ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, and PECAM-1 [43,55], which are essential for leukocytes migration into the CNS during MS lesions formation. Moreover, inflamed BECs can directly enhance both leukocytes adhesion and migration by producing several chemokines like chemokines (C-C motif) ligand 2 (CCL2), CCL5 and the chemokines (C-X-C motif) ligand 8 (CXCL8) and CXCL10 [55,56]. Furthermore, the expression and function of several efflux pumps of BBB, such as P-glycoprotein, is also altered during MS [57], illustrating that multiple aspects of proper BBB function are impaired.

It is important to note that BBB dysfunction has often been reported to be a major hallmark of MS and it is an early event during the course of disease [43,55]. Nevertheless, it is still not clear whether the BBB breakdown is a consequence of MS or if it precedes and enables the formation of MS lesions [58–60].

3. Dedifferentiation of epithelial and endothelial cells

3.1. Epithelial to mesenchymal transition

The epithelium is a protective cell lining which delineates cavities and structures such as organs, and it is formed by epithelial cells, which can provide both protection as well as transport of chemicals through specialized intercellular junctions. Thus, epithelia functions as an impermeable barrier that delineates organs [61]. The idea that epithelial cells can dedifferentiate into mesenchymal cells begins in the early 1990s by Elizabeth Hay who first described epithelial to mesenchymal transformation (EMT) using a model of chick primitive streak formation [62]. Nowadays, the term "transformation" has been replaced by the more appropriate "transition," highlighting the reversibility of the process. Indeed, it is known that mesenchymal cells can differentiate into epithelial cells in a process called mesenchymal to epithelial transition. EMT is not involved only during the developmental stage but has also been linked to pathologies like metastatic cancer and organ fibrosis [63,64]. During EMT, the most remarkable changes affecting epithelial cell phenotype include altered signaling pathways and gene expression, loss of cell polarity and cell–cell adhesion, reorganization of cytoskeleton, and ultimately gain of migratory and invasive properties [65]. The changes in gene expression which result in the repression of epithelial or endothelial phenotype and in the expression of the mesenchymal phenotype involved many different transcription factors such as Snail1, Snail2, ZEB1, ZEB2, TWIST1, and TWIST2 [65,66] in which Snail1 and Snail2 appear to be the most important transcription factors driving EMT. Snail1 and Snail2 are both members of the Snail family, which also consists of the less studied and uncharacterized Snail3 and have several conserved domains in common [66]. Since Snail3 has not been characterized and has not been linked to EMT thus far, from here on the "Snail family" will be referring to Snail1 and Snail2 throughout the review.

3.2. Endothelial to mesenchymal transition

Not only epithelial cells can dedifferentiate into mesenchymal cells but endothelial cells have also been reported to be able to undergo this transition. Similar to the epithelium in EMT, during EndoMT, the endothelium acquires mesenchymal and stem cell-like characteristics

[63,64,67]. Like EMT, also EndoMT was first thought to be a purely developmental process, and many studies on EndoMT focused on heart development, where endothelial cells dedifferentiate to form both the valves and septa of the adult heart [68]. However, recent work showed that EndoMT also takes place in adult tissue and that mature endothelial cells can acquire stem cell-like properties and differentiates into adipocytes, chondrocytes, or osteoblasts [63,67]. Moreover, EndoMT has also been described in different pathological conditions such as cardiac fibrosis, cancer, cerebral cavernous malformation (CCM), vein stenosis, retina diabetes, and others [63,69–75]. During EndoMT, similar to EMT, endothelial cells show degradation of their vascular basement membrane, loss of cell–cell contact, and acquire a migratory phenotype. Furthermore, the endothelial cells lose their endothelial phenotype due to reduced expression of specific endothelial markers like the VE-Cadherin and gain expression of mesenchymal markers like fibronectin and alpha smooth muscle actin (α-SMA) [63,67,76]. Interestingly, EMT and EndoMT drive the epithelial and endothelial cells into mesenchymal cells with a similar phenotype, suggesting that similar signaling pathways are responsible for both processes [72]. However, further studies are needed to investigate these similarities in more detail, since endothelial and epithelial cells differ in their expression of cell–cell junction proteins, cytoskeleton proteins, and the signaling machinery used [72].

3.3. Signaling pathways involved during EMT/EndoMT: A role in blood–brain barrier dysfunction in multiple sclerosis?

The transition of epithelial cells into mesenchymal cells during EMT is a process that is tightly regulated by different molecules, such as transcription factors, cell-surface proteins, cytoskeleton proteins, and proteins that degrade the ECM [64,65]. Over the last years, research has been focused on unraveling the mechanisms that initiate and influence the process of EMT, but less data exist on the regulators of EndoMT. Since both epithelial and endothelial cells show similar changes in gene expression during their transition into mesenchymal cells, several studies attempted to confirm if the signaling pathways involved in EMT are also able to induce and regulate the EndoMT (Fig. 2). Interestingly, several signaling pathways, which can induce both EMT and EndoMT,

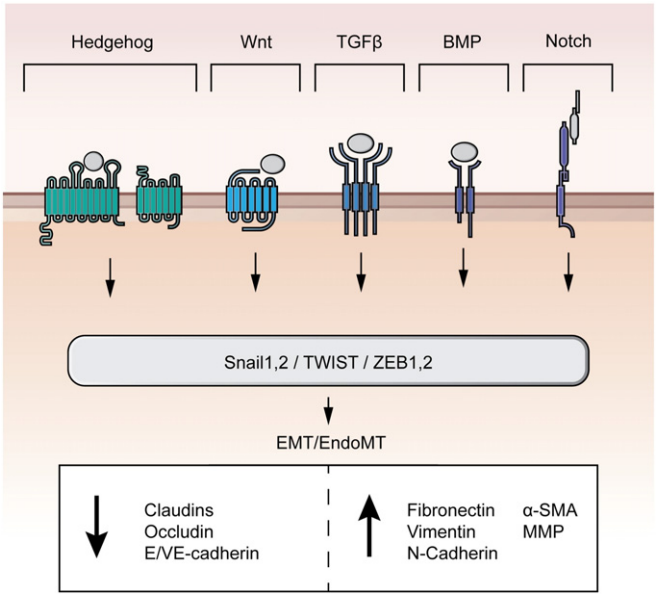

Fig. 2. Simplified scheme of the most important signaling pathways that have been shown to induce EMT and EndoMT. TGF-β, BMPs, Wnt, Notch, and Hh signaling all exert their effects on epithelial and/or endothelial cells via activation of different transcription factors. As a result, epithelial/endothelial cells dedifferentiate into mesenchymal cells, identified by loss of specific epithelial/endothelial markers and gain expression in specific mesenchymal markers.

are also involved in different CNS pathologies associated with BBB dysfunction. In the following sections, we will highlight the molecular regulators of the process of EndoMT.

3.3.1. Transforming growth factor beta

Transforming growth factor beta (TGF-β) is a cytokine which is found in most cell types and controls several processes such as cell proliferation and differentiation. After activation, TGF-β binds to the type 2 TGF-β receptor, and this interaction leads to recruitment and subsequential phosphorylation of a type 1 TGF-β receptor. In turn, intracellular proteins Smad2 and Smad3 are recruited, and after forming a complex with Smad4, it translocates to the nucleus, where it activates downstream gene transcription [77]. Since MS is considered to be an autoimmune disorder, it is important to highlight the role of TGF-β in the immune system. The major role of TGF-β in this context is to maintain immune tolerance since it can regulate lymphocyte proliferation, differentiation, and survival [78]. However, TGF-β also plays an important role under inflammatory conditions. In the presence of IL-6, TGF-β drives the differentiation of T helper 17 cells, a T-cell subpopulation involved in MS and EAE pathogenesis [79,80]. Moreover, local expression of TGF-β1 in the CNS parenchyma can enhance immune cell infiltration and amplify CNS impairment during EAE [81]. These findings are in contrast to previous observations where a suppressive role of TGF-β1 expression during EAE was demonstrated. Therefore, TGF-β may exert a dual role as either pro- or anti-inflammatory molecule based on the local environment.

Pericytes and astrocytes may also release TGF-β which contributes to BBB integrity and function [1,11,82]. Changes in the correct communication between BECs and cells from the NVU or deficiency in the TGF-β signaling results in an abnormal distribution of junctional proteins of BECs and show increased vascular permeability [82–85]. Furthermore, the cellular localization and distribution of three different isoforms of TGF-β were analyzed on frozen MS brain tissue sections with different stages of lesion activity. In active demyelinated lesions, perivascular foamy macrophages as well as hypertrophic reactive astrocytes show abundant TGF-β immunoreactivity compared to the normal control brain tissue sections where TGF-β immunoreactivity was only present on resting microglia cells. Interestingly, De Groot et al. also found that the high expression of TGF-β correlated with an astrogliotic reactivity in the lesion site. They argue that increased TGF-β expression in active MS lesions, where inflammation is elevated, might play a role in the induction of astrogliosis and overproduction of ECM [86]. However, it is important to note that the exact role of TGF-β during MS is not clear and it depends on the concentration as well as on the cellular context. While some studies on the effects of TGF-β on brain endothelial cells point toward a protective role of TGF-β, other reports state the opposite. Indeed, Dohgu et al. show that TGF-β stimulation of immortalized mouse brain capillary endothelial cells result in decrease of BBB permeability [85], whereas another study demonstrated that TGF-β1 stimulation of both bovine retinal ECs and human BECs increase BBB permeability. Particularly, the increased BBB permeability was mediated by tyrosine-phosphorylation of both claudin-5 and VE-cadherin [87].

Interestingly, TGF-β is one of the major signaling molecules that can induce EndoMT [63,70] since it is able to induce the expression of both Snail family members [72,88,89]. Moreover, a recent paper by Xiao et al. showed that EndoMT is characterized by a spectrum of intermediate and reversible mesenchymal-cell phenotype in response to TGF-β. Authors show that a subpopulation of ECs, isolated from a spontaneous mammary tumor, undergo distinct forms of EndoMT in response to TGF-β stimulation, suggesting that not all ECs respond identically to TGF-β [90].

3.3.2. Wnt/β-catenin

Proteins of the wingless-type murine-mammary-tumor virus integrations site family (Wnt) were initially discovered for their role in

carcinogenesis. It is known that the Wnt proteins control numerous different biological processes and take part in several Wnt pathways. Two non-canonical/β-catenin independent pathways co-exist together with the canonical/β-catenin dependent pathway. During the canonical Wnt signaling, Wnt ligands bind to the Frizzled receptors and thereby inhibit glycogen synthase kinase 3 beta (GSK3β) activities. GSK3β is a proline-directed serine–threonine kinase which normally phosphorylates β-catenin. Subsequently, β-catenin is ubiquinated and degraded in the proteasome [91]. In the presence of Wnt proteins, β-catenin is no longer degraded through the effects of GSK3β, but translocates to the nucleus where it forms a complex with the TCF/LEF transcription factors and activates genes transcription. The Wnt pathway has been found to play a role during CNS angiogenesis as well as in the BBB formation [11,92–94]. Initial evidence by Stenman et al. showed that a canonical Wnt signaling pathway is essential to promote formation and differentiation of the CNS vasculature [94]. Subsequently, using transgenic mice expressing LacZ specifically in cells in which Wnt/β-catenin signaling is active, Daneman et al. demonstrate the essential role of Wnt signaling in CNS, but not non-CNS, angiogenesis [92]. Moreover, Wnt signaling pathway has also been found to be important for the maintenance of the adult BBB and thus could be an important target during BBB dysfunction occurring during several brain disorders including MS [93,95,96]. Interestingly, the Wnt signaling pathway has been found to promote EMT via β-catenin-Snail1 induction [64,65]. Wnt signaling inhibits GSK3β activity, thus promoting Snail1 stability and function. GSK3β is involved in Snail1 post-transcriptional regulation, therefore regulating its localization and activity [97]. As expected, Wnt/β-catenin signaling pathway is also involved in the EndoMT process, since β-catenin-deficient mice show reduced cell–cell adhesion of vascular ECs. Moreover, vascular ECs that are β-catenin deficient show marked changes in actin cytoskeleton, as well as decreased cell–cell adhesions strength and increased paracellular permeability [98], which are hallmarks of the EndoMT process. In contrast, Bravi et al. have reported that sustained β-catenin signaling, independently from Wnt-receptor activation, also plays a pivotal role in EndoMT and in the development of brain vascular malformation in vivo [99]. Therefore, the exact role of Wnt/β-catenin signaling pathway during BECs-EndoMT remains to be investigated in more details.

3.3.3. The Notch pathway

The Notch signaling is a conserved pathway which is mediated by Notch ligand–receptor interaction between neighboring cells [100]. Briefly, the Notch signaling pathway is mediated by four transmembrane receptors (Notch 1–4) and five ligands of the Jagged (Jagged 1 and Jagged 2) and Delta-like ligand families (DLL1, DLL3, and DLL4). Upon ligand binding to its receptors, a signaling cascade is initiated, which induces the proteolytic cleavage of the Notch intracellular domain (NICD) leading to its translocation to the nucleus and subsequent expression of downstream target genes [100]. The Notch receptor family is known to regulate differentiation of various cell types and tissues during embryogenesis, but also postnatal. During embryogenesis, Notch signaling is involved in vascular development and angiogenesis [101], while in the postnatal CNS, Notch signaling regulates the formation of tip cells, thereby controlling vessel sprouting and branching in the mouse retina [102]. Moreover, Notch signaling pathway also regulates the maturation of oligodendrocyte precursor cells into mature oligodendrocytes, as well as myelination of axons and activation of microglia upon inflammation [103,104].

The role of Notch signaling during inflammatory process in MS has also been investigated. In EAE mice, the inhibition of the Notch receptor attenuated Th1 response whereas enhanced remyelination and axonal survival in lesioned sites [104,105]. Moreover, Delta1, has an opposite effect on the autoimmunity induced in the EAE model compared to Jagged1 [106].

The Notch pathway has also been linked to the integrity of the BBB. It has been demonstrated that Notch-4 and ZO-1 expression were decreased in ECs as a result of exposure to exogenous compounds as nicotine and saquinavir [107]. Moreover, a zebrafish model was used to determine the effect of Notch3 and the Notch3 receptor on pericyte proliferation and BBB integrity [108]. It was also found that zebrafish homozygous for a nonsense mutation in Notch3 developed hemorrhages specifically in the brain. Moreover, leakage of fluorescent tracers indicated that mutant embryos have enhanced permeability, suggesting that Notch3 is necessary for the functioning of the BBB. Taken together, these results suggest that a loss of proper Notch signaling leads to a reduced number of pericytes and subsequent loss of BBB integrity [108]. Mutations in the Notch3 gene are also associated with the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome, a rare autosomal dominant genetic disease characterized by recurrent stroke, headache, cognitive deficits, and hemorrhages [109].

The Notch signaling cascade may also contribute to the process of EMT [110] where the NICD can activate the expression of Snail2 and thereby induce EMT in epithelial cells [111]. In endothelial cells, the Notch signaling pathway has also been found to be involved in EndoMT [111,112]. In the study by Timmerman et al., it was shown that hyperactivation of Notch signaling in zebra fish embryos resulted in an excessive induction of EndoMT in cardiac ECs during ontogeny of the heart of the embryos. Conversely, Notch pathway mutants exhibit a collapsed endocardium, lack of mesenchymal cushion cells, as well as attenuated Snail2 expression. Subsequent analysis revealed that Notch exerts its function in inducing EndoMT through proteins of the Snail family. This finding that Notch directly targets members of the Snail family was also confirmed during experiments that showed that Snail2, but not Snail1 was induced in embryonic mice cardiac ECs by Notch during the process of EndoMT [111]. Additionally, Niessen et al. demonstrated that Snail2 expression is required for acquiring migration abilities of ECs during the process of EndoMT. Interestingly, they also showed that the Notch pathway can induce the expression of Snail1 if it acts synergistically with TGF-β, illustrating that different factors may act in concert to induce EndoMT.

3.3.4. The Hedgehog signaling pathway

The Hedgehog (Hh) pathway plays a pivotal role during embryogenesis in vertebrates and, postnatal, is involved in tissue homeostasis. Hh signaling pathway has been demonstrated to promote the integrity of the BBB and thereby prevents immune cells migration and diffusion of molecules [113]. Sonic hedgehog (Shh), the most characterized protein of the mammalian Hh pathway, was specifically upregulated and secreted by astrocytes during TNFα and IFNγ stimulation to mimic the inflammatory state observed during MS. In contrast, Shh receptors Patched-1 and Smoothened were strongly upregulated on ECs. Hh pathway activation in human ECs promoted an immune quiescence state of the BBB by decreasing the secretion of the pro-inflammatory chemokines, such as interleukin 8 (IL-8) and CCL2, as well as by reducing level of ICAM-1 [113]. Moreover, activation of the Hh pathway also reduced $CD4^+$ T-cells adhesion and migration across the BBB endothelial cells. The authors propose that the inflammatory-mediated Shh up-regulation functions to promote BBB repair and counterbalance inflammatory events, aiming to prevent further damage. Additionally, the same study showed that Hh signaling pathway posses barrier strengthening effects. Overall, the Hh signaling pathway was found to provide a barrier-promoting effect and promotes an immune quiescence phenotype of the BECs.

Importantly, the Hh pathway has also been shown to be involved in EMT since it can activate the transcription factor Gli-1 which can directly increase the expression of Snail1 [65]. In vitro experiments on murine skin cells showed that Gli-1 induction of Snail1 is an early event in EMT. Moreover, blocking the activity of Snail1 inhibited Gli-1-induced EMT, once again demonstrating the importance of Snail1 during EMT [114]. However, to date, the Hh pathway has not been reported to be involved in EndoMT, and its role remains to be established.

3.4. Linking endothelial to mesenchymal transition and altered blood–brain barrier permeability

Previous sections highlighted the role that TGF-β, Wnt, Notch, and Hh pathways have during BBB maintenance as well as during EMT/EndoMT processes. These signaling pathways have also been implicated in MS and other neuropathologies, like Alzheimer's disease (AD), and thus raise the suggestion that EndoMT might contribute to the pathogenesis of neurological diseases associated with BBB dysfunction. However, it should also be noted that most of the research regarding EndoMT was performed in the context of development of the embryo or on non-brain-derived endothelial cells. Nevertheless, recent studies have focused on the role of EndoMT during different brain pathologies. In 2013, Maddaluno et al. showed that the process of EndoMT contributed to development of vascular malformations in mice brains. Indeed, the disruption in any of the three CCM genes, named after the vascular dysplasia CCM, was able to induce EndoMT in brain microvascular ECs [71]. The authors state that in iCCM1 mice, where the CCM1 gene is specifically ablated in ECs, the loss of this gene will lead to an up-regulation of BMP-6. This up-regulation of BMP-6 can in turn activate both TGF-β and BMP signaling pathways leading to the induction of EndoMT. Conversely, inhibition of the TGF-β and BMP pathways prevented EndoMT both in vitro and in vivo. Inhibition of EndoMT also resulted in less vascular lesions in CCM1 deficient mice, confirming TGF-β-induced EndoMT in the vascular malformation found in CCM. In addition, Bravi et al. observed that activation of β-catenin precedes the initiation of TGF-β/BMP signaling in CCM3-ablated ECs both in vivo and in vitro thereby delineating a sequence of signaling steps in response to ablation of CCM3 [99]. Moreover, the mesenchymal and pluripotent stem cells marker Kruppel-like factor 4 (KLF4) was also upregulated in CCM1-deficient mice and it is known that KLF4 can be upregulated by CCL2 [115]. CCL2 expression is strongly increased during degenerative and inflammatory conditions in the CNS [116]. Therefore, production of CCL2 may also contribute to EndoMT. However, no direct evidence involving CCL2 during EndoMT has been provided yet. A more recent paper demonstrates that primary cultured rat BECs undergo EndoMT upon TGF-β stimulation [117]. Although this paper focuses on brain metastatic invasion, the results indicate that, upon dedifferentiation of BECs into mesenchymal cells, tumor cells gain access to the brain by crossing the impaired BBB.

Evidence of brain endothelial dedifferentiation was recently provided by Kim et al. [118]. Authors were the first to show that human brain microvascular endothelial cells infected with a meningeal pathogen Group B *Streptococcus* (GBS) results in the induction of Snail1 which in turn affects the expression of TJs molecules and impair the BBB functions. To further examine a functional role of Snail1 during infection in vivo, Kim et al. also showed that siRNA-mediated reduction of Snail1 resulted in decreased brain penetration of the GBS in adult zebrafish [118]. Furthermore, stimulation of primary rat ECs with activated cancer cell line conditioned medium results in a TGF-β-dependent decrease of the TEER, increase adhesion between metastatic and ECs, and enhanced transendothelial migration of melanoma cells. Overall, the provided evidence suggest that TGF-β-dependent EndoMT is a necessary mechanism for metastatic migration and may be one of the potential mechanisms leading to metastatic extravasations [117].

Moreover, inflammatory mediators which are known modulators of BBB permeability [119] have also been linked to EMT and EndoMT, highlighting also the role of transcription factor NF-κB during these processes [120–124]. This last finding strengthens the hypothesis that EndoMT may contribute to BBB breakdown during MS pathogenesis. It is known that reactive astrocytes and microglia are potent contributors to endothelial inflammation during MS since they secrete pro-inflammatory cytokines and chemokines like TNFα, IL-1β, IL12, and CCL2 [1,125,126]. Those pro-inflammatory cytokines are considered to mediate changes in gene expression in BECs toward an "inflamed" phenotype. Indeed, increased permeability and expression of cell adhesion molecules on the brain endothelium allow the trafficking of inflammatory agents and circulating leukocytes into the CNS, leading to demyelination and axonal loss [55,127]. Based on these findings, it is tempting to speculate that also in MS the process of EndoMT might be the underlying mechanism occurring during BBB dysfunction thereby playing a key role during the early phase of the disease pathogenesis (Fig. 3).

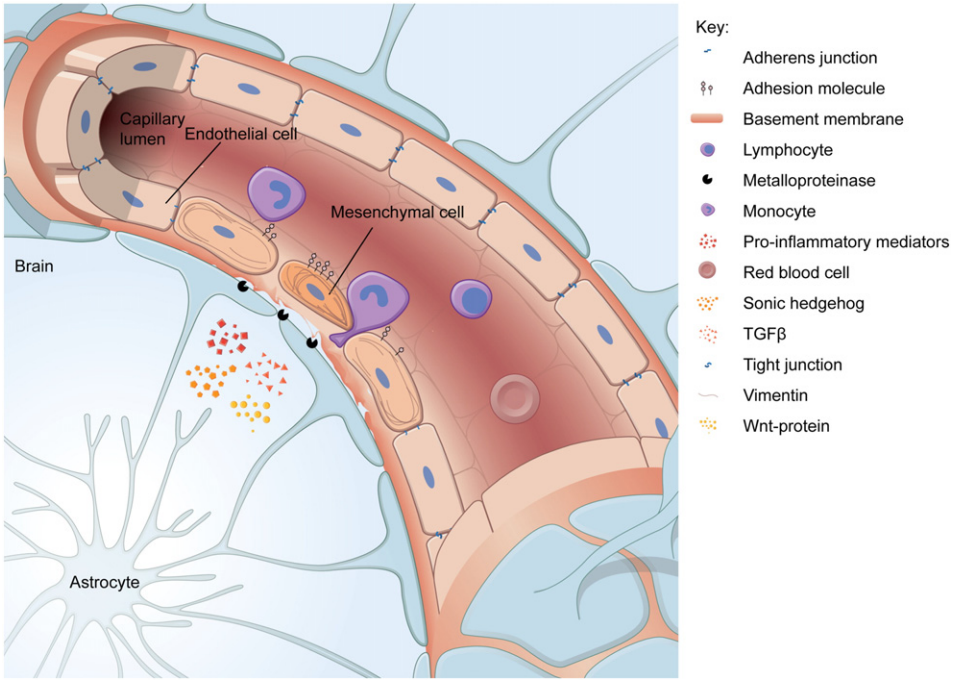

Fig. 3. Endothelial to mesenchymal transition of the human brain endothelial cells contributes to blood–brain barrier dysfunction. The figure shows a schematic representation of the EndoMT process occurring during BBB breakdown. First, activated leukocytes and CNS-resident cells contribute to inflammation via secretion of pro-inflammatory cytokines. This leads to an induction and/or an up-regulation of endothelial adhesion molecules and mediates the adhesion and transendothelial migration of circulating leukocytes. Second, the inflammatory environment, together with other key signaling molecules, induces EndoMT. As result, endothelial cells dedifferentiate into mesenchymal cell and contribute to BBB disruption.

3.5. Conclusions and future perspective

MS is a progressive neurodegenerative disorder of the CNS characterized by brain inflammation, axonal demyelination, and ultimately neuronal loss. MS is accompanied by an infiltration from the blood vessels into the CNS parenchyma and damage to myelin sheaths, which occurs after the BBB is disrupted early in the course of the disease. In healthy conditions, the BBB is composed of specialized BECs that are tightly connected by specific proteins in tight and adherens junctions. They serve as a protective barrier to prevent immune cells, pathogens, and potential molecules from diffusing into the brain from the blood circulation. In MS, the BBB is inflamed and numerous immune cells enter the brain by crossing it. The damage of the BBB may be due to a loss of structure and proper functions of ECs, a phenomenon witnessed in pathological events in other tissue linked to cancer, cardiac fibrosis, and other diseases. In those cases, ECs change their phenotype and dedifferentiate into mesenchymal cells, in a process known as EndoMT. In the past year, the dedifferentiation of ECs into mesenchymal cells has also been observed in brain disorders, showing that also BECs can dedifferentiate. None of these studies, however, have investigated a potential role of EndoMT in the specific context of BBB dysfunction during MS pathogenesis. The fact that MS pathogenesis, BBB disruption, and EndoMT have several common denominators suggests that there may well be a link. Future research is needed in order to elucidate a possible role of EndoMT in driving BECs dedifferentiation during BBB dysfunction that accompanies MS pathogenesis. Discovering such a link might offer new routs to novel therapies for MS that inhibit EndoMT, restore proper BBB integrity, and prevent the destructive effects of immune cells on brain tissue during the disease.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Transparency Document

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References

[1] N.J. Abbott, L. Ronnback, E. Hansson, Astrocyte—endothelial interactions at the blood—brain barrier, Nat. Rev. Neurosci. 7 (2006) 41–53.
[2] C.M. Van Itallie, J.M. Anderson, The molecular physiology of tight junction pores, Physiology 19 (2004) 331–338.
[3] J.M. Scherrmann, Exchanges Through the Blood–Brain Barrier, 2002 372–379.
[4] P.G. Nijland, I. Michailidou, M.E. Witte, M.R. Mizee, S. Pol, A. Reijerkerk, L. Pellerin, P. Valk, H.E. Vries, J. Horssen, Cellular distribution of glucose and monocarboxylate transporters in human brain white matter and multiple sclerosis lesions, Glia 62 (2014) 1125–1141.
[5] H. Wolburg, A. Lippoldt, Tight junctions of the blood—brain barrier: development, composition and regulation, Vasc. Pharmacol. 38 (2002) 323–337.
[6] E. Dejana, Endothelial cell—cell junctions: happy together, Nat. Rev. Mol. Cell Biol. 5 (2004) 261–270.
[7] G. Bazzoni, E. Dejana, Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis, Physiol. Rev. 84 (2004) 869–901.
[8] T. Nitta, M. Hata, S. Gotoh, Y. Seo, H. Sasaki, N. Hashimoto, M. Furuse, S. Tsukita, Size-selective loosening of the blood–brain barrier in claudin-5—deficient mice, J. Cell Biol. 161 (2003) 653–660.
[9] S. Liebner, U. Kniesel, H. Kalbacher, H. Wolburg, Correlation of tight junction morphology with the expression of tight junction proteins in blood–brain barrier endothelial cells, Eur. J. Cell Biol. 79 (2000) 707–717.
[10] A. Hartsock, W.J. Nelson, Adherens and tight junctions: structure, function and connections to the actin cytoskeleton, Biochim. Biophys. Acta Biomembr. 1778 (2008) 660–669.
[11] B. Obermeier, R. Daneman, R.M. Ransohoff, Development, maintenance and disruption of the blood–brain barrier, Nat. Med. 19 (2013) 1584–1596.
[12] J.I. Alvarez, R. Cayrol, A. Prat, Disruption of central nervous system barriers in multiple sclerosis, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1812 (2011) 252–264.
[13] B.T. Hawkins, T.P. Davis, The blood–brain barrier/neurovascular unit in health and disease, Pharmacol. Rev. 57 (2005) 173–185.
[14] A. Araque, V. Parpura, R.P. Sanzgiri, P.G. Haydon, Tripartite synapses: glia, the unacknowledged partner, Trends Neurosci. 22 (1999) 208–215.
[15] M. Nedergaard, B. Ransom, S.A. Goldman, New roles for astrocytes: redefining the functional architecture of the brain, Trends Neurosci. 26 (2003) 523–530.
[16] M. Simard, M. Nedergaard, The neurobiology of glia in the context of water and ion homeostasis, Neuroscience 129 (2004) 877–896.
[17] I. Lundgaard, M.J. Osorio, B.T. Kress, S. Sanggaard, M. Nedergaard, White matter astrocytes in health and disease, Neuroscience 276 (2014) 161–173.
[18] A.V. Molofsky, R. Krenick, E. Ullian, H.h. Tsai, B. Deneen, W.D. Richardson, B.A. Barres, D.H. Rowitch, Astrocytes and disease: a neurodevelopmental perspective, Genes Dev. 26 (2012) 891–907.
[19] N.A. Oberheim, S.A. Goldman, M. Nedergaard, Heterogeneity of Astrocytic Form and Function, Astrocytes, Springer, 2012 23–45.
[20] A. Reichenbach, A. Derouiche, F. Kirchhoff, Morphology and dynamics of perisynaptic glia, Brain Res. Rev. 63 (2010) 11–25.
[21] G. Bergers, S. Song, The role of pericytes in blood-vessel formation and maintenance, Neuro-Oncology 7 (2005) 452–464.
[22] A. Armulik, G. Genove, M. Mae, M.H. Nisancioglu, E. Wallgard, C. Niaudet, L. He, J. Norlin, P. Lindblom, K. Strittmatter, Pericytes regulate the blood–brain barrier, Nature 468 (2010) 557–561.
[23] R.D. Bell, E.A. Winkler, A.P. Sagare, I. Singh, B. LaRue, R. Deane, B.V. Zlokovic, Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging, Neuron 68 (2010) 409–427.
[24] Z. Cohen, G. Bonvento, P. Lacombe, E. Hamel, Serotonin in the regulation of brain microcirculation, Prog. Neurobiol. 50 (1996) 335–362.
[25] G. Rascher, A. Fischmann, S. Kroger, F. Duffner, E.H. Grote, H. Wolburg, Extracellular matrix and the blood–brain barrier in glioblastoma multiforme: spatial segregation of tenascin and agrin, Acta Neuropathol. 104 (2002) 85–91.
[26] H. Wolburg, S. Noell, P. Fallier-Becker, A.F. Mack, K. Wolburg-Buchholz, The disturbed blood—brain barrier in human glioblastoma, Mol. Asp. Med. 33 (2012) 579–589.
[27] D. Zenker, D. Begley, H. Bratzke, H. Rubsamen-Waigmann, H. Briesen, Human blood-derived macrophages enhance barrier function of cultured primary bovine and human brain capillary endothelial cells, J. Physiol. 551 (2003) 1023–1032.
[28] G.J. Guillemin, B.J. Brew, Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification, J. Leukoc. Biol. 75 (2004) 388–397.
[29] J. Hettinger, D.M. Richards, J. Hansson, M.M. Barra, A.C. Joschko, J. Krijgsveld, M. Feuerer, Origin of monocytes and macrophages in a committed progenitor, Nat. Immunol. 14 (2013) 821–830.
[30] C. Schulz, E.G. Perdiguero, L. Chorro, H. Szabo-Rogers, N. Cagnard, K. Kierdorf, M. Prinz, B. Wu, S.E. Jacobsen, J.W. Pollard, A lineage of myeloid cells independent of Myb and hematopoietic stem cells, Science 336 (2012) 86–90.
[31] M. Prinz, J. Priller, Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease, Nat. Rev. Neurosci. 15 (2014) 300–312.
[32] M. Ihara, H. Tomimoto, M. Kinoshita, J. Oh, M. Noda, H. Wakita, I. Akiguchi, H. Shibasaki, Chronic cerebral hypoperfusion induces MMP-2 but not MMP-9 expression in the microglia and vascular endothelium of white matter, J. Cereb. Blood Flow Metab. 21 (2001) 828–834.
[33] S. Mun-Bryce, G.A. Rosenberg, Gelatinase B modulates selective opening of the blood–brain barrier during inflammation, Am. J. Physiol. Regul. Integr. Comp. Physiol. 274 (1998) R1203–R1211.
[34] G.A. Rosenberg, L.A. Cunningham, J. Wallace, S. Alexander, E.Y. Estrada, M. Grossetete, A. Razhagi, K. Miller, A. Gearing, Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures, Brain Res. 893 (2001) 104–112.
[35] T. Kalincik, V. Vivek, V. Jokubaitis, J. Lechner-Scott, M. Trojano, G. Izquierdo, A. Lugaresi, F. Grand'Maison, R. Hupperts, C. Oreja-Guevara, Sex as a determinant of relapse incidence and progressive course of multiple sclerosis, Brain (2013) awt281.
[36] R. Milo, A. Miller, Revised diagnostic criteria of multiple sclerosis, Autoimmun. Rev. 13 (2014) 518–524.
[37] P.A. Calabresi, Diagnosis and management of multiple sclerosis, Am. Fam. Physician 70 (2004) 1935–1944.
[38] A. Prat, J. Antel, Pathogenesis of multiple sclerosis, Curr. Opin. Neurol. 18 (2005) 225–230.
[39] C. Stadelmann, C. Wegner, W. Bruck, Inflammation, demyelination, and degeneration—recent insights from MS pathology, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1812 (2011) 275–282.
[40] S.L. Hauser, D.E. Goodkin, Multiple sclerosis and other demyelinating diseases, Harrisons Princ. Intern. Med. 2 (2001) 2452–2461.
[41] C.H. Polman, S.C. Reingold, B. Banwell, M. Clanet, J.A. Cohen, M. Filippi, K. Fujihara, E. Havrdova, M. Hutchinson, L. Kappos, Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria, Ann. Neurol. 69 (2011) 292–302.
[42] W. Kamphuis, C. Derada Troletti, A. Reijerkerk, A. Romero, E.d. Vries, The blood–brain barrier in multiple sclerosis: microRNAs as key regulators, CNS Neurol. Disord. Drug Targets 14 (2015) 157–167.

[43] A. Minagar, J.S. Alexander, Blood–brain barrier disruption in multiple sclerosis, Mult. Scler. 9 (2003) 540–549.
[44] A. Prat, K. Biernacki, J.F. Lavoie, J. Poirier, P. Duquette, J.P. Antel, Migration of multiple sclerosis lymphocytes through brain endothelium, Arch. Neurol. 59 (2002) 391–397.
[45] G.A. Rosenberg, Neurological diseases in relation to the blood—brain barrier, J. Cereb. Blood Flow Metab. 32 (2012) 1139–1151.
[46] N. Silver, M. Lai, M. Symms, G. Barker, I. McDonald, D. Miller, Serial gadolinium-enhanced and magnetization transfer imaging to investigate the relationship between the duration of blood–brain barrier disruption and extent of demyelination in new multiple sclerosis lesions, J. Neurol. 246 (1999) 728–730.
[47] T. Tourdias, V. Dousset, Neuroinflammatory imaging biomarkers: relevance to multiple sclerosis and its therapy, Neurotherapeutics 10 (2013) 111–123.
[48] S. Floris, E.L.A. Blezer, G. Schreibelt, E. Dopp, S.M.A. Van der Pol, I.L. Schadee-Eestermans, K. Nicolay, C.D. Dijkstra, H.E. De Vries, Blood–brain barrier permeability and monocyte infiltration in experimental allergic encephalomyelitis a quantitative MRI study, Brain 127 (2004) 616–627.
[49] M. Rausch, P. Hiestand, C.A. Foster, D.R. Baumann, C. Cannet, M. Rudin, Predictability of FTY720 efficacy in experimental autoimmune encephalomyelitis by in vivo macrophage tracking: clinical implications for ultrasmall superparamagnetic iron oxide—enhanced magnetic resonance imaging, J. Magn. Reson. Imaging 20 (2004) 16–24.
[50] J. Kirk, J. Plumb, M. Mirakhur, S. McQuaid, Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood–brain barrier leakage and active demyelination, J. Pathol. 201 (2003) 319–327.
[51] F. Pfeiffer, J. Schafer, R. Lyck, V. Makrides, S. Brunner, N. Schaeren-Wiemers, U. Deutsch, B. Engelhardt, Claudin-1 induced sealing of blood—brain barrier tight junctions ameliorates chronic experimental autoimmune encephalomyelitis, Acta Neuropathol. 122 (2011) 601–614.
[52] D.W. Holman, R.S. Klein, R.M. Ransohoff, The blood—brain barrier, chemokines and multiple sclerosis, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1812 (2011) 220–230.
[53] G.L. Suidan, J.R. Mcdole, Y. Chen, I. Pirko, A.J. Johnson, Induction of blood brain barrier tight junction protein alterations by CD8 T cells, PLoS One 3 (2008), e3037.
[54] H. Kebir, K. Kreymborg, I. Ifergan, A. Dodelet-Devillers, R. Cayrol, M. Bernard, F. Giuliani, N. Arbour, B. Becher, A. Prat, Human TH17 lymphocytes promote blood–brain barrier disruption and central nervous system inflammation, Nat. Med. 13 (2007) 1173–1175.
[55] C. Larochelle, J.I. Alvarez, A. Prat, How do immune cells overcome the blood–brain barrier in multiple sclerosis? FEBS Lett. 585 (2011) 3770–3780.
[56] E.A. Subileau, P. Rezaie, H.A. Davies, F.M. Colyer, J. Greenwood, D.K. Male, I.A. Romero, Expression of chemokines and their receptors by human brain endothelium: implications for multiple sclerosis, J. Neuropathol. Exp. Neurol. 68 (2009) 227–240.
[57] G. Kooij, J. van Horssen, V.V.R. Bandaru, N.J. Haughey, H.E. de Vries, The role of ATP-binding cassette transporters in neuro-inflammation: relevance for bioactive lipids, Front. Pharmacol. 3 (2012).
[58] D.E. Goodkin, W.D. Rooney, R. Sloan, P. Bacchetti, L. Gee, M. Vermathen, E. Waubant, M. Abundo, S. Majumdar, S. Nelson, A serial study of new MS lesions and the white matter from which they arise, Neurology 51 (1998) 1689–1697.
[59] A.G. Kermode, A.J. Thompson, P. Tofts, D.G. MacManus, B.E. Kendall, D.P.E. Kingsley, I.F. Moseley, P. Rudge, W.I. McDonald, Breakdown of the blood–brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis pathogenetic and clinical implications, Brain 113 (1990) 1477–1489.
[60] P.K. Stys, G.W. Zamponi, J. van Minnen, J.J. Geurts, Will the real multiple sclerosis please stand up? Nat. Rev. Neurosci. 13 (2012) 507–514.
[61] R.Y.-J. Huang, P. Guilford, J.P. Thiery, Early events in cell adhesion and polarity during epithelial-mesenchymal transition, J. Cell Sci. 125 (2012) 4417–4422.
[62] E.D. Hay, An overview of epithelio-mesenchymal transformation, Cells Tissues Organs 154 (1995) 8–20.
[63] E.M. Zeisberg, O. Tarnavski, M. Zeisberg, A.L. Dorfman, J.R. McMullen, E. Gustafsson, A. Chandraker, X. Yuan, W.T. Pu, A.B. Roberts, Endothelial-to-mesenchymal transition contributes to cardiac fibrosis, Nat. Med. 13 (2007) 952–961.
[64] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Invest. 119 (2009) 1420.
[65] S. Lamouille, J. Xu, R. Derynck, Molecular mechanisms of epithelial—mesenchymal transition, Nat. Rev. Mol. Cell Biol. 15 (2014) 178–196.
[66] J.Y. Shih, P.C. Yang, The EMT regulator slug and lung carcinogenesis, Carcinogenesis 32 (2011) 1299–1304.
[67] D. Medici, E.M. Shore, V.Y. Lounev, F.S. Kaplan, R. Kalluri, B.R. Olsen, Conversion of vascular endothelial cells into multipotent stem-like cells, Nat. Med. 16 (2010) 1400–1406.
[68] E.J. Armstrong, J. Bischoff, Heart valve development endothelial cell signaling and differentiation, Circ. Res. 95 (2004) 459–470.
[69] Y. Cao, B. Feng, S. Chen, Y. Chu, S. Chakrabarti, Mechanisms of endothelial to mesenchymal transition in the retina in diabetes, Invest. Ophthalmol. Vis. Sci. 55 (2014) 7321–7331.
[70] B.C. Cooley, J. Nevado, J. Mellad, D. Yang, C.S. Hilaire, A. Negro, F. Fang, G. Chen, H. San, A.D. Walts, TGF-beta signaling mediates endothelial-to-mesenchymal transition (EndMT) during vein graft remodeling, Sci. Transl. Med. 6 (2014) 227ra34.
[71] L. Maddaluno, N. Rudini, R. Cuttano, L. Bravi, C. Giampietro, M. Corada, L. Ferrarini, F. Orsenigo, E. Papa, G. Boulday, EndMT contributes to the onset and progression of cerebral cavernous malformations, Nature 498 (2013) 492–496.
[72] S. Potenta, E. Zeisberg, R. Kalluri, The role of endothelial-to-mesenchymal transition in cancer progression, Br. J. Cancer 99 (2008) 1375–1379.
[73] E.M. Zeisberg, S. Potenta, L. Xie, M. Zeisberg, R. Kalluri, Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts, Cancer Res. 67 (2007) 10123–10128.
[74] E.M. Zeisberg, S.E. Potenta, H. Sugimoto, M. Zeisberg, R. Kalluri, Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition, J. Am. Soc. Nephrol. 19 (2008) 2282–2287.
[75] P. Zhu, L. Huang, X. Ge, F. Yan, R. Wu, Q. Ao, Transdifferentiation of pulmonary arteriolar endothelial cells into smooth muscle—like cells regulated by myocardin involved in hypoxia—induced pulmonary vascular remodelling, Int. J. Exp. Pathol. 87 (2006) 463–474.
[76] D. Medici, R. Kalluri, Endothelial—Mesenchymal Transition and Its Contribution to the Emergence of Stem Cell Phenotype, Elsevier, 2012 379–384.
[77] R. Derynck, Y.E. Zhang, Smad-dependent and Smad-independent pathways in TGF-beta family signalling, Nature 425 (2003) 577–584.
[78] A. Mirshafiey, M. Mohsenzadegan, TGF-beta as a promising option in the treatment of multiple sclerosis, Neuropharmacology 56 (2009) 929–936.
[79] L. Durelli, L. Conti, M. Clerico, D. Boselli, G. Contessa, P. Ripellino, B. Ferrero, P. Eid, F. Novelli, T—helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta, Ann. Neurol. 65 (2009) 499–509.
[80] E. Bettelli, M. Oukka, V.K. Kuchroo, TH-17 cells in the circle of immunity and autoimmunity, Nat. Immunol. 8 (2007) 345–350.
[81] T. Wyss-Coray, P. Borrow, M.J. Brooker, L. Mucke, Astroglial overproduction of TGF-beta 1 enhances inflammatory central nervous system disease in transgenic mice, J. Neuroimmunol. 77 (1997) 45–50.
[82] E.A. Winkler, R.D. Bell, B.V. Zlokovic, Central nervous system pericytes in health and disease, Nat. Neurosci. 14 (2011) 1398–1405.
[83] A. Antonelli-Orlidge, K.B. Saunders, S.R. Smith, P.A. D'Amore, An activated form of transforming growth factor beta is produced by cocultures of endothelial cells and pericytes, Proc. Natl. Acad. Sci. 86 (1989) 4544–4548.
[84] C.M. Garcia, D.C. Darland, L.J. Massingham, P.A. D'Amore, Endothelial cell—astrocyte interactions and TGF beta are required for induction of blood—neural barrier properties, Dev. Brain Res. 152 (2004) 25–38.
[85] S. Dohgu, F. Takata, A. Yamauchi, S. Nakagawa, T. Egawa, M. Naito, T. Tsuruo, Y. Sawada, M. Niwa, Y. Kataoka, Brain pericytes contribute to the induction and up-regulation of blood—brain barrier functions through transforming growth factor beta production, Brain Res. 1038 (2005) 208–215.
[86] C.J. De Groot, L. Montagne, A.D. Barten, P. Sminia, P. Van Der Valk, Expression of transforming growth factor (TGF)-[beta] 1,-[beta] 2, and-[beta] 3 isoforms and TGF-[beta] type I and type II receptors in multiple sclerosis lesions and human adult astrocyte cultures, J. Neuropathol. Exp. Neurol. 58 (1999) 174–187.
[87] W. Shen, S. Li, S.H. Chung, L. Zhu, J. Stayt, T. Su, P.O. Couraud, I.A. Romero, B. Weksler, M.C. Gillies, Tyrosine phosphorylation of VE-cadherin and claudin-5 is associated with TGF beta 1-induced permeability of centrally derived vascular endothelium, Eur. J. Cell Biol. 90 (2011) 323–332.
[88] J. Xu, S. Lamouille, R. Derynck, TGF beta-induced epithelial to mesenchymal transition, Cell Res. 19 (2009) 156–172.
[89] T. Kokudo, Y. Suzuki, Y. Yoshimatsu, T. Yamazaki, T. Watabe, K. Miyazono, Snail is required for TGF beta-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells, J. Cell Sci. 121 (2008) 3317–3324.
[90] L. Xiao, D.J. Kim, C.L. Davis, J.V. McCann, J.M. Dunleavey, A.K. Vanderlinden, N. Xu, S.G. Pattenden, S.V. Frye, X. Xu, Tumor endothelial cells with distinct patterns of TGFbeta-driven endothelial-to-mesenchymal transition, Cancer Res. 75 (2015) 1244–1254.
[91] T. Maniatis, A ubiquitin ligase complex essential for the NF-kB, Wnt/Wingless, and Hedgehog signaling pathways, Genes Dev. 13 (1999) 505–510.
[92] R. Daneman, D. Agalliu, L. Zhou, F. Kuhnert, C.J. Kuo, B.A. Barres, Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis, Proc. Natl. Acad. Sci. 106 (2009) 641–646.
[93] S. Liebner, M. Corada, T. Bangsow, J. Babbage, A. Taddei, C.J. Czupalla, M. Reis, A. Felici, H. Wolburg, M. Fruttiger, Wnt/beta-catenin signaling controls development of the blood–brain barrier, J. Cell Biol. 183 (2008) 409–417.
[94] J.M. Stenman, J. Rajagopal, T.J. Carroll, M. Ishibashi, J. McMahon, A.P. McMahon, Canonical Wnt signaling regulates organ-specific assembly and differentiation of CNS vasculature, Science 322 (2008) 1247–1250.
[95] R. Paolinelli, M. Corada, L. Ferrarini, K. Devraj, C. Artus, C.J. Czupalla, N. Rudini, L. Maddaluno, E. Papa, B. Engelhardt, Wnt activation of immortalized brain endothelial cells as a tool for generating a standardized model of the blood brain barrier in vitro, PLoS One 8 (2013), e70233.
[96] Y. Wang, A. Rattner, Y. Zhou, J. Williams, P.M. Smallwood, J. Nathans, Norrin/Frizzled4 signaling in retinal vascular development and blood brain barrier plasticity, Cell 151 (2012) 1332–1344.
[97] B.P. Zhou, J. Deng, W. Xia, J. Xu, Y.M. Li, M. Gunduz, M.C. Hung, Dual regulation of snail by GSK-3 beta-mediated phosphorylation in control of epithelial—mesenchymal transition, Nat. Cell Biol. 6 (2004) 931–940.
[98] A. Cattelino, S. Liebner, R. Gallini, A. Zanetti, G. Balconi, A. Corsi, P. Bianco, H. Wolburg, R. Moore, B. Oreda, The conditional inactivation of the beta-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility, J. Cell Biol. 162 (2003) 1111–1122.
[99] L. Bravi, N. Rudini, R. Cuttano, C. Giampietro, L. Maddaluno, L. Ferrarini, R.H. Adams, M. Corada, G. Boulday, E. Tournier-Lasserve, Sulindac metabolites decrease cerebrovascular malformations in CCM3-knockout mice, Proc. Natl. Acad. Sci. 112 (2015) 8421–8426.
[100] F. Radtke, H.R. MacDonald, F. Tacchini-Cottier, Regulation of innate and adaptive immunity by Notch, Nat. Rev. Immunol. 13 (2013) 427–437.
[101] E.A. Winkler, R.D. Bell, B.V. Zlokovic, Central nervous system pericytes in health and disease, Nat. Neurosci. 14 (2011) 1398–1405.
[102] M. Hellstrom, L.K. Phng, J.J. Hofmann, E. Wallgard, L. Coultas, P. Lindblom, J. Alva, A.K. Nilsson, L. Karlsson, N. Gaiano, Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis, Nature 445 (2007) 776–780.

[103] G.R. John, S.L. Shankar, B. Shafit-Zagardo, A. Massimi, S.C. Lee, C.S. Raine, C.F. Brosnan, Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation, Nat. Med. 8 (2002) 1115–1121.
[104] M. Jurynczyk, A. Jurewicz, B. Bielecki, C.S. Raine, K. Selmaj, Inhibition of Notch signaling enhances tissue repair in an animal model of multiple sclerosis, J. Neuroimmunol. 170 (2005) 3–10.
[105] L.M. Minter, D.M. Turley, P. Das, H.M. Shin, I. Joshi, R.G. Lawlor, O.H. Cho, T. Palaga, S. Gottipati, J.C. Telfer, Inhibitors of gamma-secretase block in vivo and in vitro T helper type 1 polarization by preventing Notch upregulation of Tbx21, Nat. Immunol. 6 (2005) 680–688.
[106] W. Elyaman, E.M. Bradshaw, Y. Wang, M. Oukka, P. Kivisakk, S. Chiba, H. Yagita, S.J. Khoury, JAGGED1 and delta1 differentially regulate the outcome of experimental autoimmune encephalomyelitis, J. Immunol. 179 (2007) 5990–5998.
[107] V.K. Manda, R.K. Mittapalli, W.J. Geldenhuys, P.R. Lockman, Chronic exposure to nicotine and saquinavir decreases endothelial Notch − 4 expression and disrupts blood − brain barrier integrity, J. Neurochem. 115 (2010) 515–525.
[108] Y. Wang, L. Pan, C.B. Moens, B. Appel, Notch3 establishes brain vascular integrity by regulating pericyte number, Development 141 (2014) 307–317.
[109] E. Tournier-Lasserve, A. Joutel, J. Melki, J. Weissenbach, G.M. Lathrop, H. Chabriat, J.L. Mas, E.A. Cabanis, M. Baudrimont, J. Maciazek, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12, Nat. Genet. 3 (1993) 256–259.
[110] I. Espinoza, R. Pochampally, F. Xing, K. Watabe, L. Miele, Notch signaling: targeting cancer stem cells and epithelial-to-mesenchymal transition, Oncol.Targets Ther. 6 (2013) 1249.
[111] K. Niessen, Y. Fu, L. Chang, P.A. Hoodless, D. McFadden, A. Karsan, Slug is a direct Notch target required for initiation of cardiac cushion cellularization, J. Cell Biol. 182 (2008) 315–325.
[112] L.A. Timmerman, J. Grego-Bessa, A. Raya, E. Bertran, J.M. Perez-Pomares, J. Diez, S. Aranda, S. Palomo, F. McCormick, J.C. Izpisua-Belmonte, Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation, Genes Dev. 18 (2004) 99–115.
[113] J.I. Alvarez, A. Dodelet-Devillers, H. Kebir, I. Ifergan, P.J. Fabre, S. Terouz, M. Sabbagh, K. Wosik, L. Bourbonniere, M. Bernard, The Hedgehog pathway promotes blood–brain barrier integrity and CNS immune quiescence, Science 334 (2011) 1727–1731.
[114] X. Li, W. Deng, C.D. Nail, S.K. Bailey, M.H. Kraus, J.M. Ruppert, S.M. Lobo-Ruppert, Snail induction is an early response to Gli1 that determines the efficiency of epithelial transformation, Oncogene 25 (2006) 609–621.
[115] Y. Hasegawa, D. Tang, N. Takahashi, Y. Hayashizaki, A.R. Forrest, H. Suzuki, CCL2 enhances pluripotency of human induced pluripotent stem cells by activating hypoxia related genes, Sci. Report. 4 (2014).
[116] M. Prinz, J. Priller, Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease, Nat. Rev. Neurosci. 15 (2014) 300–312.
[117] I.A. Krizbai, A. Gasparics, P. Nagyoszi, C. Fazakas, J. Molnar, I. Wilhelm, R. Bencs, L. Rosivall, A. Sebe, Endothelial-mesenchymal transition of brain endothelial cells: possible role during metastatic extravasation, PLoS One 10 (2015), e0119655.
[118] B.J. Kim, B.M. Hancock, A. Bermudez, N. Del Cid, E. Reyes, N.M. van Sorge, X. Lauth, C.A. Smurthwaite, B.J. Hilton, A. Stotland, Bacterial induction of Snail1 contributes to blood–brain barrier disruption, J. Clin. Invest. 125 (2015) 0.
[119] N.J. Abbott, Inflammatory mediators and modulation of blood − brain barrier permeability, Cell. Mol. Neurobiol. 20 (2000) 131–147.
[120] R.C. Bates, A.M. Mercurio, Tumor necrosis factor-alfa stimulates the epithelial-to-mesenchymal transition of human colonic organoids, Mol. Biol. Cell 14 (2003) 1790–1800.
[121] G.J. Mahler, E.J. Farrar, J.T. Butcher, Inflammatory cytokines promote mesenchymal transformation in embryonic and adult valve endothelial cells, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 121–130.
[122] F. Rieder, S.P. Kessler, G.A. West, S. Bhilocha, C. de la Motte, T.M. Sadler, B. Gopalan, E. Stylianou, C. Fiocchi, Inflammation-induced endothelial-to-mesenchymal transition: a novel mechanism of intestinal fibrosis, Am. J. Pathol. 179 (2011) 2660–2673.
[123] Y.d. Wu, B.P. Zhou, TNF-alpha/NF-kB/Snail pathway in cancer cell migration and invasion, Br. J. Cancer 102 (2010) 639–644.
[124] M. Maleszewska, J.R.A. Moonen, N. Huijkman, B. van de Sluis, G. Krenning, M.C. Harmsen, IL-1beta and TGFbeta2 synergistically induce endothelial to mesenchymal transition in an NFkB-dependent manner, Immunobiology 218 (2013) 443–454.
[125] M. Hayashi, Y. Luo, J. Laning, R.M. Strieter, M.E. Dorf, Production and function of monocyte chemoattractant protein-1 and other beta-chemokines in murine glial cells, J. Neuroimmunol. 60 (1995) 143–150.
[126] A.K. Stalder, A. Pagenstecher, N.C. Yu, C. Kincaid, C.S. Chiang, M.V. Hobbs, F.E. Bloom, I.L. Campbell, Lipopolysaccharide-induced IL-12 expression in the central nervous system and cultured astrocytes and microglia, J. Immunol. 159 (1997) 1344–1351.
[127] H. Lassmann, W. Bruck, C.F. Lucchinetti, The immunopathology of multiple sclerosis: an overview, Brain Pathol. 17 (2007) 210–218.