Seminars in Cell & Developmental Biology 38 (2015) 16–25

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

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

Review

Transmembrane proteins of the tight junctions at the blood–brain barrier: Structural and functional aspects

Reiner F. Haseloff, Sophie Dithmer, Lars Winkler, Hartwig Wolburg[1], Ingolf E. Blasig*

Leibniz Institute for Molecular Pharmacology, Robert Roessle-Str. 10, 13125 Berlin, Germany

ARTICLE INFO

Article history:
Available online 26 November 2014

Keywords:
Tight junction
Blood–brain barrier
Claudin
Occludin
Tricellulin
Endothelial cells

ABSTRACT

The blood–brain barrier (BBB) is formed by microvascular endothelial cells sealed by tetraspanning tight junction (TJ) proteins, such as claudins and TAMPs (TJ-associated marvel proteins, occludin and tricellulin). Claudins are the major components of the TJs. At the BBB, claudin-5 dominates the TJs by preventing the paracellular permeation of small molecules. On the other hand, TAMPs regulate the structure and function of the TJs; tricellulin may tighten the barrier for large molecules. This review aims at integrating and summarizing the most relevant and recent work on how the BBB is influenced by claudin-1, -3, -5, -12 and the TAMPs occludin and tricellulin, all of which are four-transmembrane TJ proteins. The exact functions of claudin-1, -3, -12 and TAMPs at this barrier still need to be elucidated.

Contents

1. Introduction

Barriers throughout the organism regulate the passage of ions, molecules, proteins and cells *via* specialized endothelial or epithelial cell layers by establishing gradients between compartments. The blood–brain barrier (BBB) is formed by endothelial cells (ECs)

* Corresponding author. Tel.: +49 30 94793 244.
E-mail address: iblasig@fmp-berlin.de (I.E. Blasig).
[1] Institute of Pathology and Neuropathology, University of Tübingen, Germany.

http://dx.doi.org/10.1016/j.semcdb.2014.11.004
1084-9521/

of brain capillaries, and it is one of the tightest barriers in higher organisms. Passage through the barrier may be transcellular or paracellular. The transcellular pathway is regulated by transporter proteins serving the nutritional demands of the brain and by outward-directed transport systems. Passive diffusion of molecules toward a concentration gradient through the paracellular gap is controlled by a multi-protein complex, the tight junctions (TJs). TJs are composed of transmembrane and cytosolic TJ-associated proteins. Interacting transmembrane proteins form a network spanning the cell circumferentially. Thus, TJs separate not only extracellular compartments, but also apical and basolateral membrane domains of barrier-forming cells. This TJ belt acts like a fence by hindering membrane proteins from floating freely between the two membrane compartments. As a consequence, the apical and basolateral plasma membranes have different protein compositions establishing a cellular polarity. By this means, TJs play a pivotal role in maintaining the homeostasis of the CNS.

1.1. Tight junction ultrastructure

In ultrathin section transmission electron microscopy (TEM), TJs at cell–cell contacts can be recognized as the *zonula occludens*, and thus distinguished from other intercellular structures (Fig. 1B). Ultrathin sections of contacts between opposing brain vascular ECs depict electron dense TJ elements, of diameter 10–20 nm and length 20–50 nm separated by interspaces of 50–200 nm. At the TJs, opposing cell membranes approach each other, so that the outer leaflets appear to fuse and thereby obliterate the intercellular space [1], leading to so-called "kissing points". In ECs, TJs and adherens junctions are intermingled over the entire interendothelial cleft [2].

In freeze-fracture electron microscopy (FFEM, a variant of TEM), the membranes are cleaved into two halves, one associated with the extracellular space and called external fracture face or E-face, the other one associated with the cytoplasm called protoplasmic fracture face or P-face. The TJ elements are about 10 nm in diameter and organized as a complicated network of strands surrounding the cell like a belt (Fig. 1C). Endothelial cell TJs outside the brain are associated mostly with the E-face while epithelial cell TJs are mostly associated with the P-face. However, BBB endothelial cell TJs are equally associated with both, the E- and the P-face [3,4]. This observation, together with the finding that BBB endothelial cell TJs lose their P-face association *in vitro*, suggested that the brain microenvironment may be responsible for the special architecture of the BBB TJs. E-face association seems to be caused by claudin-5 as it is associated with the E-face in TJ-free cells after transfection [5,6]. Other BBB claudins, *e.g.*, claudin-1 [7] or claudin-3 are P-face associated or are unable to form strands – such as claudin-12 [8]. Strands containing solely claudin-5 assemble discontinuous chains of particles, whereas strands of claudin-1 or -3 establish continuous strands. The diameter of the round-shaped meshes in a network of pure claudin-5 is larger (approx. 500 nm) than that of claudin-3 (approx. 350 nm) [9]. Although claudin-5 is the most abundant claudin molecule at the BBB [10,11], strand morphology in the BBB is strongly influenced by other TJ proteins as demonstrated by the lower diameter and the square-shaped meshes [7,8]. The rectangular low diameter strand network is probably caused by claudin-3 [9] and by TJ-associated marvel proteins (TAMPs) [12,13] such as occludin or tricellulin, which are also expressed at the BBB [14–16]. Claudin-3 [8] and TAMP interaction [7] condenses the network as demonstrated by the coexpression of a claudin forming large round-shaped meshes with any TAMP in TJ-free cells.

The E-face and P-face association changes during development from extremely low particle density in both leaflets at embryonic day E13, over predominant E-face association at E15 and E18 to predominant P-face association at postnatal day 1 in rat brain ECs [17]. In parallel to the P/E-face ratio, there is an increase in transendothelial electrical resistance up to E20 in rats [18] and a decrease in permeability during embryonic development [19]. Thus, the P/E-face ratio has been hypothesized to be a measure of barrier tightness. However, data on the predictive value of TJ particle distribution in epithelial cells are inconsistent [20].

1.2. Molecular composition of tight junctions

Although *zonula occludens* and intramembranous particle strands have been known since the 1970s, their nature remained unknown until the first transmembrane protein localized in the TJs was identified – namely occludin [21]. Occludin is expressed in virtually all TJs, and can therefore be considered as their marker protein. Two occludin-related proteins, tricellulin [22] and marvel-D3 [12,13], were subsequently identified. According to structural predictions, their termini are both cytosolic, and the four transmembrane helices are linked by one intra- and two extracellular loops (ECLs), thus forming the MARVEL (MAL and related protein for vesicle trafficking and membrane link) domain. It is hypothesized that this domain plays a crucial role in mediating the interaction of the TJs with membrane lipids [23]. A 3D structure of a complete TAMP has not yet been solved. As occludin is polyphosphorylated, a regulatory function is highly probable. Tricellulin specifically localizes at 3-cell contacts and has been shown to seal tissue barriers for macromolecules [24]. The three TAMPs may partially complement each others' functions [12].

The main structural proteins of the TJs were described in 1998 and named claudins [25]. In contrast to occludin, claudins are able to reconstitute *de novo* TJ strands in TJ-free mouse fibroblasts [26]. Claudins are a 27-gene family (human 26) with 207–305 aa [27], that codes the main protein fraction of the TJs which plays a crucial role in the formation and integrity of TJs and determines the barrier function. Claudin isoform expression depends on the tissue and developmental stage [28]. The functions of the claudins are to tighten the paracellular cleft (*e.g.*, claudin-1, -3, -5, -11), to form paracellular ion pores (*e.g.*, claudin-2, -10, -16) or to contribute to the maturation of barriers (claudin-6, -13) [29]. Distinctive charges on the extracellular surface seem to be important for paracellular interactions and enable complete tightening or ion-selective permeation. The first ECL contains a consensus motif with two cysteines conserved throughout the family: G-L-W-x-x-C-[*STDENQH*]-C (italic: 7–9 amino acids including polar/charged residues) [27].

Claudins and TAMPs can interact with each other within the plasma membrane (*cis*-interaction) and/or between opposing cells (*trans*-interaction) [7,8]. They share a four transmembrane topology with two ECLs, an intracellular loop, and cytosolic N- and C-termini. The protein assembly at the BBB is schematically illustrated in Fig. 1A.

2. Claudins

In addition to claudin-5, the expression of claudin-1, -3 and -12 has been confirmed in brain capillary ECs [30,31]. The claudin-5 mRNA level of cerebral capillaries is 600–700 times higher compared to claudin-1, -3 or -12 mRNAs [10,11,32]. The existence of claudin-1 protein is disputed as certain antibodies can exhibit cross-reactivity between claudin-1 and claudin-3 [30,33]. The sequences of claudin-1, -3, and -5 are highly homologous suggesting that they have similar properties; in contrast, the claudin-12 sequence is comparatively different (sequence similarity given as positive match scores in BLOSUM62-based alignment: claudin-1, -3, -5 among each other, 63%–68%; claudin-1, -3, -5 *vs.* claudin-12, 33%–36%). No evidence has been found that these claudins replace each other [10,31,34]. A 3D-structure of a BBB claudin has not been