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MicroRNAs regulate tight junction proteins and modulate epithelial/endothelial barrier functions

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Abbreviations: AJ, Adherens junctions; AJC, apical junctional complex; CDS, coding sequence; IBD, inflammatory bowel disease; JAM, junctional adhesion molecule; miRNA, microRNA; ROS, reactive oxygen species; TEM, transmission electron microscopy; TJ, tight junction

Tightly controlled epithelial and endothelial barriers are a prerequisite for life as these barriers separate multicellular organisms from their environment and serve as first lines of defense. Barriers between neighboring epithelial cells are formed by multiple intercellular junctions including the 'apical junctional complex—AJC' with tight junctions (TJ), adherens junctions (AJ), and desmosomes. TJ consist of tetraspan transmembrane proteins like occludin, various claudins that directly control paracellular permeability, and the 'Junctional Adhesion Molecules' (JAMs). For establishing tight barriers TJ are essential but at the same time have to allow also selective permeability. For this, TJ need to be tightly regulated and controlled. This is organized by a variety of adaptor molecules, i.e., protein kinases, phosphatases and GTPases, which in turn are regulated and fine-tuned involving microRNAs (miRNAs). In this review we summarize available data on the role and targeting of miRNAs in the maintenance of epithelial and/or endothelial barriers.

Introduction

Tight (TJ) and adherens (AJ) junctions form a decisive part of the 'Apical Junctional Complex – AJC' and regulate the paracellular permeability of epithelial layers across the apical/basolateral axis. Different groups of proteins are required to assemble the tight junction complex: transmembrane proteins like occludin, proteins of the claudin family and junctional adhesion molecules (JAM). These proteins are linked with cytosolic regulatory proteins as well as scaffolding and cytoskeletal proteins.[1-4] Interactions of barrier components have to be strictly coordinated and tightly controlled to maintain their function in homeostasis of epithelial—or endothelial—barriers including their paracellular permeability. There is increasing evidence on how this sophisticated regulation might be orchestrated by microRNAs (miRNAs), small regulatory RNAs that post-transcriptionally affect most of the assembly steps and synthesis processes of junctional complex proteins.

In this review we aim to summarize current knowledge about regulatory effects of miRNAs involved in the adaptation of tight junctions i.e., modulating epithelial barrier functions in response to environmental challenges such as inflammation or disease. Our main focus is directed toward miRNA involved in TJ of intestinal epithelial barriers, as the gut is constantly challenged by foreign antigens and is by far the largest immunological organ. In addition, we will briefly touch the endothelial blood brain barrier as toxin or drug passage across this barrier is of particular pharmaceutical interest.

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Tight Junctions and Adherens Junctions

Tight (TJ) and adherens junctions (AJ) are the major protein complexes of the Apical Junctional Complex (AJC).[5,6] The AJC complex tightly connects the polarized epithelial cells in the intestinal mucosa and maintains the homeostasis of the intestinal barrier.[7] The AJC represents the key structure for maintaining intestinal barrier functions.[8,9] Tight junctions are found in all vertebrate epithelia and represent specialized multi-protein complexes localized at the apical side of lateral membranes of polarized epithelial cells.[10] Since their discovery in 1963 more than 50 TJ-associated proteins have been identified.[1] Tight junctions allow selective permeability for molecules on the basis of charge and size restrictions.[11-13] It also became apparent that TJ are highly dynamic and may vary in different tissues giving rise to distinct barrier properties.[1,2] However, despite ever increasing numbers of TJ-associated components, the detailed mechanism (s) of how these components work together to form a controlled selectively permeable barrier has remained largely enigmatic.[1,3]

Trans-membrane proteins such as occludin, claudins, junctional adhesion molecules (JAMs) constitute the main protein

complexes of tight junctions.[8,10,11] With the exception of JAMs, all trans-membrane proteins of tight junctions are tetraspanins consisting of 4 trans-membrane domains, which form 2 extracellular loops and one intracellular loop. Adaptor proteins such as ZO-1, -2 and -3 are linked to the cytosolic C-terminus of the trans-membrane proteins. The adaptor proteins interact also with many other proteins and are anchored to the actin cytoskeleton. Interactions between the different tight junction proteins and the cytoskeleton are essential for the normal assembly and maintenance of tight junction and hence also epithelial barrier integrity.

Examination by electron microscopy of the apical junctional complex has revealed a distinct ultrastructure. At the apical side of lateral membranes of polarized epithelial cells transmission electron microscope (TEM) pictures exhibited electron-dense regions consisting of regularly punctates representing the cellular epithelial barrier (**Fig. 1**). According to freeze fracture electron microscopy tight junctions exist of an anastomosing strands of beads.[13]

Tight junctions in different epithelia are challenged by a variety of factors like toxins, pathogens or inflammation potentially leading to tissue damage.[14-16] At the same time these barriers are positively affected by probiotics and/or molecules like growth factors or anti-inflammatory cytokines, which stabilize the barrier function.[17-21] The disruption as well as the assembly of tight junctions are regulated by several signaling cascades, involving protein kinases, protein phosphatases and G-proteins.[22-25]

The second major constituents of AJC are adherens junctions (AJ). AJ are characteristic for anchoring cells to cytoplasmatic actin filaments via a tightly controlled network of adaptor proteins. Cadherins, the transmembrane spanning proteins of 2 opposing cell membranes of epithelial cells (E-cadherins) or endothelial cells (VE-cadherins) are involved in homotypical trans-interactions thereby mediating cell-cell adhesion. Interactions of cadherins to several catenins provide the linkage to the actin cytoskeleton.[26]

Impact of Tight Junction Regulation

The interactions of tight junction proteins are dynamically regulated by several regulatory mechanisms (see **Fig. 2**). This results in selectively permeable barriers that are distinct in different tissues. Various extracellular factors such as inflammatory cytokines, reactive oxygen species (ROS) or microbial pathogens and their products disrupt epithelial tight junctions by activating multiple intracellular signaling pathways. In particular, molecules involved in signaling like protein kinases are involved in disruption or assembly of tight junctions. The c-Src-dependent tyrosine phosphorylation of different AJC proteins disrupts barrier functions and intestinal and renal epithelia.[23,24]

Work from our and other laboratories showed that PKC isoforms such as PKCλ, PKCζ and PKCη are involved in the assembly of tight junctions,[25,27]: e.g., the activation of PKCζ effects the availability of occludin in TJ. Other protein phosphatases like PP2A and PP1 are able to effect TJ stability indirectly by dephosphorylating components of Par3, Par6, CDC42 polarity complex, which is essential for the integrity on TJ.[28-30]

Bacterial lipopolysaccharides (LPS) induce tight junction disruption via NF-kB and the induction of the Toll-like receptor 4 (TLR4) and LPS-binding protein (LBP) pathways. A specific knockdown of TLR4 or LBP significantly attenuates LPS-induced tight junction disruption.[31]

For several gastrointestinal inflammatory diseases, including Crohn's disease, ulcerative colitis, celiac disease, as well as many diarrheal syndromes induced by pathogenic microbes, the disturbance of TJ functions leads to a disruption of the intestinal barrier. In this way an increased penetration of microbial and other antigens is facilitated, resulting in inflammatory

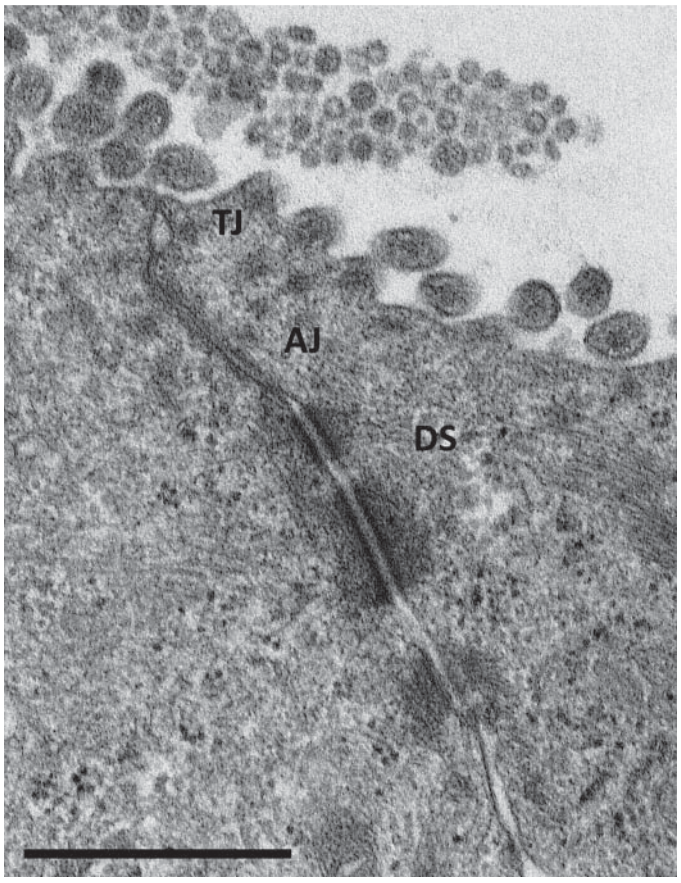

Figure 1. Transmission electron microscopy (TEM) image of an 'apical junctional complex' of polarized T84 human colorectal carcinoma epithelial cells Depicted are the 'tight junctions' (TJ), directly beneath the microvilli, 'adherens junctions' (AJ), and 'desmosomes' (DS) below the 'apical junctional complex'. Scale bar = 1 μm (courtesy of Lilo Greune, Institute of Infectiology – Center for Molecular Biology of Inflammation (ZMBE), University of Münster).

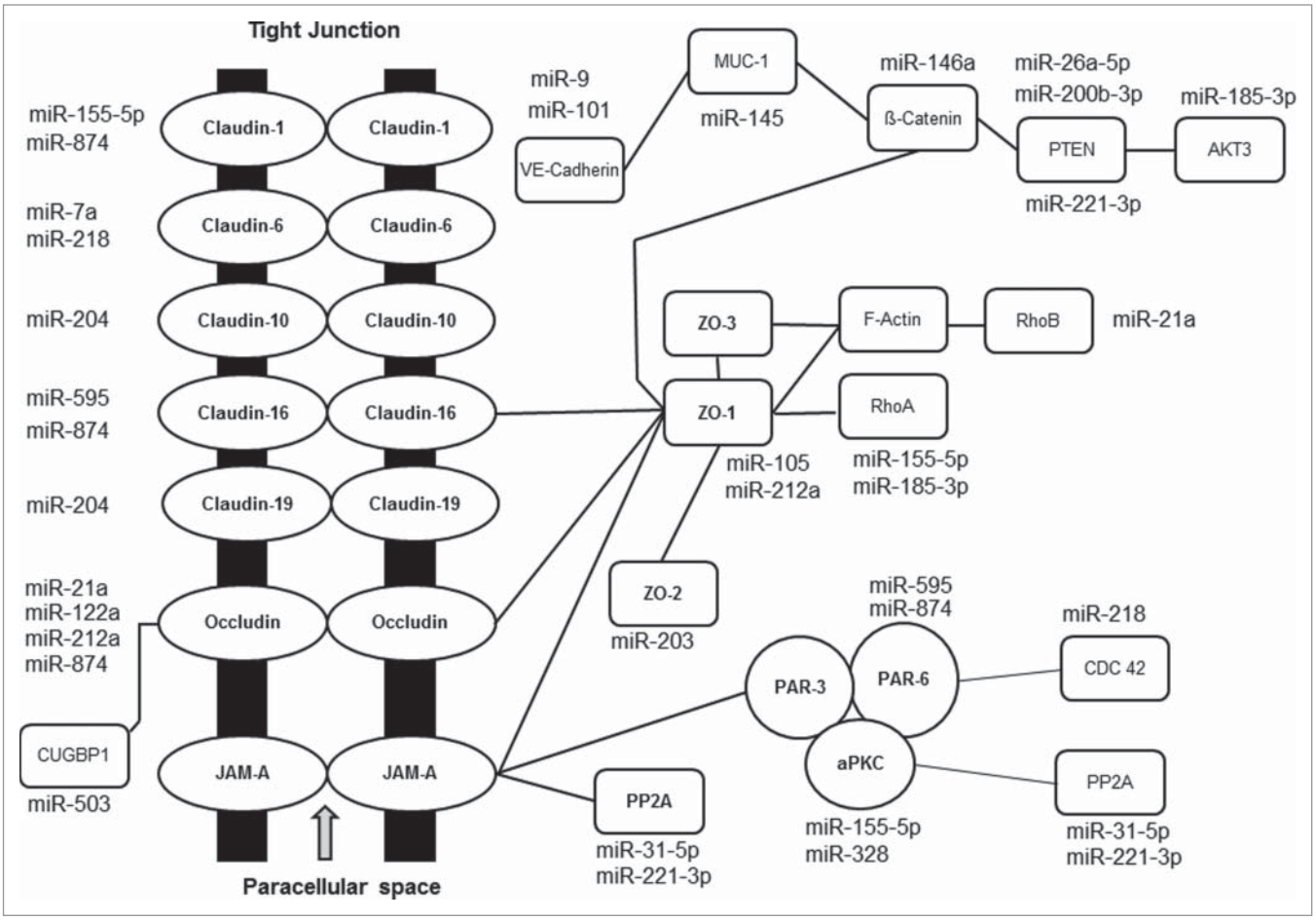

Figure 2. Schematic representation of currently known TJ pathway genes affected by miRNAs (modified from Veltman et al. 2012[93]).

responses.[23,32] Correspondingly, restoring or even enhancing the intestinal TJ barrier limits the development of intestinal inflammation and facilitates a more rapid resolution of the inflammatory disease.[33,34] However, the role of transmembrane TJ protein depletion in disorders involving increased intestinal permeability remains largely undefined. A marked decrease of occludin levels in inflammatory bowel disease has been reported in several clinical studies.[35-37] How occludin depletion causes an increase of intestinal permeability is currently under investigation.

Tumor necrosis factor α (TNF-α) represents one of the central mediators of gastrointestinal inflammation.[38,39] Expression levels of TNF-α are significantly elevated in patients with inflammatory conditions, like Crohn's disease, ulcerative colitis, or celiac disease. An effective induction of remission by anti-TNF-α therapy has been achieved in patients with severe active Crohn's disease and ulcerative colitis[39,40] as well as refractory celiac disease.[41] This suggests that the pro-inflammatory activity of TNF-α may contribute to an increase in intestinal TJ permeability.[42-45] TNF-α induces a breakdown of barrier functions, as shown *in vitro* by employing the Caco-2 monolayer model and *in vivo* in a mouse intestinal model.[45]

MicroRNA Biogenesis

MicroRNAs (miRNAs) represent a large family of short, single-stranded, non-coding RNAs (ncRNAs) of about 19–25 nucleotides in length. The human genome encodes for probably more than 2570 miRNAs (mirBase 20).[46,47] Different sets of miRNAs are found in different cell types and tissues.[48] MiRNAs are synthesized by a defined biogenesis pathway.[49,50] The relevant gene regulation and mode of action has been reviewed extensively.[46,51-53] The basic principle of gene regulation depends on the final, partial hybridization of one miRNA strand ('guide strand') within the RISC complex to partially complementary sequences, localized mostly within the 3′-UTR of target mRNAs (imperfect base-pairing). This leads to gene silencing by triggering mRNA deadenylation and degradation or—as a second option—translation inhibition. However, it is important to emphasize that several other mechanisms of the regulation of gene expression by miRNAs have also been described.[54] Since their discovery in 1993 miRNAs have emerged as a new class of gene regulators that modulate and control the activities of thousands of mRNAs and in recent years the number of protein coding genes found to be regulated involving miRNA has been

increasing steadily.[55] Actually, it has been estimated that at least 60% of all protein-encoding genes are regulated by miRNAs.[46,56] MiRNAs are well conserved in eukaryotic organisms and are supposed to be evolutionarily ancient components of gene regulation.[57] Hence, it is not surprising that miRNA-deregulation effects many physiological and/or pathological processes[58,59] particularly when one takes into account that miRNA are usually able to target different mRNA, and vice versa that a certain mRNA is supposed be targeted by multiple miRNAs.[60] Moreover, it has been discovered recently, that miRNA-binding sites might not only be localized within the 3′-UTR[46] but also within the coding sequence (CDS)[61] or the 5′-UTR of a transcript.[62] The genome-wide analyses of miRNA-binding sites, performed by Darnell and others indicate that a substantial number of miRNAs interact with these alternative binding sides.[63,64] Therefore, this implies that the number of miRNA target sequences might have been even underestimated up to now.

Nevertheless, the prevailing consent concerning miRNA-mediated regulatory mechanisms is gene silencing by transcript decay due to mRNA deadenylation. However, miRNA-based gene regulation following this mechanism may operate in certain cells differently depending on the locus of miRNA-mRNA interactions and also the physiological state of the cell.

For the identification of miRNAs and the elucidation of potential miRNA targets several algorithms have been developed including TargetScan (http://www.targetscan.org/), MicroCosm Targets (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/), DIANA LAB (http:// http://diana.cslab.ece.ntua.gr/), miRSearch (https://www.exiqon.com/ mirsearch) or GeneGlobe (http://http://www.qiagen.com/products/genes%20and%20pathways/) just to mention a few.

MiRNA and Human Disease

As miRNAs are involved in the regulation of many essential physiological processes in eukaryotic cells, dysregulation and aberrant expression of miRNAs has been associated with numerous disease states[65] Consequently, miRNA-based diagnostics and therapies are under investigation.[66] Here, only a few examples of miRNA involvement in pathologic conditions will be mentioned.

Role of miRNA in Cancer

Chronic lymphocytic leukemia was the first human disease known to be associated with miRNA deregulation.[67] Many miRNAs correlated with different types of cancer[68] are also referred to as 'oncomirs' e.g., miRNA-21 which is linked with different types of cancer such as glioblastoma.[69] In human breast cancer cells the expression of claudin-6, as a TJ protein, was shown to be regulated by miR-7 and miR-218.[70] Recent studies concerning 5 members of the microRNA-200 family (miR-200a, miR-200b, miR-200c) as well as miR-141 and miR-429 also revealed their regulatory effects during tumor progression of breast cancer.[71] Screening assays for regulated miRNAs connected to early detection of colorectal cancer have been developed and are currently undergoing clinical trials. Our own experiments revealed that a sufficient selectivity and specificity can be achieved from less than 100 μl of blood plasma samples. This is due to the fact that cell-free, circulating miRNAs are highly stable in body fluids. This indicates that certain miRNAs might be used in diagnostics to assist clinical decision-making or the monitoring and interpretation of different disease treatment regimes.

Beyond that, just to indicate the scope of miRNA dependent regulation, the impact of miRNAs during heart development in general has been proven by inhibiting miRNA maturation in mouse models and for miR-155, miR-221 and miR-222 a pivotal role for the development of obesity during of the differentiation of stem cell progenitors into adipocytes have been shown.[72,73] Furthermore let-7 inhibition might be used obesity and type 2 diabetes treatment.

MiRNA and Barrier Function

MiRNAs are involved in nearly every developmental and physiological process and play decisive roles in the differentiation, cell migration, architecture, and barrier function in intestinal epithelial cells. In recent years the important role of miRNAs in protein expression in the small intestine has been firmly established and it has become increasingly clear that expression patterns of intestinal miRNAs are altered in intestinal diseases. In different mouse models the impact of miRNAs on the impairment of epithelial barrier function was shown e.g., by Mckenna et al.[74] In a typical Dicer1-deficient mouse model the intestinal barrier function is impaired leading to spontaneous intestinal inflammation. This is due to the loss of mmu-miR-192, which is normally highly expressed in the intestinal mucosa. Furthermore, in this mutant the transcription factors Regα and Regß as well as Relmß, which play a crucial role in inflammation and infection susceptibility are up-regulated and represent targets for miR-23a and miR-23b. The "recycling perfusion in vivo mouse model" by Ye et al. is discussed in the occludin section.[75] In addition, it has been found that the intestinal miRNA signature is also influenced by the presence of microbiota.[76,77] During bacterial infections miRNA expression is altered and plays an important role in the onset and progression of intestinal disease (for review: Staedel and Darfeuille, 2013).[78] Recently, intestinal barrier dysfunction due to altered miRNA expression has been reported also in HIV and SIV infections.[79] Furthermore, miRNAs have been identified as important factors in the host's response against microbial insults.

Inflammatory bowel diseases (IBD) represent a group of chronic, idiopathic, relapsing, and remitting immune disorders of the gastrointestinal tract in genetically susceptible individuals who are exposed to environmental risk factors.[80,81] In these diseases disturbances of the intestinal barrier are major factors in aggravating and perpetuating disease pathology. Thus, restoring barrier functions greatly helps to induce remission. MiRNAs have been found to regulate tight junctions in intestinal epithelial cells and in this way also affect intestinal barrier functions.[82] Interestingly, clinical studies demonstrated that in IBD including

Crohn's disease[83] and ulcerative colitis[84] miRNA expression patterns are abnormal. From our own work as well as others we know that this differences are also reflected in mouse models which to a certain degree simulate the human diseases. In the more UC like dextran sulfate sodium (DSS) colitis model (miR-155) or in the more CD like T cell transfer model (mir-10a) different miRNAs, indicating the different origin of the disease, are upregulated during the course of the inflammation.[85-88] Wu et al. were able to show that different sets of miRNAs also represent the degree of inflammation in ulcerative colitis (UC) and Crohn´s disease (CD).[83,84] For example, they were able to characterize miR-192 as up-regulated and miR-16 as down-regulated in patients with active UC. MiR-16 has been shown to be upregulated during inactive UC and is therefore inversely correlated with the status of the disease. For active CD miR-23b has been revealed to be up-regulated and on the other hand miR-19b as downregulated. Pathophysiologically both diseases are correlated with diarrhea and the according breakdown of the barrier function. However, these miRNAs have not yet been linked to the exact target molecules during the course of disease.

Occludin

Recently, it could be shown that miR-21 is upregulated in chronic UC patients.[84] MiR-21 induces the degradation of *ras* homolog gene family member B mRNA, leading to the depletion of occludin with the resulting impairment of tight junctions. Increased levels of miR-21 levels in inflamed tissues were also shown by Takagi et al.[89] revealing its important role in pathogenesis of IBD. UC patients revealed an increase in miR-21 levels in serum samples, this was also supported by the presence of miRNAs in peripheral blood cell as it was shown by Paraskevi et al.[90] A similar increase in miR-21 levels was also reported in patients with pediatric CD.[91] After transfection with miR-21 mimics Caco-2 cells suffer the loss of tight junction proteins and the according structural changes.[83] These results indicate that circulating miRNAs such as miR-21 and others might have the potential to be used as biomarkers in clinical applications, which is also supported by our own recent results (Pott et al.; 2014; personal communication). The expression of occludin in Caco-2 cells as well as in mouse intestinal epithelial cells is TNF-α dependent as had been shown by Ye et al.[75] TNF-α is increased in patients under inflammatory conditions (anti-TNF-α therapy) which leads to the expression of miR-122a. By binding to the non-coding region of occludin mRNA, miR-122a induces specific mRNA degradation and thus occludin depletion, leading to an increase in intestinal permeability. Therefore, miRNA-122a plays a crucial role in TJ formation and barrier function during intestinal inflammation.

Zonula occludens proteins ZO-1 and ZO-2

The up-regulation of miR-212 expression after alcohol abuse was reported to induce the disruption of TJ by inhibition of ZO-1 translation.[92] Work from our own laboratory used miRNA profiling in T84 (human colorectal carcinoma cells) monolayers and employing specific miRNA inhibitors showed that miR-203, miR-483-3p, and miR-595 affect the expression of several adapter molecules of the tight junctional complexes as monitored by expression of associated proteins and transepithelial resistance (TER) in epithelial cellular barrier models (Table 1). Although ZO-2 and PKCζ were found to be affected, interestingly, a modulation of ZO-1 could not be observed.[93] Further putative activities of these miRNAs were not investigated in these studies.

Claudins

McKenna et al. demonstrated that claudin-4 and claudin-7 are not expressed in the apical membrane of intestinal epithelial cells of Dicer 1-deficient mice, which resulted in impaired intestinal barrier functions, which could not be correlated yet to a certain miRNA.[74] In contrast Zhi et al. were able to show that claudin-1 is indirectly affected by miR-874 during intestinal dysfunction after ischemic injury, which might result as a complication following intestinal disease or abdominal surgery. It was found that the level of miR-874 was inversely related to the level of aquaporin 3 (AQP3) expression which enhances intestinal permeability by down regulating claudin-1 and occludin.[94]

Looking at the retinal pigment epithelium another indirect effect by miR-204 on the upregulation of claudin-10 and -19 has been shown.[95] The direct target for miR-204 is the TGFß-R2 which in its active form reduces the claudin expression via transcriptionfactor SNAIL2. Furthermore, the dysregulation of TJ is a basic phenomenon, e.g.,: Claudin-1 expression is down-regulated by an increase of miR-155 in ovarian cancer cells.[96]

Rho-GTPases

Different members of the Rho GTPase family play significant cellular roles e.g. in organizing the actin cytoskeleton.[97] RhoB, a Ras GTPase was identified as an additional target for miR-21, which has been already identified as targeting the mRNA of occludin. Unlike RhoA and RhoC, RhoB acts as a tumor suppressor and affects cell cycle, angiogenesis, and apoptosis. On the cellular level it also influences actin organization, cell migration, and cell adhesion.[98] In colorectal cancer cell lines,[97] hepatocellular carcinoma cell lines,[99] and human umbilical vein endothelial cells (HUVECs),[100] RhoB is regulated by miR-21 via binding to 3′-UTR. Furthermore, reducing RhoB expression by siRNA treatment resulted in a decrease in TER and a destabilization of the junctional complex emphasizing the role of RhoB in the AJC. RhoA induces and regulates the assembly of the AJC. It has been shown that down-regulation of RhoA leads to disruption of junctional complexes[101,102] by interfering with the synthesis of tight junction proteins.[103] The mRNA of RhoA contains 3 miRNA binding sites in the 3′-UTR. One of these sites is specifically bound by miR-155.[104] MiR-155 plays a pivotal role in the systemic inflammatory response.[105] In addition, miR-155 interferes with the activation of Toll-like receptor (TLR) pathway in monocytic cell after LPS-induction.[106] Inflammatory cytokines, such as TNF-α and interferons are able to induce miR-155 expression.[104,107] The increase of TNF-α in inflammatory bowel disease, which can be induced by

Table 1. miRNAs quoted in this review, affecting the expression of apical junctional complex proteins

miRNA	miRNA - target	Reference
miR-7a	Claudin-6	Li et al. 2012[70]
miR-9	Aquaporin 3, Claudin-14	Zhi et al. 2014[94]
miR-21a	Occludin, RhoB	Yang et al. 2013[83]
miR-105	VE-cadherin, Tight junction protein 1	Zhou et al. 2014[122]
miR-122a	Occludin, Dicer1	Ye et al. 2011[75]
miR-145	Protein phosphatase 2 regulatory subunit, Tight junction protein 1, Mucin1	Ma et al. 2010[117]
miR-146a	ß-Catenin, Protein Kinase C	Hwang et al. 2012[121]
miR-155-5p	Claudin-1, RhoA, ß-Catenin, Protein Kinase C	Tili et al. 2007[104] O'Connell RM et al. 2007[105]
miR-200	Pten	Gregory et al. 2007[71]
miR-203	Tight junction protein 2, Claudin1	Veltman et al. 2012[93]
miR-212	Occludin, Tight junction protein 1	Tang et al. 2008[92]
miR-218	Claudin-2/6, CDC-42	Li et al. 2012[70]
miR-221	Protein phosphatase 2 regulatory subunit, Pten	Romao et al. 2011[73]
miR-223	Occludin, Protein Kinase C, Tiam1	Redell J. 2012[122]
miR-328	Tiam-1, Claudin-19	Arora et al. 2011[120]
miR-483-3p	Protein Kinase-α,	Zang Y-W et al. 2012[114]
miR-503	CUG-binding protein 1, Occludin	Yang et al. 2014[123]
miR-595	Cell-polarity protein-6	Veltman et al. 2012[93]
miR-874	Cell-polarity protein-6	Veltman et al. 2012[93]

Detailed information concerning microRNA sequences and validated or further potential target mRNA molecules can be obtained from `miRBase´or `TargetScan´ databases.

bacterial infection leads to the downregulation of ZO-1 and E-cadherin expression and the destabilization of the AJC.[108] This further emphasizes a key role of miR-155 for the regulation of barrier function in intestinal epithelial cells.

Currently validated miRNAs and their targeted proteins modulating epithelial and/or endothelial barrier integrity are summarized in **Table 1**.

MiRNA and Blood Brain Barrier

The complex blood brain barrier (BBB) ensures the barrier functions between the vascular system and the brain and consists of brain microvascular endothelial cells, astroglia and pericytes. The tight junctions and adherens junctions[109] between these cells restrict the passage of cells, bioactive molecules, including most therapeutics across this barrier.[110,111] Impairment of the homeostasis of these cellular junctions leads to barrier disruption. Experiments in several mouse models revealed the importance of Claudin-based tight junctions for the selectivity of endothelial barrier function. The knock-out of claudin-1 or -5 in different murine models was found to be lethal during embryogenesis.[112,113] In esophageal carcinoma cells the displacement of claudin-7 induces the loss of E-cadherin expression and therefore the destabilization of tight junctions.[114]

VE-cadherin, the endothelial cell-specific AJ and transmembrane protein, is a key regulator of endothelial apical junctional complexes and of endothelial barrier integrity.[115] VE-cadherin interferes with a variety of signaling molecules, which coordinate endothelial TJ organization and permeability[113] and is targeted by different miRNAs that modulate the transcriptional repression directly and also indirectly. MiR-9 was described to target VE-cadherin, inducing the reduction of ß-catenin, which enhances invasion as well as increased tumor angiogenesis.

The HI-virus is able to cross the BBB and to infect brain macrophages/microglia. The HIV-1 Tat protein induces miRNA-32. This miRNA regulates the expression level of TRAF3, which itself enables the infection of microglial cells.[113] This is also

achieved by the downregulation of VE-cadherin, due to the upregulation of miR-101 by Tat C protein. Knockdown experiments for miR-101 revealed that the degree of claudin-5 expression is depending on VE-cadherin level.

TJ are also destabilized by metastatic brain tumors. These enable circulating tumor cells to enter the brain, this indicates that the integrity of the BBB is an important barrier for melanoma cells. It has been shown that the disruption of barrier function is correlated with a reduced Claudin-5 and ZO-1 availability, which can be monitored by a loss in transendothelial electrical resistance (TER).[116,117] Recently, these findings have been supported by Zhou et al.[118] who found that miR-105 which is secreted by metastatic breast cancer cells targets the TJ protein ZO-1. In this way, miR-105 secreted and transferred via exosomes disrupts TJ and facilitates metastatic migration.

Brain metastasis has been linked to the expression of miR-145 and miR-328.[119,120] The overexpression of miR-328 has been shown to interfere with the level of protein kinase C α (PKCa) which is one of the TJ regulatory proteins. On the contrary other studies were able to show that the overexpression of miR-146 leads to an increased β-catenin level which suppresses brain invasion by migrating cells.[121]

Furthermore brain injury induces a variety of signaling pathways, which effect blood-brain barrier permeability. MiR-223 levels seem to be upregulated in cerebral microvasculature where it targets TIAM1 thereby affecting barrier integrity. It has been reported by John Redell in 2012 that blocking miR-223 leads to an improvement in barrier function.[122]

Summary and Perspective

Cellular junctions such as tight and adherens junctions play a crucial role in regulating paracellular permeability in vertebrate epithelia and endothelia. In order to fulfill this task effectively the assembly and maintenance of junctional complexes need to be properly controlled and regulated. TJ and AJ exist as a set of tetraspins, transmembrane proteins (occluding, claudins) and JAMs, accompanied by many regulatory and adapter proteins like ZO-1, -2 and -3, or kinases and phosphatases. During disease or infection the function of epithelial or endothelial cellular barriers can be severely disturbed. A cellular response mechanism to counteract an impairment of barrier function or to fine-tune and adapt the paracellular permeability are microRNAs, which are able to modulate the AJC proteins post-transcriptionally.

To demonstrate the very complex regulation of TJ proteins, Yang et al.[123] reviewed very recently the cooperative and post-transcriptional effects of RNA-binding proteins (RBPs) and miRNAs on mRNA stability coding for TJ proteins in the gastrointestinal mucosa.

The extent of gene regulation byMiRNAs will become more complex as new miRNA binding sites are likely to be discovered.[46,124] It is foreseeable that there will be many more regulatory interactions to be identified that are affecting the synthesis of junctional proteins, the proper assembly of the complexes and the fine-tuning of immunological responses. Due to the intrinsic 'musketeer strategy' ('one for all and all for one') in miRNA-mRNA interactions, the ensuing degeneration of regulatory pathways involving miRNAs will present a formidable task to distill and validate the determining interactions with target mRNAs. Defining certain interactions and regulations of miRNAs with junctional proteins will hopefully also pave the way for potential therapeutic applications that would either reinforce cellular barriers to prevent tissue damage, induced by pathogenic microbes or inflammatory reactions, or would on the contrary provide possibilities to temporarily disrupt or impair barrier functions to facilitate drug delivery.

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No potential conflicts of interest were disclosed.

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