Claudin as a Target for Drug Development

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Abstract: Tight junctions (TJs) play pivotal roles in the fence and barrier functions of epithelial and endothelial cell sheets. Since the 1980s, the modulation of the TJ barrier has been utilized as a method for drug absorption. Over the last decade, the structural and functional biochemical components of TJs, such as occludin and claudin, have been determined, providing new insights into TJ-based pharmaceutical therapy. For example, the modulation of the claudin barrier enhances the jejunal absorption of drugs, and claudin expression is deregulated in cancer cells. Claudin is a co-receptor for the hepatitis C virus. Moreover, claudin is modulated during inflammatory conditions. These findings indicate that claudins are promising drug targets. In this review, we discuss the seeds of claudin-based drug development, which may provide potential pharmaceutical breakthroughs in the future.

Keywords: Tight junction, claudin, cancer, inflammation, infection.

INTRODUCTION

Tight junctions (TJs) limit the movement of molecules through the intercellular space in epithelial and endothelial sheets, and they are located on the most apical part of cells [1, 2]. Electron microscopy has revealed that TJs appear as a series of continuous, anastomotic and intramembranous particle strands. Tsukita's group performed a series of biochemical analyses that clearly showed that the tetra-transmembrane proteins occludin and claudin are components of the TJ [3-5]. The claudin family contains more than 20 members. Interestingly, the expression profiles and the TJ-barrier function of the claudin family members are tissue-specific. For example, claudin-1 is involved in the epidermal barrier, and claudin-5 is involved in the blood-brain barrier [2, 6, 7]. It appears that claudin forms heteromeric and/or homomeric strands in TJs and that the combination and mixing ratios of different claudins determines the tissue-specific barrier properties of TJs [5, 8]. Epithelial cell sheets have bicellular TJs between adjacent cells and tricellular TJs at which three adjacent cells join together. Occludin and claudins are components of bicellular TJs. The occludin-related protein tricellulin has been recently identified to be a component of tricellular TJs [9]. Tricellulin is ubiquitously expressed in epithelial junctions of tissues and organs throughout the body. Down regulation of tricelluin mRNA by RNA interference resulted in disruption of epithelial barrier in an epithelial cell line [9]. However, human tricellulin mutations had no effect on epidermal, respiratory, renal or intestinal barrier [10]. Whether tricellulin can be a target for drug development is unclear.

Functions of TJs are classified as fence- and barrier- functions. Modulation of the TJ barrier has been a popular strategy used to promote drug absorption since the 1980s (See reviews [11, 12]). Sodium caprate is clinically used as an absorption enhancer of drug. Disturbance of either the TJ-fence function or the TJ-barrier function causes human diseases. Disturbance of the TJ-fence function followed by a loss of cellular polarity often occurs in tumorigenesis (See reviews [13-16]). TJs regulate the paracellular passage of ions, molecules, pathogens and inflammatory cells in epithelial and endothelial cell sheets [17-19]. The TJ-barrier becomes deregulated in various human diseases, including infections, inflammation and hereditary diseases (See reviews [20, 21]). Based on these findings, novel therapeutic strategies for TJ-related diseases have been proposed. In the present review, we discuss the seeds of claudin-based pharmaceutical therapies for human diseases relevant to TJs.

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CANCER AND CLAUDIN

Malignant tumors are a major cause of death. Approximately 7.6 million people worldwide died from cancer in 2007, and 90% of tumors are derived from epithelial tissue [22]. Normal epithelial tissues develop cellular polarity, whereas the epithelial polarity is often deregulated during tumorigenesis [23]. TJs are localized between adjacent epithelial cells and separate the apical and basolateral membrane domains, which vary in protein and lipid content, resulting in the maintenance of the cell polarity. Claudins are deregulated in various cancers [13-16]. Claudin may regulate cancer metastasis by modulating activation of matrix metalloproteinases [11]. In this section, we discuss recent breakthroughs in claudin-targeted cancer therapy.

Claudin as a Diagnostic Marker

Claudin proteins are frequently overexpressed in ovarian cancers. In ovarian cancer cells with a high level of claudin-4, the critical claudin-4 promoter region exhibits a low level of DNA methylation and a high level of histone H3 acetylation [24]. Claudin-4 was detected in the 32 of 63 plasma samples of patients with ovarian cancers. Among 50 patients without ovarian cancer, only one had claudin-4–positive plasma. Thus, claudin-4 has a high specificity for the detection of ovarian cancers *via* a blood test, indicating that claudin-4 may be a diagnostic marker for ovarian cancer [25]. Because of the high specificity of claudin expression patterns in cancers, claudin might be a novel non-invasive diagnostic marker for cancer therapy.

Anti-Claudin Antibody

One of the most popular strategies for claudin-targeted cancer therapy is the preparation of antibody against the extracellular region of claudin. However, attempts to prepare anti-claudin antibodies have had little success because claudin has low antigenicity and is highly conserved in various species. A strain of autoimmune mice, BXSB, was immunized with a human pancreatic cancer cell line, resulting in the successful preparation of anti-claudin-4 monoclonal antibody that recognizes the extracellular region of claudin-4 [26, 27]. Moreover, the antibody mediated antibody-dependent cell cytotoxicity (ADCC) and *in vivo* anti-tumor activity. ScFv against the extracellular region of claudin-3 was isolated by using the ETH-2 Gold phage display library, which is a synthetic human recombinant antibody library that contains $>10^9$ possible antibody combinations in an scFv format [28, 29]. Immunization with DNA encoding the first extracellular loop of claudin-18 made success on preparation of anti-claudin-18 monoclonal antibody [30]. These successes in the preparation of anti-claudin antibody are likely to lead to a breakthrough in the development of claudin-targeted cancer therapy.

Clostridium Perfringens Enterotoxin

Another approach to targeting claudin in cancer therapy is the use of *Clostridium perfringens* enterotoxin (CPE) . CPE is a single-chain polypeptide of 35 kDa that causes food poisoning in humans. The functional domains of CPE consist of the N-terminal cytotoxic region and the C-terminal receptor-binding region [31]. Claudin-3 and -4 serve as the receptors for CPE. CPE binds to the second extracellular loop of claudin-3 and -4 [32] (Fig. **1**). We previously prepared a claudin-targeting molecule (C-CPE-PSIF) by fusion of the C-terminal fragment of CPE (C-CPE) with the protein synthesis inhibitory factor (PSIF) derived from *Pseudomonas aeruginosa* exotoxin. C-CPE-PSIF, but not PSIF, is cytotoxic to claudin-4 expressing cells. TJ-undeveloped cells are more sensitive to C-CPE-PSIF than TJ-developed cells. Polarized epithelial cells are sensitive to the basolaterally applied C-CPE-PSIF, but they are less sensitive to the apically applied C-CPE-PSIF. A claudin-targeting molecule may recognize the cellular polarity. Intratumoral injection of C-CPE-PSIF reduced tumor growth. These findings indicate that C-CPE may be a novel molecule for drug delivery and cancer therapy [33]. The receptor-binding region of C-CPE fused to TNF was cytotoxic in human ovarian cancer cells [34]. Thus, CPE fragments might be a tool for claudin-targeting therapy. Treatment of mice with claudin-3 siRNA suppressed ovarian tumor growth and metastasis [35]. Claudin gene silencing with siRNA is also potent anti-tumor agents.

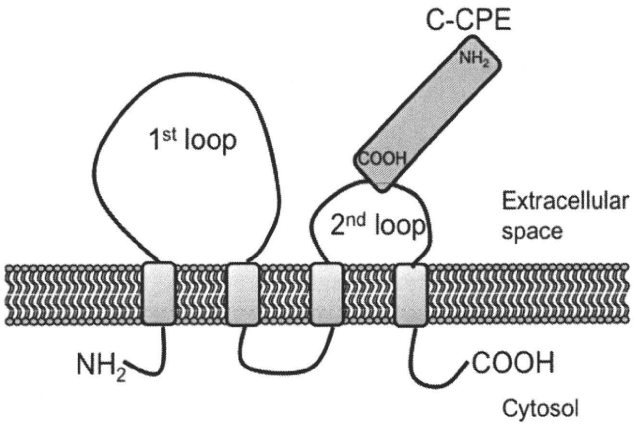

Fig. (1). Schematic illustration of interaction of C-CPE and claudin. Claudin is a tetra-transmembrane protein. C-CPE interacted with the 2nd loop region of claudin *via* its C-terminal domain [32, 76].

INFECTION AND CLAUDINS

Twenty million people die from infectious diseases each year. Most pathogens enter the body through nasal, pulmonary, intestinal and genital mucosa, and the mucosal epithelial cell sheets play a pivotal role as the first line of defense against the pathogens. Invading pathogens are distributed throughout the organ *via* endothelial cells of the blood vessels. TJs seal intercellular spaces between adjacent cells, preventing entry of the pathogens into the body and into the organ across the paracellular spaces. Disruption of mucosal TJ seals allows pathogens to enter into the body and the organ. In this section, we review the recent findings on the relationship between infections and claudins.

West Nile Virus (WNV) and Claudin

WNV, a neurotropic flavivirus, is a human pathogen that targets neurons and causes potentially lethal encephalitis in 1% to 2% of WNV-infected febrile patients [36]. No therapeutic agents or vaccines have been approved for use against WNV infection. Langerhans cells in the skin become infected with WNV by the bite of a carrier mosquito. WNV replicates in the regional tissues and lymph nodes, which results in the dissemination of the virus into the bloodstream. The following second replication proceeds at several sites in the host, including epithelial cells in the skin, kidney, intestine and testis, and then WNV may ultimately invade the brain [37]. The infection of the nervous system is characteristic of the most severe cases of WNV disease, and it often results in death or long-term neurologic sequelae [38]. Understanding the mechanism of the second infection and the viral entry into the brain is critical for the development of therapies against WNV. In WNV-infected epithelial cells, claudin-1, -2, -3 and -4 are degraded, followed by a disruption in the TJ barrier without cell death. The capsid of the WNV was responsible for the modulation of the TJ barrier [39]. These findings suggest that an inducer of claudin may be a promising candidate for pharmaceutical agents to inhibit the dissemination of WNV. Whether or not the WNV modulates the blood-brain barrier *via* the modulation of claudin-5 is an unsettled question.

Human Immunodeficiency Virus (HIV) and Claudin

HIV encephalitis (HIVE), including behavioral, motor, and cognitive impairments, is a common condition in the late stage of HIV-associated dementia [40]. Invasion of HIV into the brain and the transmigration of HIV-infected lymphocytes into the brain are the major causes of HIVE [41]. The blood-brain barrier (BBB), which is responsible for the regulation of solutes and cells between the peripheral circulation and the central nervous system, is comprised of the brain microvascular endothelial cells. Adjacent brain microvascular endothelial cells are connected by TJs that limit paracellular flux and restrict permeability [42]. The BBB frequently breaks down in patients with HIVE [41]. Claudin-5 plays a pivotal role in the BBB [7]. Treatment of human brain microvascular endothelial cells with HIV Gp120 envelope glycoprotein decreased the claudin-5 levels, followed by a disruption of the TJ barrier [43]. Claudin-5 levels were lower in brain microvessels from HIV patients with HIVE compared with brain microvessels from HIV patients without HIVE [44]. The deregulation of the claudin-5 barrier by HIV may be responsible for the breakdown of the BBB in HIV patients. Cannabinoids, the active ingredients in marijuana, reduce pain and improve the quality of life in HIV patients [45]. HIV activates signal transducers and activators of transcription-1 (STAT-1) [46]. Cannabinoids and an inhibitor of STAT-1 prevented the down-regulation of claudin-5 in the HIV Gp120- and HIV-treated human brain microvascular endothelial cells, respectively [43, 44]. These findings indicate that an inducer of claudin-5 may be a pharmaceutical agent for HIVE.

Hepatitis C Virus (HCV) and Claudin

Approximately 170 million people worldwide are infected with HCV. More than 80% of acute infections become persistent, resulting in liver fibrosis, cirrhosis, and hepatocellular carcinoma [47]. HCV infects human hepatocytes but not murine hepatocytes, and the detailed mechanism responsible for this difference has remained obscure. There is no pharmaceutical agent that prevents HCV infection. HCV attaches to tetraspanin CD81 and scavenger receptor class B type I (SR-BI) on host cells through its envelop glycoprotein [48, 49]. However, when CD81 and SR-BI were expressed in non-primate cell lines, the cells were still resistant to HCV entry [50, 51]. Recent studies to identify the additional factors that are needed to render non-human cells susceptible to HCV entry revealed that claudin-1 and occludin are co-receptors for HCV entry [51, 52]. HCV envelop proteins interact with the first extracellular loop region of claudin-1 and the second extracellular loop region of occludin [51, 52]. Binders to CD81, SR-BI, claudin-1 or/and occludin are expected to inhibit HCV entry. The HCV genome is frequently mutated; thus, pharmaceutical agents that recognize host molecules, such as the receptors, may be promising candidates for the prevention of HCV infection.

INFLAMMATORY BOWEL DISEASE (IBD) AND CLAUDIN

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is characterized by an activated mucosal immune system that leads to impaired epithelial barrier function and tissue destruction with relapsing diarrhea [53, 54]. Ulcerative colitis is characterized by chronic inflammation and ulcers in the colon, while Crohn's disease causes ulcers and swelling of the mucosa on all areas of the digestive tract from the mouth to the anus. A common feature of IBD is enhanced permeability of the intestinal epithelium and disruption of the epithelial barrier. In this section, we summarize the recent findings on the relationship between IBD and claudins.

Changes of Claudins in IBD

The epithelial barrier function is impaired in ulcerative colitis, and ulcerative colitis is associated with decreased numbers of TJ strands in the epithelial barrier [55]. Biochemical analysis of TJ components in rectal biopsy specimens from patients with active ulcerative colitis revealed that the protein and mRNA levels of claudin-4 and -7 were decreased, whereas the protein and mRNA levels of claudin-2 were increased, as compared with control patients [56]. Overexpression of claudin-2 led to a decrease in the TJ barrier in an epithelial cell line, whereas claudin-4 or -7 transfection elevated the epithelial barrier function [57, 58]. Thus, the down-regulation of claudin-4/7 and the up-regulation of claudin-2 can lead to altered TJ structure, resulting in impaired epithelial function in active ulcerative colitis. However, claudin-deficient mice or claudin-overexpressing mice did not reproduce the pathology of IBD. Whether change in claudins is cause of IBD or result from IBD remains to be proved.

Although the precise etiology of IBD remains unknown, it is well accepted that IBD results from a deregulated mucosal immune response to environmental factors in genetically susceptible hosts. In IBD patients, the primary defect may be due to an abnormal intestinal epithelial barrier function [59]. The SAMP1/YitFc (SAMP) mouse strain is a spontaneous model of IBD that closely resembles Crohn's disease due to its histological features and localization to the terminal ileum [60]. The deregulated epithelial barrier function in SAMP mice is accompanied by an increase in claudin-2 and a decrease in occludin [61, 62].

FoxO4 is a member of the forkhead box transcription factor O (FoxO) subfamily, which has unique cell type–specific functions that regulate target genes and are involved in the regulation of immune responses [63, 64]. FoxO4-null mice were more susceptible to trinitrobenzene sulfonic acid–induced colitis [65]. FoxO4 deficiency increased the intestinal epithelial permeability and down-regulated the TJ proteins ZO-1 and claudin-1. Immunohistochemical analysis revealed that epithelial expression of FoxO4 was significantly down-regulated in patients with active ulcerative colitis as compared to patients with inactive ulcerative colitis [66]. Thus, FoxO4 might be a target for ulcerative colitis therapy.

A Potent Pharmaceutical Agent for IBD

Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ, are key mediators for the disruption of the epithelial barrier associated with Crohn's disease [55, 66, 67]. Expression of claudin-2 was increased by TNF-α in epithelial cells [68]. Experimental colitis model mice showed the down-regulation of occludin and up-regulation of claudin-2. Deletion of TNF-α receptor attenuated these changes of occludin and claudin-2 in the experimental colitis model. Importantly, anti-TNF treatment infliximab, which is currently used in Crohn's disease and ulcerative colitis, suppressed the reduction of occludin and elevation of claudin-2 in the experimental colitis model [69].

n-3 polyunsaturated fatty acids (PUFAs), which are abundant in fish oil and include eicosapentaenoic acid and docosahexaenoic acid, have beneficial effects on IBD [70-72]. In an experimental IBD model induced by treatment with trinitrobenzene sulfonic acid, the distribution of TJ proteins, including occludin and claudin-1, was affected; however, the administration of n-3 PUFAs prevented this redistribution of TJ proteins [73].

Probiotics are living bacteria that, when ingested in sufficient quantity, improve the health of the host beyond their inherent basic nutrition [74]. Probiotics have anti-inflammatory effects in IBD. VSL#3, a mixture of 8 probiotic bacterial strains, provided protection against intestinal inflammation in an experimental colitis model. Probiotics also attenuated the enhancement of epithelial permeability and the reduction of TJ components, including occludin, claudin-1 and -4 in the experimental model [75]. Therefore, compounds that enhance the TJ barrier function are candidates for IBD therapy.

CONCLUSIONS

Epithelium and endothelium are located between the outer and inner components of the body or tissues. Most malignant tumors are derived from epithelium. Moreover, epithelium and endothelium are also barriers that prevent invading pathogens and inflammatory cells from entering into the body and tissues. Therefore, the epithelium and endothelium are excellent targets for drug delivery systems, anti-tumor agents, anti-infection agents and anti-inflammatory agents.

Recent studies have revealed the involvement of claudin in some human diseases relevant to TJs (Table **1**). Claudin is often overexpressed in human cancers [13-16]. Therefore, a cancer therapy approach that uses claudin ligands is sought. Suzuki *et al.* used autoimmune mice to successfully prepare an anti-claudin-3 monoclonal antibody that mediated ADCC [26]. We anticipate that a novel claudin-targeted cancer therapy will be forthcoming. TJ components are also associated with infections. Claudin-1 and occludin are co-receptors for HCV [51, 52]. The claudin-5 level was reduced in brain microvessels of patients with HIVE [44], and cannabinoids, a clinically used agent for HIV patients, prevented the down-regulation of claudin-5 [43]. These findings indicate that a

Table 1. Perspective on Claudin-Targeted Therapies

Applications	Claudins	References
A diagnostic marker for ovarian cancers	Claudin-4	[25]
Inhibitor of WNV dissemination	Claudin-1~4	[39]
Inhibitor of HIV encephalitis	Claudin-5	[43-45]
Inhibitor of HCV infection	Claudin-1	[51]
Inhibitor of intestinal inflammation in IBD	Claudin-1~4	[69, 73, 75]

WNV, west nile virus; HIV, human immunodeficiency virus; HCV, hepatitis C virus; IBD, inflammatory bowel disease.

claudin/occludin binder and an inducer of claudin-5 may be an inhibitor of HCV infection and a therapeutic agent for HIVE patients. Disruption of the intestinal epithelial barrier is a common feature in patients with IBD. A chemical compound that strengthens the claudin barrier function will be a promising drug for IBD.

Biochemical and functional information regarding TJs has accumulated since the identification of occludin in 1993, and the deregulation of claudins has been observed in several human diseases [16, 20, 21]. The potential of TJ-based therapies is promising. We believe that TJ-targeted therapies might provide a breakthrough in pharmaceutical therapy in the future.

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ABBREVIATIONS

TJ	=	Tight junction
ADCC	=	antibody-dependent cell cytotoxicity
CPE	=	*Clostridium perfringens* enterotoxin
C-CPE	=	the C-terminal fragment of CPE
PSIF	=	protein synthesis inhibitory factor
WNV	=	West Nile virus
HIV	=	human immunodeficiency virus
HIVE	=	HIV encephalitis
BBB	=	blood-brain barrier
STAT-1	=	signal transducers and activators of transcription-1
HCV	=	hepatitis C virus
SR-BI	=	scavenger receptor class B type I
IBD	=	inflammatory bowel disease
FoxO	=	forkhead box transcription factor O
TNF	=	tumor necrosis factor
PUFAs	=	n-3 polyunsaturated fatty acids

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