# Properties of Claudin-2

Subjects: Biochemistry & Molecular Biology

Contributor: Katalin Szaszi

Claudin-2 is a member of the claudin family of epithelial tight junction proteins expressed mostly in the kidneys and gastrointestinal tract. Its role as a cation-selective and water permeable paracellular channel is well studied. In addition, newly emerging strong evidence also shows that it can modulate proliferation, migration, and cell fate determination. These effects appear to be due to its interactions with cytosolic adapters, that connect it to key signaling pathways. A multitude of new data document dysregulated claudin-2 expression in many pathologies including cancer, inflammation, and fibrosis. Thus, changes in claudin-2 expression may contribute to the generation, maintenance, and/or progression of diseases through both permeability-dependent and -independent mechanisms. Based on this, efforts are underway to develop therapies targeting claudin-2 with the hope of benefiting patients with a variety of disease.

tight junctions epithelial cells kidney tubules intestinal epithelium paracellular permeability cell proliferation cell migration signalling pathways inflammation cancer

## 1. Overview of claudins

Claudin-2 is a member of the claudin family of tight junction (TJ) membrane proteins, which consists of 26 (human) or 27 (rodents) small (20-25 kDa) tetraspan proteins expressed in a tissue-specific manner in epithelial and endothelial cells [1]. They are responsible for sealing the paracellular space and generating a permeability pathway for ions and water [2][3]. Each member of the family has unique properties. The TJs contain a mosaic of claudins, and the permeability of the paracellular pathway in each epithelial layer is determined by the type of claudins expressed. The function of claudins appear to go beyond controlling permeability. Many members of the family are increasingly found to have key effects on vital cell functions, such as proliferation, migration and differentiation [4]. These effects are likely mediated by interactions of claudins with TJ-localized cytosolic adapter proteins, that generate large signaling complexes and bind to the cytoskeleton [5]. Claudin-2 is one of the earliest described claudins. It forms paracellular cation and water permeable channels and affects cell proliferation and migration [1][6].

## 2. Expression, structure and Interactions of claudin-2

Claudin-2 is enriched in the kidney proximal tubules and in the gastrointestinal tract [7][8]. Although it is mostly an epithelial protein, there are reports that it is also present in endothelial cells and macrophages [9][10]. However, the role of claudin-2 outside of epithelium remains to be established.

Claudin-2 is a 230 amino acid tetraspan membrane protein [3]. Its calculated molecular mass is 24.5 kDa. Like other members of the family, it has two extracellular domains (ECL1 and ECL2), a small intracellular loop connecting the second and third transmembrane sections and short intracellular N- and C-terminal portions (Figure 1). ECL1 is responsible for paracellular charge selectivity and permeability [11], while the role of ECL2 remains undetermined.

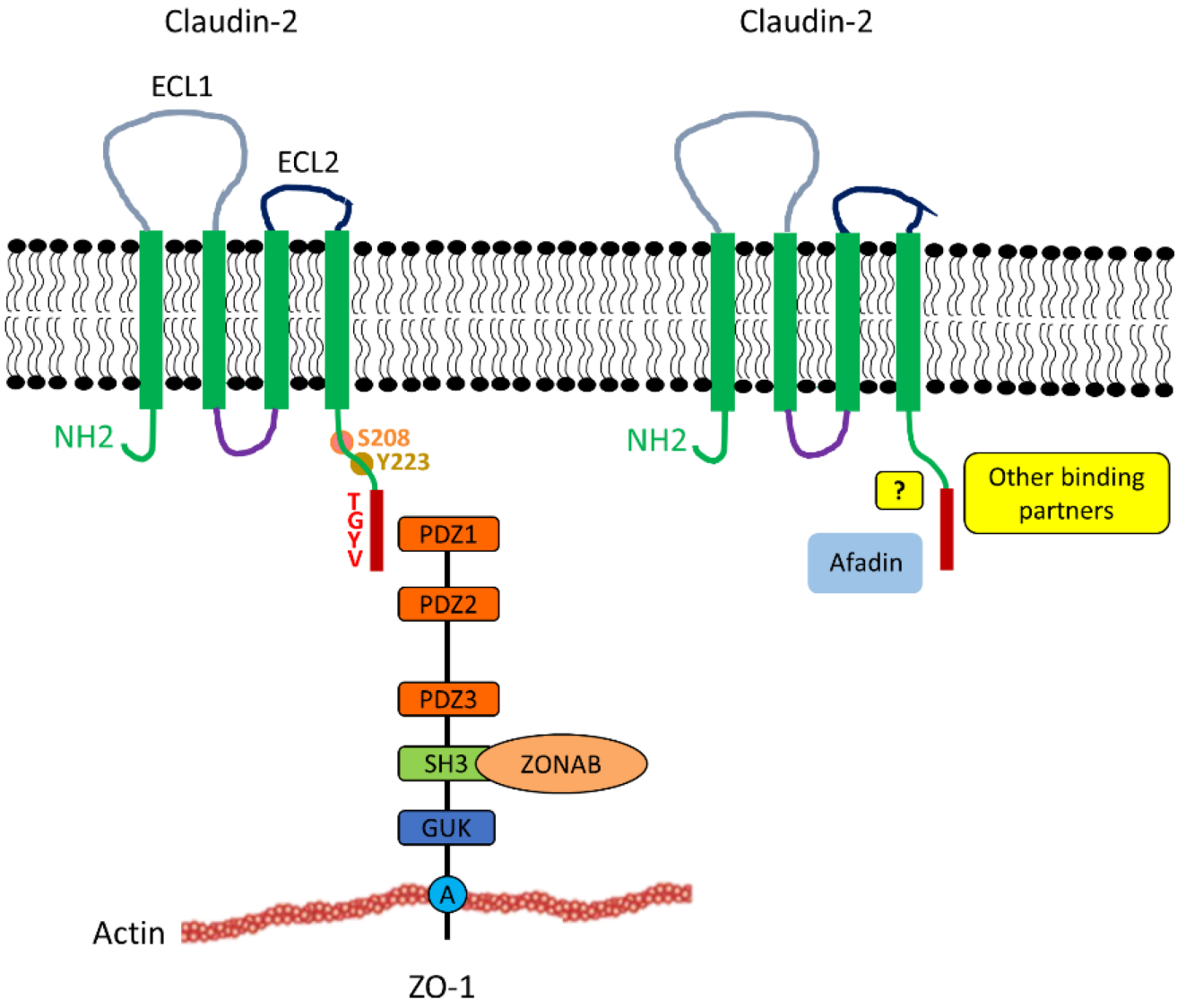


**Figure 1. The structure of claudin-2 and its interactions with cytosolic multidomain adapters**. Claudin-2 consists of two extracellular loops, ECL1 (grey) and ECL2 (black), 4 transmembrane domains (green box), a cytoplasmic loop (purple), a short N-terminal and a longer C-terminal cytosolic portion (green). Claudin-2 interacts with the ZO family of TJ plaque proteins (for clarity only ZO1 is depicted) through its PDZ-binding motif (TGYV) located at the end of the C-terminus (red box). ZO1 is a multidomain adapter, containing two PDZ and an SH3

domain that mediate interactions with a variety of proteins to create large multiprotein complexes. The guanylate kinase (GUK) domain likely mediates interactions with cytoskeletal components. “A” indicates the actin binding segment. The SH3 domain binds to the transcription factor ZONAB, which may play a role in the proliferative effects of claudin-2. Afadin is a recently identified claudin-2 partner. The mode of coupling (direct biding or indirect association through adapters) remains to be established. Other newly identified candidate binding partners for claudin-2 include Scrib, Arhgap21, PDLIM2/7, and Rims-2. The claudin-2 tail contains many potential phosphorylation sites. Among these, phosphorylation at Y223 affects the affinity of the PDZ binding domain, and phosphorylation at S208 appears to be a switch for membrane retention and lysosomal or nuclear localization.

Claudin-2 can be found predominantly in the TJs, but is also present in cytoplasmic vesicles [6][12], and can translocate to the nucleus[13]. It can homodimerize with claudin-2 expressed in opposing cells, which likely promotes cell-cell adhesion. Further, claudin-2 can bind to claudin-3 and probably occludin (heterotypic interactions). The intracellular interactions of claudin-2 are starting to emerge (Figure 1). These are likely pivotal for signal-modulating effects. The last four C-terminal amino acids (T-G-Y-V, N- to C-term) correspond to a PDZ domain-binding motif, that mediate association with TJ plaque scaffold proteins including the membrane-associated guanylate kinase (MAGUK) family adapters ZO-1-3. Recently, claudin-2 was also shown to bind to the junctional adapter afadin (AF-6/MLLT4), which may have central roles in mediating effects on proliferation [14].

## 3. Regulation of claudin-2

Claudin-2 expression is affected by a large array of inputs including hormones, growth factors [15], cytokines [12][16][17][18], physical parameters [19][20][21][22] and drugs [23][24], via a complex and context-dependent network of signaling pathways. Importantly, expression of claudin-2 is altered by many pathological conditions, including cancer and inflammatory diseases. Regulation of claudin-2 expression happens both at the level of synthesis and turn-over . The claudin-2 promoter has binding sites for several transcription factors, including Caudal-related homeobox (cdx) proteins, hepatocyte nuclear factor (HNF)-1, GATA 2 and -4, Activator protein 1 (AP1), Vitamin D Receptor, T-cell factor/lymphoid enhancer binding factor 1 (TCF/LEF1) and signal transducer and activator of transcription (STAT) [25][26][27][28][29]. Some of these factors act in a tissue-specific manner. Importantly, an array of signalling pathways converge on these factors, and the combined activity of all inputs determines claudin-2 levels in a highly tissue and stimulus-dependent manner. Many of the regulating pathways are altered in diseases, leading to changes in claudin-2 expression in a variety of conditions. Several of the regulating signaling pathways also affect turnover of the claudin-2. Its degradation requires endocytosis from the TJs and is likely mediated by the lysosomes [30]. Localization at the TJs in turn is affected by post-translational modifications of the C-terminal intracellular portion of claudin-2, including phosphorylation [30] (Figure 1), sumoylation[31], and nitration[32]. The specific effects of posttranslational modification on claudin-2 turn-over and interactions are not fully understood.

## 4. Permeability functions of claudin-2

Claudin-2 forms paracellular cation and water permeable channels. Interestingly, water transport is mediated by the same pore that allows cation transport [33] . These permeability properties are well verified in cell lines and knockout mouse models. Claudin-2 overexpression and silencing in various cell lines decreases and increases TER, respectively (e.g.[12][34][35]). Further, knockout mouse models revealed that the selective, site-specific localization and cation channel properties of claudin-2 are essential for highly specialized functions in the kidneys and the bile duct [36]. In the kidneys, claudin-2 is fundamental for $Na^+$ and water transport, and therefore it plays a key role in blood pressure regulation [37][38]. In the liver and bile system, claudin-2 plays a central role in water transport associated with bile generation [39].

## 5. Signal-modulating functions of claudin-2

Evidence is mounting that claudin-2 is not only a paracellular channel protein, but also acts as a signal modulator and integrator. This conclusion is derived in part from correlation between claudin-2 expression and altered outcomes. More importantly, overexpression and silencing studies verified a causal relationship, as revealed that primary changes in claudin-2 expression have major impact on cell behavior. Accordingly, dysregulation of claudin-2 expression appears to be an important event in several diseases.

Emerging new data suggest that claudin-2 can affect signalling pathways via its intracellular interactions [14]. Claudin-2 is bound to adapters that connect it to the junctional acto-myosin belt and the microtubules. The cytoskeleton has a bidirectional relationship with claudin-2: they are central regulators of TJ proteins including claudin-2 but acto-myosin is also modulated by claudin-2. The pathways affected by claudin-2 include MEK/ERK1/2 signalling and Rho family small GTPases [40][18]. The exact connection between these and claudin-2 remain to be defined.

Many studies implicate claudin-2 in the control of proliferation. A strong correlation was shown between proliferation/cell viability and altered claudin-2 expression. Importantly, experimentally altered claudin-2 levels impact proliferation. While the underlying mechanisms are still not fully known, several mechanisms have been brought forward. First, claudin-2 impacts cell cycle progression and expression of cell cycle regulators both in cultured cells and in a transgenic mouse model [18][41]. Second, altered claudin-2 expression affects the above-mentioned signaling pathways, and several transcription factors with proliferative effects. These include the ZO-1–associated Y-box factor (ZONAB), that shuttles between the TJs and the nucleus[13]; Sp1, a zinc finger transcription factor belonging to the Sp/KLF family [42]; and c-jun, part of the AP1 early response complex. Finally, claudin-2 was also shown to translocate to the nucleus [13].

Effects of claudin-2 on migration are also reported. In different cells claudin-2 overexpression or silencing both resulted in enhanced migration, pointing to context-dependent factors [43][42][44][45]. Like effects on proliferation, mechanism connecting claudin-2 with migration remain incompletely understood. Potential mechanisms include effects of claudin-2 on matrix metalloproteases (MMP)[42][43], a family of zinc-dependent proteases that degrade extracellular matrix proteins, thereby promoting migration. Claudin-2 was also shown to alter integrin expression[46] and to affect Rho proteins[18], that control the cytoskeleton. Finally, claudin-2 might also affect epithelial-

mesenchymal transition (EMT) [18], a process that involves a coordinated genetic reprogramming, during which cells lose their epithelial characteristics and gain mesenchymal properties, including enhanced motility.

## 6. Claudin-2 in diseases

A growing number of studies document altered claudin-2 expression, phosphorylation and/or localization in various pathological conditions. In the past years, strong evidence accumulated in support of a causal link between claudin-2 dysregulation and functional alterations, the key points of which are the following. First, signalling pathways that are known to be overactivated in diseases can alter claudin-2 expression, and a good correlation exists between disease stage and claudin-2 levels. Second, loss-of-function and gain-of-function studies recapitulate some aspects of the functional changes. Studies demonstrating that primary changes in claudin-2 expression can alter cell behavior prompted a paradigm shift, favoring a pathogenic role for claudin-2. Although dysregulation of claudin-2 is likely not a primary cause, pathological changes in claudin-2 abundance and/or localization can be significant in the maintenance and/or progression of diseases.

An expanding body of literature documents dysregulated claudin-2 expression in various carcinomas, including gastric, colorectal, lung, breast and renal carcinomas and in osteosarcoma[46][47][48]. Claudin-2 is highly expressed in gastric and colorectal cancers and its expression level shows a good correlation with the development of these tumors. Initial upregulation of claudin-2 could be due to the overactivation of the above-discussed pathways, and this may promote carcinogenesis. Indeed, overexpresison of exogenous claudin-2 in colorectal cancer cell lines promoted colonocyte proliferation and anchorage-independent colony formation and stimulated tumor formation in colorectal cancer xenografts[41]. Further, claudin-2 expression was reported as a negative predictor for post-chemotherapy disease-free survival of colon cancer patients. Claudin-2 was also shown to promote self-renewal of colorectal cancer stem-like cells [49].

Claudin-2 expression in normal bronchial epithelium is low, but lung adenocarcinoma samples were found to overexpress claudin-2, in part likely due to elevated EGFR signalling [50]. Rapid proliferation of a human lung adenocarcinoma cell line (A549) required claudin-2, and manipulations that reduced claudin-2 levels decreased proliferation[51][23][52]. Further, studies provided strong evidence that targeting claudin-2 can mitigate proliferation.

In some tumors, e.g. osteosarcoma and some breast cancers, claudin-2 expression is decreased. In osteosarcoma cells, lower claudin-2 expression is associated with enhanced migration [40].

The importance of claudin-2 is also emerging in metastasis formation, and this might be due to effects on migration and adhesion. One prominent example was described in breast cancer cells, where claudin-2 overexpression was shown to augment metastasis to the liver[53] .

Claudin-2 levels are also altered by inflammation. Cytokines elevate claudin-2 levels in inflammatory bowel disease (IBD), a condition that inludes ulcerative colitis and Crohn's disease [54]. Claudin-2 is thought to increased gut permeability in IBD, that is a key contributor to the disease. The role of claudin-2 in IBD however might be more

complex, as recent studies suggest protective effects of claudin-2 against tissue injury. A transgenic mouse with targeted overexpression of claudin-2 in the colon had reduced colitis-associated injury [41]. This could be due to reduced apoptosis and augmented regeneration resulting from faster proliferation. While this is a beneficial effect, this is a double-edged sword, as augmented proliferation might also promote carcinogenesis. Thus, elevated claudin-2 expression might be a crucial connection between inflammation and cancer, which could protect against injury and enhance regeneration, but also raises the risk of cancer[55].

In the kidney, claudin-2 is necessary for energy efficient tubular $Na^+$ transport [56]. Accordingly, its loss increases susceptibility to hypoxia-induced injury. Of note, claudin-2 expression in the kidney tubules was shown to be reduced by a large variety of potentially harmful stimuli, and in various kidney disease animal models (e.g. [20][57][12][58][8][59][21]. Thus, pathological loss of claudin-2 might promote injury and augment the progression of kidney disease. The exact connection, however, remains to be established.

Taken together, altered claudin-2 expression is emerging as a possible pathogenic factor in a a number of conditions. Indeed, several studies have now demonstrated that targetting claudin-2 might have beneficial effects in cancer[60][61][52]. Future work will have to address the details of the mechanisms that connect claudin-2 with signalling pathways and various outcomes. The design and testing of new therapeutic interventions to interfere with this protein is also an important next step.

## References


1. Shruthi Venugopal; Shaista Anwer; Katalin Szászi; Claudin-2: Roles beyond Permeability Functions. *International Journal of Molecular Sciences* **2019**, *20*, 5655, 10.3390/ijms20225655.
2. Sachiko Tsukita; Hiroo Tanaka; Atsushi Tamura; The Claudins: From Tight Junctions to Biological Systems. *Trends in Biochemical Sciences* **2019**, *44*, 141-152, 10.1016/j.tibs.2018.09.008.
3. Rothee Günzel; Alan S. L. Yu; Claudins and the Modulation of Tight Junction Permeability. *Physiological Reviews* **2013**, *93*, 525-569, 10.1152/physrev.00019.2012.
4. Amar B. Singh; Srijayaprakash B. Uppada; Punita Dhawan; Claudin proteins, outside-in signaling, and carcinogenesis.. *Pflügers Archiv - European Journal of Physiology* **2016**, *469*, 69-75, 10.1007/s00424-016-1919-1.
5. L Gonzalezmariscal; Tight junction proteins. *Progress in Biophysics and Molecular Biology* **2002**, *81*, 1-44, 10.1016/s0079-6107(02)00037-8.
6. Mikio Furuse; Claudin-1 and -2: Novel Integral Membrane Proteins Localizing at Tight Junctions with No Sequence Similarity to Occludin. *The Journal of Cell Biology* **1998**, *141*, 1539-1550, 10.1083/jcb.141.7.1539.

7. Phyu Phyu Aung; Yoshitsugu Mitani; Yuichi Sanada; Hirofumi Nakayama; Keisuke Matsusaki; Wataru Yasui; Differential expression of claudin-2 in normal human tissues and gastrointestinal carcinomas. *Virchows Archiv* **2005**, *448*, 428-434, 10.1007/s00428-005-0120-2.
8. Katalin Szászi; Yasaman Amoozadeh; New Insights into Functions, Regulation, and Pathological Roles of Tight Junctions in Kidney Tubular Epithelium. *International Review of Cell and Molecular Biology* **2013**, *308*, 205-271, 10.1016/b978-0-12-800097-7.00006-3.
9. Xiaohua Tan; Ngmei Li; Xiaobo Wang; Yan Zeng; Yutao Yan; Lei Yang; Claudin-2 downregulation by KSHV infection is involved in the regulation of endothelial barrier function. *Journal of Cutaneous Pathology* **2014**, *41*, 630-639, 10.1111/cup.12332.
10. J. Van Den Bossche; D. Laoui; Y. Morias; K. Movahedi; Geert Raes; P. De Baetselier; J. A. Van Ginderachter; Claudin-1, Claudin-2 and Claudin-11 Genes Differentially Associate with Distinct Types of Anti-inflammatory Macrophages In vitro and with Parasite- and Tumour-elicited Macrophages In vivo. *Scandinavian Journal of Immunology* **2012**, *75*, 588-598, 10.1111/j.1365-3083.2012.02689.x.
11. Alan S.L. Yu; Mary H. Cheng; Susanne Angelow; Rothee Günzel; Sanae A. Kanzawa; Eveline E. Schneeberger; Michael Fromm; Rob D. Coalson; Molecular Basis for Cation Selectivity in Claudin-2–based Paracellular Pores: Identification of an Electrostatic Interaction Site. *The Journal of General Physiology* **2008**, *133*, 111-127, 10.1085/jgp.200810154.
12. Yasaman Amoozadeh; Qinghong Dan; Jenny Xiao; Faiza Waheed; Katalin Szászi; Tumor necrosis factor-α induces a biphasic change in claudin-2 expression in tubular epithelial cells: role in barrier functions.. *American Journal of Physiology-Cell Physiology* **2015**, *309*, C38-50, 10.1152/ajpcell.00388.2014.
13. Akira Ikari; Ryo Watanabe; Tomonari Sato; Saeko Taga; Shun Shimobaba; Masahiko Yamaguchi; Yasuhiro Yamazaki; Satoshi Endo; Toshiyuki Matsunaga; Junko Sugatani; et al. Nuclear distribution of claudin-2 increases cell proliferation in human lung adenocarcinoma cells. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1843*, 2079-2088, 10.1016/j.bbamcr.2014.05.017.
14. Sébastien Tabariès; Alexander McNulty; Véronique Ouellet; Matthew G. Annis; Mireille Dessureault; Maude Vinette; Yasmina Hachem; Brennan Lavoie; Atilla Omeroglu; Hans-Georg Simon; et al.Logan A. WalshSiker KimbungIngrid HedenfalkPeter M. Siegel Afadin cooperates with Claudin-2 to promote breast cancer metastasis.. *Genome Research* **2019**, *33*, 180-193, 10.1101/gad.319194.118.
15. Amar B. Singh; Raymond C. Harris; Epidermal Growth Factor Receptor Activation Differentially Regulates Claudin Expression and Enhances Transepithelial Resistance in Madin-Darby Canine Kidney Cells. *Journal of Biological Chemistry* **2003**, *279*, 3543-3552, 10.1074/jbc.m308682200.

16. Yaya Wang; John Brian Mumm; Ronald Herbst; Roland Kolbeck; Yue Wang; IL-22 Increases Permeability of Intestinal Epithelial Tight Junctions by Enhancing Claudin-2 Expression. *The Journal of Immunology* **2017**, *199*, 3316-3325, 10.4049/jimmunol.1700152.
17. Rana Al-Sadi; Dongmei Ye; Michel Boivin; Shuhong Guo; Mariam Hashimi; Lisa Ereifej; Thomas Y. Ma; Interleukin-6 Modulation of Intestinal Epithelial Tight Junction Permeability Is Mediated by JNK Pathway Activation of Claudin-2 Gene. *PLOS ONE* **2014**, *9*, e85345, 10.1371/journal.pone.0085345.
18. Qinghong Dan; Yixuan Shi; Razieh Rabani; Shruthi Venugopal; Jenny Xiao; Shaista Anwer; Mei Ding; Pam Speight; Wanling Pan; R. Todd Alexander; et al.András KapusKatalin Szászi Claudin-2 suppresses GEF-H1, RHOA, and MRTF, thereby impacting proliferation and profibrotic phenotype of tubular cells. *Journal of Biological Chemistry* **2019**, *294*, 15446-15465, 10.1074/jbc.ra118.006484.
19. Naoko Fujii; Yukinobu Matsuo; Toshiyuki Matsunaga; Satoshi Endo; Hideki Sakai; Masahiko Yamaguchi; Yasuhiro Yamazaki; Junko Sugatani; Akira Ikari; Hypotonic Stress-induced Down-regulation of Claudin-1 and -2 Mediated by Dephosphorylation and Clathrin-dependent Endocytosis in Renal Tubular Epithelial Cells*. *Journal of Biological Chemistry* **2016**, *291*, 24787-24799, 10.1074/jbc.M116.728196.
20. Jeannette E. Gonzalez; Robert J. DiGeronimo; D'ann E. Arthur; Jonathan M. King; Remodeling of the tight junction during recovery from exposure to hydrogen peroxide in kidney epithelial cells.. *Free Radical Biology and Medicine* **2009**, *47*, 1561-9, 10.1016/j.freeradbiomed.2009.08.024.
21. Damien Maggiorani; Romain Dissard; Marcy Belloy; Jean-Sébastien Saulnier-Blache; Audrey Casemayou; Laure Ducasse; Sandra Grès; Julie Bellière; Cécile Caubet; Jean-Loup Bascands; et al.Joost P. SchanstraBénédicte Buffin-Meyer Shear Stress-Induced Alteration of Epithelial Organization in Human Renal Tubular Cells. *PLOS ONE* **2015**, *10*, e0131416, 10.1371/journal.pone.0131416.
22. Yasaman Amoozadeh; Shaista Anwer; Qinghong Dan; Shruthi Venugopal; Yixuan Shi; Emily Branchard; Elisabeth Liedtke; Menachem Ailenberg; Ori D. Rotstein; Andras Kapus; et al.Katalin Szaszi Cell confluence regulates claudin-2 expression: possible role for ZO-1 and Rac. *American Journal of Physiology-Cell Physiology* **2018**, *314*, C366-C378, 10.1152/ajpcell.00234.2017.
23. Hiroyuki Sonoki; Asami Tanimae; Satoshi Endo; Toshiyuki Matsunaga; Takumi Furuta; Kenji Ichihara; Akira Ikari; Kaempherol and Luteolin Decrease Claudin-2 Expression Mediated by Inhibition of STAT3 in Lung Adenocarcinoma A549 Cells. *Nutrients* **2017**, *9*, 597, 10.3390/nu9060597.
24. Natalia Martin-Martin; Gavin Ryan; Tara McMorrow; Michael P. Ryan; Sirolimus and cyclosporine A alter barrier function in renal proximal tubular cells through stimulation of ERK1/2 signaling and

claudin-1 expression. *American Journal of Physiology-Renal Physiology* **2010**, *298*, F672-F682, 10.1152/ajprenal.00199.2009.

25. Takanori Sakaguchi; Cloning of the Human Claudin-2 5'-Flanking Region Revealed a TATA-less Promoter with Conserved Binding Sites in Mouse and Human for Caudal-related Homeodomain Proteins and Hepatocyte Nuclear Factor-1alpha. *Journal of Biological Chemistry* **2002**, *277*, 21361-21370, 10.1074/jbc.m110261200.
26. F. Escaffit; F. Boudreau; J.-F. Beaulieu; Differential expression of claudin-2 along the human intestine: Implication of GATA-4 in the maintenance of claudin-2 in differentiating cells. *Journal of Cellular Physiology* **2005**, *203*, 15-26, 10.1002/jcp.20189.
27. Yong-Guo Zhang; Shaoping Wu; Rong Lu; David Zhou; Jingsong Zhou; Geert Carmeliet; Elaine Petrof; Erika C. Claud; Jun Sun; Tight junction CLDN2 gene is a direct target of the vitamin D receptor. *Scientific Reports* **2015**, *5*, 10642, 10.1038/srep10642.
28. Akira Ikari; Naoko Fujii; Shinya Hahakabe; Hisayoshi Hayashi; Masahiko Yamaguchi; Yasuhiro Yamazaki; Satoshi Endo; Toshiyuki Matsunaga; Junko Sugatani; Hyperosmolarity-Induced Down-Regulation of Claudin-2 Mediated by Decrease in PKCβ-Dependent GATA-2 in MDCK Cells. *Journal of Cellular Physiology* **2015**, *230*, 2776-2787, 10.1002/jcp.25004.
29. Michael J. Rosen; Rupesh Chaturvedi; M. Kay Washington; Lindsay A. Kuhnhein; Preston D. Moore; Scott S. Coggeshall; Elizabeth M. McDonough; Jörn-Hendrik Weitkamp; Amar B. Singh; Lori A. Coburn; et al.Christopher S. WilliamsFang YanLuc Van KaerR. Stokes PeeblesKeith T. Wilson STAT6 deficiency ameliorates severity of oxazolone colitis by decreasing expression of claudin-2 and Th2-inducing cytokines. *The Journal of Immunology* **2013**, *190*, 1849-1858, 10.4049/jimmunol.1201373.
30. Christina M. Van Itallie; Amber Jean Tietgens; Kirsten LoGrande; Angel Aponte; Marjan Gucek; James M. Anderson; Phosphorylation of claudin-2 on serine 208 promotes membrane retention and reduces trafficking to lysosomes.. *Journal of Cell Science* **2012**, *125*, 4902-12, 10.1242/jcs.111237.
31. Christina M. Van Itallie; Laura L. Mitic; James M. Anderson; SUMOylation of claudin-2. *Annals of the New York Academy of Sciences* **2012**, *1258*, 60-64, 10.1111/j.1749-6632.2012.06541.x.
32. Eduardo Molina-Jijón; Rafael Rodríguez-Muñoz; María Del Carmen Namorado; José Pedraza-Chaverri; José L. Reyes; Oxidative stress induces claudin-2 nitration in experimental type 1 diabetic nephropathy. *Free Radical Biology and Medicine* **2014**, *72*, 162-175, 10.1016/j.freeradbiomed.2014.03.040.
33. Rita Rosenthal; Rothee Günzel; Susanne M. Krug; Jörg-Dieter Schulzke; Michael Fromm; Alan S.L. Yu; Claudin-2-mediated cation and water transport share a common pore.. *Acta Physiologica* **2016**, *219*, 521-536, 10.1111/apha.12742.

34. Shinsaku Tokuda; Mikio Furuse; Claudin-2 Knockout by TALEN-Mediated Gene Targeting in MDCK Cells: Claudin-2 Independently Determines the Leaky Property of Tight Junctions in MDCK Cells. *PLOS ONE* **2015**, *10*, e0119869, 10.1371/journal.pone.0119869.

35. Mikio Furuse; Kyoko Furuse; Hiroyuki Sasaki; Shoichiro Tsukita; Conversion ofZonulae Occludentesfrom Tight to Leaky Strand Type by Introducing Claudin-2 into Madin-Darby Canine Kidney I Cells. *The Journal of Cell Biology* **2001**, *153*, 263-272, 10.1083/jcb.153.2.263.

36. Hiroo Tanaka; Atsushi Tamura; Koya Suzuki; Sachiko Tsukita; Site-specific distribution of claudin-based paracellular channels with roles in biological fluid flow and metabolism. *Annals of the New York Academy of Sciences* **2017**, *1405*, 44-52, 10.1111/nyas.13438.

37. Shigeaki Muto; Masaki Hata; Junichi Taniguchi; Shuichi Tsuruoka; Kazumasa Moriwaki; Mitinori Saitou; Kyoko Furuse; Hiroyuki Sasaki; Akio Fujimura; Masashi Imai; et al.Eiji KusanoShoichiro TsukitaMikio Furuse Claudin-2–deficient mice are defective in the leaky and cation-selective paracellular permeability properties of renal proximal tubules. *Proceedings of the National Academy of Sciences* **2010**, *107*, 8011-8016, 10.1073/pnas.0912901107.

38. Michael Fromm; Jörg Piontek; Rita Rosenthal; Dorothee Günzel; Susanne M. Krug; Tight junctions of the proximal tubule and their channel proteins. *Pflügers Archiv - European Journal of Physiology* **2017**, *469*, 877-887, 10.1007/s00424-017-2001-3.

39. Kengo Matsumoto; Mitsunobu Imasato; Yuji Yamazaki; Hiroo Tanaka; Mitsuhiro Watanabe; Hidetoshi Eguchi; Hiroaki Nagano; Hayato Hikita; Tomohide Tatsumi; Tetsuo Takehara; et al.Atsushi TamuraSachiko Tsukita Claudin 2 Deficiency Reduces Bile Flow and Increases Susceptibility to Cholesterol Gallstone Disease in Mice. *Gastroenterology* **2014**, *147*, 1134-1145.e10, 10.1053/j.gastro.2014.07.033.

40. Xiaowei Zhang; Haiming Wang; Qian Li; Tao Li; CLDN2 inhibits the metastasis of osteosarcoma cells via down-regulating the afadin/ERK signaling pathway. *Cancer Cell International* **2018**, *18*, 160, 10.1186/s12935-018-0662-4.

41. Rizwan Ahmad; Rupesh Chaturvedi; Danyvid Olivares-Villagómez; Tanwir Habib; Mohammad Asim; Punit Shivesh; D B Polk; Keith T. Wilson; Mary K. Washington; Luc Van Kaer; et al.Punita DhawanAmar B. SinghBrent D. Polk Targeted colonic claudin-2 expression renders resistance to epithelial injury, induces immune suppression, and protects from colitis.. *Mucosal Immunology* **2014**, *7*, 1340-53, 10.1038/mi.2014.21.

42. Akira Ikari; Tomonari Sato; Ayumi Takiguchi; Kosuke Atomi; Yasuhiro Yamazaki; Junko Sugatani; Claudin-2 knockdown decreases matrix metalloproteinase-9 activity and cell migration via suppression of nuclear Sp1 in A549 cells. *Life Sciences* **2011**, *88*, 628-633, 10.1016/j.lfs.2011.02.002.

43. Akira Ikari; Ayumi Takiguchi; Kosuke Atomi; Tomonari Sato; Junko Sugatani; Decrease in claudin-2 expression enhances cell migration in renal epithelial madin-darby canine kidney cells. *Journal*

*of Cellular Physiology* **2011**, *226*, 1471-1478, 10.1002/jcp.22386.

44. Shinji Mima; Masaya Takehara; Hiroko Takada; Tomoko Nishimura; Tatsuya Hoshino; Tohru Mizushima; NSAIDs suppress the expression of claudin-2 to promote invasion activity of cancer cells. *Carcinogenesis* **2008**, *29*, 1994-2000, 10.1093/carcin/bgn134.
45. Masaya Takehara; Tomoko Nishimura; Shinji Mima; Tatsuya Hoshino; Tohru Mizushima; Effect of claudin expression on paracellular permeability, migration and invasion of colonic cancer cells.. *Biological and Pharmaceutical Bulletin* **2009**, *32*, 825-831, 10.1248/bpb.32.825.
46. S Tabariès; Z Dong; M G Annis; A Omeroglu; F Pepin; V Ouellet; C Russo; Mazen Hassanain; P Metrakos; Z Diaz; et al.M BasikN BertosM ParkC GuettierR AdamM HallettP M Siegel Claudin-2 is selectively enriched in and promotes the formation of breast cancer liver metastases through engagement of integrin complexes. *Oncogene* **2010**, *30*, 1318-1328, 10.1038/onc.2010.518.
47. Siker Kimbung; Anikó Kovács; Pär-Ola Bendahl; Per Malmström; Mårten Fernö; Thomas Hatschek; Ingrid Hedenfalk; Claudin-2 is an independent negative prognostic factor in breast cancer and specifically predicts early liver recurrences. *Molecular Oncology* **2013**, *8*, 119-128, 10.1016/j.molonc.2013.10.002.
48. Song Xin; Chen Huixin; Shen Benchang; Bai Aiping; Wang Jinhui; Li Xiaoyan; Wong Benjamin Chun Yu; Chen Minhu; Expression of Cdx2 and Claudin-2 in the Multistage Tissue of Gastric Carcinogenesis. *Oncology* **2006**, *73*, 357-365, 10.1159/000135351.
49. Sophie Paquet-Fifield; Shir Lin Koh; Lesley Cheng; Laura Marie Beyit; Carolyn Shembrey; Christina Mølck; Corina Behrenbruch; Marina Papin; Meritxell Gironella; Sophie Guelfi; et al.Ramona NasrFanny GrilletMichel PrudhommeJean-François BourgauxAntoni CastellsJean-Marc PascussiAlexander G. HeriotAlain PuisieuxMelissa J. DavisJulie PannequinAndrew F. HillErica K. SloanFrédéric Hollande Tight Junction Protein Claudin-2 Promotes Self-Renewal of Human Colorectal Cancer Stem-like Cells. *Cancer Research* **2018**, *78*, 2925-2938, 10.1158/0008-5472.can-17-1869.
50. Akira Ikari; Tomonari Sato; Ryo Watanabe; Yasuhiro Yamazaki; Junko Sugatani; Increase in claudin-2 expression by an EGFR/MEK/ERK/c-Fos pathway in lung adenocarcinoma A549 cells. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2012**, *1823*, 1110-1118, 10.1016/j.bbamcr.2012.04.005.
51. Asami Hichino; Miki Okamoto; Saeko Taga; Risa Akizuki; Satoshi Endo; Toshiyuki Matsunaga; Akira Ikari; Down-regulation of Claudin-2 Expression and Proliferation by Epigenetic Inhibitors in Human Lung Adenocarcinoma A549 Cells*. *Journal of Biological Chemistry* **2017**, *292*, 2411-2421, 10.1074/jbc.M116.762807.
52. Akira Ikari; Saeko Taga; Ryo Watanabe; Tomonari Sato; Shun Shimobaba; Hiroyuki Sonoki; Satoshi Endo; Toshiyuki Matsunaga; Hideki Sakai; Masahiko Yamaguchi; et al.Yasuhiro YamazakiJunko Sugatani Clathrin-dependent endocytosis of claudin-2 by DFYSP peptide causes

lysosomal damage in lung adenocarcinoma A549 cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2015**, *1848*, 2326-2336, 10.1016/j.bbamem.2015.07.003.

53. Sébastien Tabariès; Fanny Dupuy; Zhifeng Dong; Anie Monast; Matthew G. Annis; Jonathan Spicer; Lorenzo E. Ferri; Atilla Omeroglu; Mark Basik; Eitan Amir; et al.Mark ClemonsPeter M. Siegel Claudin-2 Promotes Breast Cancer Liver Metastasis by Facilitating Tumor Cell Interactions with Hepatocytes. *Molecular and Cellular Biology* **2012**, *32*, 2979-2991, 10.1128/MCB.00299-12.
54. Liguo Zhu; Jing Han; Li Li; Ying Wang; Ying Li; Shenghong Zhang; Claudin Family Participates in the Pathogenesis of Inflammatory Bowel Diseases and Colitis-Associated Colorectal Cancer.. *Frontiers in Immunology* **2019**, *10*, 1441, 10.3389/fimmu.2019.01441.
55. Christopher R. Weber; Sam C. Nalle; Maria Tretiakova; David T. Rubin; Jerrold R. Turner; Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation.. *Laboratory Investigation* **2008**, *88*, 1110-20, 10.1038/labinvest.2008.78.
56. Lei Pei; Glenn Solis; Mien T.X. Nguyen; Nikhil Kamat; Lynn Magenheimer; Min Zhuo; Jiahua Li; Joshua Curry; Alicia A. McDonough; Timothy A. Fields; et al.William J. WelchAlan S.L. Yu Paracellular epithelial sodium transport maximizes energy efficiency in the kidney.. *Journal of Clinical Investigation* **2016**, *126*, 2509-18, 10.1172/JCI83942.
57. Daniel F. Balkovetz; Phillip Chumley; Hassane Amlal; Downregulation of claudin-2 expression in renal epithelial cells by metabolic acidosis. *American Journal of Physiology-Renal Physiology* **2009**, *297*, F604-F611, 10.1152/ajprenal.00043.2009.
58. Natalia Martin-Martin; Qinghong Dan; Yasaman Amoozadeh; Faiza Waheed; Tara McMorrow; Michael P. Ryan; Katalin Szászi; RhoA and Rho kinase mediate cyclosporine A and sirolimus-induced barrier tightening in renal proximal tubular cells. *The International Journal of Biochemistry & Cell Biology* **2011**, *44*, 178-188, 10.1016/j.biocel.2011.10.014.
59. Amar B. Singh; Keisuke Sugimoto; Punita Dhawan; Raymond C. Harris; Juxtacrine activation of EGFR regulates claudin expression and increases transepithelial resistance. *American Journal of Physiology-Cell Physiology* **2007**, *293*, C1660-C1668, 10.1152/ajpcell.00274.2007.
60. Mutsumi Takigawa; Manami Iida; Shotaro Nagase; Hidehiko Suzuki; Akihiro Watari; Minoru Tada; Yoshiaki Okada; Takefumi Doi; Masayoshi Fukasawa; Kiyohito Yagi; et al.Jun KunisawaMasuo Kondoh Creation of a Claudin-2 Binder and Its Tight Junction–Modulating Activity in a Human Intestinal Model. *Journal of Pharmacology and Experimental Therapeutics* **2017**, *363*, 444-451, 10.1124/jpet.117.242214.
61. Yosuke Hashimoto; Tomoyuki Hata; Minoru Tada; Manami Iida; Akihiro Watari; Yoshiaki Okada; Takefumi Doi; Hiroki Kuniyasu; Kiyohito Yagi; Masuo Kondoh; et al. Safety evaluation of a human chimeric monoclonal antibody that recognizes the extracellular loop domain of claudin-2. *European Journal of Pharmaceutical Sciences* **2018**, *117*, 161-167, 10.1016/j.ejps.2018.02.016.