

REVIEW



## The claudin–transcription factor signaling pathway

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### ABSTRACT

Claudins (CLDNs) represent major transmembrane proteins of tight junctions and contribute to the barrier function. They also serve as anchors for several signaling proteins, but the underlying molecular basis has yet to be established. The present review covers the recent progress in our understanding of the CLDN signaling pathway in health and disease. We discuss the functional relevance of phosphotyrosine motifs in the C-terminal cytoplasmic domain of CLDNs and define mutual regulation between CLDNs and Src-family kinases (SFKs). In addition, we focus on the crosstalk between CLDN and transcription factor signaling. We also describe how aberrant CLDN–transcription factor signaling promotes or inhibits cancer progression. We propose that a link between various cell adhesion molecules and transcription factors coordinates a range of physiological and pathological events via activation or suppression of target genes.

### ARTICLE HISTORY

Received 17 December 2020  
Revised 17 March 2021  
Accepted 22 March 2021

### KEYWORDS

Tight junction; CLDN; cell adhesion signal; signal transduction; PI3K; AKT; nuclear receptor; Src-family kinase; transcription factors



### Introduction

The claudin (CLDN) family is capable of forming tight-junction strands and thereby represents the backbone of tight junctions in vertebrate epithelial and endothelial cells, as well as in other types of cells.<sup>1,2</sup> It is composed of 27 members in mammals, and a specific combination of CLDNs is expressed in a given cell/tissue type. CLDNs are tetraspan transmembrane proteins that include two extracellular loops (EC1 and EC2) and N- and C-terminal cytoplasmic domains. The CLDN-EC1 creates paracellular barriers or pores for selective ions and solutes,<sup>3–7</sup> whereas both CLDN-EC1 and CLDN-EC2 contribute to *cis*- and *trans*-interactions between CLDNs.<sup>7–11</sup> On the other hand, the C-terminal cytoplasmic domain of many CLDN subtypes contains specific sequence motifs such as PDZ domain-binding motifs and phosphorylation consensus sites<sup>5, 12–16</sup> and receives or propagates a magnitude of intracellular signals as platforms; however, it remains poorly defined how CLDN signaling reaches the nucleus and regulates gene expression.<sup>17</sup>

CLDNs are absolutely required for human health, and their dysregulations are involved in the pathogenesis of diverse diseases.<sup>4–6, 18–16</sup> For instance, it is well known that mutations in several *CLDN* genes cause

various human hereditary diseases. In addition, CLDNs frequently show aberrant expression and/or localization in a wide variety of cancers, resulting in either promotion or repression of tumor progression, most probably by dysregulated CLDN signaling.<sup>22, 26–32</sup> Moreover, recent studies have established the region-selective CLDN5 breakdown in brain disorders, such as schizophrenia, depression, Alzheimer's disease, and multiple sclerosis.<sup>33–39</sup> In this respect, we and others have previously reported how endothelial CLDN5 expression is regionally disrupted in these psychiatric disorders.<sup>40, 41</sup>

In the current review, we focus on the link between CLDN and transcription factor signaling because it is theoretically attributed to the organization of a broad range of cellular processes, including cell growth, survival, differentiation, polarity, migration, and metabolism, via regulation of the expression of corresponding target genes. We also discuss aberrant CLDN–transcription factor signaling in cancer. We do not describe recent progress in our understanding of numerous aspects of other tight-junction players, such as junctional adhesion molecules (JAMs), tight junction-associated MARVEL domain-containing proteins (TAMPs: occludin, tricellulin, and MarvelD3), and the ZO family of scaffolding proteins.<sup>42–55</sup>

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### **The CLDN/SFK/PI3K/AKT/transcription factor signaling cascade**

We previously reported that retinoid X receptor  $\alpha$  (RXR $\alpha$ )/retinoic acid receptor  $\gamma$  (RAR $\gamma$ ) and another member of the nuclear receptor superfamily, hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ), trigger the formation of mature cell–cell junctions and microvilli, expression of tight-junction markers (CLDN6, CLDN7, occludin, and ZO-1 $\alpha$ + variant) and a microvilli marker (ezrin/radixin/moesin-binding phosphoprotein 50 [EBP50]), as well as morphological differentiation into epithelial cells from stem cells.<sup>56–59</sup> These effects are incredibly similar to the CLDN6-triggered ones,<sup>60</sup> implying a possible crosstalk between them. Along this line, we have recently identified the CLDN–transcription factor signaling pathway.<sup>61</sup>

### **Reciprocal regulation between CLDNs and SFKs**

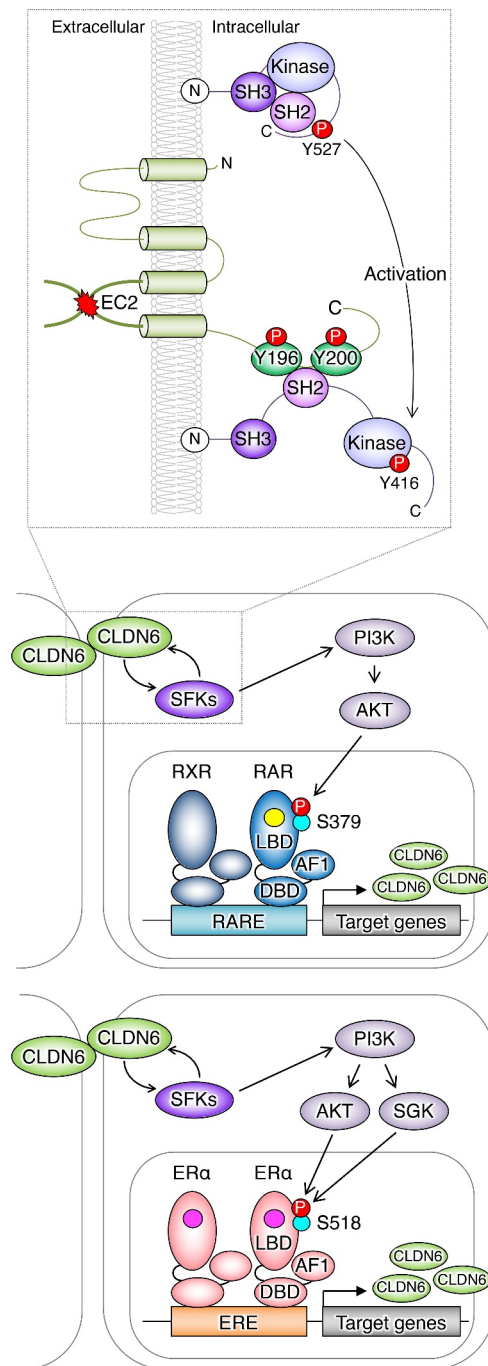
First, we showed, by using the corresponding deletion mutants, that the CLDN6–adhesion signal is transduced through the EC2 domain and the first half C-terminal cytoplasmic domains but not through the EC1 domain (Figure 1). Second, we paid attention to the four tyrosine residues in the C-terminal domain of CLDN6, which are completely conserved among vertebrates, and revealed that Y196/200, but not Y213/218, are definitely required for CLDN6–signaling ability. Third, we disclosed that CLDN6 recruits and activates Src-family kinases (SFKs) in EC2-dependent and Y196/200-dependent manners, and SFKs in turn phosphorylate CLDN6 at Y196/200 in an EC2-dependent fashion. The functional relevance of the EC2 in the CLDN6–adhesion signal was also supported by using the C-terminal half of *Clostridium perfringens* enterotoxin (C-CPE).<sup>60,61</sup> Moreover, we have identified SFK members associated with CLDN6 and obtained evidence showing that recombinant proteins corresponding to the Src homology 2 (SH2) domains of certain SFKs directly bind to the C-terminal cytoplasmic domain of CLDN6 and the Y196/200-containing peptide (our unpublished results).

Through a careful search for amino acid sequence of human and mouse CLDN1–20, both

Y196 and Y200 in the C-terminal cytoplasmic domain of CLDN6 are conserved in CLDN2/4/12 (Figures 2 and 3). It is also noteworthy that CLDN6Y196 and CLDN6Y200 are conserved in CLDN3/7/8/10/14/16 and CLDN1/5/9/17/18, respectively.

We have found mutual regulation between certain CLDN subtypes (tyrosine residues corresponding to CLDN6Y200) and SFKs (our unpublished results). In addition, Li et al.<sup>62</sup> have recently reported that CLDN11 is phosphorylated at two adjacent tyrosine residues, which are positioned at different sites from CLDN6Y196/200, as described above, and activates SRC. The C-terminal domain of CLDN1 is also associated with SRC although the involvement of the pY motifs is not determined.<sup>63</sup> It should also be noted that amino acids around pY, in particular 3–5 residues at the C-terminal side, influence the binding specificity of the SH2 domain.<sup>64–67</sup> For instance, the C-terminal amino acids of the CLDN6Y200–corresponding tyrosine residue in human and mouse CLDN18 are very different from others; therefore, it does not seem to function as the pY motif. CLDN18 is known to decrease PI3K and AKT activities,<sup>68,69</sup> suggesting this notion. Further studies are required to determine whether other CLDN subtypes, similar to CLDN6, couple with SFKs via the pY motifs in the C-terminal cytoplasmic domains.

SFKs are known to be activated by several cell–cell and cell–matrix adhesion proteins lacking intrinsic kinase activity, such as E-cadherin, integrins, and cellular prion protein.<sup>62,70–76</sup> In addition, engagement of JAMs, the JAML (junctional adhesion molecule-like) and CAR (coxsackie and adenovirus receptor), stimulates PI3K,<sup>77</sup> which is the major downstream signal of SFKs. Besides, it is well known that certain signaling proteins, which contain the SH2, phosphotyrosine-binding (PTB), Hakaityrosine binding (HYB), C2, and pyruvate kinase M2 domains, bind to the pY motifs.<sup>66,67,78–81</sup> Taken together, these findings strongly suggest that the pY motifs in the C-terminal cytoplasmic domains of diverse cell–cell and cell–matrix adhesion molecules generally serve as the signaling landscapes for SFKs and other pY motif-binding proteins.



**Figure 1.** Schematic model for regulation of the nuclear receptor activity by the CLDN–adhesion signaling. The schema is modified from that reported previously (Sugimoto et al., 2019). SH2/3: Src homology 2/3 domain; Kinase: kinase domain; AF1: activation function-1; DBD: DNA-binding domain; LBD: ligand-binding domain; RARE: retinoic acid response element; ERE: estrogen response element; RA: yellow circle; estrogen: pink circle.



**Figure 2.** Amino acid sequences of a part of the C-terminal cytoplasmic domain in human CLDN1-20. Tyrosine residues corresponding to CLDN6Y196/200 are highlighted and the conserved ones are indicated in red.

### A link between CLDN/SFK and transcription factor signalings

Importantly, the CLDN6/SFK/PI3K/AKT axis targets the AKT-phosphorylation sites in the RAR $\gamma$  and the estrogen receptor  $\alpha$  (ER $\alpha$ ) and stimulates their activities, thereby regulating the expression of respective target genes. This conclusion was drawn from the following results: (1) the CLDN6-induced cellular events were hindered in three distinct F9: *Rxra*<sup>-/-</sup>:*Rarg*<sup>-/-</sup>:*Cldn6* cell lines, despite SFKs being activated; (2) AKT formed a complex with either

RXR $\alpha$ /RAR $\gamma$  or ER $\alpha$ ; and (3) characterization of F9: *Rxra*<sup>-/-</sup>:*Rarg*<sup>-/-</sup>:*Cldn6*:*iRxra-Rarg2S379A* (hereafter, “i” means doxycycline-inducible expression of a give gene) and F9: *Rxra*<sup>-/-</sup>:*Rarg*<sup>-/-</sup>:*Cldn6*:*iRxra-Rarg2S379E* cells, as well as MCF-7: *ESR1S518E* cells, revealed that CLDN6 signaling directs S379 and S518 in mouse RAR $\gamma$  and human ER $\alpha$ , respectively. In addition, CLDN6-provoked RAR $\gamma$ S379 phosphorylation in mice resulted in releasing the nuclear receptor corepressor (NCoR) from several retinoic acid response elements (RAREs) of three



**Figure 3.** Amino acid sequences of a part of the C-terminal cytoplasmic domain in mouse CLDN1-20. Tyrosine residues corresponding to CLDN6Y196/200 are highlighted and the conserved ones are indicated in red.

distinct RA target genes, including *Cldn6*. Since RXR $\alpha$ /RAR $\gamma$  heterodimer appears to induce *Cldn6* gene expression,<sup>56,61</sup> the positive loop of the CLDN6-RAR $\gamma$  cascade could contribute not only to triggering but also to the maintenance of CLDN6-initiated cellular events. Intriguingly, the AKT-consensus phosphorylation motifs are conserved in 14 of 48 members of human nuclear receptors, implying the biological relevance of this phosphorylation site.

### Aberrant CLDN-transcription factor signaling in cancer

We have recently reported that aberrant CLDN6 expression in endometrial cancer tissues is significantly associated with several clinicopathological variables, such as surgical stage III/IV, histological type, histological grade 3, lymphovascular space involvement, lymph node metastasis, and distant metastasis.<sup>82</sup> Additionally, we showed that the high CLDN6 expression in endometrial cancer represents an independent

prognosis marker, and the 5-y survival rate was approximately 30%, which was one-third of that in the low-expression group.

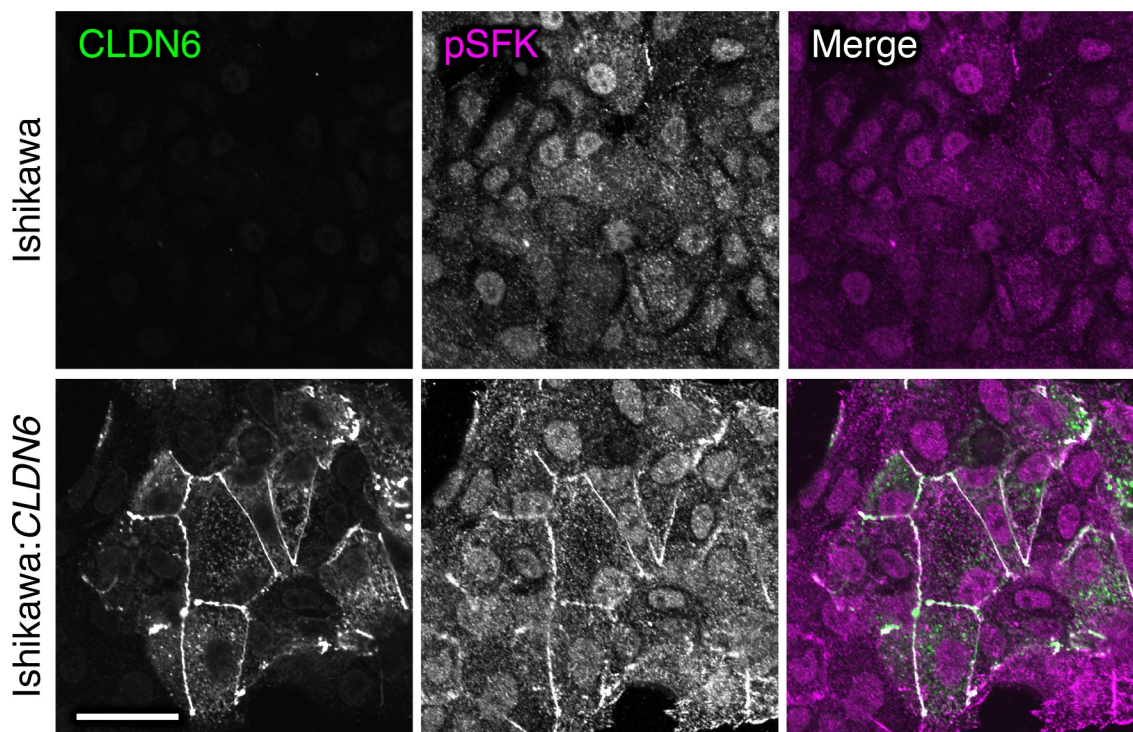
In an additional study, we found that aberrant CLDN6 expression promotes the malignant phenotypes of endometrial cancer *in vitro* and *in vivo* via hijacking the CLDN6-ER $\alpha$  axis.<sup>83</sup> For instance, we demonstrated that abnormal CLDN6-ER $\alpha$  signaling stimulates not only cell proliferation but also collective cell migration in the leading front of endometrial cancer cells. It is noteworthy that activated SFKs appear to be concentrated at the cell borders together with CLDN6 in Ishikawa:CLDN6 cells but not in parental Ishikawa cells (Figure 4). The EC2 domain and Y196/200 of CLDN6 were required to recruit and activate SFKs and to stimulate malignant phenotypes of endometrial cancer cells. In addition, the CLDN6/SFK/PI3K pathway propagates both AKT and serum- and glucocorticoid-regulated kinase (SGK), which share a high degree of homology and the same consensus phosphorylation motif,<sup>84</sup> resulting in targeting S518 in human ER $\alpha$  and activating target genes in a ligand-independent manner. Furthermore, RNaseq and RT-

qPCR analyses indicated the presence of not only ER $\alpha$ -dependent but also ER $\alpha$ -independent CLDN6 signaling (Figure 5). The identification of this machinery highlights the regulation of transcription factor activity by cell adhesion to advance tumor progression.

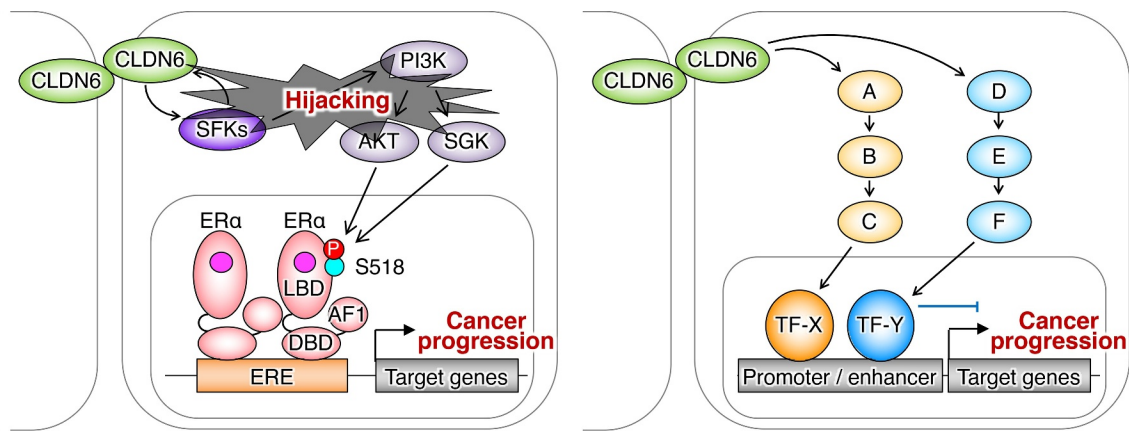
Another issue that should be mentioned is other abnormal CLDN-transcription factor signaling in health and disease. The CLDN18/Yes-associated protein (YAP) pathway regulates the homeostasis of normal lung stem and progenitor cells, and its deficiency promotes tumorigenesis and progression of lung and gastric adenocarcinoma.<sup>69,85-87</sup> By contrast, CLDN2 activates YAP, leading to self-renewal of human colorectal cancer stem-like cells.<sup>88</sup> Since CLDN2 has two conserved pY in the C-terminal cytoplasmic domain as described above, it should also be verified whether CLDN2/SFK signaling is involved in the self-renewal of various cancer stem-like cells.

## Conclusions and future directions

The work discussed in the present review highlights the CLDN-transcription factor signaling pathway,



**Figure 4.** Aberrant SFK activation in human endometrial cancer cells by CLDN6. Scale bar, 20  $\mu$ m.



**Figure 5.** The ER $\alpha$ -dependent and independent CLDN6 signaling in endometrial cancer. A–F indicate signaling proteins. TF, transcription factor.

notably the CLDN/SFK/PI3K/AKT/nuclear receptor cascade. Cell–cell and cell–matrix adhesion molecules are indispensable not only for proper tissue integrity but also for signaling properties that coordinate a wide range of cell behaviors. In other words, appropriate tissue formation connected by various cell adhesion proteins should be pre-requested for normal cell-adhesion signal. Since cell–cell and cell–matrix adhesion proteins are broadly expressed in distinct cell types, we propose that various combinations of cell adhesion molecules and transcription factors coordinate diverse physiological and pathological processes, including cancer. The cell-adhesion signals most probably lead to posttranslational modification of transcription factors, thereby regulating their activities. In future, it would be interesting to generalize the cell adhesion–transcription factor signaling pathway in health and disease.

### Author Contributions

H.C. wrote the paper, and H.C. and K.S. prepared figures and agreed to the final version of the manuscript.

### Acknowledgments

We thank the former and present members of our laboratory for a helpful discussion. We also thank the English Editing Service of the Medical Research Promotion Office, Fukushima Medical University, for their assistance with the manuscript.



### Disclosure of Potential Conflicts of Interest

The authors declare no conflict of interest.

### Funding

This work was supported by Japan Agency for Medical Research and Development [JP201m0203010]; Japan Society for the Promotion of Science [19K16615]; Japan Society for the Promotion of Science [17K08699]; Japan Society for the Promotion of Science [17K17978]; the Uehara Memorial Foundation; the Takeda Science Foundation; and the Ichiro Kanehara Foundation for the Promotion of Medical Sciences and Medical Care.

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