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Claudins: emerging targets for cancer therapy

Scott L. Kominsky

The claudin (CLDN) family of transmembrane proteins plays a critical role in the maintenance of epithelial and endothelial tight junctions. In addition to their function in preserving the structure of tight junctions, CLDNs might also play a role in the maintenance of the cytoskeleton and in cell signalling. Interestingly, several studies have recently reported specific CLDN family members to be overexpressed in a wide variety of cancer types. Although their functional role in cancer progression remains unclear, the differential expression of these proteins between tumour and normal cells, in addition to their membrane localisation, makes them prime candidates for cancer therapy. Preclinical studies have shown that tumour cells overexpressing CLDNs can be successfully targeted via several approaches, including the use of anti-CLDN antibodies as well as the cytolytic enterotoxin from *Clostridium perfringens*. Further studies are needed to determine the potential systemic toxicity of this approach considering the ubiquitous expression of CLDNs in the body, but CLDN-targeted therapeutics appear to have promise in the treatment of cancer.

Cancer is the second leading cause of death for all ages in the USA, and the primary cause in individuals under the age of 85 (Ref. 1). Unfortunately, despite advancements in the diagnosis and treatment of cancer, mortality rates have remained essentially unchanged over the past 50 years (Ref. 1). Recent advances in genetic and proteomic analyses have allowed the identification of tumour-specific targets for cancer therapy, ushering in the era of targeted therapeutics. This approach takes advantage of our increased knowledge of cancer cell biology, which enables us to target unique features of cancer cells in order to increase treatment specificity and reduce systemic toxicity relative to conventional radiation and chemotherapy. To date, the majority of targeted therapy has been directed against growth factor receptors, such as ErbB2, which are overexpressed in cancers and easily accessible because of their cell-surface localisation. Recently, a new family of tight-junction proteins named claudins (CLDNs) was discovered. The increased expression of various CLDN family members in cancer combined with their membrane localisation could make these proteins attractive targets for cancer therapy.

Claudins and cell biology

The CLDN family of proteins plays an integral role in the formation and function of tight junctions. Tight junctions are one of four main

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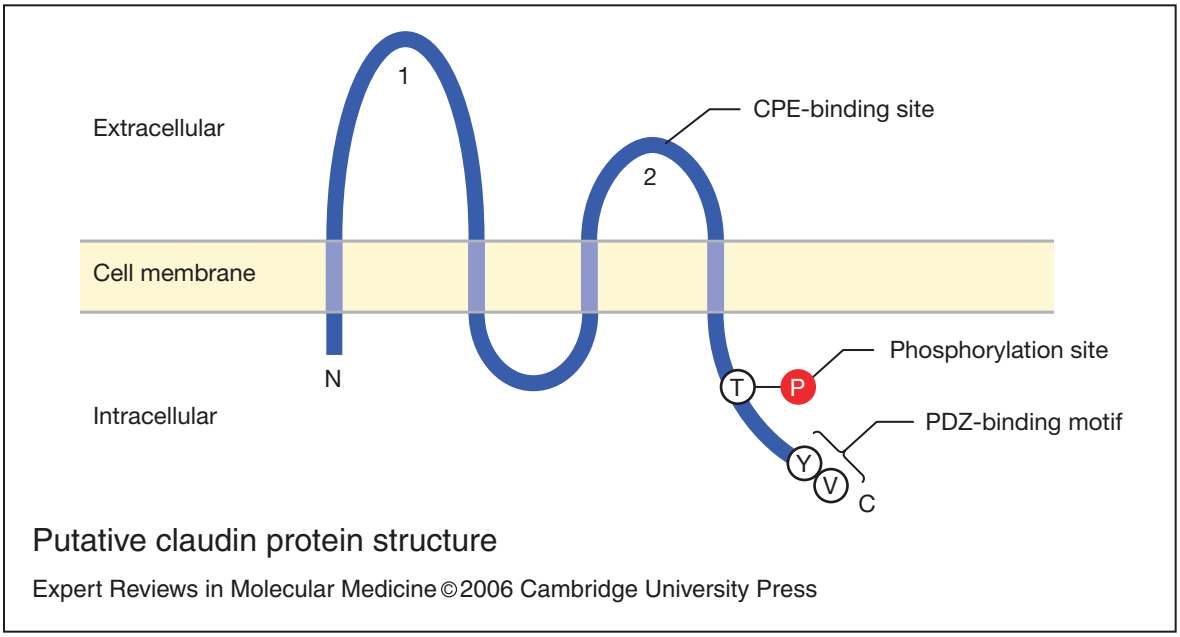


Figure 1. Putative claudin protein structure. The claudin (CLDN) protein consists of intracellular amino (N) and carboxy (C) termini, four transmembrane domains (shaded), and two extracellular loops (1 and 2). The second extracellular loop serves as a binding site for *Clostridium perfringens* enterotoxin (CPE) in CLDN-3 and -4. The C-terminus of the CLDN protein contains a phosphorylation site at threonine 192 (T) and a PDZ-binding motif (YV) consisting of the final two amino acids, through which it can bind to PDZ-containing proteins.

structures regulating cell-to-cell interactions in epithelial and endothelial cell layers. While adherens junctions and desmosomes are primarily involved in cell-to-cell adhesion, and gap junctions in cell-to-cell communication, tight junctions provide a seal between the apical most portions of adjacent basolateral cell membranes (Ref. 2). This seal functions as a barrier to the paracellular transport of solutes and ions in order to maintain concentration gradients regulating transcellular transport. In addition, tight junctions help to maintain cell polarity by preventing the diffusion of membrane proteins from the apical to the basolateral cell membrane.

Tight junctions are composed of cytoplasmic proteins, such as zonula occludens (ZO) 1–3, in addition to three transmembrane proteins: occludin, junctional adhesion molecules (JAMs) and CLDNs (Ref. 3). The CLDN family of proteins was discovered in 1998 (Ref. 4). Since that time, at least 23 human CLDN family members have been identified (Refs 5, 6). CLDNs are expressed in both epithelial and endothelial cells and thus are found throughout the body. Expression of individual CLDN family members can be ubiquitous or cell-type-specific (Ref. 7). Furthermore, individual cells can express either multiple CLDN family members or a single CLDN species (Refs 7, 8). The expression of some CLDNs (e.g. CLDN-6) has also been reported to be developmentally regulated (Ref. 9).

The CLDN protein structure is predicted to consist of cytoplasmic N- and C-termini, four transmembrane domains, and two extracellular loops through which interactions with CLDNs on adjacent cells can occur (Ref. 10) (Fig. 1). Although the tight-junction protein occludin has the same basic tetraspanning structure as CLDN, evidence suggests that CLDNs function as the main sealing proteins of the tight junction (Ref. 11). CLDN family members can interact in both a homo- and heterotypic fashion, likely enabling diversity in the structure and function of tight junctions (Ref. 10). The sealing function of CLDNs at tight junctions is also probably mediated, in part, through phosphorylation events. The cytoplasmic C-terminus of CLDNs contains several phosphorylation sites. Phosphorylation of CLDNs in this region may be mediated by

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various proteins such as mitogen-activated protein kinase and cyclic-AMP-dependent protein kinase, resulting in increased permeability of tight junctions, potentially through the removal of CLDN from the tight junction (Refs 12, 13).

In addition to phosphorylation sites, the cytoplasmic C-terminal region of CLDN contains a PDZ-binding motif, through which PDZ-domain-containing proteins such as the tight-junction proteins ZO-1, -2 and -3 can bind. ZO proteins function as scaffolding proteins and have been shown to bind to cytoskeletal proteins such as β-actin as well as the transcription factor ZONAB, which regulates ErbB2 expression (Ref. 14). In addition, CLDNs may bind to the PDZ-domain-containing proteins partitioning-defective protein 3 (Ref. 15), which functions in asymmetric cell division, and membrane-associated guanylate kinase inverted 2, implicated in the regulation of the tumour suppressor PTEN (Ref. 16). Thus, in addition to their specialised role in cell–cell adhesion, CLDNs may play an indirect role in cytoskeletal and cell signalling events, perhaps by regulating the localisation of proteins involved in these processes.

Claudins and cancer

The role of CLDNs in carcinogenesis and progression to metastasis is an active area of investigation as a result of the frequent finding of altered CLDN expression in cancer. The association between altered CLDN expression and cancer was first reported shortly after the discovery of CLDNs: in one of the earliest reports, CLDN-1 mRNA expression was found to be reduced in human breast cancer cell lines relative to normal human mammary epithelial cells (Ref. 17). Currently, the over- or underexpression of at least one of eight CLDN family members has been reported in more than 12 cancer types (Table 1). With more than 20 known CLDN family members, the number of CLDN species found to be aberrantly expressed in cancer is likely to increase.

To date, the majority of studies have focused on CLDN-1, -3, -4, -7 and -10. For some CLDN

Table 1. Differential expression of claudins in human cancer

Cancer type	Claudin expression	Change	Refs
Breast	3, 4	↑	26
	1, 2, 4, 7	↓	17, 18, 65, 66
Prostate	3, 4	↑	25
Ovarian	3, 4, 16	↑	31, 32, 54, 67
Pancreatic	4	↑	27, 28, 29, 30
Colon	1	↑	24, 43
Gastric	4, 7	↑	20, 40
	4, 18	↓	68, 69
Thyroid	10	↑	33
Head and neck	7	↓	19
Hepatocellular	10	↑	34
Cervical	1, 7	↑	21
Glioblastoma multiforme	1	↓	22
Melanoma	1	↓	23

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species, the shift in CLDN expression observed between normal and cancerous states is cancer-specific. For example, CLDN-7 expression is lost in cancers of the breast (Ref. 18) and head and neck (Ref. 19), but increased in gastric cancer (Ref. 20) and cervical cancer (Ref. 21). Similarly, CLDN-1 expression is lost in breast cancer (Ref. 17), glioblastoma multiforme (Ref. 22) and melanoma (Ref. 23), although increased in colon cancer (Ref. 24) and cervical cancer (Ref. 21). By contrast, CLDN-3, -4 and -10 are predominantly reported as overexpressed in cancers. CLDN-3 and -4 are overexpressed in primary and metastatic cancers of the prostate (Ref. 25), breast (Refs 18, 26), pancreas (Refs 27, 28, 29, 30) and ovary (Refs 31, 32), while CLDN-10 expression is increased in both thyroid (Ref. 33) and hepatocellular carcinoma (Ref. 34).

The mechanisms responsible for the differential expression of CLDNs in cancer are largely unknown; however, recent studies have identified several growth factors, cytokines and transcription factors that affect CLDN expression. The tumour-promoting factors hepatocyte growth factor and epidermal growth factor have been shown to decrease CLDN-7 expression and increase CLDN-1, -3 and -4 expression, respectively (Refs 18, 35). Exposure to the cytokines tumour necrosis factor α, interleukin 1β (IL-1β) and IL-17 can cause increased CLDN-1 expression (Refs 36, 37). The transcriptional repressor Snail, known to induce epithelial–mesenchymal transition (EMT), has been shown to directly bind to E-boxes in the CLDN promoter, completely repressing gene expression (Ref. 38). In addition to the observed changes in CLDN expression, CLDNs may also be mislocalised in poorly differentiated cancers as a result of the lack of formation of tight junctions. Immunohistochemical analysis shows that CLDN-4 is expressed on the basolateral cell membrane at sites of cell–cell contact in normal mammary epithelium, but is circumferentially expressed in poorly differentiated human breast cancers even at sites lacking cell–cell contact (Scott L. Kominsky, unpublished).

While the aberrant expression of CLDNs in cancer has been well established, whether CLDNs play a functional role in tumourigenesis and cancer progression remains unclear. Based on the role of CLDNs in the formation of tight junctions, it has been hypothesised that the loss of CLDN expression may reduce cell adhesion and thereby increase tumour cell motility and invasive potential. In line with this hypothesis, a correlation between the loss of CLDN-7 expression and cellular discohesion has been reported in breast cancer (Ref. 18). In addition, EMT, associated with increased motility and invasiveness, has been associated with a loss of CLDN expression (Refs 38, 39). EMT may be initiated by factors such as transforming growth factor β, whose stimulation of the extracellular-signal-regulated kinase pathway and the transcriptional repressor Snail has been implicated in the loss of CLDN expression (Ref. 39). In addition, in vitro studies in gastric and pancreatic cancer show that overexpression of CLDN-4 in cancer cell lines decreases motility, invasiveness and anchorage-independent growth (Refs 40, 41). In animal experiments, pancreatic cancer cells overexpressing CLDN-4 formed fewer pulmonary metastases following intravenous injection in mice (Ref. 41).

Paradoxically, overexpression of CLDN-3 or -4 in human ovarian surface epithelial cells was reported to increase motility and invasiveness, whereas small interfering RNA (siRNA) knockdown of CLDN-3 and -4 in ovarian cancer cell lines inhibited invasive ability (Ref. 42). Consistent with these findings, overexpression of CLDN-1 in colon cancer cells resulted in increased tumour growth and metastasis in vivo, whereas siRNA knockdown of CLDN-1 in metastatic colon cancer cells inhibited migration and invasion (Ref. 43). Interestingly, CLDN-1 overexpression induced morphological changes characteristic of EMT concordant with the expression of vimentin and fibroblast-specific protein 1. In addition, the overexpression of CLDN-1, -3 and -4 in these studies was reported to increase the activity of the matrix metalloproteinases MMP-2 and MMP-9. The apparently contradicting role of CLDNs in cancer cell biology suggests that CLDNs may have cell-specific effects. It is also possible that a balance of CLDN expression is physiologically necessary, with a shift in either direction resulting in the disruption of the function of tight junctions and alterations in cellular behaviour. This is supported by studies in transgenic mice where both CLDN-1 knock-out and CLDN-6 overexpression were lethal due to a defect in epithelial barrier function (Refs 44, 45).

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Claudins and therapeutics

Irrespective of their role in cancer progression, CLDNs hold great promise as targets for therapeutic intervention. The potential value of CLDNs in this regard has been the subject of a number of investigations and is based on several key points. First, CLDNs are expressed at the cell surface and contain two extracellular domains that serve as potential target sites (Fig. 1). Second, at least one CLDN species has been reported to be overexpressed in nine of 12 cancer types, creating a differential expression between tumour and normal cells. Third, CLDNs might be more accessible in tumours, where tight-junction permeability is increased relative to that in normal tissues (Ref. 46); thus, a therapeutic window may be achieved even in the absence of CLDN overexpression in tumour tissue.

Antibody-based therapy

One approach to targeting CLDNs in cancer therapy is the use of antibody-based therapeutics. This approach has gained favour in recent years owing to the potential for increased tumour specificity and lower toxicity profiles. Tumour lysis can be achieved by the attachment of radionuclides or toxins, or by stimulating immune mechanisms (Fig. 2). Anti-CLDN antibodies used for cell biology studies routinely target the C-terminus since this region is one of the least conserved among the CLDN family members. Recently, the first antibodies to the extracellular domain of CLDN-3 and -4 were generated (Ref. 47). These antibodies were capable of binding to the surface of nonpermeabilised breast and colon cancer cells that express CLDN-3 and -4, but not to the surface of cells of monocytic origin, which lack CLDN expression. Furthermore, detection of disseminated breast cancer cells in bone marrow by immunocytochemical staining using CLDN-3 antibody was equivalent to that by a cytokeratin-specific monoclonal antibody commonly used for this purpose. These data not only confirm the continued expression of CLDNs by disseminated tumour cells, but also demonstrate the feasibility of targeting surface CLDN. Whether CLDN-3 and -4 antibodies are taken up into cells following binding remains unknown, although claudins have been shown to undergo endocytosis during tight-junction remodelling (Ref. 48). The endocytosis of CLDN-targeted antibody would expand the therapeutic possibilities to include conjugation to agents such as *Pseudomonas* exotoxin, which require cellular uptake to elicit cytotoxic effects.

Clostridium perfringens enterotoxin-based therapy

Another approach to targeting CLDNs for cancer therapy involves the use of *Clostridium perfringens* enterotoxin (CPE). CPE is commonly associated with *C. perfringens* type A food poisoning and is capable of inducing cytolysis of mammalian cells in as little as 5 min (Ref. 49). Interestingly, it was recently discovered that CLDN-3 and -4 serve as the receptors for CPE (Refs 50, 51). CPE binding to the second extracellular domain of CLDN-3 or -4 triggers the formation of a large multiprotein membrane-pore complex, which disrupts cellular osmotic equilibrium, causing cell lysis (Ref. 52). Although CPE has been shown to bind to other CLDN family members, albeit with lower affinity, only binding to CLDN-3 or -4 results in membrane-pore formation. This is illustrated by the fact that cells lacking CLDN-3 and -4 are completely unaffected by the toxin (Ref. 53).

The consistent presence or overexpression of CLDN-3 and/or -4 in pancreatic, prostate, breast and ovarian cancers makes them ideal candidates for CPE-based therapy. Furthermore, CPE might be particularly useful in the setting of chemotherapy resistance because chemotherapy-resistant ovarian tumours have been reported to express elevated levels of CLDN-3 and -4 relative to chemotherapy-naive tumours (Refs 54, 55). CPE treatment of human cancer cell lines in vitro has been shown to cause cytolysis in a dose-dependent fashion, killing virtually 100% of cells in as little as 30 min (Refs 26, 27, 54). As might be expected, cells expressing high levels of CLDN-3 or -4 were more sensitive to CPE-mediated cytolysis, whereas those lacking CLDN-3 and -4 were unharmed. The cytolytic effect of CPE does not appear to be unique to cancer cell lines, as increased necrosis has been observed in freshly resected breast tumour tissue following CPE treatment (Ref. 26). In addition, the in vivo efficacy of CPE therapy has been investigated in several cancer types using murine xenograft models. Intra-tumoural administration of CPE in pancreatic and breast cancer models

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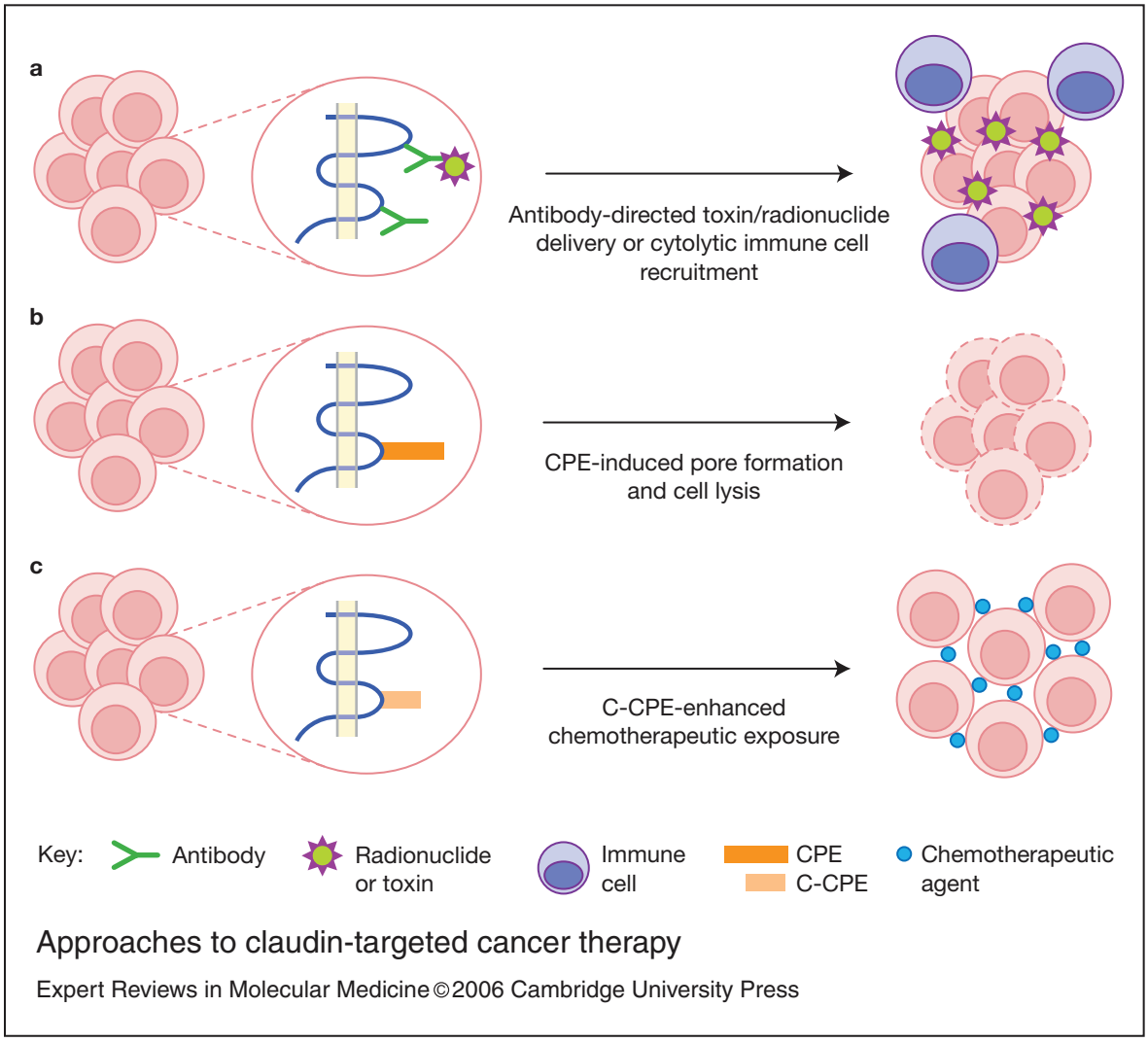


Figure 2. Approaches to claudin-targeted cancer therapy. Tumour cytolysis may be achieved directly via: (a) antibody-directed delivery of cytotoxic agents; or (b) binding of *Clostridium perfringens* enterotoxin (CPE), which binds to the second extracellular domain of claudin (CLDN)-3 and -4 and induces the formation of pores in the cell membrane. Indirect cytolysis may be achieved by: (a) stimulation of an antibody-mediated immune response; or (c) binding of C-terminal CPE fragments (C-CPE), which increase tight-junction permeability and thus enhance exposure to standard chemotherapeutic agents.

significantly inhibited tumour growth or caused tumour regression concomitant with a large degree of tumour necrosis (Refs 26, 27). In a more clinically relevant model, intraperitoneal administration of CPE significantly inhibited the growth of freshly explanted human ovarian cancer in severe combined immunodeficiency (SCID) mice (Ref. 54). The efficacy of CPE treatment was dependent on the initial tumour burden. In the case of low tumour burden, 100% of mice treated with CPE remained tumour-free for the study duration, of more than 17 weeks, whereas no mice survived beyond 9 weeks in the absence of treatment.

In addition to its ability to directly induce cytolysis, CPE may be useful in targeting cytotoxic agents such as radionuclides and toxins to tumour cells expressing CLDN-3 and -4. The C-terminal 30 amino acids of CPE have been shown to be sufficient for binding to CLDN-3 and -4, but insufficient to induce cytolysis (Ref. 56). Attachment of the cytotoxic protein synthesis inhibitory factor (PSIF) to such a C-terminal fragment of CPE (C-CPE)

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resulted in a fusion protein capable of inducing cytolysis in breast cancer cells expressing CLDN-3 and -4 (Ref. 57). Furthermore, C-CPE–PSIF had no effect on cells lacking CLDN-3 and -4 expression despite expression of other CLDN family members. Importantly, since PSIF must enter the cytosol to induce cytolysis, these data suggest that C-CPE is internalised following binding to CLDN-3 or -4, likely through clathrin-mediated endocytosis (Ref. 58). Another potential use of C-CPE is in the enhancement of standard chemotherapy. Exposure of MDCK I cells to C-CPE resulted in the removal of CLDN-4, disintegration of tight-junction strands, and an increase in tight-junction permeability (Ref. 59). Thus, when combined with chemotherapy, C-CPE might allow greater penetration of solid tumours, thereby increasing therapeutic efficacy. This effect might also be achieved through the use of tight-junction-altering absorption enhancers such as sodium caprate (Ref. 60), although the more global effect of these agents may be undesirable.

Clinical implication and applications

Advances in molecular biology and proteomics have given rise to the development of targeted therapeutics. The ability to direct therapy to specific molecular targets on cancer cells provides several advantages over standard chemotherapy, including higher specificity and reduced systemic toxicity. Indeed, therapeutics targeting growth factor receptors (e.g. Herceptin®) and cell signalling proteins (e.g. Gleevec®) have shown clinical success in the treatment of cancer (Ref. 61).

The large number of cancer types that overexpress various CLDN family members, along with the cell-surface localisation of CLDNs, makes CLDNs attractive molecular targets for cancer therapy. However, the clinical application of CLDN-targeted therapeutics faces several obstacles. Common to all currently conceived CLDN-targeted therapeutics is the issue of systemic toxicity. Since CLDNs are expressed throughout the body in the epithelia of both vital and non-vital organs, long-term toxicity studies must be conducted. Furthermore, agents designed to disrupt tight junctions, such as C-CPE, might be double-edged swords: whereas the resulting increase in tight-junction permeability may be beneficial in providing enhanced uptake of chemotherapeutic agents, it may also support tumour progression by permitting greater nutritional uptake or by enhancing tumour cell shedding.

To date, CPE has been the most frequently studied CLDN-targeted therapeutic and its ability to rapidly lyse tumour cells of several cancer types has been demonstrated. However, in addition to the potential dose-limiting toxicity to organs expressing CLDN-3 or -4, such as the colon and lung, systemic exposure to CPE may induce toxic shock. Studies in mice show that intravenous administration of as little as 0.75 mg/kg CPE can result in death within 1–2 h, concordant with an increase in the pro-inflammatory cytokines interferon γ, IL-1 and IL-6 (Ref. 62). Therefore, CPE might be best suited to local administration such as in the intraductal treatment of breast carcinoma in situ (Ref. 63) or intraperitoneal treatment of ovarian cancer (Ref. 54). Another potential complication in the use of CPE-based therapeutics is the development of a neutralising antibody response. Since all preclinical studies of CPE efficacy in cancer therapy to date have utilised immunodeficient mice, this event could not be examined. Interestingly, a sole study in healthy human volunteers found that ingestion of CPE did elicit an antibody response, although this provided no protection against subsequent CPE challenge (Ref. 64). While this finding is promising, the development of neutralising antibodies following CPE exposure by other routes must be tested.

Outstanding research questions and future prospects

Although several cancers may be amenable to local administration of CPE, its utility in the treatment of others, such as those of the prostate and pancreas, necessitates systemic administration. Thus, the key question to be addressed is how to administer CLDN-targeted therapeutics while avoiding systemic toxicity. Evidence suggests that the acute shock response elicited following systemic exposure to CPE might be due to the stimulation of macrophages (Ref. 62). Currently the nature of this interaction is unknown, but it is not likely to involve CLDN-3 and -4 because macrophages have not been reported to express these molecules. Thus, it would be interesting to determine the region of CPE responsible for macrophage stimulation

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and whether it could be mutated or deleted such that the ability of CPE to induce cytolysis of cells expressing CLDN-3 and -4 remains intact while macrophage stimulation is inhibited. Another possible means of permitting systemic administration of CPE-based therapeutics is to use a prodrug approach. Here, a latent drug may be targeted to CLDNs via attachment to C-CPE and activated at the tumour site by a tumour-specific enzyme. Although this approach promises a high level of therapeutic specificity, its utility is complicated by the challenge of identifying suitable tumour-specific enzymes for drug activation. If systemic toxicity cannot be avoided, perhaps it can be lessened. For example, C-CPE, innocuous alone, may be used to block CLDN-3 and -4 at potential sites of toxicity by local administration (Ref. 56).

While CLDNs have great potential as targets for cancer therapy, studies examining their expression pattern in cancer and normal tissues, as well as their clinical utility, are in their infancy. Several studies examining CLDN expression have utilised cell lines or relied on microarray analysis followed by validation in a small number of tissue samples. Examination of CLDN expression in cell lines can be misleading since placing cells in culture has been reported to affect CLDN expression profoundly (Ref. 18). Furthermore, expression of most of the 23 human CLDN family members has yet to be examined in cancer or normal tissues. Therefore, further studies are needed to examine more precisely the expression of CLDNs in normal tissues and during the course of cancer progression. Although the concept of CLDN-targeted therapy has been demonstrated in animal models, given the ubiquitous expression of CLDNs, continued preclinical testing is required to examine more closely the systemic toxicity associated with this approach and to determine whether it can be applied practically and safely in a clinical setting.

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References

1 National Center for Health Statistics (2005) Health, United States, 2005, with Chartbook on Trends in the Health of Americans, Centers for Disease Control and Prevention, Hyattsville, MD, USA
2 Mitic, L.L. and Anderson, J.M. (1998) Molecular architecture of tight junctions. Annu Rev Physiol 60, 121-142
3 Mitic, L.L., Van Itallie, C.M. and Anderson, J.M. (2000) Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. Am J Physiol Gastrointest Liver Physiol 279, G250-G254
4 Furuse, M. et al. (1998) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141, 1539-1550
5 National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov.
6 Katoh, M. and Katoh, M. (2003) CLDN23 gene, frequently down-regulated in intestinal-type gastric cancer, is a novel member of CLAUDIN gene family. Int J Mol Med 11, 683-689
7 Van Itallie, C.M. and Anderson, J.M. (2006) Claudins and epithelial paracellular transport. Annu Rev Physiol 68, 403-429
8 Morita, K. et al. (1999) Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. J Cell Biol 145, 579-588
9 Turksen, K. and Troy, T.C. (2001) Claudin-6: a novel tight junction molecule is developmentally regulated in mouse embryonic epithelium. Dev Dyn 222, 292-300
10 Furuse, M., Sasaki, H. and Tsukita, S. (1999) Manner of interaction of heterogeneous claudin species within and between tight junction strands. J Cell Biol 147, 891-903
11 Furuse, M. and Tsukita, S. (2006) Claudins in occluding junctions of humans and flies. Trends Cell Biol 16, 181-188
12 Tanaka, M., Kamata, R. and Sakai, R. (2005) EphA2 phosphorylates the cytoplasmic tail of Claudin-4 and mediates paracellular permeability. J Biol Chem 280, 42375-42382
13 D'Souza, T., Agarwal, R. and Morin, P.J. (2005) Phosphorylation of claudin-3 at threonine 192 by cAMP-dependent protein kinase regulates tight junction barrier function in ovarian cancer cells. J Biol Chem 280, 26233-26240

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14 Balda, M.S. and Matter, K. (2000) The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. Embo J 19, 2024-2033
15 Lin, D. et al. (2000) A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat Cell Biol 2, 540-547
16 Wu, Y. et al. (2000) Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. J Biol Chem 275, 21477-21485
17 Swisshelm, K. et al. (1999) SEMP1, a senescence-associated cDNA isolated from human mammary epithelial cells, is a member of an epithelial membrane protein superfamily. Gene 226, 285-295
18 Kominsky, S.L. et al. (2003) Loss of the tight junction protein claudin-7 correlates with histological grade in both ductal carcinoma in situ and invasive ductal carcinoma of the breast. Oncogene 22, 2021-2033
19 Al Moustafa, A.E. et al. (2002) Identification of genes associated with head and neck carcinogenesis by cDNA microarray comparison between matched primary normal epithelial and squamous carcinoma cells. Oncogene 21, 2634-2640
20 Johnson, A.H. et al. (2005) Expression of tight-junction protein claudin-7 is an early event in gastric tumorigenesis. Am J Pathol 167, 577-584
21 Lee, J.W. et al. (2005) Increased expressions of claudin-1 and claudin-7 during the progression of cervical neoplasia. Gynecol Oncol 97, 53-59
22 Liebner, S. et al. (2000) Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. Acta Neuropathol (Berl) 100, 323-331
23 Cohn, M.L. et al. (2005) Loss of claudin-1 expression in tumor-associated vessels correlates with acquisition of metastatic phenotype in melanocytic neoplasms. J Cutan Pathol 32, 533-536
24 Miwa, N. et al. (2000) Involvement of claudin-1 in the beta-catenin/Tcf signaling pathway and its frequent upregulation in human colorectal cancers. Oncol Res 12, 469-476
25 Long, H. et al. (2001) Expression of Clostridium perfringens enterotoxin receptors claudin-3 and claudin-4 in prostate cancer epithelium. Cancer Res 61, 7878-7881
26 Kominsky, S.L. et al. (2004) Clostridium perfringens enterotoxin elicits rapid and specific cytolysis of breast carcinoma cells mediated through tight junction proteins claudin 3 and 4. Am J Pathol 164, 1627-1633
27 Michl, P. et al. (2001) Claudin-4: a new target for pancreatic cancer treatment using Clostridium perfringens enterotoxin. Gastroenterology 121, 678-684
28 Ryu, B. et al. (2002) Relationships and differentially expressed genes among pancreatic cancers examined by large-scale serial analysis of gene expression. Cancer Res 62, 819-826
29 Nichols, L.S., Ashfaq, R. and Iacobuzio-Donahue, C.A. (2004) Claudin 4 protein expression in primary and metastatic pancreatic cancer: support for use as a therapeutic target. Am J Clin Pathol 121, 226-230
30 Sato, N. et al. (2004) Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. Am J Pathol 164, 903-914
31 Hough, C.D. et al. (2000) Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. Cancer Res 60, 6281-6287
32 Rangel, L.B. et al. (2003) Tight junction proteins claudin-3 and claudin-4 are frequently overexpressed in ovarian cancer but not in ovarian cystadenomas. Clin Cancer Res 9, 2567-2575
33 Aldred, M.A. et al. (2004) Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. J Clin Oncol 22, 3531-3539
34 Cheung, S.T. et al. (2005) Claudin-10 expression level is associated with recurrence of primary hepatocellular carcinoma. Clin Cancer Res 11, 551-556
35 Singh, A.B. and Harris, R.C. (2004) Epidermal growth factor receptor activation differentially regulates claudin expression and enhances transepithelial resistance in Madin-Darby canine kidney cells. J Biol Chem 279, 3543-3552
36 Han, X., Fink, M.P. and Delude, R.L. (2003) Proinflammatory cytokines cause NO*-dependent and -independent changes in expression and localization of tight junction proteins in intestinal epithelial cells. Shock 19, 229-237
37 Kinugasa, T. et al. (2000) Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology 118, 1001-1011
38 Ikenouchi, J. et al. (2003) Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression

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https://doi.org/10.1017/S1462399406000056 Published online by Cambridge University Press

of claudins/occludin by Snail. J Cell Sci 116, 1959-1967
39 Medici, D., Hay, E.D. and Goodenough, D.A. (2006) Cooperation between snail and LEF-1 transcription factors is essential for TGF-beta1-induced epithelial-mesenchymal transition. Mol Biol Cell 17, 1871-1879
40 Mima, S. et al. (2005) Induction of claudin-4 by nonsteroidal anti-inflammatory drugs and its contribution to their chemopreventive effect. Cancer Res 65, 1868-1876
41 Michl, P. et al. (2003) Claudin-4 expression decreases invasiveness and metastatic potential of pancreatic cancer. Cancer Res 63, 6265-6271
42 Agarwal, R., D'Souza, T. and Morin, P.J. (2005) Claudin-3 and claudin-4 expression in ovarian epithelial cells enhances invasion and is associated with increased matrix metalloproteinase-2 activity. Cancer Res 65, 7378-7385
43 Dhawan, P. et al. (2005) Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer. J Clin Invest 115, 1765-1776
44 Furuse, M. et al. (2002) Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 156, 1099-1111
45 Turksen, K. and Troy, T.C. (2002) Permeability barrier dysfunction in transgenic mice overexpressing claudin 6. Development 129, 1775-1784
46 Soler, A.P. et al. (1999) Increased tight junctional permeability is associated with the development of colon cancer. Carcinogenesis 20, 1425-1431
47 Offner, S. et al. (2005) Epithelial tight junction proteins as potential antibody targets for pancarcinoma therapy. Cancer Immunol Immunother 54, 431-445
48 Matsuda, M. et al. (2004) A peculiar internalization of claudins, tight junction-specific adhesion molecules, during the intercellular movement of epithelial cells. J Cell Sci 117, 1247-1257
49 McClane, B.A. and McDonel, J.L. (1979) The effects of Clostridium perfringens enterotoxin on morphology, viability, and macromolecular synthesis in Vero cells. J Cell Physiol 99, 191-200
50 Katahira, J. et al. (1997) Clostridium perfringens enterotoxin utilizes two structurally related membrane proteins as functional receptors in vivo. J Biol Chem 272, 26652-26658
51 Morita, K. et al. (1999) Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc Natl Acad Sci U S A 96, 511-516
52 McClane, B.A. (2001) The complex interactions between Clostridium perfringens enterotoxin and epithelial tight junctions. Toxicon 39, 1781-1791
53 Horiguchi, Y., Uemura, T., Kozaki, S., Sakaguchi, G. (1985) The relationship between cytotoxic effects and binding to mammalian culture cells of Clostridium perfringens enterotoxin. FEMS Microbiol Let 28, 131-135
54 Santin, A.D. et al. (2005) Treatment of chemotherapy-resistant human ovarian cancer xenografts in C.B-17/SCID mice by intraperitoneal administration of Clostridium perfringens enterotoxin. Cancer Res 65, 4334-4342
55 Stewart, J.J. et al. (2006) Proteins associated with Cisplatin resistance in ovarian cancer cells identified by quantitative proteomic technology and integrated with mRNA expression levels. Mol Cell Proteomics 5, 433-443
56 Hanna, P.C. et al. (1991) Localization of the receptor-binding region of Clostridium perfringens enterotoxin utilizing cloned toxin fragments and synthetic peptides. The 30 C-terminal amino acids define a functional binding region. J Biol Chem 266, 11037-11043
57 Ebihara, C. et al. (2006) Preparation of a claudin-targeting molecule using a C-terminal fragment of Clostridium perfringens enterotoxin. J Pharmacol Exp Ther 316, 255-260
58 Ivanov, A.I., Nusrat, A. and Parkos, C.A. (2004) Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. Mol Biol Cell 15, 176-188
59 Sonoda, N. et al. (1999) Clostridium perfringens enterotoxin fragment removes specific claudins from tight junction strands: Evidence for direct involvement of claudins in tight junction barrier. J Cell Biol 147, 195-204
60 Soderholm, J.D. et al. (1998) Reversible increase in tight junction permeability to macromolecules in rat ileal mucosa in vitro by sodium caprate, a constituent of milk fat. Dig Dis Sci 43, 1547-1552
61 Kumar, C.C. and Madison, V. (2001) Drugs targeted against protein kinases. Expert Opin Emerg Drugs 6, 303-315
62 Wallace, F.M. et al. (1999) Evidence for Clostridium perfringens enterotoxin (CPE) inducing a mitogenic and cytokine response in vitro and a cytokine response in vivo. Curr Microbiol 38, 96-100

Accession information: DOI: 10.1017/S1462399406000056; Vol. 8; Issue 18; August 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000056 Published online by Cambridge University Press

63 Murata, S. et al. (2006) Ductal access for prevention and therapy of mammary tumors. Cancer Res 66, 638-645
64 Skjelkvale, R. and Uemura, T. (1977) Experimental diarrhoea in human volunteers following oral administration of Clostridium perfringens enterotoxin. J Appl Bacteriol 43, 281-286
65 Soini, Y. (2005) Expression of claudins 1, 2, 3, 4, 5 and 7 in various types of tumours. Histopathology 46, 551-560
66 Tokes, A.M. et al. (2005) Claudin-1, -3 and -4 proteins and mRNA expression in benign and malignant breast lesions: a research study. Breast Cancer Res 7, R296-305
67 Rangel, L.B. et al. (2003) Characterization of novel human ovarian cancer-specific transcripts (HOSTs) identified by serial analysis of gene expression. Oncogene 22, 7225-7232
68 Lee, S.K. et al. (2005) Loss of the tight junction protein claudin 4 correlates with histological growth-pattern and differentiation in advanced gastric adenocarcinoma. Oncol Rep 13, 193-199
69 Sanada, Y. et al. (2006) Down-regulation of the claudin-18 gene, identified through serial analysis of gene expression data analysis, in gastric cancer with an intestinal phenotype. J Pathol 208, 633-642

Further reading, resources and contacts

Van Itallie, C.M. and Anderson, J.M. (2006) Claudins and epithelial paracellular transport. Annu Rev Physiol 68, 403-429

Schaedel, O. and Reiter, Y. (2006) Antibodies and their fragments as anti-cancer agents. Curr Pharm Des 12, 363-378

Information on murine orthologs of CLDNs are collected at the Mouse Genome Informatics website:

http://www.informatics.jax.org/mgihome/nomen/genefamilies/claudin.shtml

Author's institute URL: www.hopkinsmedicine.org/som/index.html

Features associated with this article

Figures
Figure 1. Putative claudin protein structure.
Figure 2. Approaches to claudin-targeted cancer therapy.

Table
Table 1. Differential expression of claudins in human cancer.

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