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Tight Junction Proteins: From Barrier to Tumorigenesis

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Abstract

The tight junction is a multi-protein complex and is the apical most junctional complex in certain epithelial and endothelial cells. A great deal of attention has been devoted to the understanding of these proteins in contributing to the barrier function - that is, regulating the paracellular flux or permeability between adjacent cells. However, tight junction proteins are now recognized as having functions beyond the barrier. The focus of this review is to discuss the barrier function of the tight junction and to summarize the literature with a focus on the role of tight junction proteins in proliferation, transformation, and metastasis.

Keywords

occludin; claudin; tight junction; cancer; metastasis

1. Introduction

The tight junction (TJ) complex is the apical most junctional complex in many types of epithelial and endothelial cells. The TJ can be sub-divided into the integral membrane and cytoplasmic proteins. Occludin, tricellulin, marvelD3, and the claudins (of which there are 27 members [1]) are tetra-spanning membrane proteins whose N- and C-termini reside in the cytosol and each possesses two extracellular loop regions. Occludin, tricellulin, and marvelD3 each contain a MARVEL (MAL-related proteins for vesicle trafficking and membrane link) domain, whereas the claudins do not. The junctional adhesion molecules (JAMs) are single pass membrane proteins with two IgG-like motifs. The cytoplasmic adaptor proteins are the zonula occludens or ZO proteins, and are designated ZO-1, -2, and -3. These proteins link the membrane proteins to the actin cytoskeleton. Collectively, the TJ imparts two functions in the cell: a barrier function, namely regulating the permeability of solutes between adjacent cells, and a fence function, controlling the lateral diffusion of proteins within the lipid bilayer [2, 3]. Traditionally, research efforts focused on the barrier and fence functions; however, there is a new movement in the field, which is to understand how TJ proteins participate in cell proliferation, transformation, and metastasis suppression.

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Conflict of Interest Statement

None

The focus of this review shall be to briefly orient the reader with TJ proteins by describing their traditional roles followed by a summary of the aforementioned novel functions of this class of proteins. In addition to the aspects of TJ proteins to be discussed in this review, these proteins are also key components to cell signaling events; for a review of the role of TJ proteins with regards to signaling and gene expression, please see Balda and Matter [4].

2. Traditional functions of tight junction proteins

2.1. ZO proteins

ZO-1 was the first TJ protein described and ZO-2 and -3 were subsequently identified by co-immunoprecipitation studies [5–9]. ZO proteins are classified as members of the membrane associated guanylate kinase (MAGuK) family and are composed of three postsynaptic density 95/disc-large/zona occludens (PDZ) domains and one Src homology (SH3) and GuK domain [10]. Via fusion of biotin ligate to either the N- or C-terminus of ZO-1, it was found that the ends of the ZO-1 protein are embedded in different functional sub-compartments of the TJ [11]. The manner in which the ZO proteins interact with the membrane proteins appears to be specific to each type of membrane protein. The unique-5 (U5) region of ZO-1, located between the SH3 and GuK domains, is responsible for ZO-1 localization to the TJ and for the interaction with the distal C-terminus of occludin [12–14]. With the exception of claudin-12, the conserved C-terminal YV motif of claudins interact with the PDZ domains of ZO proteins through a conserved C-terminal YV motif [15]. Moreover, the C-terminus of JAMs contains a PDZ domain-binding motif, which interacts with ZO-1 [16]. ZO-1 and ZO-2 are critical to junction assembly [17, 18] and permeability [19], respectively, and in the absence of ZO-1 and -2, cells fail to form TJs [20]. Both ZO-1 and -2 knockouts are embryonic lethal in mice due to apoptosis and reduced yolk sac angiogenesis and proliferation [21, 22].

2.2. Occludin

Occludin was the first transmembrane TJ protein discovered [23] and its function in the TJ remains to be completely understood. Occludin overexpression increases electrical resistance, implying a pro-barrier phenotype; yet, occludin overexpression increases permeability to small molecule tracers [24]. While the occludin-null mouse forms intact TJs, the mice exhibited a variety of abnormal phenotypes including postnatal growth retardation, thinning of compact bone, calcification in the brain, testicular atrophy, male infertility, loss of cytoplasmic granuoles in salivary epithelial cells, females not suckling their young, and gastric inflammation and hyperplasia [25]. Silencing of occludin *in vitro* increases permeability to divalent organic cations and also to small molecules under hydrostatic pressure [26, 27]. Furthermore, in the microvascular endothelial cells of the retina, occludin regulates vascular endothelial growth factor (VEGF)-induced permeability through its phosphorylation and subsequent ubiquitination *in vitro* and *in vivo* [28, 29]. Shen and Turner demonstrated a clear network between the actin cytoskeleton and the TJ as actin depolymerization results in a loss of barrier function mediated through caveolae-dependent occludin internalization [30]. Furthermore, caveolin-1-dependent occludin endocytosis is necessary for the tumor necrosis factor-1 mediated loss of barrier function [31]. Clearly, occludin is a dynamic protein at the TJ.

2.3. Claudins

The claudin family is regarded as the backbone of the TJ [32]. Interestingly, multiple claudin family members are able to co-exist in the same tight junction strand while other combinations of claudins fail to do so [33]. Claudins interact with other claudins in the same cell through their N-terminal extracellular loops (*cis*-interactions) while claudins interact with claudins in adjacent cells through their C-terminal extracellular loops (*trans*-

interactions) [34]. This *cis*- and *trans*-interaction leads to the formation of a “zipper”-like structure, thus describing the claudin-driven barrier.

Support for claudins as the major drivers of TJ formation is derived largely in part from the fact that the occludin null mouse is viable and genetic ablation of claudin proteins results in deleterious barrier-specific phenotypes. Deletion of claudin-1 compromises the epidermal barrier and is lethal within one day due to excessive water loss [35]. The claudin-5 knockout mouse has severe brain hemorrhaging and dies within 10 hours after birth [36]. The claudin-11 knockout is viable; however, the mouse has hind limb weakness, slowed conductive velocities of the central nervous system, and male sterility [37]. In humans, mutations in claudins-16 and 19 are associated with hypomagnesemia [38] and renal magnesium wasting [39], respectively. Analysis of claudin-11 [40] and -14 [41] knockout mice revealed that these proteins are indispensable for maintenance of endocochlear potential and cochlear hair cells, respectively, and loss of either leads to deafness. The claudin-15 knockout will be discussed in the following section. Undeniably, claudins are essential to the formation of the TJ and proper TJ function is of the upmost importance.

2.4. Junctional adhesion molecules (JAMs)

The JAMs (~40kDa) are a group of proteins that are subdivided as JAM-1 (or A), -2 (or B), and -3 (or C). JAM-A is a mediator of barrier formation [42, 43] and function [44, 45]. JAM-A is also crucial to polarity [46], potentially through interactions with the polarity protein PAR-3 [47, 48]. Further, Laukoetter *et al.* found that JAM-A knockout mice exhibit increased polymorphonuclear leukocyte infiltration and, consistent with the *in vitro* study, increased mucosal permeability [49].

2.5. Tricellulin

Tricellulin is concentrated at regions where three cells form a contact or at the tricellular TJ (tTJ), thus the name tricellulin. Silencing tricellulin disrupts the tTJ and reduces barrier integrity to small molecule tracers [50]. In humans with nonsyndromic deafness, there is an association between hearing loss and four recessive mutations at splice sites of the tricellulin gene [51, 52]. Furthermore, loss of occludin shifts tricellulin from the tTJ to the bicellular TJ (bTJ) to compensate for the loss of occludin at bTJ. Thus, these proteins collectively support the epithelial barrier at bi- and tricellular points [53]. Importantly, tricellulin integrates into claudin-based TJs independent of binding with ZO-1 [53], although tricellulin can interact with ZO-1 [51]. Finally, when localized at TJs, tricellulin expression increases electrical resistance values and decreases permeability; while when expressed exclusively at tTJs, tricellulin decreases solute permeability to macromolecules but not ions [54].

2.6. MarvelD3

Recently, the third member of the MARVEL containing proteins, MarvelD3, was described. To date, very little is known about marvelD3. Two independent studies using RNAi demonstrate phenotypes for marvelD3. Steed and colleagues show that silencing of marvelD3 does not affect TJs as assessed by immunofluorescence of occludin and ZO-1 nor does it affect the kinetics of TJ assembly as measured by Ca^{2+} switch assay. However, in the Ca^{2+} switch assay and under normal Ca^{2+} conditions, marvelD3 silencing eventually resulted in higher resistance readings [55]. Conversely, Raleigh and colleagues found that silencing of marvelD3 delays the assembly of TJs [56]. In a recent genome-wide association study, an intergenic single nucleotide polymorphism near the *MARVELD3* gene was linked to resistance to severe malaria [57]. The interactions between marvelD3 with occludin and tricellulin is influenced by claudins and these interactions further modulate the function of claudins [58].

3. Tight junctions and proliferation

3.1. ZO proteins

Matter and Balda initially conceived the notion that TJ proteins could participate in cell cycle regulation upon their discovery of the ZO-1 interacting protein ZONAB (ZO-1-associated nucleic acid-binding protein) [59]. ZONAB interacts with the promoters of cell cycle regulatory proteins [59] and regulates the ErbB-2 promoter activity and endogenous ErbB-2 expression [59]. Silencing ZONAB or expressing ZO-1 peptides that bind ZONAB reduces proliferation rates while ZONAB overexpression increases cell density [5]. ZONAB also regulates the cell cycle through a direct interaction with PCNA and cyclin D1 [60]. Finally, ZONAB impedes differentiation by direct binding and repression of the megalin and cubilin promoters [61], whose gene products are large (~600 and 460kDa, respectively) glycoproteins involved in the absorption of glomerular-filtered substrates in differentiated tubules (reviewed in [62]). Clearly, the ZO-1-ZONAB protein complex is critical to regulation of cell proliferation and differentiation.

Intriguingly, ZO-2 appears to participate in proliferation control as a result of its nuclear accumulation in sub-confluent cultures [63, 64]. ZO-2 interacts with the DNA-binding protein scaffold attachment factor-B (SAF-B) [64] along with the AP-1 transcription factors Jun and Fos, and the CCAAT/enhancer binding protein (C/EBP). ZO-2, but not ZO-1, negatively regulates the promoters of AP-1 target genes [65]. Huerta and colleagues identified a complex consisting of ZO-2 and c-Myc in which c-Myc binds directly to an E-box within the cyclin D1 promoter and this complex recruits histone deacetylase 1, thus repressing cyclin D1 [66]. A follow-up study supports ZO-2 suppression of cyclin D1 through the finding that ZO-2 inhibits the cell cycle at G_1/S and shuttles into the nucleus during G_1 and leaves during mitosis, thus providing a model whereby ZO-2 is present in the nucleus in sub-confluent (i.e. proliferating) cells, but absent from the nucleus and at the TJ in confluent (i.e. quiescent) cells [67, 68]. Conversely, ZO-2 nuclear accumulation causes an increase in the M2 type of pyruvate kinase, which is associated with increased proliferation [69].

3.2. Occludin

While the occludin null mouse exhibited no gross barrier abnormalities, the finding that these mice exhibit mucus cell hyperplasia [70] suggests that occludin may be involved in cell proliferation. This supposition is supported by the observations of Phillips *et al*, who noted that loss of occludin increases proliferation rates [27]. Surprisingly, occludin was identified in centrosomes and mutational analysis revealed that occludin may regulate mitotic entry via centrosome separation in a phosphorylation-dependent manner [71]. Finally, in a cell culture model of uveal melanoma, blood vessel epicardial substance overexpression lead to an increase in ZO-1 and occludin, which correlates with decreased cell proliferation [72].

3.3. Claudins

Gene deletion studies in claudin-15 null mice revealed that these mice, which were viable and developed normally, exhibited a phenotype described as megaintestine [73]. Tamura and colleagues reported that, when compared with normal littermates, the claudin-15 (−/−) mouse was found to have an approximate two-fold increase in the size of the upper small intestine. The authors further characterize this phenomenon and demonstrate an increase in proliferation of the crypts of the upper small intestine with no changes in apoptosis. Importantly, there were no changes in the expression of other claudins and the mice did not exhibit any disease phenotype such as cancer. In ovarian cancer, miR-155 inhibits the proliferation of ovarian tumor initiating cells by targeting *CLAUDIN-1* 3′ untranslated

region (UTR) [74]. However in hepatoma cells, miR-198 upregulates the expression of claudin-1 and E-cadherin and this regulation contributes to cell growth retardation conferred by miR-198 overexpression [75].

4. Tight junctions and tumorigenesis

4.1. Epithelial to mesenchymal transition

Epithelial to mesenchymal transition, or EMT, like many physiological processes, is an essential feature to both pathological and physiological events [76]. The EMT is an important feature of development, cancer, fibrosis, and pathology [77] and there are a number of features that distinguish epithelial and mesenchymal cells. Epithelial cells are characterized by well-developed junctions, an apical-basolateral polarization which is seen at the cell-cell junction, and the ability to become motile. However, motility is a feature that is rarely seen under normal physiological conditions [77, 78]. On the contrary, mesenchymal cells lack polarization due to the loss of an organized junctional layer. Reorganization of the cytoskeleton and organelles is generally not associated with a lamina [77–80]. Furthermore, transforming growth factor-beta (TGF-β) treated cells undergo EMT and are subsequently resistant to apoptosis [81].

In MDCK cells, TGF-β treatment induces EMT concurrent with the loss of claudin-1, -2, occludin, and the adherens junction protein E-cadherin [82]. Moreover, expression of the homeodomain protein HOXB7 in MCF10A and MDCK cells represses claudins-1 and -7 and mis-localizes claudin-4 while HOXB7 expressing MDCK cells form tumors in mice [83]. The pro-EMT repressor Snail directly interacts with E-boxes within the promoters of occludin and claudins-3, -4, and -7, but not ZO-1, and suppresses their promoter activities thus reducing mRNA and protein content [84]. The mechanistic manner in which TJ proteins are repressed during EMT was clarified through the finding that the TGF-β effector proteins SMAD-3 and -4 complex with the EMT repressor protein SNAIL1 and this SNAIL1-SMAD-3/4 complex represses occludin and claudin-3 in breast epithelial cells. The repression of TJ molecules is relieved upon SNAIL-1 or SMAD-4 siRNA-mediated silencing [85]. While it does not appear that ZO-1 is modified in the same manner that the claudins and occludin are during EMT, there is evidence that ZO-1 is involved in dedifferentiation and tumor formation. Reichert and colleagues expressed the PDZ domains of ZO-1 leading to a lack of TJ localization [86]. Functionally, MDCK cells stably expressing the ZO-1 PDZ domains fail to differentiate in collagen type I gel cultures, form tumors in nude mice, and decrease epithelial markers (with a corresponding increase in mesenchymal markers) [86]. Finally, recent studies have unveiled the findings that tricellulin [87] and marvelD3 [88] are silenced in EMT settings of gastric carcinoma and pancreatic cancer cells, respectively.

4.2 Transformation and metastasis

4.2.1. Occludin—Occludin emerged as a critical mediator of transformation from the discovery that it is transcriptionally repressed following constitutive *Raf-1* expression and subsequent re-expression sufficiently rescued the transformed phenotype [89]. The *Raf-1* induced occludin repression is mediated through a direct interaction between activated Slug and the E-box in the occludin promoter [90]. Congruently, in addition to occludin, claudin-1, -2 and ZO-1 are repressed by the active Ras signaling cascade, and chemical inhibition of MEK restores TJs [91–93]. Domain analyses of occludin revealed that the N- and C-terminal halves along with the second extracellular loop are essential for occludin reversal of *Raf-1* transformation *in vitro* and tumor formation *in vivo*, whereas the first extracellular loop is dispensable to repression of transformation [94]. Loss of the tumor suppressor von Hippel-Lindau (VHL) down-regulates occludin and claudin-1, independent of E-cadherin. In

clear cell renal cell carcinoma (CCRCC) cell lines, occludin is reduced in early lesions in patients with germline *VHL* mutations [95]. Occludin is epigenetically silenced through promoter hypermethylation in murine melanoma cells and forced expression of occludin also reduces migration in melanoma cells. Stable occludin expression in melanoma and breast cancer cells followed by injection into the craniolateral thorax and mammary fat pad, respectively, reduces the size of lung metastases [96]. Further, occludin induces premature senescence in breast cancer cells, which was blocked by chemical inhibition of the MEK pathway [97].

4.2.2. Claudins—While the current literature suggests that occludin would be an anti-transformation protein, the verdict on the claudin family is less clear. A great deal of effort in regards to understanding changes in claudin content in a wide range of cancers is well-documented in clinically based association studies. In the interest of brevity, the focus of this section shall be only on those studies where claudin content has been experimentally manipulated. For a comprehensive review of claudins and cancer, including association studies, please see Singh et al. [98]. Increased claudin-1 content was reported in human primary colon carcinomas and metastases as well as in cell lines derived from primary and metastatic tumors while genetic manipulation of claudin-1 *in vitro* and *in vivo* followed by cellular and rodent based assays supported these observations [99]. On the other hand, claudin-1 positivity is associated with better patient outcome in lung adenocarcinomas and genetic manipulation in lung carcinoma cell lines supports claudin-1 as a negative regulator of metastatic phenotypes and metastasis *in vitro* and *in vivo* [100]. Claudin-4 over-expression in invasive pancreatic cancer cells reduces invasion and survival in soft agar growth assays and reduces the number of lung metastases following tail vein injection in mice [101]. Surprisingly, claudins-3 and -4 are overexpressed in ovarian cancer [102, 103] and these findings are substantiated in human ovarian surface epithelial cells where overexpression of claudins-3 and -4 increases cell invasion and motility and siRNA studies support the invasion findings [104]. In ductal carcinoma in situ and invasive ductal carcinoma of the breast, claudin-7 is down regulated, which correlates with promoter hypermethylation in breast cancer cell lines; however, this was not the case in invasive ductal carcinomas [105]. Claudin-2 is increased in colorectal cancer and inflammatory bowel disease-associated colorectal cancer and expression of claudin-2 in colon cells which lack claudin-2 expression increases cell proliferation, anchorage-independent growth, and tumor volume *in vivo* [106].

4.2.3. Connection of occludin and claudins to stem cell-like phenotype and lineage/cancer genes—Breast cancers with low gene expression of claudins 3/4/7, occludin, and E-cadherin were termed "claudin^{low}" breast cancer by Herschkowitz et al. [107]. This subtype of breast cancer is mostly triple negative (HER2^{-}, ER^{-}, and PR^{-}) and shows poor prognosis; furthermore, the claudin^{low} breast cancer loosely resembles the mammary epithelial stem cell [108]. With regard to other cancer types, it remains to be determined if the claudin^{low} phenotype also correlates with stem cell-like potential.

Thyroid transcription factor 1 (*TTF-1* or *NKX2-1*) is a lung lineage gene that controls pulmonary development and maturation [109, 110]; it is also the most recurrently amplified gene in lung adenocarcinomas [111–114]. The gene amplification of *TTF-1* suggests a pro-oncogenic function for *TTF-1*. However, mouse models implicate tumor-suppressive and anti-metastatic activities of TTF-1 [115–117]. Taken together, *TTF-1* is a cancer gene with context-dependent functional multiplicity. We recently discovered that both occludin and claudin-1 are under direct transcriptional control by TTF-1 [118]. *TTF-1* knockdown conferred human lung cancer cells resistance to anoikis, and expression of occludin restored cellular sensitivity to anoikis. Furthermore, overexpression of occludin impeded migration and induced anoikis in lung carcinoma cells [118]. Interestingly, analysis of metastatic and

non-metastatic lung cancer cells from mice revealed that occludin content is associated with TTF-1 content, whereas loss of TTF-1 has no effect on claudin-1 protein levels. Collectively, we suggest that the TJ proteins mediate the anti-metastatic activity of TTF-1 and predict that other tissue lineage master regulators may also be functionally linked to the TJ constituents.

4.3. Genetic evidence linking tight junction molecules to cancer

While the literature is replete with data demonstrating the expression changes of occludin and claudins in a wide range of cancer types, expression alterations may or may not be indicative of genetic causes. The experimental observations reviewed so far convincingly suggest that TJ proteins play positive and negative functional roles in the tumorigenic process. However, direct evidence of genetic alterations of TJ genes in human cancers has not been explicitly noted in the literature. In view of the robust cancer genomic studies in the recent years, we wonder if an inkling of TJ gene mutations could be detected in the cancer genomic data. To this end, we limit our analysis to the two human TJ genes (*OCCLUDIN* and *CLAUDIN-1*). Using the cBio Cancer Genomics Portal [119], we probed 28 datasets for mutations and DNA copy number alterations (CNAs) of these two TJ genes. The results, as shown in Fig. 1, are rather intriguing. First of all, CNAs are the predominant form of genetic alterations associated with both genes. In the case of occludin, the general trend is a loss of DNA copy number. This is in line with the common decrease of occludin expression seen in multiple tumor types [120]. However, for claudin-1, it appears that *CLAUDIN-1* undergoes DNA copy number increases frequently in cervical squamous cell carcinoma and endocervical adenocarcinoma (30.6%) and lung squamous cell carcinoma (29.2%). These observations may be counter-intuitive initially. However, a significant overexpression of claudin-1 was indeed reported in cervical [121] and lung squamous cell carcinomas [122]. Therefore, gene dosage alterations are likely a factor shaping the expression patterns of TJ molecules.

By functional cancer genomics, TJ-related molecules as a whole are considered a significant pathway. The evidence came from a transposon-directed mutagenesis study to search for cooperating mutations with an oncogenic *K-Ras* allele in promoting murine pancreatic adenocarcinomas [123]. The results identify the TJ signaling pathway as a cellular process that is enriched in candidate cancer genes scored positive in the screen. Since the majority of the genes identified in the transposon insertional mutagenesis screen (90%) are predicted to be disrupted based on the orientation of the transposon with respect to the gene, an implication of this study is that most of the candidate cancer genes found by the study to be associated with the TJ pathway are putative tumor suppressors. Combining this observation and the CNAs affecting *OCCLUDIN* and *CLAUDIN-1*, we suggest that there is putative genetic evidence linking TJ genes to tumorigenesis. Our simplistic analyses via the cBio Portal are only meant to provoke researchers to initiate further studies to examine all the TJ genes for genetic and epigenetic aberrations.

5. Conclusion

The last several years have detailed new insights into the role for TJ proteins in cell proliferation, transformation, and metastasis. In view of the considerable functional data and putative genetic evidence connecting TJ factors to the tumorigenic process, we have come a long way from the early demonstration of TJ attenuation in tumors more than 30 years ago [124]. While instinctively one would expect TJ dissolution and concomitant downregulation of TJ factors as a prerequisite for cellular transformation, the reality is that TJ molecule expression patterns, claudins in particular, are more complex than originally anticipated. In terms of the intricate balance of regulation of individual TJ factors, we are only beginning to build a fundamental framework for a holistic understanding. Nevertheless, experimental

evidence increasingly suggests that TJ proteins are players in initiation and progression of cancers. Still, a multitude of questions remain. For example, while there have been advances as to how occludin and claudins come to be lost in highly proliferative settings or during transformation, little is known with regards to the precise mechanism that these proteins utilize to intervene on the transformation process. Similarly, it is not yet known if cellular localization is critical. The ZO proteins have been described at the TJ, but also in the nucleus where several regulatory events are occurring [67, 68]. A recent study did demonstrate that occludin is present at centrosomes [71], but this study lacked a mechanistic understanding of the function of occludin's presence at these organelles. The use of cell culture models has been extremely valuable to solidify the claim that TJ proteins participate in cellular events beyond barrier regulation. Clearly, it is imperative to revisit the knockout models, perhaps in an inducible setting, to definitively attribute these protein functions to transformation and metastasis.

Acknowledgments

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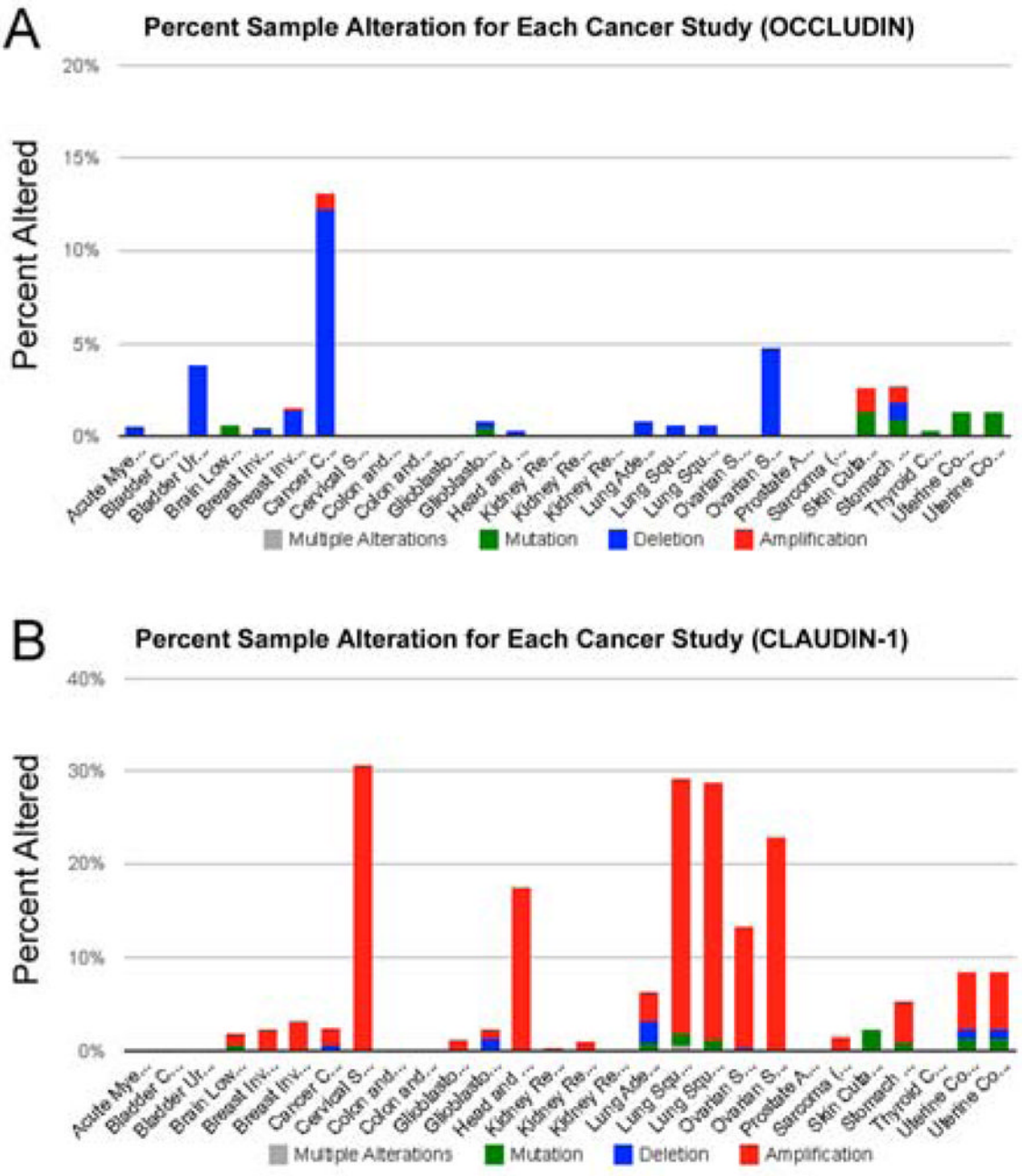

Fig. 1. Cancer genetic alteration profiles of *OCCLUDIN* and *CLAUDIN-1*

(A) The *OCCLUDIN* gene profile was interrogated in these studies: Acute Myeloid Leukemia (TCGA, Provisional) altered in 0.5% of 187 cases; Bladder Cancer (MSKCC, JCO 2013) altered in 0% of 97 cases; Bladder Urothelial Carcinoma (TCGA, Provisional) altered in 3.8% of 26 cases; Brain Lower Grade Glioma (TCGA, Provisional) altered in 0.6% of 169 cases; Breast Invasive Carcinoma (TCGA [125]) altered in 0.4% of 482 cases; Breast Invasive Carcinoma (TCGA, Provisional) altered in 1.6% of 760 cases; Cancer Cell Line Encyclopedia [126] altered in 13.2% of 882 cases; Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (TCGA, Provisional) altered in 0% of 36 cases; Colon and Rectum Adenocarcinoma [127] altered in 0% of 212 cases; Colon and Rectum Adenocarcinoma (TCGA, Provisional) altered in 0% of 221 cases; Glioblastoma [128]

altered in 0% of 91 cases; Glioblastoma Multiforme (TCGA, Provisional) altered in 0.8% of 236 cases; Head and Neck Squamous Cell Carcinoma (TCGA, Provisional) altered in 0.3% of 302 cases; Kidney Renal Clear Cell Carcinoma (TCGA, Provisional) altered in 0% of 290 cases; Kidney Renal Clear Cell Carcinoma (TCGA, in revision) altered in 0% of 418 cases; Kidney Renal Papillary Cell Carcinoma (TCGA, Provisional) altered in 0% of 100 cases; Lung Adenocarcinoma (TCGA, Provisional) altered in 0.8% of 129 cases; Lung Squamous Cell Carcinoma [129] altered in 0.6% of 178 cases; Lung Squamous Cell Carcinoma (TCGA, Provisional) altered in 0.6% of 177 cases; Ovarian Serous Cystadenocarcinoma [130] altered in 0% of 316 cases; Ovarian Serous Cystadenocarcinoma (TCGA, Provisional) altered in 4.8% of 311 cases; Prostate Adenocarcinoma [131] altered in 0% of 103 cases; Sarcoma [132] altered in 0% of 207 cases; Skin Cutaneous Melanoma (TCGA, Provisional) altered in 2.7% of 225 cases; Stomach Adenocarcinoma (TCGA, Provisional) altered in 2.6% of 115 cases; Thyroid Carcinoma (TCGA, Provisional) altered in 0.3% of 318 cases; Uterine Corpus Endometrioid Carcinoma [133] altered in 1.2% of 240 cases; Uterine Corpus Endometrioid Carcinoma (TCGA, Provisional) altered in 1.2% of 240 cases. (B) The *Claudin-1* gene profile was interrogated in these studies: Acute Myeloid Leukemia (TCGA, Provisional) altered in 0% of 187 cases; Bladder Cancer (MSKCC, JCO 2013) altered in 0% of 97 cases; Bladder Urothelial Carcinoma (TCGA, Provisional) altered in 0% of 26 cases; Brain Lower Grade Glioma (TCGA, Provisional) altered in 1.8% of 169 cases; Breast Invasive Carcinoma (TCGA [125]) altered in 2.1% of 482 cases; Breast Invasive Carcinoma (TCGA, Provisional) altered in 3% of 760 cases; Cancer Cell Line Encyclopedia [126] altered in 2.3% of 882 cases; Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (TCGA, Provisional) altered in 30.6% of 36 cases; Colon and Rectum Adenocarcinoma [127] altered in 0% of 212 cases; Colon and Rectum Adenocarcinoma (TCGA, Provisional) altered in 0% of 221 cases; Glioblastoma [128] altered in 1.1% of 91 cases; Glioblastoma Multiforme (TCGA, Provisional) altered in 2.1% of 236 cases; Head and Neck Squamous Cell Carcinoma (TCGA, Provisional) altered in 17.5% of 302 cases; Kidney Renal Clear Cell Carcinoma (TCGA, Provisional) altered in 0.3% of 290 cases; Kidney Renal Clear Cell Carcinoma (TCGA, in revision) altered in 1% of 418 cases; Kidney Renal Papillary Cell Carcinoma (TCGA, Provisional) altered in 0% of 100 cases; Lung Adenocarcinoma (TCGA, Provisional) altered in 6.2% of 129 cases; Lung Squamous Cell Carcinoma [129] altered in 29.2% of 178 cases; Lung Squamous Cell Carcinoma (TCGA, Provisional) altered in 28.8% of 177 cases; Ovarian Serous Cystadenocarcinoma [130] altered in 13.3% of 316 cases; Ovarian Serous Cystadenocarcinoma (TCGA, Provisional) altered in 22.8% of 311 cases; Prostate Adenocarcinoma [131] altered in 0% of 103 cases; Sarcoma [132] altered in 1.4% of 207 cases; Skin Cutaneous Melanoma (TCGA, Provisional) altered in 2.2% of 225 cases; Stomach Adenocarcinoma (TCGA, Provisional) altered in 5.2% of 115 cases; Thyroid Carcinoma (TCGA, Provisional) altered in 0% of 318 cases; Uterine Corpus Endometrioid Carcinoma [133] altered in 8.3% of 240 cases; Uterine Corpus Endometrioid Carcinoma (TCGA, Provisional) altered in 8.3% of 240 cases. The analyses were conducted using the cBio Cancer Genomics Portal [119]. TCGA, The Cancer Genome Atlas.