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Claudin proteins, outside-in signaling, and carcinogenesis

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Abstract

Environment affects an individual's development and disease risk which then suggest that the environmental cues must have ways of reaching to the cellular nuclei to orchestrate desired genetic changes. Polarized and differentiated epithelial cells join together by cell-cell adhesions to create a protective sheet which separates body's internal milieu from its environment, albeit in highly regulated manner. Among these cell-cell adhesions, a key role of tight junction, the apical cell-cell adhesion, in maintaining epithelial cell polarity and differentiation is well recognized. Moreover, significant changes in expression and cellular distribution of claudin proteins, integral component of the tight junction, characterize pathophysiological changes including neoplastic growth and progression. Studies have further confirmed existence of complex claudin-based interactomes and demonstrated that changes in such protein partnering can influence barrier integrity and communication between a cell and its environment to produce undesired outcome. Cell signaling is the process by which cells respond to their environment to make dynamic decisions to live, grow and proliferate, or die. Thus, pivotal role of the deregulated tight junction structure/function in influencing cellular signaling cascades to alter cellular pheno-type can be envisaged, however, is not well understood. Needless to mention that advanced knowledge in this area can help improve therapeutic considerations and preventive measures. Here, we discuss potential role of the tight junction in the regulation of Outside-in[^] signaling to regulate cancer growth, with specific focus upon the claudin family of proteins.

Keywords

Tight junction; Signaling; Stem cell; Claudin; Cancer progression

Introduction

Proper functioning of the living organisms depends upon maintenance of the body's homeostasis which is defined as dynamic constancy of the body's internal environment. To

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Compliance with ethical standards

Conflict of interest

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maintain this internal constancy, the vertebrate body constantly monitors the extracellular conditions via bodily sensors, and preserves regulated separation of its internal tissues and organs from its environment. Epithelial cells serve this sentry role by forming a protective barrier between body's interior milieu and its surrounding environment [41, 58]. Cell-cell adhesions adjoining neighboring epithelial cells help create this protective barrier, and to uphold its proper functioning [21]. Tight junction, also known as occluding junction or zonula occludens; the most apical cell-cell adhesion, has been widely studied for its critical role in helping to maintain this barrier property, and is believed to influence the Boutside-in[^] signaling [20, 54]. Importantly, tight junctions are important for regulating permeability properties of the epithelial barriers as they restrict diffusion along the paracellular space [11]. In accordance, disintegration of tight junction integrity and increased paracellular permeability characterize unhealthy vertebrate organs including inflamed tissues and cancers [13, 65]. Ongoing studies including studies from our laboratory have further revealed that tight junction integral proteins can also help regulate epithelial cell proliferation and differentiation [9, 10, 59, 63]. Emerging evidence further suggest that tight junction harbors and recruits evolutionarily conserved protein complexes and signaling cascades which, in turn, may help connect internal milieu of the vertebrate body with its environment, to help maintain normal homeostasis [18, 29]. Identification of novel integral components of the tight junction, the claudin family of transmembrane proteins, and the findings that these apical proteins can partner with basolaterally located integrins of epithelial cells have further helped to unravel a potential role of tight junctions in the dynamic facilitation of "Boutside-in" and "Binside-out" communication/signaling [14, 16, 69, 71] (Fig. 1). These dynamic regulations are now considered hallmark of human health and disease. Here, we will review the recent knowledge describing how alteration of tight junction composition may influence cellular signaling to modify epithelial cell pheno-type to help facilitate neoplastic growth and progression.

Cellular signaling and carcinogenesis

Cellular signaling is the mechanism by which stimuli are transmitted via signaling cascade/s to effector molecule/s to orchestrate desired cellular responses/s. However, cellular signaling pathways are often interconnected to form complex signaling networks and can be initiated by diverse mechanisms including stimulation of the growth factor receptors, cell-matrix interactions, and/or cell-cell contact. Eventually, cells integrate the diverse signaling events to regulate cell growth, motility, polarity, differentiation, and death. However, same signaling components can be used differentially within different signaling complexes or at different intracellular locations, and activation of same signaling molecule may have distinct consequences, depending on the cellular context. This intricacy of cellular signaling networks has major implications for our understanding of tumor cell behavior and for our ability to use this knowledge for cancer therapy. Thus, understanding how diverse complex signaling networks are initiated and function in vivo and how they are altered in cancer cells represent a major intellectual challenge for the success of the anti-cancer therapies. In this regard, key role of tight junction/s in regulating cellular communication and signaling by maintaining cell polarity and differentiation has long been recognized [2, 15, 75]. The loss of cell polarity, due to tight junction deregulation, can therefore abrogate the normal checkpoints for receptor activation by bringing otherwise spatially separated ligand and

receptors in proximity for uninhibited activation, for example, EGF receptor and its ligands [2, 72]. Moreover, recent research indicates that myriads of signaling proteins and transduction pathways reside at the tight junction and interact with its constituent proteins [54, 75]. Considering the key role of the environmental stimuli in stimulating neoplastic changes in individuals not genetically predisposed, it is important that we understand how changes in the composition and behavior of this apical guard of cellular homeostasis and its constituents affect cellular signaling associated with cancer growth and progression.

Claudin proteins and cancer

Studies led by the pioneer discovery of Tsukita and colleagues that claudin family of transmembrane proteins is integral to the structure and function of the tight junction has led to the identification of 27 members [16, 60]. Despite strong genetic and structural similarities between different claudin family members, they are expressed in tissue- and cell-specific manner, and help regulate diverse biological processes [17]. A growing body of data indicates that claudin expression is altered in numerous epithelial cancers in a stage- and tumor-specific manner, and in tissue-specific manner [62]. However, this is not surprising considering that majority of the cancers originate from an epithelium including the cancer originating from the oral cavity, esophagus, stomach, colon, rectum, prostate, ovary, bladder, kidney, lung, pancreas, breast, and liver [7]. Due to the tissue-specific expression, initial investigations explored the potential of claudin proteins for serving as diagnostic and/or prognostic biomarkers, which has shown immense promise [10, 28, 32]. However, the initial euphoria quickly morphed into exploring the causal role of claudin proteins in neoplastic growth and progression. These queries were fueled by generation of mice, genetically modified for specific claudin proteins, and subjected to mouse models of carcinogenesis [23, 24, 47, 48]. Outcome from these investigations and from in vitro manipulations in cancer cells has landed strong support for the causal role of claudin proteins in regulating carcinogenic processes [9, 27, 30, 51]. Notably, deregulation of claudin expression and/or cellular distribution has inherent potential of altering the cellular homeostasis by compromising the barrier integrity of the epithelium [31, 50]. However, a question remained on how claudin dysregulation contributes to the dysfunctional cell polarity and barriers in different tumor types and how these changes are regulated. In this regard, recent studies have demonstrated novel protein-protein interactions involving claudin proteins including with proteins expressed in lateral and basal domains of the cells, and its influence on cellular signaling cascade/s. Moreover, genetic changes in claudin proteins, in vitro and in vivo, are now demonstrated to modulate multitude of cellular functions including cell proliferation, migration, and by altering key signaling processes including the Notch signaling, the Wnt/ β -catenin signaling, and JAK/STAT-3 signaling [26, 39, 47, 48].

Claudins and epithelial to mesenchymal transition

While the advent of novel screening technologies and bio-markers have drastically improved clinical management of cancer patients, cancer progression remains the major cause of cancer-associated deaths. Increasing evidence suggest that the loss of the epithelial traits results in aggressive cancer cells and hard to treat cancers [45]. Of note, Epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells acquire mesenchymal properties, and increased ability to invade and metastasize [33, 70]. Moreover, studies have

now linked EMT with the acquisition of stem-cell characteristics and demonstrated important role of specific claudin proteins in regulating the EMT process, and cancer progression [3, 52]. In this regard, the key role of claudin-1 in regulating the EMT process is well documented. Here, contrasting an expected loss of claudin-1 protein expression in cancer cells, claudin-1 protein expression is highly augmented in specific cancer types including colorectal cancer (CRC) though membrane localization of the protein is highly compromised. Here, it is noteworthy that overexpression or knockdown of claudin-1 in colon cancer cells, low in claudin-1 expression (SW480 cells) or with robust expression (SW620 cells), was sufficient to impact the EMT process and ability of the cells to form tumor and/or metastasize in vivo [10]. Multiple other studies found similar dysregulation of claudin-1 expression and/or cellular distribution in other cancer types, and causal association with cancer progression [4, 64]. Similar parallel association of claudin-2 expression with EMT and cancer progression is now demonstrated in colorectal and breast cancer [9, 67]. These studies also determined a previously unrecognized role of claudin-2 in facilitating cell-cell adhesion between breast cancer cells and hepatic cells, when metastasizing to the liver [68]. However, these associations are not always linear and highly increased expression of claudin-3 and -4 in prostate and ovarian cancers, in correlation with cancer aggressiveness, is reported [57]. Interestingly, tumor suppressor role of claudins-3 and -4 has been also reported [26, 35]. They affect the E-cadherin expression and suppress β -catenin signaling, increase in vitro cell migration, invasion, and corresponding metastasis in vivo [26, 55, 56]. However, downregulation of claudin-3 or claudin-4 in ovarian cancer promotes tumor growth and metastatic behavior in vivo [55]. In breast cancer, low expression of claudin-3, -4, and -7 and E-cadherin corresponds to high malignancy [19, 38]. In pancreatic cancer, claudin-4 expression suppresses cell invasion and metastasis [42] while it inhibits migration and invasion of gastric cancer cells without affecting cell growth [25]. Claudin-6 has been demonstrated to regulate gastric cancer [74] and breast cancer [73]. Decreased expression of claudin-6 enhances anchorage-independent growth and promotes invasiveness in breast cancer cells [46]. Claudin-6 expression was associated with decreased anchorage-independent growth, invasion, and increased in breast carcinoma cells while its overexpression increased invasion, migration, and proliferation potentials in gastric cancer cell lines. Similarly, claudin-7 has a dual role of both tumor suppressor and tumor promoter. While it enhances cell growth and metastatic behavior of esophageal squamous cell carcinoma, it inhibits colon cancer progression and invasiveness [5, 36]. Taken together, it appears that the deregulated barrier properties due to modified claudin composition in any given epithelial cells sheet may modify the outside-in signaling; however, associated changes in protein partnering and resultant signaling may help regulate the overall outcome. However, due to the lack of appropriate model system and comprehensive analysis, these postulations remain ill justified and will require further investigation.

Cross-talk between claudin proteins and signaling pathways in cancer

In addition to regulating the barrier properties, the tight junctions also serve as the hub for a multitude of signaling proteins including known tumor suppressor molecules like APC (adenomatous polyposis coli), PTEN (phosphatase and tensin homolog), and polarity proteins like Par-3 [8, 43, 49]. Silencing of the expression and/or function of these proteins, either due to genetic alterations or epigenetic regulation, modulates claudin expression, and

induces loss of polarity and EMT [8, 22, 53]. Interestingly, genetic modulation of claudin proteins in mice or cancer cells can similarly affect these signaling cascades, suggesting a feedback regulation. In this regard, over-activation of Wnt/ β -catenin signaling in colon cancer cells induces claudin-1 and claudin-2 expression [40, 44]. Similarly, overexpression of claudin-1 induces Wnt/ β -catenin signaling [6]. We have further reported that claudin-1 overexpression modulates Notch signaling in MMP-9 dependent manner to modulate barrier properties and immune homeostasis to promote susceptibility to inflammation-associated colon cancer (CAC) [48]. In human liver cells, claudin-1 promotes invasive behavior by activating the c-Abl-PKC signaling pathway [66]. Moreover, partnering between claudins and integrins helps regulate outside-in and inside-out signaling to regulate cell-matrix interaction, immune homeostasis, and cell death [12]. Integrin-mediated cell attachment activates Akt through the phosphatidylinositol-3 kinase (PI3K) and promotes cell survival [34]. On the other hand, claudin-1 regulates PI-3 kinase/Akt signaling to regulate Wnt/ β -catenin signaling [63]. Moreover, a novel protein partnering between claudin-1 and Src proteins in a complex with ZO-1 helps regulate ability of colon cancer cells to undergo anoikis presumably in claudin-1/Src/PI3k-Akt/Bcl-2-dependent manner [61]. This association influences the invasive behavior and metastasis of colon cancer cells. Overexpression of claudin-2, potentially dependent on EGFR/ERK1/2 signaling, in CRC patient samples also correlated with cancer progression, and similar trend was observed in IBD-associated CRC samples [9]. Overexpression of claudin-2 in CRC cells resulted in increased cell proliferation, anchorage-independent growth, and tumor growth and increased Bcl-2 expression [1]. These studies apparently indicate that coordination between Bcl-2 and claudins has a definitive role in tumorigenesis. However, it should not be overlooked that this coordination may be more specific to colon cancer as in breast cancer cell lines MCF-7 with over expression of claudin-1 resulted in anti-apoptotic function on treatment with tumor necrosis factor (TNF)- α with simultaneous accumulation of β -catenin in cytoplasm [37].

Conclusion

Changes in cellular DNA caused by mutagens can have a range of effects, and therefore, the role of the gene interactions in the etiology of human disease is a critical area of study. Moreover, response of a tumor cell to an inhibitor or drug depends on its particular genetic and epigenetic status. Tumor cells acquire resistance to apoptosis during the course of tumor progression, and enhanced survival signaling may be important in promoting resistance to chemotherapeutic agents. The generally accepted concept that tumorigenesis is associated with loss of function of TJs implies that claudin expression is downregulated during tumor progression. Such a notion would imply facilitated communication, due to compromised barrier properties, between the cellular milieu and outside environment which, in turn, can modulate internal homeostasis and orchestrate genetic alterations. However, claudin expression can be decreased or increased in human cancer in a tissue-specific manner, and a role for claudins in tumor progression has been suggested based on their effects on the migration and invasion of cancer cells and metastasis. Furthermore, claudin expression is significantly associated with patient survival or recurrence in some cancers, suggesting that these TJ proteins could be prognostic markers and promising therapeutic targets. Moreover, it is now believed that most epithelial tumors are maintained by a subpopulation of cells

called cancer stem cells (CSCs) with self-renewal and multi-lineage differentiation capacity. Recently, new insights into this population have emerged in certain epithelial tumor types, including their claudin (low) phenotype and its importance to the epithelial-mesenchymal transition (EMT) process in breast cancer. Taken together, claudins' role in EMT and CSC appears to constitute an axis-of-evil in cancer, possibly by altering cellular communications and outside-in signaling, and improved understanding of these regulations may help lead to new therapeutic platforms.

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Fig. 1.

A cartoon demonstrating how interactions between tight junction and integrins may help regulate “Boutside-in” and “BInside-out” signaling. Environmental stress enforces epigenetic and genetic changes to modulate expression of specific claudin proteins resulting in loss of polarity, free flow of communication between environment and cellular milieu, and cancer phenotype. Herein, we have included claudin-1 and claudin-7 regulation in colon tumorigenesis as the model. APC mutation induces Wnt/ β -catenin signaling which induces claudin-1 expression and loss of its membrane expression in colonic epithelial cells. Increased claudin-1 expression suppresses claudin-7 expression. Loss of claudin-7 expression, in turn, disrupts association between claudin-1, claudin-7, and β 1-integrin. Deregulated barrier properties and cell-matrix adhesion then allow free flow of “Boutside-in” and “BInside-out” signaling, modifies cellular signaling, and induces gene expression promotive of cancer promotion