Review Article

iMedPub Journals
www.imedpub.com

Cancer Biology and Therapeutic Oncology

2018
Vol.2 No.1:1

Role of Tight and Adherens Junctional Complexes in Tumor Suppression

Grace Ramena* and Yathish Ramena

Department of Pathology, University of Arkansas, Arkansas, Pine Bluff, USA

***Corresponding author:** Grace Ramena

ramenagrace@gmail.com

Assistant Professor in Fish Pathology, Department of Aquaculture and Fisheries, University of Arkansas at Pine Bluff 1200 N. Arkansas-71601, USA.

Tel: +1 870-575-8137

Citation: Ramena G, Ramena Y (2018) Role of Tight and Adherens Junctional Complexes in Tumor Suppression. Cancer Biol Ther Oncol. Vol.2 No.1:1

Abstract

Epithelial cells are connected to each other and the basemen membrane by junctional complexes. These complexes are made up of transmembrane proteins and they play important role in tumor suppression. Dysregulation of the junctional proteins at tight and adherens junctions results in the loss of epithelial integrity and epithelial phenotype. This leads the cells to undergo EMT because of various signaling events, activating the transcription factors that upregulate mesenchymal genes. Cancer cells utilize EMT to gain invasive and migratory properties. In this review we have focused on the various TJ and AJ proteins, their regulation and various signaling events at these junctions that contribute to tumor suppression.

Keywords: Junctional complexes; Tight junctions; Claudins; Occludins; Nectins

Received: December 30, 2017, **Accepted:** January 10, 2018 **Published:** January 17, 2018

Introduction

There multiple types of epithelial cells depending on the organ and the function they perform. They are squamous, cuboidal or glandular and columnar epithelium and they can be single or multiple layered. The cells in epithelial tissues are tightly packed together with very little intercellular and extracellular space. The epithelial cells are connected to one another and to the basement membrane by junctional complexes. They are tight junctions (TJs), adherens junctions (AJs), gap junctions, desmosomes and hemidesmosomes. AJs are mediated by E-cadherin. Desmosomes are mediated by desmoglein and desmocollin that also belong to cadherin family. GJs constitute of tube like cylinder formed by transmembrane proteins called connexins and the hemidesmosomes consist of integrins that help the cells adhere to the basement membrane. In this review, we focus on the TJ and AJ proteins and their role in tumor suppression and the various signaling events at these junctions [1-3].

Tight Junctions

TJs are present the apical most region at the cell-cell junctions, followed by AJs. TJs consist of four types of transmembrane proteins: Claudins, Occludins, Nectins and Junctional Adhesion Molecules. TJs help maintain cell polarity, perform barrier function, hold the cells together, and regulate the movement of ions, and other molecules. They perform these functions either by homotypic or heterotypic interactions, by binding to peripheral proteins with their cytoplasmic tail that link them to the actin cytoskeleton [4-6].

Claudins

Claudins (CLDN) are tetra span proteins expressed at the tight junctions. However, unlike occludins they are expressed in cells that do not form TJs but are highly expressed in cells that form TJs. There 27 claudins 1 to 24, and 3 CLDNs from splice variants of CLDN10, 18 and 19. In general they are about 20 to 34 KD in size. Like occludins their N and C- termini extend into the cytoplasm and c-terminus is longer to be able to bind to different scaffolding proteins. Claudins act as para-cellular barrier in the absence of occludin. Like occludins the two claudin extracellular loops interact with the extracellular loops of claudin on adjacent cells. This interaction and cell-cell adhesion is Ca+2 independent. Claudins bind to ZO-1, ZO-2, ZO-3, PAR3 and PAR6 at their cytoplasmic tail. Mutations in different types of claudins are indicative of disease and some CLDNs are abnormally expressed in cancers while some are downregulated. CLDN1 is known as a tumor suppressor for breast cancer. It is also known as senescence-associated epithelial membrane protein1. Claudins play a role in regulation of paracellular permeability of specific ions to maintain physiological homeostasis. CLDN3 and 4 are upregulated in several cancers and play a role in motility, invasion and anoikis resistance. Basal type breast cancers are referred to a claudin-low type which is a very aggressive subtype [7-9].

Junctional Adhesion Molecules

JAMs are about 40KD in size. There are six types of JAMs that are so far identified. They are JAM-A, JAM-B, JAM-C, CAR (coxsackie

and adenovirus receptor), ESAM (endothelial cell-selective adhesion molecule) and JAM4. JAMs are expressed on leukocytes, platelets, epithelial and endothelial cells. In the epithelial cells they are found to be localized at tight junctions. JAM-c is highly expressed during embryogenesis, lymphatic vessels, lymph nodes, endothelial venules and vascular structures in the kidney [10-12]. JAMs have membrane distal V-type Ig-domain and membrane-proximal C2-type Ig-domain in the N-terminus and a PDZ domain in the c-terminu. Both v-type Ig domain and C2-type Ig domain have conserved disulphide bridges. They undergo homotypic and heterotypic interactions. JAMs interact with occludins and this interaction is important for TJs assembly. The JAMs interact with leukocytes β2, α4β1, β1, LFA1 and Mac-1 through their extracellular domains. The cytoplasmic tail of JAMs anchor or bind to various tight junction proteins like zonula occludens (ZO-1), PAR-3 (cell polarity protein), AF-6 and MUPP1 that link JAMs to the actin cytoskeleton. Therefore, JAMs play a role in regulating immune cells and endothelial cell association in the immune surveillance and also maintain cell polarity by tight junction formation in endothelial and epithelial cells [13,14].

Cell Signaling Events at TJs

Bves is a transmembrane cell-cell adhesion protein expressed at TJs that is known to play a role in TJ formation and function. Bves has been shown to interact with ZO-1. Patricia et al. used human corneal epithelial cells (HCE) to study how Bves regulates TJ associated signaling. ZO-1 binds to GEF-H1 via cingulin and directly to ZONAB; and GEF-H1 activates RhoA at TJs. Patricia et al. found that Bves regulates TJ formation and function by sequestering ZO-1 at the TJs. In the presence of Bves, GEF H1 and ZONAB were localized to the membrane but its loss caused an increase in free ZO-1; cytosolic GEF-H1 and nuclear ZONAB. This resulted in high RhoA by GEF-H1 and thus increases in phosphorylated myosin light chain (MLC). MLC is a target of Rock which is a Rho kinase that is down stream of RhoA. P-MLC causes cell contraction and important for motility [15-17].

It is known that ZONAB is a transcriptional factor that regulates cell proliferation. To identify the genes that are induced by ZONAB activity Tony et al. overexpressed ZONAB or the SH3 domain of ZO-1 to inhibit ZONAB activity in MCF10A cells. They found that ZONAB binds to SH3 domain of ZO-1 but not to SH3 domains of Abl, Src, Crk, Grb2, ZO-2 or ZO-3. MCF10A cells showed increased proliferation in ZONAB overexpressed cells but not in cells that express both ZO-1 and ZONAB. With increase in ZONZAB, there was an increase in transcription factors like c-jun and c-myc and a significant increase in mRNA levels of proliferating cell nuclear antigen (PCNA) and cyclin D1. Chromatin immunoprecipitation assays show that both PCNA and cyclin D1 promoters have ZONAB binding sequences. The similar results for ZONAB activity are seen in MDCK cells.

The above are few of much evidence showing that TJs not only perform barrier function and maintain cell polarity of epithelial cells but also play a role in cell signaling. TJs regulate the transcriptional activity of multiple genes and as a result regulate cell proliferation which is important or tumor suppression.

Occludins

It is a 504 aa long polypeptide with about 60 to 65 KD. It has four transmembrane domains, with both N-terminus and C-terminus in the cytoplasm. The hydrophicity plot shows that its primary structure is similar to connexin, a gap junctional protein. The extracellular loops interact with the extracellular loops of other occludin on the neighboring cell in a Ca+2 independent way. This allows occludins to seal the para-cellular space [18,19]. Its cytoplasmic tail interacts with zonula occludens-1 (ZO-1) and this interaction is important for occludin to maintain its extracellular cell-cell adhesion [20-45]. Occludin is not expressed in cells that don't form TJs but only in epithelial and endothelial cells that do form TJs. Occludin molecules are important for TJ assembly which is regulated by its phosphorylation. Smales et al. have showed that CK2 phosphorylates c-terminus of occludin in crude brain cell extracts [46]. Yu et al. showed that KD of occludin caused increased permeability to large cations.

Adherens Junctions

AJs are mediated by E-cadherin. E-cadherin is expressed on the lateral sides at cell-cell contacts special beneath the TJs. The human E-cadherin gene (*CDH1*) is located on chromosome 16q22.1. E-cadherin was first identified by Hyafil et al. They called it as uvomorulin (UM) because anti-uvomorulin antibodies were able to bind to E-cadherin. Electron microscopic images show that E-cadherin is localized to the adherens junctions of intestinal epithelium. Nagafuchi et al. first cloned E-cadherin cDNA from mouse and showed that it is a calcium cell-cell adhesion molecule. In addition to mediating cell-cell adhesion, E-cadherin is also important for maintaining apico-basal polarity and it is a type I transmembrane glycoprotein which is 728 amino acids long. It has a single transmembrane domain, cytoplasmic tail and five extracellular repeats. The extracellular domain binds calcium and E-cadherin forms a homodimer with E-cadherin on adjacent cells via histidine-alanine-valine (HAV) motif in its amino-terminus. Even though the extracellular domain mediates cell-cell adhesion, the intracellular cytoplasmic domain is required for the same function. The cytoplasmic tail of E-cadherin connects the adherens junction to actin cytoskeleton via many adaptor proteins like α, β, γ catenin's and p120 [47-49]. Therefore E-cadherin along with the adaptor molecules is important for adherens junction integrity and also many signaling pathways.

Cell Signaling Events at TJs

Beta catenin is a transcription factor that is sequestered at the AJs and it is an important molecule in Wnt signaling. On the other hand, loss of alpha catenin causes hyper activation of MAPK signaling and p120 catenin regulates NF-kβ and Rho pathways [50-53]. The loss of E-cadherin activates specific downstream signaling pathways that allow epithelial cells to become mesenchymal like via epithelial to mesenchymal transition (EMT). The loss or downregulation of E-cadherin can occur due to several reasons. They are somatic mutations, chromosomal deletions, promoter silencing by DNA hypermethylation or by transcription factors like Snail, Slug and Twist and proteolytic cleavage [54]. Chen et al. showed that under normal circumstances beta catenin is required

for E-cadherin transport to the cell membrane, which otherwise causes proteosomal degradation of E-cadherin. On the otherhand, cytosolic beta-catenin is phosphorylated at the serine residues of its N-terminus by GSK-3β, axin and adenomatous polyposis coli (APC) complex. This results in beta-catenin ubiquitination and proteosomal degradation. But wnt signaling allows beta-catenin to accumulate in the cytosol and translocate to the nucleus to activate TCF/LEF transcription factors, c-myc and cyclin D1 that effects cell adhesion, tumor development, increase in cell migration and invasion. Phosphorylation of serine/threonine kinase on E-cadherin or beta-catenin stabilizes their complex but if beta-catenin gets phosphorylated at its tyrosine kinase site by intracellular signaling it disrupts E-cadherin complex and results in EMT [55]. Onder et al. showed that loss of E-cadherin cause's increase in cytosolic beta-catenin, mesenchymal markers like N-cadherin, fibronectin and vimentin but a decrease in epithelial markers like cytokeratins. To assess if beta-catenin is the main downstream signaling molecule causing EMT upon E-cadherin loss, Onder et al. made double KD of E-cad and beta-catenin in HMLE cells. They found that there is a significant increase in mesenchymal markers like N-cad, vimentin and fibronectin but the overexpression of beta-catenin alone was not enough to induce EMT. So they compared the gene expression profiles of *shEcad* and *DNEcad* that still had E-cadherin cytoplasmic tail. They found that DN E-cad downregulated epithelial markers but no induction of EMT. Interestingly, they found that there is an increase in Twist and Zeb-1 that are actually TFs known to repress E-cadherin expression. Twist was upregulated in ShEcad but not in DNEcad. There was an increase in invasion and anoikis resistance with Twist induction in ShEcad cells. When Twist was inhibited there was a decrease in invasion and anoikis resistance. Therefore E-cadherin loss promotes EMT through induction of several downstream signaling pathways.

Nectins

Like JAMs, nectins are also immunoglobulin like cell-cell adhesion molecules. There are 4 members in nectin family; they have 3 immunoglobulin loops in their extracellular domain, one transmembrane domain and a cytoplasmic tail. They play a role in TJ formation by recruiting cell-cell adhesion molecules like JAMs, claudins and occludins. Nectin is connected to actin cytoskeleton via afidin. Nectin has been shown to recruit E-cadherin –β catenin complex and ZO-1 to nectin based cell-cell adhesion sites via afidin [55-57].

Discussion and Conclusion

E-cadherin binds to RhoA via p120 catenin and this inhibits Smad3 phosphorylation by TGFβ via RhoA. But loss of E-cadherin causes upregulation of TGFβ and its downstream targets. In human retinal pigment epithelial cells that have undergone EMT, there is an increase in TGFβ that upregulates anti-apoptotic protein survivin and thus inhibits apoptosis. Therefore loss of E-cadherin expression is enough to induce EMT in mammary epithelium.

Once the cancer cells undergo EMT they enter blood stream, survive there, extravasate and form colonies at a distant sites, called micro and macro metastasis. Usually the detached cells undergo apoptosis and it is called anoikis. But cancer cells gain resistance to anoikis by co-opting themselves with blood platelets to promote metastasis. Overexpression of anti-apoptotic proteins like BCL2, BCL-Xl and XIAP can make cancer cells resistant to anoikis and become more metastatic. Onder et al. showed that loss of E-cadherin caused a decrease in apoptotic marker Annexin V, suggesting that E-cadherin loss enhances anoikis resistance and increased invasiveness. Cancer cells that undergo EMT are more resistant to chemo and radiation therapies, because these cells gain stem like properties [56,57]. Piyush et al. showed that conventional chemotherapy helps select for these cancer stem cells. Therefore, EMT requires loss of cell-cell adhesion molecule E-cadherin that helps cancer cell to become more migratory, invasive and resistant to anoikis.

Walia et al. found that loss of CLCA2, downregulates E-cadherin and induces EMT in HMEC. We found that CLCA2 is expressed at basolateral membranes in epithelial cells and it interacts with EVA1. EVA1 is a transmembrane protein similar to JAMs structurally except it has only one Ig-V-like domain with disulphide bridges in the N-terminus and no PDZ domain in the c-terminus unlike JAMs [58]. However, EVA1 is also expressed on lymphocytes, epithelial and endothelial cells. EVA1 expression caused CHO cells to aggregate in suspension suggesting that it undergoes homotypic interaction. It has been also shown to regulate immune surveillance at blood-CSF barrier. However, we found that EVA1 is expressed at the AJs along with *CLCA2* and E-cadherin. Interestingly, its loss leads to cell morphology change and thus cells undergo EMT. Both *CLCA2* and *EVA1* are tumor suppressor genes expressed at lateral junctions along with E-cadherin [59]. *CLCA2* and *EVA1* form a complex with ZO-1 and CLCA2 sequesters beta-catenin by itself, therefore inhibits EMT via multiple mechanisms.

On the other hand, TJs sequester transcription factors like ZONAB at cell membrane and inhibit cell proliferation. TJs are not shown to play a role in EMT. However, breast cancers with loss of claudins at TJs are characterized as the most aggressive and recurrent subtypes. Therefore TJs also might play a role in EMT. Junichi et al. showed that ZO-1 is required not only for TJ formation but also belt-like adherens junction formation during epithelial polarization. Anastasios et al. showed that ZO-1 knockdown caused an increase in cell proliferation as well as induced EMT in retinal pigment epithelial cells [60]. There is enough evidence that the proteins present at cell-cell junctions that can inhibit EMT in cancer cells are possible tumor suppressors.

Conflict of Interest

No conflict of interest.

References

1 Anastasios G, Marion T, James WBB, Kamaljit SB, Freya M, et al. (2010) The tight junction associated signalling proteins ZO-1 and ZONAB regulate retinal pigment epithelium homeostasis in mice. PLoS ONE 5: e15730.

2 Agarwal R, D'Souza T, Morin PJ (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.

3 Aijaz S, Balda MS, Matter K (2006) Tight junctions: Molecular architecture and function. Int Rev Cytol 248: 261-298.

4 Aurrand LM, Duncan L, Ballestrem C, Imhof BA (2001) JAM-2, A novel immunoglobulin superfamily molecule, expressed by endothelial and lymphatic cells. J Biol Chem 276: 2733-2741.

5 Balda MS, Matter K (2000) The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. Embo J 19: 2024–2033.

6 Barclay, AN, Brown MH, Law SKA, McKnight AJ, Tomlinson MG, et al. (1997) The leukocyte antigen factsbook. Academic Press, London.

7 Batlle E, Sancho E, Franci C (2000) The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2: 84–89.

8 Bazzoni G, Martinez-Estrada OM, Orsenigo F, Cordenonsi M, Citi S, et al. (2000) Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. J Biol Chem 275: 20520–20526.

9 Berx G, Becker KF, Hofler H, van Roy F (1998) Mutations of the human E-cadherin (CDH1) gene. Hum Mutat 12: 226–237.

10 Blaschuk OW, Sullivan R, David S, Pouliot Y (1990) Identification of a cadherin cell adhesion recognition sequence. Dev Biol 139: 227-229.

11 Boller K, Vestweber D, Kemler R (1985) Cell-adhesion molecule uvomorulin is localized in the intermediate junctions of adult intestinal epithelial cells. J Cell Biol 100: 327–332.

12 Bolos V, Peinado H, Perez-Moreno MA, Fraga MF, Esteller M, et al. (2003) The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. J Cell Sci 116: 499–511.

13 Chatterjee G, Carrithers LM, Carrithers MD (2008) Epithelial V-like antigen regulates permeability of the blood-CSF barrier. Biochem Biophys Res Commun 372: 412-417.

14 Chavakis T, Preissner KT, Santoso S (2003) Leukocyte trans-endothelial migration: JAMs add new pieces to the puzzle. Thromb Haemost 89: 13–17.

15 Chen YT, Stewart DB, Nelson WJ (1999) Coupling assembly of the E-cadherin/beta-catenin complex to efficient endoplasmic reticulum exit and basal-lateral membrane targeting of E-cadherin in polarized MDCK cells. J Cell Biol 144: 687-699.

16 Cohen CJ, Shieh JT, Pickles RJ, Okegawa T, Hsieh JT, et al. (2001) The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. Proc Natl Acad Sci USA 98: 15191-15196.

17 Douma S, Van Laar T, Zevenhoven J, Meuwissen R, Van Garderen ES (2004) Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. Nature 430: 1034–1039.

18 Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM (1998) The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J Biol Chem 273: 29745–29753.

19 Farshori P, Kachar B (1999) Redistribution and phosphorylation of occludin during opening and resealing of tight junctions in cultured epithelial cells. J Membr Biol 170: 147–156.

20 Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S (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.

21 Guttinger M, Sutti F, Panigada M, Porcellini S, Merati B, et al. (1998) Epithelial V-like antigen (EVA), a novel member of the immunoglobulin superfamily, expressed in embryonic epithelia with a potential role as homotypic adhesion molecule in thymus histogenesis. J Cell Biol 141: 1061-1071.

22 Halbleib JM, Nelson WJ (2006) Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. Genes Dev 20: 3199-3214.

23 Hirabayashi S, Tajima M, Yao I, Nishimura W, Mori H, et al. (2003) JAM4, a junctional cell adhesion molecule interacting with a tight junction protein, MAGI-1. Mol Cell Biol 23: 4267-4282.

24 Hyafil F, Morello D, Babinet C, Jacob F (1980) A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. Cell 21: 927–934.

25 Itoh M, Bissell MJ (2003) The organization of tight junctions in epithelia: Implications for mammary gland biology and breast tumorigenesis. J Mammary Gland Biol Neoplasia 8: 449–462.

26 Je Cho YW, Kim CY, Han EH, Kim, RA Anderson, et al. (2010) E-cadherin antagonizes transforming growth factor β1 gene induction in hepatic stellate cells by inhibiting RhoA-dependent samd3 phosphorylation. Hepatology 52: 2053- 2064.

27 Jiang WG, Bryce RP, Horrobin DF, Mansel RE (1998) Regulation of tight junction permeability and occludin expression by polyunsaturated fatty acids. Biochem Biophys Res Commun 244: 414–420.

28 J Lee, Choi JH, CK Joo (2013) TGF-β1 regulates cell fate during epithelial-mesenchymal transition by upregulating survivin. Cell Death and Disease 4: e714.

29 Junichi I, Kazuaki U, Sachiko T, Mikio F, Shoichiro T (2007) Requirement of ZO-1 for the formation of belt-like adherens junctions during epithelial cell polarization 176: 779–786.

30 Kavanagh E, Buchert M, Tsapara A, Choquet A, Balda MS, et al. (2006) Functional interaction between the ZO-1-interacting transcription factor ZONAB/DbpA and the RNA processing factor symplekin. J Cell Sci 119: 5098–5105.

31 Liu Y, Nusrat A, Schnell FJ, Reaves TA, Walsh S, et al. (2000) Human junction adhesion molecule regulates tight junction resealing in epithelia. J Cell Sci 113: 2363-2374.

32 Mani S, Guo W, Liao M, Eaton E, Ayyanan A, et al. (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133: 704-715.

33 Maretzky T, Reiss K, Ludwig A, Buchholz J, Scholz F, et al. (2005) ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and h-catenin translocation. Proc Natl Acad Sci U S A 102: 9182–9187.

34 Martin SS, Ridgeway AG, Pinkas J, Lu Y, Reginato MJ, et al. (2004) A cytoskeleton-based functional genetic screen identifies Bcl-xL as an enhancer of metastasis, but not primary tumor growth. Oncogene 23: 4641–4645.

35 McNeill H, Ozawa M, Kemler R, Nelson WJ (1990) Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. Cell 62: 309-316.

36 Mikio F, Tetsuaki H, Masahiko I, Akira N (1993) Occludin: A novel integral membrane protein localizing at tight junctions. J Cell Biol 123: 1777-1788.

37 Mrsny RJ, Brown GT, Gerner-Smidt K, Buret AG, Meddings JB (2008) A key claudin extracellular loop domain is critical for epithelial barrier integrity. Am J Pathol 172: 905–915.

38 Nagafuchi A, Shirayoshi Y, Okazaki K, Yasuda K, Takeichi M (1987) Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. Nature 329: 341–343.

39 Nasdala I, Wolburg BK, Wolburg H, Kuhn A, Ebnet K, et al. (2002) A transmembrane tight junction protein selectively expressed on endothelial cells and platelets. J Biol Chem 277: 16294-16303.

40 Onder TT, Piyush BG, Senduarai AM, Jing, Y, Eric SL, et al. (2008) Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res 68: 3645-3654.

41 Osler ME, Chang MS, Bader DM (2005) Bves modulates epithelial integrity through an interaction at the tight junction. J Cell Sci 118: 4667–4678.

42 Patricia KR, Christopher JP, Christopher SW, David MB, Frederick RH, et al. (2011) Bves modulates tight junction associated signaling. PLoS ONE 6: e14563.

43 Ramena G, Yin Y, Yu Y, Walia V, Elble RC (2016) CLCA2 interactor EVA1 is required for mammary epithelial cell differentiation. PLoS ONE 11: e0147489.

44 Swisshelm K, Machl A, Planitzer S, Robertson R, Kubbies M (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.

45 Sakakibara A, Furuse M, Saitou M, Ando-Akatsuka Y, Tsukita S (1997) Possible involvement of phosphorylation of occludin in tight junction formation. J Cell Biol 137: 1393–1401.

46 Smales C, Ellis M, Baumber R, Hussain N, Desmond H, et al. (2003) Occludin phosphorylation: identification of an occludin kinase in brain and cell extracts as CK2. FEBS Lett 545: 161–166.

47 Strathdee B, Kemler R (2002) Protein kinase CKII regulates the interaction of β-catenin with α-catenin and its protein stability. J Cell Sci 115: 4743–4753.

48 Kouichi T, Hiroyuki N, Kenji M, Kumi O, Wataru I, et al. (2000) Two cell adhesion molecules, nectin and cadherin, interact through their cytoplasmic domain-associated proteins. J Cell Biol 150: 1161– 1176.

49 Van Itallie CM, Anderson JM (1997) Occludin confers adhesiveness when expressed in fibroblasts. J Cell Sci 110: 113–1121.

50 Vasioukhin V, Bauer C, Degenstein L, Wise B, Fuchs E (2001) Hyperproliferation and defects in epithelial polarity on conditional ablation of a-catenin in skin. Cell 104: 605–617.

51 Walia V, Yang Y, Cao D, Cheng J, Rao K, et al. (2012) Loss of breast epithelial marker hCLCA2 promotes epithelial to mesenchymal transition and metastasis. Oncogene 31: 2237-2246.

52 Wildenberg GA, Dohn MR, Carnahan RH, Davis MA, Lobdell NA, et al. (2006) p120-catenin and p190RhoGAP regulate cell-cell adhesion by coordinating antagonism between Rac and Rho. Cell 127: 1027–1039.

53 Williams AF, Barclay AN (1988) The immunoglobulin superfamily – domains for cell surface recognition. Annu Rev Immunol 6: 381-405.

54 Williams LA, Martin PI, Dejana E, Hogg N, Simmons DL (1999) Identification and characterization of human Junctional Adhesion Molecule (JAM). Mol Immunol 36: 1175-1188.

55 Wong V (1997) Phosphorylation of occludin correlates with occludin localization and function at the tight junction. Am J Physiol 273: C1859–1867.

56 Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, et al. (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117: 927–939.

57 Yokoyama S, Tachibana K, Nakanishi H (2001) α-Catenin-independent recruitment of ZO-1 to nectin based cell-cell adhesion sites through afadin. Mol Biol Cell 12: 1595–1609.

58 Yoshimi T, Hiroyuki N (2003) Nectin and afadin: novel organizers of intercellular junctions. J Cell Sci 116: 17-27.

59 Yoshiura K, Kanai Y, Ochiai A, Shimoyama Y, Sugimura T, et al. (1995) Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas. Proc Natl Acad Sci U S A 92: 7416-7419.

60 Yu AS, McCarthy KM, Francis SA, McCormack JM, Lai J, et al. (2005) Knockdown of occludin expression leads to diverse phenoltypic alterations in epithelial cells. Am J Physiol Cell Physiol 288: C1231-1241.