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Systematic Review

Tight Junctions and Prostate Cancer

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Abstract: In this review proteins associated with tight junctions (TJs) is described with an emphasis on prostate cancer. Overall tight junctional proteins do not seem to play a decisive role in prostate carcinoma pathogenesis. Of TJ proteins, expression of some claudins show an association with clinical behaviour of the tumors. Claudin 1 expression appears to be related to a better prognosis partly due to its involvement in EMT abrogation. Claudin 3 and 4 are highly expressed in prostate cancer and their expression is associated with aggressive behaviour. Inhibition of claudin 8 promotes prostate carcinoma invasion and spread but studies are few. CPE has been known to bind to especially claudins 3 and 4 and cause cell lysis. Several experiments with modified CPE have been made in prostate cancer cell lines. Regardless of this effective CPE based human treatment for prostate cancer have not yet been developed.

Keywords: Prostate, Carcinoma, Claudin, Tight Junction

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1. Tight junctions

Tight junctions (TJ) are structures connecting adjacent cells and they are located at the apicolateral border of epithelial cells [1,2]. They regulate the paracellular permeability of cells (barrier function) regulating the movement of ions and solutes between adjacents cells in the epithelium and maintain cellular polarity isolating the apicolateral membrane area from the other parts (fence function) so that membrane proteins and lipids from separate membrane areas do not mix with each other [1,2]. In earlier ultrastructural studies defective tight junctional strands have been described in prostate carcinoma cells compared to benign hyperplasia [3]. Additionally, freeze fraction electron microscopy showed several isolated short strands which usually were situated away from the main tight junctional complex [3].

The proteins forming TJs are divided to single span proteins which include junctional adhesion molecules (JAM), angulin, and members of the superfamily of immunoglobulins, and tetraspan proteins including claudins and TJ associated marvel proteins (TAMPs) [4, 5, 6]. The latter include occludin, tricellulin or MarvelD2 and Marvel D3 and the protein crumbs homolog 3 which forms a complex with PATJ and Pals1 which are necessary for the development of apico-basal polarity in epithelial cells [4, 5, 6). Tricellulin is a TJ protein present in corner areas where three cells meet [7]. It has four transmembrane domains while angulins 1, 2 and 3 which also are present in these areas, are type I transmembrane proteins with one immunoglobulin-like domain [8].

Scaffolding proteins of TJs include ZO1 (Zonula occludens 1), ZO2 and ZO3 proteins, cingulin, cell polarity protein Par3, afadin, and the multi PDZ domain proteins MUPP1 and PAT which mediate signals from TJ structures to the interior of the cell [4]. ZO proteins belong to the membrane-associated guanylate kinase (MAGUK) homologue family with guanylate kinase domain, SH-binding domain and PDZ domains [9]. ZO proteins

have a molecular weight between 195 and 240kDa, and the carboxyterminal part associates with the cytoskeleton [9]. With PDZ domains ZO proteins may dimerise with each other and the N-terminal domain associates with tight junctional and cytoplasmic proteins such as occludin [9]. The absence of ZO1 and ZO2 proteins leads to an absence of tight junctional structures and a loss of barrier function of cells and leads to embryonic lethality [6, 2, 9]. ZO proteins evidently recruit claudins and occludin thus taking part in formation of TJs [Otani]. Afadin interacts with JAM A influencing cell motility but also affects the function of TJs [6]. ZO1, 2 and 3 have three PDZ domains and claudins bind to ZO1 by its PDZ1 domain [8]. Moreover, ZO1 binds JAM A by its PDZ3 domain and to ZO2 by its PDZ2 domain [8]. Presence of cytoplasmic and nuclear ZO1 induces expression of vimentin and MMP-14 promoting invasion and EMT [9]. It binds to angulin 1 with the PDZ2 domain [8].

Cingulin is a 140-160 kDa protein which interacts with its N terminal globular structure with ZO1, ZO2 and ZO3 and myosin and acting filaments [9]. The molecule has also a globular COOH terminal unit and a rodlike structure in between [9]. The COOH unit also interacts with myosin and ZO3 and the rodlike middle part interacts with a guanine nucleotide exchange factor, GEF-H1, (which is an activator of small GTPase) and acts as a component for dimerization of the molecule [9]. Cingulin strengthens tight junctional sealing, and its absence increases expression of occludin, ZO3 and claudins 2 and 7 [9]. Tight junctional structures are preserved in mice with knockdown of cingulin [9].

Angulin is present in tricellular junctions and recruits tricellulin to these locations [8, 10]. Interestingly, knockdown of angulin 1 destroyed the 10nm tricellular contacts, but they were not affected in case of tricellulin knockdown [8]. These contacts were also retained in quinclaudin (lacking claudins 1, 2, 3, 4 and 7) knockdown or JAM A knockdown indicating that angulin 1 is responsible for the barrier function in tricellular junctions [8]. In colon carcinoma cell lines knockdown of angulin 1 (also known as lipolysis-stimulated lipoprotein receptor) results in smaller tumor cell size and promoted apoptosis and necrosis [11]. Palmitoylation of amino acid residues is essential for localisation of angulin 1 in tricellular junctions [12]. It has been suggested that tricellulin in a similar manner like occludin mediates end to side connections in tight junctional strands which is probably related to their structural similarity [8]. The role of angulin 2 and 3 is unclear. Mutations in angulin 1 gene cause intrahepatic cholestasis and lack of the gene in embryonic mice is lethal [8]. Lack of angulin 2 causes renal concentrating defects and hearing loss and the latter also occurs in tricellulin deficiency [8, 12]. Tricellulin, claudins 3, 4, 7, MarvelD3 and TJP3 show a similar expression pattern in different types of tumor cell lines indicating epithelial differentiation [13]. When comparing epithelial differentiation to specific expression of tight junctional proteins correlation to epithelial phenotype of tumors were highest with MarcelD3, claudin 7 and ZO3, and tricellulin had a high correlation too (r=0.773) [13].

Transport of water through an epithelial cell layer can take place by a transcellular or paracellular way [10]. Aquaporins are responsible for transcellular pathway while in TJs claudins 2 and 15 regulate water permeability through the paracellular space [10]. In tricellular junctions both angulin 1 and tricellulin regulate water permeability depending on whether the renal cell lines are of a tight epithelial (MDCK C7) or intermediate cell type (HT-29/B6) the latter not being affected by the regulation [10]. Knockdown of angulin 1 decreases expression of claudin 1, 4 and occludin in MDCK C7 cells but levels of angulin 2 or 3 do not change [10]. On the other hand, in HT-29/B6 cells angulin knockdown increased the level of several claudins and water efflux is not affected [10]. Treatment of MDCKII cells with staurosporine leads to apoptosis and analysis of protein lysates show that tricellulin is a target of caspases [14]. Tricellulin was observed to be degraded three hours after staurosporine treatment [14]. The caspase target for cleavage of tricellulin is located at the carboxyterminal part of the molecule and cleavage abolished contact with

angulin 1 which was degraded in four fragments due to staurosporine treatment [14]. Tricellulin cleavage sites are located at D487 and D441 of which the former is preferred and has to be cleaved first [14]. In addition to tricellulin, ZO1, ZO2 and occludin are caspase targets [14, 15]. Occludin is cleaved to 31 and 55 kDa fragments after staurosporine treatment [15]. Of claudins 1 and 2, claudin 2 showed a 11 kDa cleavage fragment after staurosporine treatment while claudin 1 was not affected [15]. ZO1 was degraded to five and ZO2 to three fragments [15].

2. Junctional adhesion molecules

The JAMs (junctional adhesion molecules) include JAM A, B, C and JAM4 which display a 15-36 % amino acid homology with each other and belong to a part of a larger entity of cell surface immunoglobulin-like molecules [16, 4, 17, 18]. These proteins exist only in vertebrates [4]. In addition to barrier function they take part in interepithelial leucocyte migration [18]. There are also other immunoglobulin-related proteins such as JAML, CAR, CLMP, ESAM, Coxsackie- and Adenovirus Receptor (CAR) and CAR-like membrane protein (CLMR) [16, 4]. JAM A, B, C and JAM4 are expressed in many cell types, and they are present in epithelial and endothelial barriers, cells of the male reproductive system, and cells of the central and peripheral nervous system [17]. JAM A, JAM C, CAR, CLMR and ESAM associate with tight junctional complexes [4]. The function of TJs is impaired by lack of the extracellular domain of JAM A in epithelial cells [4]. JAM A contains two immunoglobulin-like domains, a transmembrane domain, and a cytoplasmic tail [6]. Adhesion of JAM A to corresponding molecules of adjacent cells or for cis linkage takes place via the extracellular part [6]. JAM A binds to β2 integrin and LFA-1, JAM B interacts with α4β1 integrin, and JAM C acts as a counter-receptor for the leukocyte integrin Mac-1 [18]. ZO1, ZO2, PAR-3, and MUPP1 belong to the tight junctional scaffolding proteins with which JAM A interacts [6]. For a fully developed TJ JAM A needs to be phosphorylated at Ser285 site and JAM A itself recruits molecular components which are needed for development of cellular polarity and TJ formation [4]. JAM A, through interaction with scaffolding proteins, influences the actomyosin contractility both in epithelial and endothelial cells which is critical for the barrier function of TJs [4]. In epithelial junctions JAM A retains membrane apposition of 6-7 nm and may hinder penetration of larger non-charged solutes through paracellular spaces in the absence of claudins [4]. Knockout of JAM A abolishes this macromolecule-involved barrier function from TJs [2]. In addition to epithelial or endothelial cells JAM A is also found in leukocytes, Sertoli cells, hematopoietic stem cells, and cells of the nervous system such as glial cells and neuronal progenitor cells and consequently it is involved in various functions such as angiogenesis, hematopoesis, inflammation, immunity and the development of nervous system [6]. The cytoplasmic domain in the C-terminal part contains phosphorylation sites and PDZ-binding sites which interact with other proteins making it possible for JAM A to function in different ways [6].

JAM A deficient mice develop non-alchoholic steatohepatitis which is due to increased leakiness of gut enterocytes for lipids [4]. The expression of JAM A is decreased in chronic inflammation of the colon such as IBD and ulcerative colitis and in experimental dextran sulfate solute induced colitis in mice where phosphorylation of Tyr280 disturbs the interaction of JAM A with Rap2 leading to decreased actomyosin contractility and disturbed TJ barrier function [4]. JAM A downregulation also contributes to increased expression of claudins 10 and 15 leading to increased paracellular permeability [4]. In lung alveolar cells, depletion of JAM A leads to abrogation of claudin 15 and ZO1 and disturbed distribution of claudins 18 and 4 and ZO2 leading to disposition of oedema formation in the alveoli [4]. JAM A has been reported to induce secretion of PSA in association with claudin 7 [19]. SNARE (soluble N-ethylmaleimide-sensitive factor associated receptor) proteins are involved in trafficking of proteins from ER and Golgi apparatus to cell mem-

brane [20]. Ykt6, one of these proteins, downregulates JAM A expression in prostate carcinoma cell line DU145 resulting in abrogation of cell migration [20]. Knockdown of Ykt6 leads to overexpression of JAM A and activation of Rap1 which induces cell motility by β1-integrin mediated mechanism [20]. Rap also activates Rac which stimulates formation of actin rich lamellopodia thus promoting cell migration [20]. Ykt6 also appears to control expression of Mir125 which regulates JAM A translation [20]. Ykt6 expression appears to vary in different carcinomas being low in prostate cancer [20].

JAM B for its part plays a role in blood brain barrier (BBB) formation [4]. Also JAM C appears to contribute to the function of BBB since its abnormal splicing leads to brain hemorrhage and its loss associates with hydrocephalus [4]. Evidently it is also present in epithelial cells and is recruited to TJs by ZO1 or Par3 [4]. JAM proteins can form homodomers and heterodimers with each other through trans interaction, however, later studies claim that JAM C also forms homophilic tetramers, JAM B forms octamers, and JAM4 forms decamers [21, 17]. Favored heterodimers include JAM A and B especially during embryonic development, JAM B and C, and JAM C and JAM4 the latter displaying the strongest strength of interaction [17]. JAM B and C help leucocytes penetrate the endothelial barrier, and they can adhere to specific leucocyte integrins [21]. JAM C is also present in human hematopoetic stem cells, lymphocytes and platelets [21]. KLN205 lung squamous carcinoma cells which do not express JAM C showed decreased proliferation with forced expression of JAM C E66R and K68E mutants and these JAM C mutants also prevented metastasis [21]. The mutation affects dimerization with JAM B which declines by 30 % [21]. Further analysis showed that dimerization is not necessary for the loss of metastatic capacity [21]. The E66R mutation was responsible for reduced migration and K68E mutation for reduced metastasis of lung cancer cells [21]. KLN205 cells with wild type JAM C produced metastases and the authors concluded that the mutations disturbed cellular polarisation which blocked the capability of the KLN205 cells to form metastases in xerographic mice [21]. The mutated cell line induced phosphorylation of p38 MAPK and ERK1/2 after stimulation with the growth factor EGF and, surprisingly, induced expression of snail, LEF1 and ADAM10 contrary to the wild type [21]. JAM C has also been shown to promote metastasis in HT1080 fibrosarcoma cells [18]. JAM A or JAM C are not related to survival in prostate cancer, but high expression of JAM C is an unfavourable sign in urothelial and renal cancer.

3. Coxsackie and adenovirus receptor

CAR (coxsackie and adenovirus receptor) can heterodimerise with JAM C and it is found in all kinds of tissues especially testis, brain, heart, lung, liver, pancreas or kidney [22, 23, 24]. CAR associated with proteins containing a PDZ domain [22]. CAR functions as a receptor for adenoviruses (especially 3 and 5), coxsackieviruses and some other viruses and it is necessary in heart and pancreatic development [22]. Because of its function as a receptor for adenovirus it has been used in experiments concerning treatment of cancer with adenovirus vectors. Human CAR is located at chromosome 21 (21q21.1) and it forms splice variants [23]. Like other JAM proteins, it contains two extracellular IgG-components needed for dimerization and contains glycosylation and palmitoylation sites in the transmembrane and cytoplasmic areas which influence its function [23]. Of stromal proteins, CAR interacts with tenascin-R, agrin and fibronectin, and of other junctional adhesion molecules with JAM C and JAM L, the latter interaction activating the phosphoinositide-3-kinase pathway in T cells which activates cell proliferation and cytokine production [23]. C-terminal PDZ domain binds ZO1, MUPP1 and other molecules by which CAR is connected to actin cytoskeleton [23]. CAR also strenghtens TJs through trans homodimerization and plays a role in inflammation and immunity [23]. Additionally, in tumors, CAR may either promote or suppress tumorigenesis depending on tumor type [23, 24]. In prostate cancer and cancer cell lines, the expression of CAR is lower than in normal cells which appear to express it constantly [24, 25]. The average expression in prostate

cancer is 80 % while in glioblastoma it was 59 % and in lung neuroendocrine carcinoma 58 % [26]. The expression is decreased along with a higher Gleason score but is re-established in metastatic sites [27]. In urogenital cancer cell lines CAR expressions was shown to be regulated by histone acetylation [25]. In line with this cell lines showed increased CAR expression when exposed to histone deacetylase inhibitors [25]. The level of CAR expression differs in different cell lines and in prostate cancer cell line PC3 it is low [28]. Transfection of CAR into this cell line appeared to diminish proliferation and migration [28]. In prostate cell lines ALVA-31, DU-145, and LNCaP histone acetylase treatment increased CAR expression and apoptosis mediated by an adenovirus vector stimulating TRAIL expression [29]. A combination of lovastatin with adenoviral-TRAIL vector appears to increase CAR expression and viral intake leading to a significant lowering of tumor growth in xenografic mice compared to adenovirus-TRAIL vector alone [30]. With a chimeric mouse-human antibody attempts have been made to treat cancer in xenograft mice with a positive response observed in lung cancer [26]. In xenograft mouse models bigenic PSA/CAR+TRAMP mice appeared to obtain a larger adenovirus intake and replication than controls [31]. With histone deacetylase inhibitors such as FK228, expression of CAR has been increased in prostate carcinoma cell lines [32]. Administration of adenovirus linked to CD40L antigen vaccine followed by FK228 increased adenovirus intake in prostate cell lines [32]. However, due to complicating factors such as changes in cell differentiation to neuroendocrine lineage this mode of treatment did not seem effective [32].

4. Occludin

Occludin like claudins has four transmembrane domains between two extracellular loops and a cytoplasmic carboxyterminal and aminoterminal end [33]. Removing the N-terminal end of occludin removes its sealing properties and the carboxyterminal part is needed for tight junctional assembly and signal transduction and it has a role in intracellular trafficking of ZO1 [33, 34]. Extracellular loops have a sealing function and are needed for attachment to the neighbouring cell [33]. N-terminus and extracellular regions are important for the TJ sealing/barrier properties, and mutations of occludin cause increased TJ permeability. Transmembrane domains co-operate with claudins, especially claudin 4 and the extracellular loop are important for occludin/tight junctional assembly [9]. Carboxyterminal part of the molecule communicates with intracellular protein and takes also part in tight junctional assembly and recruitment of occluding to TJs [9]. Occludin has splice variants including one where the fourth transmembrane domain is lacking [33]. Consequently, the carboxyterminal part is located extracellularly and ZO1 cannot bind to occludin [34]. There also exist splice variants lacking exon 1a or 9 [34]. Occludin can be degraded by MMP2/9 and MMP3 which in endothelial cells leads to damaged and leaky BBB and contributes to cerebral edema and hemorrhage in diabetic retinopathy [34]. In epithelial cells increased degradation of occludin leads to leakier barriers and increased occludin expression leads to tightening of the paracellular space [34, 9]. Forced expression of occludin in skin and cervix carcinoma cells leads to promotion of apoptosis [9]. Posttranslational modifications influence occludin function [34]. Phosphorylation of especially tyrosine influences interactions with zonula occludens and other tight junctional molecules and affects barrier function with also serine and threonine phosphorylation having its effects [34]. Occludin knockdown mice have TJs, but tight junctional stability and barrier function is affected [34]. Knockdown mice show growth retardation and reproductive defects and histological changes in various cells such as in intestine. VEGF decreases occludin phosphorylation increasing vascular permeability and high glucose concentration activates VEGF and IGF1 leading to downregulation of occluding and disruption of TJ in retinal cells similar to TGFβ [9]. Occludin expression is not related to survival in prostate cancer.

5. Claudins

Claudins are the tight junctional proteins expressed in epithelial, mesothelial and endothelial cells [35,36,37]. They were discovered when it was shown that their forced expression led to formation of tight junctions in fibroblasts [2] . Claudins show tissue specific expression which in a restricted sense can be used in differential diagnosis of tumors [35,36,37,38]. As an example of this we showed that claudins 3 and 4 can be used in differential diagnosis between pleural mesothelioma and metastatic adenocarcinoma to the pleura [37]. In Paget's disease of the breast, claudin 3 and 4 can also be used to detect neoplastic cells in the squamous epithelium of the skin [35]. Claudin 4 is strongly expressed in intrahepatic cholangiocarcinoma and can in this way be discriminated from hepatocellular carcinoma [38]. Claudins are differentially expressed already in tissue specific fibroblastic stem cells where urinary tract bladder epithelial iPSCs differed in their claudin 1, 5 and 7 expression from skin epithelial iPSCs [39]. Prostate lineage iPSCs were also analysed but only through their expression of AR and PSA with which they differed from urothelial iPSCs in an early stage [39].

Claudins are situated apicolaterally in epithelial cell membranes and serve as transepithelial barriers which are selective for molecular size and charge [40]. They separate apical and basolateral cell membranes from each other and participate in cell growth and differentiation [41]. There are 27 members of claudins that are unique in their tissue-specific expression, and their molecular weight range from 20 to 34 kDa [41, 42, 43]. Knockdown of claudins 1, 2, 3, 4, and 7 in MDCKII cells at the same time leads to lack of formation of TJs [2]. Lipids and cholesterol also play part in TJ assembly and tight junctional proteins are palmitoylated which promotes their association with lipid rafts in the cell membrane [2]. Hypothetically zonula occludens proteins by attaching to tight membrane proteins can then trigger a clustering of tight junctional proteins which, however, is a stepwise and gradual process [2]. Claudin attachment to TJs is probably associated with their phosphorylation due to the Par-3/aPKC complex [2].

Claudins are transmembrane proteins with two extracellular loops and one intracellular area of 20 protein residues in between [41, 9]. The aminoterminal and carboxyterminal sites are intracytoplasmic. There are four domains which perforate the cellular membrane demarcating the extracellular loops from the intracellular part. Thus, the molecule is like a two-wave structure perforating the plasma membrane. The extracellular loops have highly conserved regions with 60 and 24 protein residues [9]. Even though the basic structure is like those of tetraspanins, claudins do not belong to this group [44, 6]. The loop which is nearest to the aminoterminal part is larger. Claudin function is affected by post-translational modification, mainly phosphorylation which occurs in the carboxyterminal part but there are also some sites for palmitoylation [41]. The carboxyterminal part also contains the PDZ motives which mediate signalling to the interior of the cell through the ZO1-3 molecules [41]. The larger loop contains the receptor for HCV and the smaller loop for clostridium perfringens toxin (CPE) in claudin types where such areas exit [41]. The extracellular segments as well as the transcellular domains are responsible for the side-by-side assembly (cis) as well as head-to-head interactions (trans) of the claudin molecule [40]. In claudin 15 residues 39–42 on two opposing (extracellular) ECS1 loops and 146–155 on the ECS2 loops interact to form channels made by eight claudin monomers which are filled with water and ions. The pore diameter is about 6 Å and it is 50 Å long running parallel to the cell membranes, and the pore density is about 300 pores per μm in tight junctional strands [40]. In experiments with claudin 15, certain amino acids at loop sides determine the selectivity of ions, for instance flux of Na+ or K+ through the pore as indicated by experiments with replacing mutations [40]. Oxygenated Na + is about 7 Å but interaction of Na+ with oxygen is replaced by interaction with charged amino acids in pores [40]. Claudins do not only homodimerize but form heterodimers in both cis and trans positions [40, 42]. When keeping in mind the relatively large number of claudins and

RNA splicing also taking place in some of them, formation of different kind of pores can be very complex even though all claudins do not exist in same cells. Also, mutation in claudins which affect the binding for instance during heterodimerisation, can modulate pore function [40]. Additionally, other factors, such as cholesterol concentration in cell membranes may influence the trans interaction of claudins because the presence of cholesterol reduces trans interactions [40].

6. Claudins and EMT

Epitheliomesenchymal transition (EMT) is a process whereby cancer cells invade and metastasise. EMT is triggered by cytokines and growth factors such as transforming growth factor-beta (TGF-β), epidermal growth factor (EGF) and insulin-like growth factor (IGF), and signaling pathways such as mitogen-activated protein kinase (MAPK) and Phosphatidylinositol 3-Kinase (PI3K) which regulate snail, slug, twist1, twist2 and ZEB1 and ZEB2 transcription factors which in turn regulate epithelial or mesenchymal type genes such as on one hand E-cadherin, occludin and claudins, and on the other hand, vimentin, N-cadherin or α-smooth muscle actin, respectively [45, 43]. Expression of matrix metalloproteinases and their inhibitors are also a part of this process. The opposite process to EMT is mesenchymoepithelial transition (MET) which supposedly occurs when tumor cells start growing and forming epithelial sheets at distant metastatic sites [46].

Snail belongs to a family of conservative zinc finger transcription factors [47]. Other members of the group are slug and smug [47]. Snail transcription factors react with its zinc finger motives with E-box sequences of the target gene promoter or regulatory regions [47]. Snail induces the expression of mesenchymal genes, such as vimentin, fibronectin, matrix metalloproteinases MMP2, and MMP9 [47]. It has been shown that Snail and slug downregulate claudin 1 [48]. There are about 200 HLH transcription factors which can be divided in seven groups [49]. Twist1 and 2 belong to group II [49]. Twist, Slug, and Snail are associated with aggressive features and disease progression in prostate cancer [50]. Twist1 has been shown to downregulate claudin 4 in an esophageal cell line [51]. In castrate-resistant prostate carcinoma, VNLG-152, a retinamide compound which interferes with the AR pathway, downregulates slug, snail and twist in tissue xenografts [52]. MPRSS2/ERG fusion appears to induce the expression of both ZEB1 and ZEB2 and induce EMT in prostate cancer cells [53]. Many of the transcription factors involved in EMT such as snail1, slug, twist1, and ZEB1 are upregulated in prostate cancers with poor prognosis [54]. Interestingly, cholesterol lowering rosuvastatin has been shown to downregulate ZEB1 and vimentin in prostate carcinoma PC-3 cell line thus opposing EMT (55). On the other hand, ZEB1 downregulates miR200 which inhibits ETS1, ZEB1 and ZEB2 thus promoting EMT through a positive loop [54].

In Caco-2 cells which are colon carcinoma cells claudin 4 appeared to increase the activity of MMP2 and MMP9 thus increasing their invasion [56]. A similar association is also present in gastric cancer [57]. Similarly, claudin 1 activates MMP2 and MT-MMP1 in oral cancer cell lines resulting in cleavage of laminin γ2 chain [58]. Overexpression of claudin 1 in hepatocellular carcinoma also induces MMP2 [9]. However, also decreased expression of claudins 1, 4 and 7 may increase invasion in some types of cancer [57]. The relation of prostate carcinoma to the complex interaction of claudins to MMPs has not been thoroughly studied.

As components of TJs claudins participate in cell polarity and cellular adhesion [41, 43]. In cancer, cellular adhesion is decreased. In line with this many cancers show downregulation of some claudins, such as claudins 1 and 7 [41, 43]. On the other hand, expression of some claudins, such as claudins 3 and 4, may be increased in some tumors also in prostate cancer [41, 43]. Abrogation of cellular adhesion is a part of EMT. Thus, claudins contribute to this element in tumor spread and probably also to MET even though this has been studied less [41]. In prostate cancer elevated levels of ETS1 worsens prognosis [54]. Of ETS1 splice variants ETS1p51 is highly expressed in prostate cancer tissues compared

to ETS1p42 and the former induces expression of TGFβmRNA which induces Smad2 phosphorylation and promotes tumor cell migration [54]. Through TGFβ ETS1p51 stimulated EMT by inducing ZEB1 mRNA and elevating protein levels of snail and slug leading to downregulation of claudin 1 and upregulation of EMT related genes like vimentin [54]. Some ATP sensing P2X and P2Y receptors are activated in prostate cancer and after P2Y2 or P2X7 knockdown extracellular ATP mediated influence on snail, E-cadherin or claudin 1 expression was lost in prostate cancer cell lines [59, 60]. Knockdown of P2Y2 or P2X7 receptor decreases snail1 and increased expression of claudin 1 and E-cadherin in tumor cells indicating that it plays a role in prostate cancer EMT [59, 60]. Similar to this Ephrin-A2, a ligand for Eph family receptors promotes EMT by upregulating snail, slug, vimentin an N-cadherin and downregulating claudin 1 and E-cadherin in prostate cancer cell lines [61].

7. Claudins, immunology and scaffolding proteins

Claudins contribution to immune surveillance is also less studied. Through TJs claudins take part in the formation in development and function of blood brain and blood testis barrier and also contribute to that the eyes are not reached by the immune system [62]. They also regulate access of immune mediators to cells [7]. Downregulation of claudin 2 in association with starvation increases gut epithelial tightness which is regulated by autophagy associated genes [63]. Moreover, TJs prevent gut bacteria from entering the tissues and causing infection [63]. On the other hand, claudin 1 is a co-receptor for HCV virus entry to hepatocytes [64]. Covid-19 has been reported to destroy the blood testis barrier and downregulate claudin 11 in a study done on autopsy cases [65].

Claudins are connected to the cytoskeleton of the cell by scaffolding proteins. The ZO proteins (ZO 1, 2 and 3) are most important for TJs and take part in their assembly [66]. Double ZO1/ZO2 knockout cells do not form TJs [66]. Claudins are linked to ZO proteins by structural PDZ domains and attachment to tight junctional area also requires ZO protein dimerization [66]. The ZO proteins for their part are linked to actin cytoskeleton whereby changes of cytoskeletal tension is mediated to TJs [66]. Cingulin and paracingulin are rod-shaped molecules with globular ends and they are found as scaffolding proteins in TJs and adherens junctions [66]. They interact with ZO-1, and cingulin also with ZO2, ZO3 and actin microtubules [66]. Cingulin and paracingulin also mediate interaction between microtubule and actin filament [66].

8. Claudins in prostate cancer

In mouse prostate tissue, claudins 1 and 7 are located in basolateral cells while claudins 3, 4. 5. 8 and 10 are located apically in the glands [67]. In human non-neoplastic prostate at least claudins 1, 3, 4 and 7 are present [68]. In the Human protein Atlas, immunohistochemically prostate epithelial cells are positive for claudin 1, 3, 4, 5, 7 (luminal cells positive, basal cells negative), 8, 10, 11, and 12, but expression may vary depending on antibody [69]. Claudins 3 and 4 are highly expressed in non-neoplastic prostate and claudin 3 mRNA is detected in acinar cells by in situ hybridisation [70]. In some cases, a truncated form of claudin 7 is found in addition to the normal-sized claudin in non-neoplastic prostate tissue [70]. In benign prostate hyperplasia the number of TJs is decreased [71]. The expression of E-cadherin was decreased in BHPrE1 and BPH-1 prostate hyperplasia epithelial cells leading to an increase in TJ permeability in in vitro experiments [71]. TGF-β1 decreases epithelial barrier function by downregulating claudin 1 in benign prostate hyperplasia which is lower than in normal prostate [72]. Claudin 1 in cells of BPH is downregulated by TGFβ1 but snail and slug are upregulated along with increased phosphorylation of ERK1/2 [72]. Inhibition of ERK1/2 restored claudin 1 expression while it had no effect on snail or slug [72].

In prostate adenocarcinoma, increased claudin 1 expression associates with a better prognosis [73]. Claudin 1 expression was frequently associated with a positive ETS-related gene (ERG) status [73]. The ERG-TMPRSS2 fusion is one of the most common genetic alterations in prostate cancer with a frequency of about 50 % [74]. Even though mutually present immunohistochemically claudin 4 is not associated to ERG while there is an inverse association between claudin 5 and ERG in prostate cancer and hyperplasia [75]. In fact, ERG appears to be involved in claudin 5 gene regulation [76]. ERG knockdown, however, leads to decreased expression of claudin 5 in endothelial cells probably reflecting a different path of regulation of claudin 5 in endothelial cells [77]. ERG itself is related to more aggressive prostate tumors [75]. In prostate cancer gene fusions are also found between TMPRSS2 and ETV1, ETV4, and ETV5 [78]. Also, FLI1 can be a member of a fusion protein producing a SLC45A3-FLI1 rearrangement [79].

On the other hand, loss of claudin-1 expression is associated with prostate cancer invasion, progression, high grade, biochemical recurrence and metastatic transformation [80, 81, 82]. Decreased claudin 1 protein expression independently predicted disease recurrence [82]. Seo et al found a high expression of claudin 1 in 54 % of cases and low expression group had a higher Gleason score and higher PSA values [81]. While claudin 1 is located in basal cells, its low or negative expression according to some authors can be used as a marker of malignancy in prostate tissue [80]. Not surprisingly siRNA inhibition of snail leads to upregulation of claudin1 and E-cadherin in prostate cancer [83]. Ephrin-A2 which promotes prostate carcinoma spread downregulates claudin 1 as well as ZO-1 and E-cadherin [61].

In prostate cell lines HGF downregulates expression of claudins 1 and 5 and moves ZO1, ZO2 and ZO3 away from TJ thus disrupting their structure [84]. Chemoresistance of prostate carcinoma cell lines were induced by downregulation of E-cadherin and claudin 1 and upregulation of vimentin and snail through notch signaling [85]. As TGFβ promotes EMT it upregulates Par-4 (prostate apoptosis response-4) tumor suppressor gene thus leading to phosphorylation of Smad2 and IκB-α showing involvement of NF-kB and smad pathways in TGFβ/Par-4 mediated EMT in cancer [86]. Prolonged TGF-β3 exposure leads to downregulation of claudin 1 and E-cadherin, upregulation of snail and vimentin, and silencing of Par-4 by inhibitory RNAs leads to inhibition of TGFβ mediated EMT [86]. In this scenario claudin 1 of course acts as only one component of EMT- involved changes reflecting the central role of TGFβ in EMT regulation. Expression of claudins and tight junctional permeability in prostate carcinoma cell line LNCaP appears to be regulated partly by the phosphoinositide 3-kinase (PI3K)/Akt pathway, the inhibition of which leads to claudin upregulation and increase of tight junctional permeability [87].

Weak expression of claudin 2 has been reported in prostate adenocarcinoma [88]. Downregulation of Toll like receptor 9 (TLR9) by siRNA results in upregulation of claudin 2 [89]. High TLR9 expression was associated with a poor prognosis of the patients [89]. Toll like receptors influence claudins at least in other cells. TLR4 ligand upregulates claudin 4 in keratinocytes [90]. Interestingly H influenzae and S pneumoniae bacteria induce TLR2 and 4 which downregulate claudin 7 and 10 through upregulation of snail in upper laryngeal epithelium [91]. TLRs like TLR3, 4 and 9 are expressed in prostate cancer cells [92]. All these observations suggest associations between bacterial infections and tight junctional proteins and their linkage through TLRs. In experiments with mice bacterial infection of the gut has been associated with development of prostate carcinoma [93].

Claudin 3 and 4 function as receptors for clostridium perfingens enterotoxin (CPE) [42]. Also, other claudins contain CPE receptors so that claudin-3, -4, -6, -7, and -9 are regarded as high-affinity receptors and claudin-1, -2, -5, -8, -14, and -19 low-affinity receptors, while other claudins do not attach to CPE [42]. CPE is known to cause cell death in prostate and ovarian carcinoma cells due to this fact and CPE analogues have been considered promising targets for therapy of prostate and some other cancers [94]. The cytotoxity is associated to the N-terminal part of CPE which opens a pore leading to Ca influx

in the cell while CPE is recognised by the smaller loop in the C-terminal part [95]. The problem of using CPE in treatment is of course that non-malignant cells may also contain CPE binding claudins. Thus modified CPEs have been used in cancer treatment experiments where the molecule has been changed by mutation [95]. These mutant CPEs have, however, been targeted on cancers expressing other claudins than claudins 3 or 4, such as claudin 1 in thyroid cancer [95]. On the other hand, experimental treatment on claudin 3 expressing colon carcinoma cell lines and animal models with gene transfer experiments have appeared promising [96]. The value of CPE associated treatment in cancers, like prostate cancer is, however, still only apparent on an experimental level.

In prostate carcinoma patients, higher serum levels of claudin 3 were detected [97]. On the other hand, Worst et al showed that expression of claudin 3 was higher in tumors with Gleason score 8 or higher compared with Gleason score 6-7 or benign prostate hyperplasia [98]. In another study expression of claudin-3 correlated with advanced stage tumors and recurrence while expression of claudin 4 correlated with advanced stage [82]. Claudin 3 and PTEN were also associated with a positive metastatic status and a higher stage of patients [97]. Knockdown of claudin 3 and 4 leads to decreased growth, survival and invasion of prostate adenocarcinoma cells [99]. In the study of Launders et al claudin 4 expression was higher in the low Gleason group than high Gleason group ($\geq$7) but then also showed higher expression in prostate adenocarcinoma metastatic sites [100]. The authors explain the diminished claudin 4 expressions in higher Gleason score group by diminished cohesion and organisation of cells in high grade tumors [100]. In another study claudin 4 expression was associated with mean vascular density in prostate cancer [101]. Claudin 4 expression was also associated with a high tumor grade, lymphovascular invasion and lymph node metastasis [101]

Claudin 2, 3 and 5 expressions are higher in prostate adenocarcinoma compared to prostate hyperplasia while some investigators report lower or higher claudin 4 expressions [102, 88]. Claudins 1 and 7 showed a similar level in both conditions while claudin 11 was not detected in prostate epithelial cells [102]. Claudin 5, even though present in prostate adenocarcinoma, is characteristically expressed in endothelial cells. Strong claudin 5 expression was found in 65 % of cases and was associated with lower PSA values [81]. Patients who are smokers tend to have a worse prognosis of prostate cancer. In a methylome analysis of such patients, claudin 5 (promoter and body areas included) appeared to be one methylated site of ten most frequently methylated genetic regions [103]. In prostate cancer cell line PC3, inhibition of methylome preserving enzyme DNMT1, however, leads to increased claudin 3 expression [104].

In a cell line study downregulation of claudin 7, like α-catenin, appears to be related to aggressive tumor cell types with putative metastatic potential [105]. In another study decreased claudin 7 expression in prostate adenocarcinoma was found to be related to high tumor grade [82]. Stage specific embryonic antigen 4 (SSEA4) induces EMT and cell migration which is reflected by downregulation of claudin 7 and E-cadherin and upregulation of snail, slug, vimentin, ZEB1 and ZEB2 [106].

Claudin 8 expression is higher in prostate carcinoma than in benign prostate [107]. In the promoter region of claudin 8 there are two androgen-sensitive regions, and the expression is blocked by inhibiting the androgen receptor suggesting that it takes part in the regulation of claudin 8 [107]. Knockdown of claudin 8 mRNA inhibits prostate cancer proliferation and migration [107].

Claudin 1 expression is downregulated in older males suggesting that an increased transmembrane permeability of prostate epithelial cells may increase inflammation in prostate tissues contributing to prostate hyperplasia [108]. The leakiness might contribute to that intraglandular proteins might escape to the intestitium thus recruiting inflammatory T cells and macrophages to the site [108]. Similarly age-related lowering of testos-

terone levels leads to lower expression of claudins 4 and 8 and increased leakiness of epithelial barrier promoting inflammation and an autoimmune humoral response towards prostatic antigens [109].

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