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Review

Finding the junction between claudins and endometrial carcinoma

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ABSTRACT

Endometrial carcinoma (EC) defines a heterogeneous group of neoplastic diseases originating from the transformation of endometrial cells that constitute the internal lining of the uterus. To date several molecular targets have been analysed to describe the natural course of the disease, claudins being among these. Claudins are the main components of tight junctions (TJs), and their main functions are ascribed to the compartmentalization of tissues and cell-cell communication by means of intracellular ions diffusion: these features are typical of epithelial cells. Their overexpression, mis-localization or loss contribute to the malignancy of EC cells. This review collected all available data regarding the expression, regulation and claudin-related signaling pathways to provide a comprehensive view on the influence of claudin in EC progression. Further, the translational potential of claudin differential expression was explored, indicating that their role in personalized medicine could also contribute to EC therapy besides their employment for diagnosis and prognosis.

1. Structure and function of claudins

Claudins are the major component of tight junctions (TJs) strands, and main determinant of the barrier properties of epithelia and endothelia [1]. Forming a tight sealing between cellular sheets, TJs control paracellular ion flux and create a barrier to solute diffusion across the epithelia, therefore maintaining the tissue homeostasis [2]. Besides, TJs play a crucial role in the determination of cell polarity by preventing the lateral diffusion of membrane proteins and lipids, thus creating a boundary between the apical and basolateral plasma membrane domains [3]. First identified in 1998 by Furuse and colleagues in a purified fraction of membrane junctions isolated from chicken liver, claudins appeared as novel strand forming proteins since they did not show any similarity with occludin, the first integral membrane protein described in TJs [4]. The term claudin derives from the Latin word "claudĕre", which means "to close". The name anticipated its structural contribution to the formation and maintenance of the tight junctions, whose functional role in the regulation of barrier properties was progressively clarified in the following decades by the same authors and from other groups [5].

The claudin family includes 27 related members expressed in epithelia, in a tissue-specific manner [6–8]. Structurally, claudins are small transmembrane proteins with four alpha-helical membrane-spanning domains, two highly conserved extracellular loops and N and C termini oriented towards the cytoplasm [1]. Claudins mediate homo- and hetero-typical interactions in cis- and trans- manners. These different interactions result in joints with the same degree of tightness, and this different binding mode explains the strength and the selectivity of a given tissue [3,9]. Further, claudins transduce signals by the interaction of their C-terminal domain with the post synaptic density

Abbreviations: ASPP2, apoptosis-stimulating p53 protein 2; DFS, Disease-Free Survival; DSS, Disease-Specific Survival; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique (the international federation of gynecology and obstetrics); HDAC, histone deacetylase; IL, interleukin; LSR, lipolysis-stimulated lipoprotein receptor; MAPK, mitogen-activated protein kinase; OS, Overall Survival; PDZ, post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), zonula occludens-1 protein (zo-1); PFI, Progression-Free Interval; PI3K, phosphoinositide-3-kinase; scFv, single-chain Fragment variable; SFK, Src family of protein tyrosine kinases; STAT, signal transducer and activator of transcription; TCGA, the cancer genome atlas; TGF- β, transforming growth factor β; TJ, tight junction; TLR, toll-like receptor; TSA, trichostatin A; USPC, uterine serous papillary carcinoma; YAP, yes-associated protein; ZO, zonula occludens..

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protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), zonula occludens-1 protein (zo-1) (PDZ) binding domain with cytoplasmic zonula occludens (ZO) proteins. These interactions lead to the activation of a mechanotransduction connecting claudins to the cytoskeleton [10]. Alternative signaling pathways may be activated by the interaction between the extracellular loop 2 and other tight junction proteins. These interactions are triggered by the phosphorylation of tyrosines in the C-terminal domain, which recruit Src-family kinase (SFK) and PI3K pathway, leading to the activation of nuclear receptors RARγ and ERα [11].

Claudin expression is modulated by several extracellular molecules including growth factors, micronutrients, cytokines and vitamins such as EGF, HGF, Retinoic acid, T/EBP/NKX2.1, Vitamin D, Mg2+, HIV-1 Tat protein, IL-1β, IL-17. Also, several intracellular pathways have been described as claudin modulators, including TNFα/NF-κB, TGFβ-Smad/Snail, PPARγ, SP1, HNF-1α, CDX1, CDX2, GATA-4, Ghrl2 [12] in in vitro and in vivo models. Further, bacteria and LPS modulate claudin expression via TLR2 [13,14].

The dysregulation of claudins' structure, localization and expression is involved in the pathogenesis of several diseases. Point mutations along the primary structure of claudin-1 [15], −10 [16], −14 [17], −16 [18] and – 19 [19] have been reported as causal of genetic diseases gathered under the name of "claudinopathies", which usually result in the impairment of renal-dependent salt balance, followed in some cases by deafness, ocular defects and dermatological manifestations. At the blood-brain barrier level, claudin-5 dysregulation is reported to be associated with several diseases: its downregulation appears to be a necessary event prior to the development of neurologic and psychiatric disorders [20,21], indicating that tissue architecture, including that of the aforementioned barrier, might play a protective role for several pathologies.

2. Role of claudins in tumorigenesis

Claudins −1, −3, −4, −6, −7 and − 10 are the family members whose expression has been most frequently described to be abnormal in several tumors, either primary or metastases, including breast, kidney, liver, ovary, uterus, lung, pancreas, colon and stomach [22]. The abnormal expression of claudins is likely a process downstream of more relevant driving processes that lead to cell transformation, and it might be considered a marker of cell transformation as well as a functional contributor to tumor cell phenotype. In cancers, claudin expression is tightly controlled by a wide range of regulatory mechanisms such as genetic and epigenetic changes, transcriptional regulation and post-translational modifications [22]. Beside molecular events, extrinsic factors like tumor microenvironment and inflammation can also influence the dynamic expression of claudin patterns. An inflammation-induced claudin dysregulation contributes to the pathogenesis of colorectal cancer [23], however little is known about these events in other cancers.

The altered expression of claudins, both in term of up- and down-regulation, influences cell signaling, proliferation, paracellular permeability, transport of growth factor, cell-cell adhesion, cellular polarization, detachment and invasion of parenchyma, and affects the release of soluble pro-metastatic factors that can modify the stromal tissue [24].

A number of transcription factors regulate claudin expression, the most well characterized including the Snail/Slug and the ZEB families of transcriptional repressors. Loss of claudin expression can indeed mark the occurrence of epithelial-to-mesenchymal transition (EMT), an event that occurs physiologically during cell development and differentiation but that is mostly described as one of the hallmarks of cancer [25,26], and can determine the increase of tumor cells aggressive and invasive phenotype. Experimentally, the ectopic induction of EMT factors in cancer cell lines determine downregulation of TJ proteins [27] and the acquisition of metastatic behavior [28].

On the other hand, overexpression of claudins may result in protein mis-localization (i.e. to cytoplasm or nucleus [29,30] and leading to overall alteration of cell-cell contacts. This situation determines a loss of cell polarity, tissue disorganization and increases the surface of the cell membrane exposed to several extracellular signals, such as growth factors. Also, lacking apical-basal polarity, growth factor-overexposed epithelial cells tend to proliferate and divide on different spatial planes because of the mis-orientation of the mitotic axis, resulting in clonal bulks [31].

To date none of the mutations identified in claudin sequences have been described as driver mutation for endometrial carcinomas, even though a pan-cancer analysis highlights endometrium as second tumor type by alteration frequency of overall claudins genes in terms of genomic mutations (Fig. 1). However, the growing number of NGS data may make some positions stand out. Of note, the allelic frequencies observed for such mutations account from 0 to 1.2% within the TCGA Firehose Legacy Uterine Corpus Endometrial Cancer ($n = 548$) and the CPTAC Endometrial Carcinoma datasets ($n = 95$) (Fig. 2).

2.1. The evolution of EC classification

In the last 40 years pathologists have changed the histological classification of endometrial carcinomas (ECs), formerly distinguished between cervical and uterine corpus carcinomas [34]. The first classification by Bockman divided ECs into endometrioid low-grade estrogen dependent Type I ECs and non-endometrioid high grade serous non-estrogen dependent Type II ECs [35]. Currently the WHO classification of endometrial carcinoma distinguishes between serous, endometrioid, clear cell, undifferentiated/dedifferentiated and mixed histology classes. During the following years several proteins were proposed as prognostic biomarkers, including PTEN [36], ARID1A [37], HER2 [38], PIK3CA [39], CTNNB1 [40] and L1CAM [41]. A panel of 6 markers for high-grade ECs, namely p16, p53, ER, PR, IMP3 and PTEN, was reported by Alkushi et al. for histotype discrimination in immunohistochemistry: while p53 expression significantly differed between low-grade and high-grade ECs, its sensitivity and specificity was not enough to help discriminating between histotypes. ER and PR expression discriminated between endometrioid histology and others, while p16, IMP3 and PTEN were differentially expressed between serous and endometrioid carcinomas [42]. In 2013 The Cancer Genome Atlas (TCGA) consortium identified 4 prognostically distinct classes of ECs [43]: the ultramutated class, characterized by mutations in the exonucleasic domain of polymerase epsilon (POLE), the hypermutated class, characterized by microsatellite instability, the copy number high class, characterized by extensive copy number alterations and frequent p53 mutations, and the copy number low class, carrying fewer copy number alterations and p53 mutations but characterized by frequent mutations in genes such as ARID1A, KRAS, PIK3CA, CTNNB1, PTEN and ARID5B [43].

Since TCGA molecular classification publication, researchers focused on finding a simplified and more affordable surrogate with comparable prognostic power. The Proactive Molecular Risk Classifier for Endometrial Cancer [44–46] is currently demonstrating itself as a valid algorithm for risk stratification.

In the next sections, we reviewed all the advances about claudins and endometrial cancer, focusing on claudin-1, −2, −3, −4, −6, −7 and − 9, whose altered expression in some EC classes could be associated with a specific phenotype.

To have a panoramic view of the expression of each claudin, in Fig. 3 we gathered basal RNA expression data, while Supplementary Table S1 contains protein expression data of healthy female tissues, taken from The human protein atlas [47].

2.2. Claudin-1

The pilot study describing the differential expression of claudin-1 in the landscape of what was the most up-to-date histological classification

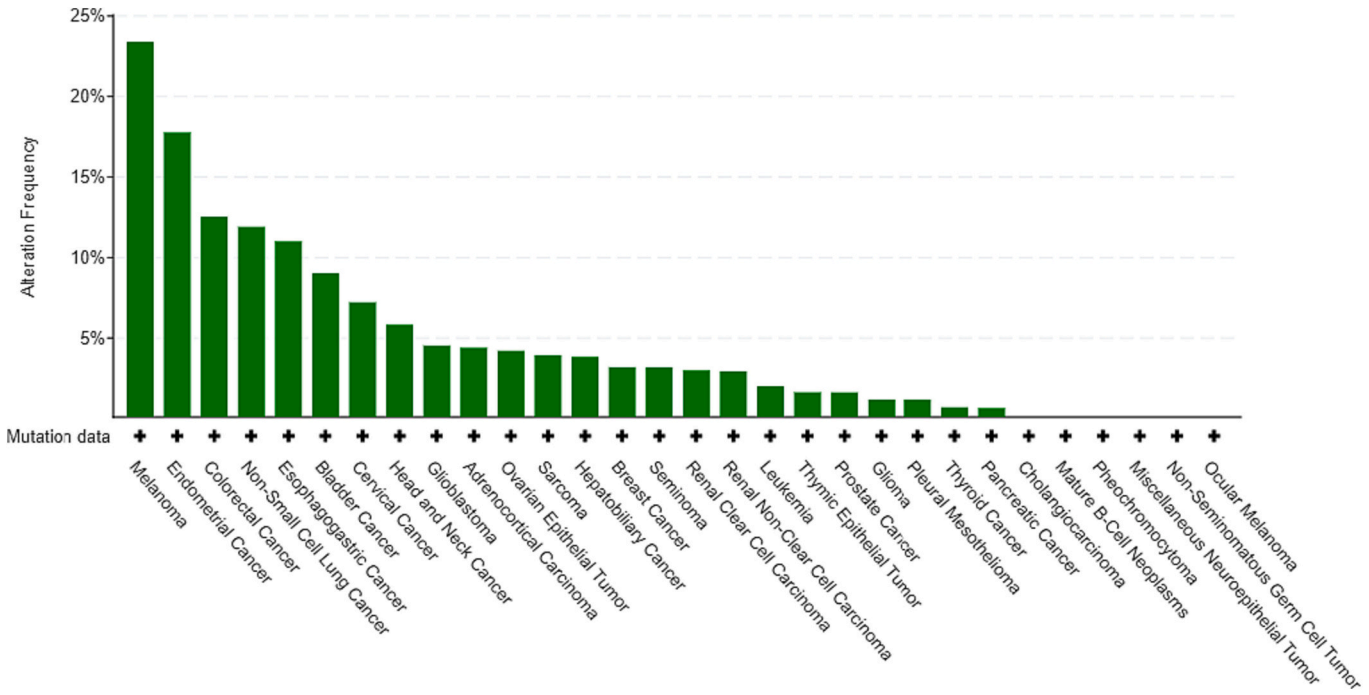

Fig. 1. C-Bioportal datasets reveal a generally low occurrence of mutations in claudins genes in cancer [32,33]. Access: 24 May 2023.

of EC was conducted by Sobel et al. in 2006. Claudin-1 was upregulated significantly in Type II ECs ($n = 15$) compared to all the controls and to Type I ECs ($n = 17$) [48]. In cisplatin-resistant EC Sawano characterized the role of claudin-1 intracellular cell junctions and invasiveness. Indeed the knockdown of Lipolysis-Stimulated Lipoprotein Receptor (LSR), which localizes at the tricellular junction, in Sawano cells increases claudin-1 and matrix-metalloproteinases expression via Sp1 activation supporting the cell invasiveness, while concomitant knockdown of *CLDN1* and *LSR* reduces their invasive phenotype [49]. To further support this evidence, the localization of claudin-1 was analysed by immunohistochemistry in 15 Type I EC. Claudin-1 relocates at the invasive front of the tissue while LSR is in the gland-like structures [49]. In 2020 the expression of LSR was correlated to the expression and activity of Apoptosis-Stimulating p53 protein 2 (ASPP2), whose knockdown or antibody-mediated blockade led to a decrease in LSR expression, with consequent upregulation of *CLDN1* [50].

2.3. Claudin-2

In addition to claudin-1, Sobel et al. identified a significant increase of claudin-2 expression in Type I ECs compared to Type II ECs [48]. More recently, Okada et al. integrated claudin-2 characterization describing its expression and localization in Type I EC by immunohistochemistry, and its role and function in Sawano cells. The expression of claudin-2 in patients, although conducted on a small cohort, indicated a direct correlation between claudin expression and tumor grade. siRNA-mediated knockdown of *CLDN2* arrests cells to G1 phase and reduces the invasiveness and migratory capacity of Sawano cells [51]. Histone deacetylase (HDAC) targeting drug Trichostatin A (TSA), which was already demonstrated to downregulate *CLDN2* expression in lung adenocarcinoma cell line A549 [52], exerted similar effects in Sawano cells, as well as inhibiting processes such as proliferation, invasion and migration [51]. We can speculate that TSA effects may be due at least in part by the down-modulation of CLDN2.

2.4. Claudin-3 and -4

Claudins 3 and 4 have been studied together in almost all the original articles found during the editing of the present manuscript, likely because their pattern of expression appears highly coordinated in most epithelial tissues and by virtue of their common role as *Clostridium Perfringens* Enterotoxin (CPE) receptors [53]. Claudin-3 and -4 alone were independently evaluated just in one and two articles, respectively.

In 2005 Santin et al. suggested a heavy upregulation of claudin-3 and claudin-4 in Uterine Serous Papillary Carcinomas (USPCs, $n = 10$) compared to Normal Endometrial Cells ($n = 5$) [54]. The upregulation of claudin-3 and -4 was confirmed by Pan et al. in 2007, in endometrioid EC (EEC) samples ($n = 30$) compared to atypical hyperplastic endometrium ($n = 15$), complex hyperplastic endometrium ($n = 12$), simple hyperplastic endometrium ($n = 20$), secretory phase endometrium ($n = 25$) and proliferative phase endometrium ($n = 25$) [55]. During the same year, Santin et al. characterized the expression of claudin-3 and -4 in endometrial carcinosarcomas and suggested the use of CPE as claudin-3 and -4 agonist in such overexpressing tumors [56,57]. In 2008, the expression of claudin-3 and -4 was analysed in a cohort of 287 patients including 137 USPC and clear cell EC. Again, serous and clear cell ECs expressed significantly higher levels of claudin-3 and -4 than EECs. Further, it was observed that the expression rate of both claudins increased with FIGO stage, and that a high expression of claudin-3 and/or − 4 associated to a significantly worse survival, both overall (OS) and disease-free (DFS), when compared to absent or weak expression [58].

Next, Pan et al. focused on claudin-4, questioning whether EECs ($n = 62$) abnormally expressed this protein compared to healthy endometria ($n = 60$), where claudin-4 almost evenly distributed by phase (proliferative $n = 34$, secretory $n = 26$), and finding significantly higher expression in tumor samples [59]. Ishikawa cells were used as model of claudin-4 highly expressing line, and the treatment with progestin drug megestrol acetate showed growth reduction and claudin-4 down-regulation both at transcript and protein levels, as well as its relocation from the membrane to cytoplasm and nucleus, allowing to speculate that progesterone might exert its anticancer effect by these mechanisms [60]. When subcutaneously grafted into nude BALB/c nu/nu mice and then treated either with antineoplastic agents such as cisplatin and paclitaxel or with saline, only cisplatin-treated Ishikawa cells formed a significantly smaller tumoral mass with significantly less claudin-4 expression, both at RNA and protein level, compared to both saline-treated controls

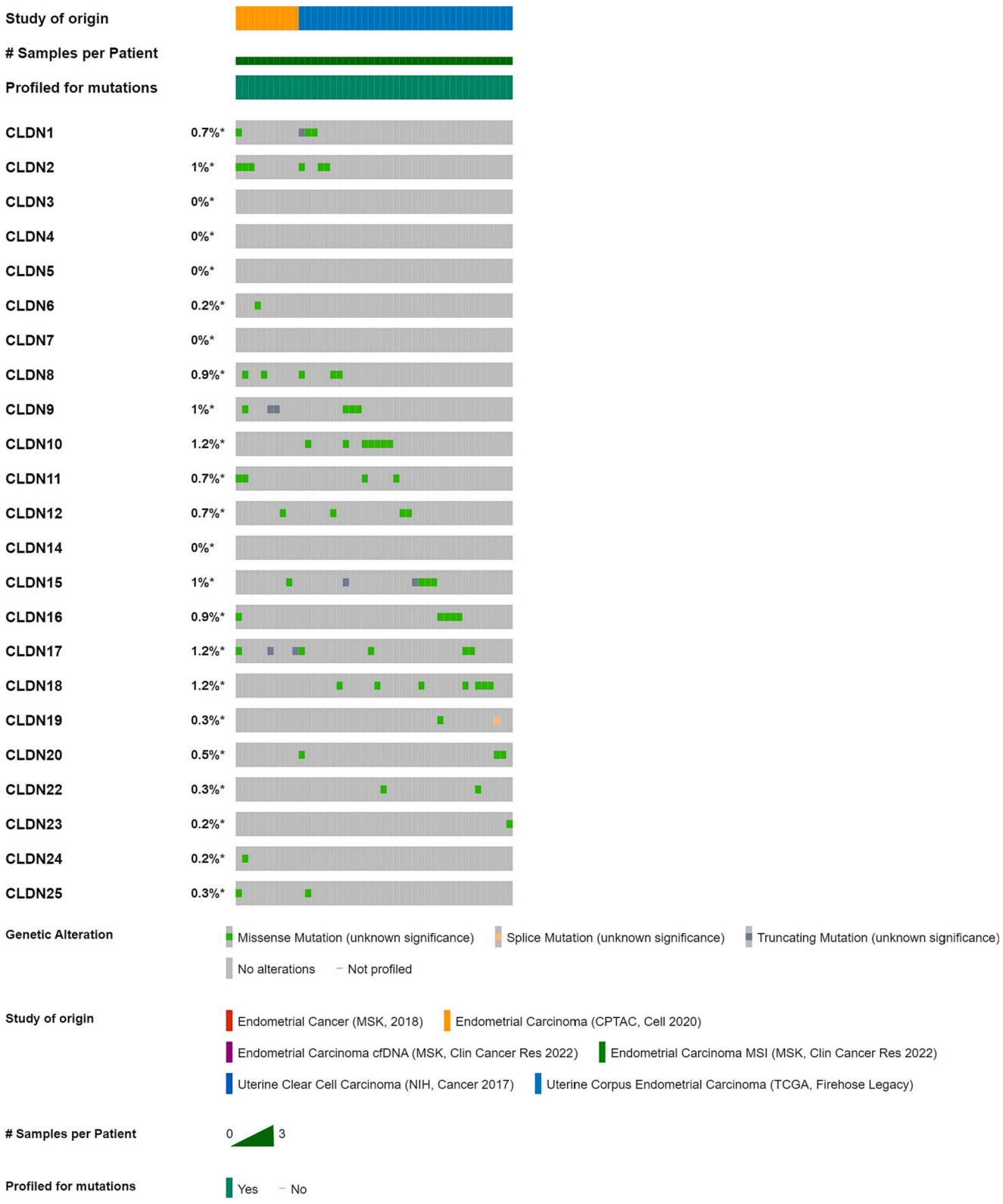

Fig. 2. Oncoprint report of ECs. Missense or truncating mutations of claudin genes show a 0 to 1.2% allelic frequency [32,33]. Access: 23 May 2023.

and paclitaxel-treated group [59].

Claudin-3 and -4 expression in progesterone- and estradiol-treated Sawano cells was investigated in 2013, and only estradiol induces their upregulation [61]. This finding apparently diverges from the just cited Pan et al. work, where megestrol acetate treatment induced downregulation of claudin-4, but we need to consider that Ishikawa and Sawano cells might model two different molecular classes of EC, and therefore stimulations of homologous targets with different ligands might exert different outputs.

Corsini et al. analysed the expression and localization of Claudin-3 in different epithelial-derived gynecological tumor samples, including uterine serous carcinoma, comparing them to healthy tissues. The

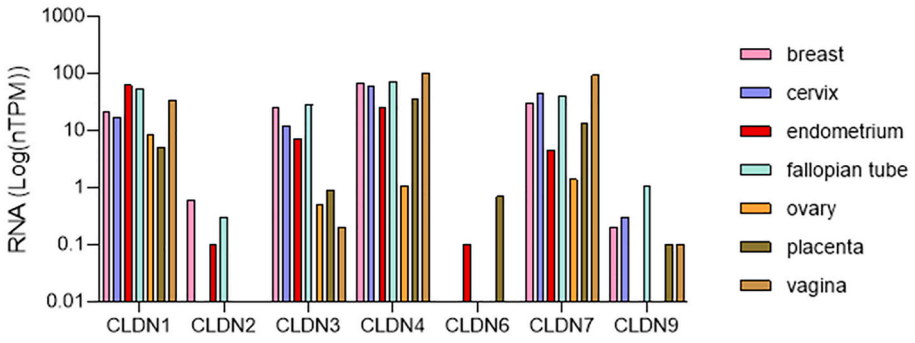

Fig. 3. RNA expression of claudin-1, −2, −3, −4, −6, −7 and − 9 in healthy female tissues. Taken from The human protein atlas [47]. Accessed on 10 Oct 2023, https://proteinatlas.org/ .

authors proved the presence of extra-junctional Claudin-3 in transformed epithelia and demonstrated that in gynecological tumors, unlike their respective healthy tissues, Claudin-3 is not confined only to the TJs, independently of its expression levels [30].

In 2020, claudin-4 was described to behave as claudin-1 following LSR knockdown, but its role in cell signaling or tight junction physiology has not been investigated [50].

2.5. Claudin-6

Claudin-6 is one of the most recently identified family members involved in EC, as its description in this pathology dates back only to 2018. Starting from the wide documentation of claudin-6 expression dysregulation in several types of cancers, Cao et al. investigated whether this would apply as well to the EC using data available on The Cancer Genome Atlas (TCGA) portal [62]. Comparing gene expression of 552 EC patients to 35 healthy endometria, claudin-6 was found upregulated in EC samples, regardless of the histology. Studying claudin-6 in 82 EC patients of an internal cohort, Cao et al. found that the comparison of its expression in cancerous lesions to the adjacent normal resected tissue revealed the same differences seen in the wider TCGA cohort. Moreover, the integration of clinicopathological data led to the conclusion that high expression of claudin-6 correlates to non-endometrioid histology and higher grade and stage, and in turn to a worse prognosis in terms of OS.

The same group employed then HEC-1-B cells as a model for claudin-6 high EC, and gene expression knockdown of this claudin induced inhibition of growth, invasiveness, migration capacity and suppression of mTOR, pAKT and pPI3K signaling pathways [62]. More recently, Kojima et al. managed to purify and validate a monoclonal antibody anti-claudin-6 with a better specificity than the commercial polyclonal one. By means of this new antibody, immunohistochemistry of 173 EC samples was performed and claudin-6 expression correlated to a significantly poorer prognosis, both in terms of OS and of disease-free survival (DFS), together with a higher surgical stage, higher histological grade and distant metastasis [63]. Further, the molecular mechanism consequent to the aberrant expression of claudin-6 in influencing endometrial cancer progression was described. The overexpression of *CLDN6* in Ishikawa EEC cells promoted pro-malignant features such as resistance to apoptosis, and increased growth in vitro and in vivo. The expression of functional claudin-6 in the membrane recruited the kinase SFK to the cytosolic face of the membrane. This interaction could be prevented by the treatment with the C-terminal domain of *Clostridium Perfringens* Enterotoxin or by the mutagenesis of the Y196/200 residues. Activated SFK led to a ligand-independent activation of ERα receptor via the PI3K/AKT pathway [64].

2.6. Claudin-7

Claudin-7 expression in ECs was first described by Li and colleagues in 2013 in a small cohort of patient samples ($n = 31$) and endometrial cell lines, revealing a substantial downregulation of claudin-7 in tumor compared to normal tissue, regardless of histological type. These first findings stated that decreased claudin-7 expression was associated with higher tumor stage and grade, and designated Ishikawa cells, KLE and RL 95–2 as *CLDN7* expressing, and AN3CA as *CLDN7*-negative cells. In vitro, silencing *CLDN7* in Ishikawa cells increased its proliferation rate and invasiveness, while *CLDN7* overexpression in AN3CA decreased these same properties compared to the original cell line, leading the authors to the conclusion that loss of claudin-7 in EC models might be responsible of an increase of the disease aggressiveness [65].

In 2020 Konno et al. described claudin-7 as one of the family members that behaves as claudin-1 consequently to the knockdown of tricellular junction receptor LSR and upstream regulators, without focusing on its role in cell signaling or tight junction physiology [50].

2.7. Claudin-9

The most recent family member described to have a role in EC was Claudin-9. Endo et al. quantified expression of Claudin-9 in endometrial cancer tissues by immunohistochemical staining, using new in-house generated monoclonal antibodies. They found high expression of Claudin-9 in 43 out of 248 endometrial carcinoma cases and a significant association with adverse clinicopathologic factors and poor prognosis, highlighting for the first time a potential role of Claudin-9 as a novel prognostic biomarker of endometrial cancer. In the same samples, the high expression of Claudin-9 correlates with the increased protein expression of claudin-6 determined using a selected monoclonal antibody, suggesting possible combination of both biomarkers to predict prognosis in endometrial cancer [66].

3. Discussion

Cancer progression is generally believed to be associated with loss of TJ and decreased expression of claudins. However, years of research have clearly shown that claudin expression is much more complex, and depends on the type of tissue, the advancement of the disease, the subtype of cancer and its origin. Overall, in reviewing several studies, it is evident that claudins are dysregulated in a variety of tumors where the most commonly identified members to have an altered expression are Claudin-1, −3, −4, −6, −7 and 11. Claudins value as prognostic marker is indeed recently being object of investigation in ovarian, gastric, breast and bladder carcinomas as well, where a claudin-low phenotype has been associated to worse prognosis and aggressive biological behavior, reflected in the higher expression of EMT and mesenchymal markers, increased invasiveness and stem cell like characteristics [67–71]. These features might also distinguish those endometrial neoplasms that downregulate the expression of specific claudins, such as those described in the section about claudin-7 [65], which could benefit from targeted therapeutic approaches with anti-EMT drugs, currently under

development [72].

All authors involved in this research field agree with a dangerous role for claudins in cancer. Both the up or down regulation of specific members of the claudin family, including claudin-1, increased the tumor malignancy in different in vitro cancer types [73]. Among the elements that may indicate the development of a biologically aggressive phenotype in epithelial-derived neoplasms is the loss of apical-basal polarity. Membranes structural mis-organization, as seen for Claudin-3-expressing ovarian and endometrial serous carcinoma cells [30], can have the double effect of allowing an out-of-plane fashioned cell proliferation and expose a greater cell membrane surface to microenvironmental molecules such as growth factors, contributing to the growth of the tumor mass.

Dysregulation of Claudin-6 expression, in particular its upregulation, has been recently reported to be a potential promising prognostic biomarker for several tumor types, ECs being among them. Indeed, Claudin-6 expression was correlated with worse OS, DSS and Progression-Free Interval (PFI), as well as clinicopathological features such as tumor stage and tumor grade, in the study of Zhang and colleagues [74], which corroborates previous similar findings of Kojima et al. [63], and strengthens the concept that Claudin-6 would represent a good marker of poor prognosis [63].

Consistent with the hypothesis that the loss of cell-cell junctional adhesion supports the metastatic abilities of cancer cells, claudin-7 expression is reported to be decreased in endometrial cancer.

Extending the look to another gynecological disease, such as high-grade serous ovarian carcinoma (HGSOC), our group showed that *CLDN7* downregulation, both in terms of transcript and protein, was significantly associated with the development of distant metastases. In particular, in tumor samples with reduced expression of claudin-7, immunohistochemical analysis highlights a discontinuous staining along the cell border, indicative of a dis-cohesive architecture that might facilitate the detachment of tumor cells from the primitive mass [71].

The authors speculated that the quantification of the expression levels of claudin-7 could be a useful tool to identify patient deserving a personalized follow-up in terms of clinical and radiological assessment: indeed, the identification of molecular biomarkers that can discriminate at diagnosis tumors that can potentially spread at distance is of the utmost importance, for a better prognostic classification of the disease and to further tailor long-term surveillance, particularly with regard to the risk of central nervous system (CNS) metastasis [71]. Since in endometrial cancer tissues the reduced expression of claudin-7 correlates with aggressive phenotype, it could be interesting to investigate its association with the risk of distant metastasis too.

Being the most recently reported claudin family member with a good prognostic capability of poor EC outcome, we don't have yet further information about how Claudin-9 expression could mechanistically impact cell signaling and therefore push EC cells towards a more malignant phenotype. Even though further independent cohort studies are still missing, this first report demonstrated Claudin-9 potentialities as an interesting marker of poor prognosis [66].

Taken together, we could conclude that claudins expression dysregulation appears to have a specific meaning in EC, particularly associated with the progression towards a more malignant phenotype and a poorer prognosis. A very intriguing topic is the analysis of the expression of claudins in the context of molecular classification of endometrial cancer. Unfortunately, in the literature there is limited information regarding this issue. Discussing the molecular subgroups of EC, Urick and Bell [75] emphasized the role of specific genomic alterations, such as PTEN and TP53, in driving different histopathological subtypes, suggesting that the expression of specific claudins may be associated with EC molecular subtypes. More research is needed to validate these assumptions. However, the predicted differential expression of claudins among molecular subtypes in EC support their role as potential biomarkers of additional prognostic classification, especially in NSMP subgroup which lacks of specific molecular markers.

Considering those family members whose expression increases, namely −3, −4, −6 and − 9, their mis-localization outside of TJs might make them accessible and targetable by high affinity molecules, such as monoclonal antibodies. This approach has been attempted by targeting Claudin-3 using a phage-display isolated scFv recombinant antibody that targets the minor extracellular domain of such antigen [76]. The same group then demonstrated that this scFv in vitro can enter the cells expressing Claudin-3 [77], speculating on a possible therapeutic rationale for the treatment of Claudin-3 overexpressing tumors based on the selective delivery of drug-loaded scFv, which those patients with poor prognosis ECs could benefit of. More recently, a humanized anti-human Claudin-6 linked to monomethyl auristatin E, an antimitotic agent, was tested in preclinical models of ovarian and endometrial carcinoma cell lines and of ovarian carcinoma patient-derived xenografts [78]. Results were encouraging and confirmed not only the specific internalization of antibodies, but also the antineoplastic activity of antibody-bound drugs. Since the anti-CLDN6 antibody showed great translational potential, a phase I clinical trial has been launched in patients with Claudin-6 expressing advanced tumors, including ovarian, endometrial and other solid tumors (NCT05103683).

In summary, the investigation of Claudins differential expression in ECs has revealed an association with the progression status, a good predictive value upon the severity of the disease, and finally constituted a good candidate target for drug development and subsequent clinical studies. Their inclusion into more modern prognostic algorithms and therapeutic strategies might contribute to efficiently stratify prognostic subgroups and to develop more personalized treatments for EC patients.

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CRediT authorship contribution statement

Davide Capoferri: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Eliana Bignotti:** Conceptualization, Supervision, Writing – review & editing. **Antonella Ravaggi:** Conceptualization, Supervision, Writing – review & editing. **Stefania Mitola:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Chiara Romani:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

We have taken claudin RNA expression data from the Human protein Atlas databases, available at the reported link https://proteinatlas.org/ Human Protein Atlas (Reference data)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbcan.2023.189019.

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