The role of tight junction proteins in ovarian follicular development and ovarian cancer

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Abstract

Tight junctions (TJ) are protein structures that control the transport of water, ions and macromolecules across cell layers. Functions of the transmembrane TJ protein, occluding (OCLN) and the cytoplasmic TJ proteins, tight junction protein 1 (TJP1; also known as zona occludens protein-1), cingulin (CGN) and claudins (CLDN) are reviewed, and current evidence of their role in the ovarian function is reviewed. Abundance of OCLN, CLDNs and TJP1 mRNA changed during follicular growth. In vitro treatment with various growth factors known to affect ovarian folliculogenesis indicated that CGN, OCLN and TJP1 are hormonally regulated. The summarized studies indicate that expression of TJ proteins (i.e., OCLN, CLDN, TJP1 and CGN) changes with follicle size in a variety of vertebrate species but whether these changes in TJ proteins are increased or decreased depends on species and cell type. Evidence indicates that autocrine, paracrine and endocrine regulators, such as fibroblast growth factor-9, epidermal growth factor, androgens, tumor necrosis factor-α and glucocorticoids may modulate these TJ proteins. Additional evidence presented indicates that TJ proteins may be involved in ovarian cancer development in addition to normal follicular and luteal development. A model is proposed suggesting that hormonal downregulation of TJ proteins during ovarian follicular development could reduce barrier function (i.e., selective permeability of molecules between theca and granulosa cells) and allow for an increase in the volume of follicular fluid as well as allow additional serum factors into the follicle that may directly impact granulosa cell functions.


Introduction

Follicular development is a coordinated series of events regulated by endocrine feedback systems of the hypothalamus–pituitary–gonad axis and is fine-tuned by autocrine and paracrine communication signals within follicular cells of the ovary involving the maturation of the oocyte, proliferation and differentiation of granulosa cells (GC) and theca cells (TC) (Richards 1980, Buccione et al. 1990, Hsueh et al. 2015). In cattle, increased follicle-stimulating hormone (FSH) recruits waves of small antral follicles (Fortune et al. 1990, Mihm & Bleach 2003, Spicer 2004) and maintains their survival (Silva et al. 2006). During development, the follicular antrum will enlarge and form the microenvironment for further maturation of the oocyte and follicular cells (Fahiminiya & Gerard 2010, Hsueh et al. 2015). This review will evaluate the current knowledge on tight junctions (TJ) and their associated proteins in follicular development. Because little is known about the function of TJ proteins in the ovary, evidence highlighting function of these TJ proteins is reviewed in the subsequent sections.

Epithelial cells usually develop specified adhesive structures to allow the communication and interaction with each other and to control paracellular permeability. TJ, adherens junctions (AJ) and desmosomes form the apical junctional complex and are abundantly expressed in epithelial and endothelial cells to provide cell–cell contacts and seal the paracellular space of the cell layer (Paris & Bazzoni 2008, Van Itallie & Anderson 2014). Among all the three junctional complexes, only TJ control the paracellular permeability for ions, water and other molecules (Balda et al. 1996, Suzuki 2013). TJ also help maintain the cell polarity by restricting the diffusion of the membrane components between apical and basolateral areas (Cereijido et al. 2008, Paris & Bazzoni 2008). In addition to ovarian follicular cells (Mora et al. 2012, Zhang et al. 2017), TJ have been characterized in primate fibroblasts (Raviola et al. 1987, Van Itallie & Anderson 1997, Furuse et al. 1998), mouse Sertoli cells (Morita et al. 1999) and mouse embryonic brain neurons (Bauer et al. 1999). Desmosomes are a type of intercellular junctions that provide strong adhesion between cells and are made of several proteins including cadherins, desmoglein and desmocollin (Owen & Stokes 2010, Hatzfeld et al. 2017). AJ are a type of adhesive junctions also involving cadherins and...
are common in epithelial cells (Yap et al. 2015, Rübsam et al. 2017).

Besides the classic barrier and fence functions, emerging studies show that TJ act as signaling complexes, mediating the exchange of the information from inside and outside the cells to regulate its own assembly and protein expression and to control intracellular gene expression and subsequent cellular responses such as proliferation and differentiation (Bordin et al. 2004, Balda & Matter 2009). TJ contains integral membrane proteins (e.g., claudins (CLDN), occludin (OCLN), junctional adhesion molecules (JAMs)) that interact with the same proteins of the adjacent cells to control the barrier functions (Citi 1993, Balda et al. 1996, Bamforth et al. 1999). Intracellular adaptor/scaffold proteins (e.g., tight junctional proteins (TJP)/zonula occludens (ZO), cingulin (CGN)) stabilize the TJ strands by linking the TJ membrane proteins to the cytoskeleton structures (e.g., actin, myosin) and regulate gene expression and subsequent cellular responses, such as proliferation and differentiation by recruiting key modulators of certain intracellular signaling pathways (Balda & Matter 2000, Balda et al. 2003, Citi et al. 2012). For example, tumorigenesis is accompanied by disrupted TJ structure, decreased expression of TJ proteins, loss of differentiation and increased proliferation and migration (Rachow et al. 2013). Gap junctions differ from TJ in that gap junctions mediate the intercellular communication by forming highly regulated channels connecting the cytoplasm of two adjacent cells to allow direct exchange of small diffusible molecules between cells (Hervé et al. 2007, Yeager & Harris 2007), and in particular, between the oocyte and cumulus cells within the follicle (Eppig et al. 1997, Winterhager & Kidder 2015). The integral membrane proteins that form gap junctions include over 20-member connexin family proteins (Evans 2015). The subsequent sections of this review will first describe these TJ proteins and later describe how they may be involved in ovarian function.

**TJ proteins**

**Occludin (OCLN)**

The first TJ protein identified, OCLN, is localized at TJ and is a highly conserved (e.g., 90% amino acid homology between human and bovine; 59–68 kDa molecular weight) 4-transmembrane protein with different domains responsible for various biological functions (Furuse et al. 1993). The N-terminus and extracellular regions are essential to provide TJ with sealing/barrier properties, as mutants cause increased TJ permeability (Wong & Gumbiner 1997) and gaps in the P-face-associated TJ strands (Bamforth et al. 1999). The extracellular loops, especially the second loop, are essential for the stable assembly of OCLN in TJ (Medina et al. 2000). Transmembrane domains are crucial for maintaining selective paracellular permeability of canine kidney epithelial (MDCK) cells (McNeil et al. 2006) by connecting and cooperating with claudins, especially CLDN4 (Balda & Matter 2000). The COOH-terminal domain of OCLN in the cytoplasm is essential for the correct assembly and barrier function of TJ (Chen et al. 1997), recruitment of OCLN to TJ (Balda et al. 1996) and the direct association of OCLN and many other peripheral membrane molecules, such as TJP1 and actin cytoskeleton (Furuse et al. 1994, Wittchen et al. 1999), and CGN (Cordenonsi et al. 1999). Expression of C-terminal mutated or normal OCLN in MDCK cells results in increased paracellular flux of small size tracers (Balda et al. 1996, 2000). Also, OCLN-knockout mice (Table 1) show normal barrier function and transepithelial resistance in epithelia of different organs (e.g., colon, stomach, urinary bladder) (Schulzke et al. 2005), but exhibit histological abnormalities in several tissues (e.g., intestinal epithelium), postnatal growth retardation and reproductive defects (Saitou et al. 2000). Increased OCLN expression is linked to increased tight epithelia (González-Mariscal et al. 2000) and increased transepithelial resistance (Balda et al. 1996, McCarthy et al. 1996). Using knockdown techniques, a study showed in vivo (mouse intestine) and in vitro (human Caco-2 monolayers) that OCLN was important for limiting macromolecule (10–70 kDa) flux across the epithelia without effecting transepithelial resistance (Al-Sadi et al. 2011) and increased TJ permeability to monovalent and divalent inorganic cations in MDCK cells (Table 1; Yu et al. 2005). Cell adhesion and apoptosis are also regulated by OCLN. For example, expression of OCLN in rat kidney fibroblasts confers cells with adhesiveness (Van Itallie & Anderson 1997) and forced expression of OCLN in human cutaneous squamous carcinoma cells (Rachow et al. 2013) and in human cervical carcinoma cells (Osanai et al. 2006) increases the sensitivity of the cells to different apoptogenic factors. Also, downregulation of OCLN in human keratinocytes results in reduced cell–cell adhesion (Rachow et al. 2013) and is a frequent observation in various tumors (Tobioka et al. 2004, Osanai et al. 2006, Orbán et al. 2008, Martin et al. 2010). The role of OCLN in ovarian function and cancer is just beginning to unfold and will be discussed in a later section.

**TJPs/ZOs**

TJPs/ZOs are members of the membrane-associated guanylate kinase (MAGUK) homologue family, characterized by a motif containing conserved protein-binding domains (one or more post-synaptic density protein 96 (PSD-96)/discs large-1 (Dlg)/ZO-1 (PDZ) domains, a Src-homology-3 (SH3) domain and a guanylate kinase (GUK) domain) (Fanning et al. 2002, Funke et al. 2005). Three ZOs (ZO-1, ZO-2 and ZO-3;
also known as tight junction protein (TJP)-1, -2 and -3 are identified as ubiquitous peripheral membrane TJ proteins (Stevenson et al. 1986, Wittchen et al. 1999). The amino acid sequence of ZO-1/TJP1 is fairly well conserved among vertebrates, with human and bovine ZO-1/TJP1 having 80% homology and range between 195 and 240kDa molecular weight. The N-terminus of ZO-1/TJP1 directly interacts with transmembrane TJ proteins (e.g., GUK domain with C-terminal of OCLN) and cytoplasmic proteins recruited to TJ; the PDZ domains mediate the dimerization of the three TJP/ZOs, which is important in strand assembly; and the C-terminal tail of ZO-1/TJP1 binds to F-actin and CGN (Fanning et al. 2007). The molecular structure makes ZO-1/TJP1 a scaffold protein that connects the TJ membrane proteins (e.g., OCLN) with cytoskeletons to regulate dynamic TJ assembly (Furuse et al. 1994). Deletion of ZO-1/TJP1 from mouse mammary epithelial cells markedly slowed TJ assembly (Umeda et al. 2004). When ZO-1 and its homolog ZO-2 were both depleted, TJ were unable to assemble in mouse mammary epithelial cells (Umeda et al. 2006). ZO-1 or ZO-2 disruption in mice (Table 1) caused embryonic lethality due to disrupted paracellular barrier and cell junctions (Katsuno et al. 2008, Xu et al. 2008). The permeability of established TJ for large solutes increased in ZO-1/TJP1 knockdown canine MDCK cells with altered cell morphology and reorganized apical actin and myosin (Van Itallie et al. 2009). In nonepithelial cells, by direct interaction with β-catenin and actin filaments at the N-terminal and C-terminal regions respectively, ZO-1/TJP1 crosslinks cadherin/catenin complex and actin cytoskeleton, and this is involved in the cadherin-based cell adhesion (Itoh et al. 1997, Van Itallie & Anderson 2014). Relocalization of ZO-1/TJP1 from the junctional complex to cytosol/nucleus causes the upregulation of genes such as vimentin or matrix metalloproteinase-14, which are part of the epithelial–mesenchymal transition and contribute to tumor invasion (Polette et al. 2007). Collectively, these studies show that TJP/ZO proteins stabilize the TJ complex at the cell membrane by passively linking junctional membrane proteins to the cytoskeleton. Moreover, TJP/ZOs also directly affect assembly of the cytoskeleton, thus regulating TJ function, cell adhesion and morphological change (Fanning & Anderson 2009). Whether ZO-1/TJP1 is involved in ovarian function and cancer will be reviewed in a later section.

### Table 1  CLDN, CGN, OCLN and TJP gene knockout/knockdown models and their phenotypes.

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<td>TJP3</td>
<td>Knockout</td>
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<td>Viable and fertile</td>
<td>Xu et al. (2008)</td>
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*CGN, cingulin; CLDN, claudin; OCLN, occludin; TJP, tight junction protein.*
**Cingulin (CGN)**

Like OCLN, CGN with a molecular weight range of 140–160 kDa is highly conserved across vertebrates (e.g., bovine and human are 87% homologous in amino acids sequence) and organized as a homodimer with each subunit containing a globular head and tail, and a central alpha-helical rod domain (Cordenonsi et al. 1999). Being first identified in avian brush-border cells (Citi et al. 1988), CGN is localized in the endofacial surfaces of TJ (Citi et al. 1989) and within the cytoplasm (Steveson et al. 1989). Most of the CGN interactions with other proteins are through its N-terminal globular head and are involved in regulating TJ assembly, cell growth and gene expression (Citi et al. 1988, 1989, Balda & Matter 2009). The N-terminus head domain of CGN interacts with ZO-1, ZO-2, ZO-3, myosin microtubules and actin filaments-6 (AF-6) (D’Atria & Citi 2001, Yano et al. 2013); the COOH-terminal interacts with myosin and ZO-3 (Cordenonsi et al. 1999); and the rod domain is involved in dimerization and interaction with a guanine nucleotide exchange factor, GEF-H1 (activator of the small GTPase, Rho member A) (Aijaz et al. 2005). The interaction between CGN and actin in vitro suggests a role of CGN in linking TJ components with actomyosin cytoskeleton, which as mentioned earlier, is important for the assembly of TJ and modulation of paracellular permeability (D’Atria & Citi 2001, D’Atri et al. 2002). CGN strengthens barrier function of endothelial cells, especially toward small-molecular-weight substances (Schossleitner et al. 2016). In addition, si-RNA-induced knockdown in human pulmonary artery endothelial cells increased thrombin-induced permeability (Tian et al. 2016). In mice, disruption of the CGN gene expression in embryoid body did not block TJ formation but altered the gene expression of other TJ proteins, such as increased OCLN, CLDN2 and CLDN7 (Guillemot et al. 2004). CGN-knockout mice (Table 1) have normal TJ structure and barrier function at the organism level, but have increased CLDN2 protein level in the duodenum and a depressed response to duodenal mucosa injury (Guillemot et al. 2012). Deletion of CGN in MDCK cells resulted in increased CLDN2 and ZO-3 mRNA and protein levels, increased activation of G1/S phase transition and increased cell density (Guillemot & Citi 2006). However, overexpression of CGN did not show the expected negative effects in MDCK cells due to some unknown compensatory mechanism (Paschoud & Citi 2008). Perhaps paracingulin, another adaptor protein that shows structural homology and dynamic behavior similar to CGN (Paschoud et al. 2011) was downregulated to compensate for overexpression of CGN, but further work will be needed to verify this suggestion. CGN has been detected at TJ, while paracingulin has been detected in both TJ and AJs, but the two distinctly interact with actin and microtubule cytoskeletons (Paschoud et al. 2011). A knockdown study in MDCK cells showed that deletion of both proteins resulted in unexpected downregulation of TJ proteins (CLDN2, CLDN3, ZO-3) without causing changes in junctional structure although junction assembly was delayed (Guillemot et al. 2013, 2014). CGN is also regulating neural crest cell migration where both knockdown and overexpression caused increased cell migration (Wu et al. 2011) an important trait in cancer cells. Thus, CGN is important in regulating TJ function and intracellular activity, such as cell proliferation, differentiation and migration. The role of CGN in ovarian function and cancer will be reviewed below.

** Claudins**

Claudins (CLDN) are tetraspan integral membrane proteins that belong to the peripheral myelin protein 22 (PMP22)/CLDN superfamily, and firstly named by Furuse et al. (1998). They are important components of tight junctions in regulating paracellular permeability and maintaining cell polarity in endothelial and epithelial cells (Furuse et al. 1998, Lal-Nag & Morin 2009). In mice, rats and other mammals, a total of 24 CLDN genes (CLDN1–24) were found, whereas humans and chimpanzees had 23 known CLDN genes (absent CLDN23). The 24 mammalian members of CLDN protein family are 20–24 kDa in size, most of them are 22–24 kDa, which span the cellular membrane 4 times with the N-terminus and C-terminus both located in the cytoplasm. The two extracellular loops are highly conserved with 60 and 24 residues, respectively. The only intracellular loop is about 20 residues (Lal-Nag & Morin 2009). The amino acid sequences of the second and third transmembrane regions are more diverse, while the sequences of the first and fourth are highly conserved. Several charged amino acids in the 60-residue extracellular loop could have an effect on paracellular ion selectivity (Lal-Nag & Morin 2009). The carboxyterminal tail among CLDN proteins show the most sequence and size heterogeneity, which contains a PDZ-domain-binding motif (Hamazaki et al. 2002, Roh et al. 2002, Rüffer & Gerke 2004, Van Itallie & Anderson 2006), targeting various posttranslational modifications (Van Itallie et al. 2005, González-Mariscal et al. 2008) to determine the structure and function of CLDN proteins. Like other TJ proteins, the CLDN proteins show a high range of sequence similarity (e.g., 88–94% amino acid homology between CLDN1–5 of human and bovine).

Overexpression of CLDN2 in rat umbrella cells using in situ adenoviral transduction increased the permeability of the paracellular route toward ions rather than large organic molecules in vitro and initiated an inflammatory process (Montalbetti et al. 2015). Human prostate cancer cells overexpress CLDN3 and CLDN4 to make these cells particularly sensitive to cytolysis by clostridium perfringens enterotoxin (CPE) resulting from the claudins aberrant distribution.
over the plasma membrane (Romanov et al. 2014). Overexpression of CLDN1-induced expression of matrix metalloproteinase-2 (MMP-2) and cell invasion and migration in normal liver and non-invasive human hepatocellular carcinoma (HCC) cells (Yoon et al. 2010). On the contrary, RNA interference of CLDN1 completely suppressed cell invasion in invasive HCC cells (Yoon et al. 2010). Knockout or knockdown models of CLDN genes and their phenotypes are listed as Table 1. Collectively, expression disorder of claudins could bring dysfunction of endothelial and epithelial cells and consequently lead to functional deficiency, tumors and/or cancers. Evidence for the role of CLDNs in normal ovarian function and cancer will be reviewed in a later section.

Regulation of TJ proteins

Expression of TJ proteins (e.g., OCLN, ZO-1) and their mRNAs were shown to be regulated by various growth and differentiation factors (e.g., TGFβ, GDF9, IGF1, estradiol) in different mammalian tissues, such as mouse retina (Walshe et al. 2009), rat colon (Braniste et al. 2009) and rat Sertoli cells (Nicholls et al. 2009). Aberrations in hormonal regulation of TJ proteins is suggested to be involved in some blood/tissue barrier dysfunctions, such as diabetic retinopathy and metastasis in certain invasive human epithelial cancers (Osanai et al. 2006, Martin et al. 2010, Akimoto et al. 2016). A reduction in OCLN protein phosphorylation by vascular endothelial growth factor (VEGF) was shown to contribute to the increased vascular permeability of human retinal endothelial cells (Harhaj et al. 2006). In a diabetic retinopathy study, high glucose levels activated VEGF and IGF1 receptor signaling, which resulted in the disruption of TJ by downregulating OCLN protein content in cultured human retinal endothelial cells (Sperri et al. 2006). Bovine mammary epithelial cells transfected with IGF1 had reduced OCLN protein content and provided no paracellular transport barrier in a phenol red transport test (Paye et al. 2007). TGF-β also increased bovine retinal endothelial cell permeability by reducing OCLN protein content (Behzadian et al. 2001). Using the same model system of bovine retinal endothelial cells, cortisol increased barrier properties with increased OCLN and CLDN5 protein content (Felinski et al. 2008, Keil et al. 2013). Glucocorticoids also increased ZO-1 protein levels in human mammary epithelial cells (Singer et al. 1994) and human corneal endothelial cells (Underwood et al. 1999). Androgens are of great importance in initiating and maintaining the normal function of TJ in the male rats (McCabe et al. 2010, Kolasa et al. 2011). IFNγ increased monolayer permeability and decreased ZO-1 protein and mRNA in T84 human intestinal epithelial cells (Youakim & Ahdieh 1999). TNFα disrupted TJ barrier function, downregulated TJ proteins (i.e., OCLN, ZO-1) in brain vascular endothelial cells of humans (Abdullah et al. 2015), mice (Lv et al. 2010) and cattle (Anda et al. 1997), as well as in rat intestinal cells (Song et al. 2009, Shen et al. 2013), bovine retinal endothelial cells (Aveleira et al. 2010) and corneal endothelial cells (Rajashkekhkar et al. 2014). Collectively, these studies indicate that TJ are dynamic structures, and gene expression and function of various TJ proteins are under regulation of a variety of hormones, including cytokines, steroids and growth factors.

In addition to the protein expression level, phosphorylation status of TJ proteins by kinase and phosphatase and other cellular and extracellular factors (e.g., EGF, VEGF, cytokines) also regulate some functions of TJ and the recruitment of TJ proteins to the cell–cell contact sites (Feldman et al. 2005, Harhaj et al. 2006) in different tissues, such as human intestinal epithelial cells (Yoshida et al. 2005), bovine retinal endothelial cells (Murakami et al. 2012) and rat mammary epithelial tumor cells (Buse et al. 1995, Woo et al. 1996).

In summary, TJ can serve as functional structures that receive and distribute signals from both inside and outside of cells to control formation of cell–cell contacts and paracellular permeability and to modulate intracellular activities, such as cell growth and migration and gene expression (Balda & Matter 2009, Spadaro et al. 2012). The TJ proteins (i.e., OCLN, CLDN, TJP1 and CGN) are key regulators that carry out these functions, and these TJ proteins are hormonally regulated. The role of these TJ proteins in ovarian function will be discussed later.

Ovarian follicular antrum formation

One characteristic of follicular growth is the formation and enlargement of an antrum. Follicular fluid (FFL) is derived from the systemic circulation through thecal capillaries, and fluid or molecules transported from microvessels to the antrum need to cross the blood–follicle barrier (BFB), which is a size- and charge-selective structure composed of the vascular endothelium, subendothelial basement membrane, theca interfissium, basement membrane and membrana granulosa (Si & Cheng 2012). FFL shares the low-molecular-weight components with serum, but shares fewer of the large-molecular-weight components (>100 kDa) (Rodgers & Irving-Rodgers 2010), because of the basement membrane acting as a molecular sieve to blood-borne proteins (Shalgi et al. 1973, Ohno et al. 2016). Concentrations of large proteins increase with increasing follicle size in cattle (Andersen et al. 1976) and mice (Zhou et al. 2007). Water transport across the follicular wall exists at both transcellular and paracellular levels and GC express aquaporins to facilitate this transcellular water transport (Huang et al. 2006, Rodgers & Irving-Rodgers 2010). Different aquaporins exist in GC of different species with aquaporin 1, 5 and 9 in porcine (Skowronska et al. 2009), aquaporin 7, 8 and/or 9 in rats (McConnell et al. 2002), aquaporin 1, 2, 3, 4 and 9 in women (Qu et al. 2010, Thoroddsen et al. 2011).
and aquaporin 3 and 9 in sheep (Sales et al. 2016). To date, no study has evaluated which aquaporins exist in GC of cattle.

The osmotic potential within the follicular antrum also drives the paracellular water transport, in addition to the transcellular transport of water. The greater osmotic potential inside the antrum is maintained by high-molecular-weight molecules (up to 300 kDa) produced by GC (e.g., hyaluronan, chondroitin sulfate), and these proteins cross-linking and recruiting other relatively smaller proteins from circulation (i.e., inter-α-inhibitor, proteoglycan link protein-1) (Rodgers & Irving-Rodgers 2010). Hyaluronan exists in the FFL of cattle (Clarke et al. 2006) and humans (Saito et al. 2000). Bovine GC in vitro produce hyaluronan (Schoenfelder & Einspanier 2003) and this protein is localized adjacent to and between GC in human antral follicles (Saito et al. 2000). Hyaluronan associates with proteoglycan-linked proteins (Sun et al. 1999, 2003) and inter-α-inhibitor (Rugg et al. 2005), thus retains them in the follicular antrum. Inter-α-inhibitor was shown in the FFL of large antral and preovulatory follicles in pigs (Nagyova et al. 2004), mice (Zhou et al. 2007) and cattle (Clarke et al. 2006). These studies indicate BFB contains paracellular junctional structures that control molecule transport, especially large proteins across the follicular wall and limit the escape of these proteins from the follicular antrum. The TJ protein, OCLN was shown responsible for paracellular permeability towards large-sized molecules in the mouse intestine (Al-Sadi et al. 2011). Therefore, it is reasonable to speculate that cellular junctions, especially TJ within ovarian follicular cells will lessen so that FFL accumulation becomes faster during development of late-stage antral follicles. However, further research will be required to verify this suggestion. The role of TJ proteins in ovarian function will be discussed next.

**TJ proteins and ovarian function**

Little work has been done to understand the role of TJ proteins in ovarian function. In the male gonad, TJ formed in Sertoli cells are important components of the blood-testis barrier (BTB) and serve as a barrier to separate adluminal germ cells from the circulation, a requirement for normal spermatogenesis (Mruk & Cheng 2010). Knockout of TJ proteins (i.e., CLDN11, OCLN) showed impaired Sertoli cell TJ permeability and compromised fertility in mice (Saitou et al. 2000, Mazaud-Guittot et al. 2010). Also, expression of TJ proteins (i.e., claudins, OCLN) and the permeability of TJ in rat Sertoli cells are regulated by many different hormones, including androgens (Kolasca et al. 2011), interleukin 6 (Pérez et al. 2012), GDF9 (Nicholls et al. 2009), TGFB (Lui et al. 2001), activin and inhibin (Wong et al. 2004). Of all knockout/knockdown studies with altered CLDNs, only one (i.e., CLDN3; Table 1) showed an altered ovarian phenotype (Sun et al. 2011). However, the study of TJ in the female reproductive system, especially during follicular development has not been thoroughly investigated.

The localization and function of TJ in ovarian follicles depend on the species being studied (Fig. 1). In mice, TJ are located in the theca externa and thecal vascular endothelial cells (Toshimori & Oura 1982), and AJ but not TJ exist in oocytes and GC (Mora et al. 2012). Protein localization of ZO-1/TJP1 in GC and oocytes of mice is related to AJ instead of TJ and may have a role in oocyte–GC interaction through cytoskeleton and AJ (Mora et al. 2012). In whole ovarian homogenates of mice, CGN and TJP1 mRNA (but not OCLN mRNA) were detected, and CGN protein was localized around GC and oocytes (Fig. 1A and B; Fleming et al. 1993, Mora et al. 2012). Also in mice, both CGN and TJP1 mRNA abundance in isolated (pooled) follicles was less in antral vs primary follicles (Mora et al. 2012) (Table 2). A recent study in cattle reported that GC contain greater CGN mRNA abundance than do TC (Zhang et al. 2017). Another study in mice showed high CGN protein content in fertilized
Table 2  Chronological summary of the changes in tight junction proteins during ovarian follicular growth.

<table>
<thead>
<tr>
<th>References</th>
<th>Species and cell typea</th>
<th>Protein or mRNAb</th>
<th>Directional change</th>
<th>Follicular stages evaluatedc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schuster et al. (2004)</td>
<td>Avian GC</td>
<td>OCLN</td>
<td>Decrease</td>
<td>Small to large</td>
</tr>
<tr>
<td>Rodewald et al. (2007)</td>
<td>Marmoset GC</td>
<td>OCLN</td>
<td>Decrease</td>
<td>Secondary to antral</td>
</tr>
<tr>
<td></td>
<td>Marmoset TC</td>
<td>CLDN5</td>
<td>Increase</td>
<td>Secondary to antral</td>
</tr>
<tr>
<td>Clelland and Kelly (2010)</td>
<td>Zebrasfish WF</td>
<td>OCLN</td>
<td>No change</td>
<td>Small to large vitellogenic</td>
</tr>
<tr>
<td>Mora et al. (2012)</td>
<td>Mice WF</td>
<td>CGN</td>
<td>Decrease</td>
<td>Primary to antral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TJP1</td>
<td>Decrease</td>
<td>Primary to antral</td>
</tr>
<tr>
<td>Hatzirodos et al. (2014)</td>
<td>Bovine TC</td>
<td>CLDN11</td>
<td>Increase</td>
<td>Small to large antral</td>
</tr>
<tr>
<td>Zhang et al. (2017)</td>
<td>Bovine GC</td>
<td>OCLN</td>
<td>Decrease</td>
<td>Small to large antral</td>
</tr>
<tr>
<td></td>
<td>Bovine GC</td>
<td>TJP1</td>
<td>Increase</td>
<td>Small to large antral</td>
</tr>
<tr>
<td></td>
<td>Bovine GC</td>
<td>CGN</td>
<td>No change</td>
<td>Small to large antral</td>
</tr>
<tr>
<td></td>
<td>Bovine TC</td>
<td>OCLN</td>
<td>Decrease</td>
<td>Small to large antral</td>
</tr>
<tr>
<td></td>
<td>Bovine TC</td>
<td>TJP1</td>
<td>Decrease</td>
<td>Small to large antral</td>
</tr>
<tr>
<td></td>
<td>Bovine TC</td>
<td>CGN</td>
<td>No change</td>
<td>Small to large antral</td>
</tr>
</tbody>
</table>

aGC, granulosa cells; TC, theca cells; WF, whole follicle; bOCLN, cingulin; CLDN, claudin; OCLN, occludin; TJP1, tight junction protein 1; c‘primary, preantral follicles with a single layer of granulosa cells; secondary, preantral follicles with multiple layers of granulosa cells.

Oocytes, which decreased during early cleavage stage until the embryo started to form TJ, indicating a role of CGN in oogenesis and embryogenesis (Javed et al. 1993). However, CGN’s role in folliculogenesis remains to be clarified.

In contrast to CGN, OCLN protein is located on the plasma membrane of GC in primate ovaries and its content decreased during follicular development in primates (Fig. 1C; Rodewald et al. 2007) and chickens (Schuster et al. 2004) (Table 2). Also in humans, OCLN protein is localized to the GC and endothelial cells but not TC and was downregulated by hCG administration in vivo (Groten et al. 2006). In zebrafish mid-vitellogenic follicles, GDF9 decreased OCLN mRNA whereas activin A, BMP15 nor TGFβ had no effect (Clelland & Kelly 2011). Gene expression for the TJ proteins OCLN and TJP1 are more prevalent in bovine TC than GC, and both are decreased with follicular development, indicating a role of these TJ proteins in bovine ovarian folliculogenesis (Zhang et al. 2017) (Table 2). In contrast, in zebrafish ovaries, OCLN protein was found in both GC and TC and its content did not change during follicular development but was regulated by GDF9 in different follicular stages (Clelland & Kelly 2010) (Table 2). A recent study in cattle revealed that GC gene expression of TJ proteins OCLN, TJP1 and CGN are regulated by autocrine, paracrine and endocrine regulators, such as fibroblast growth factor 9 (FGF9), epidermal growth factor (EGF), dihydrotestosterone (DHT), tumor necrosis factor α (TNFα), VEGFA and glucocorticoids in vitro (Zhang et al. 2017). Specifically, FGF9 significantly decreased OCLN and CGN mRNA abundance, whereas TNFα and VEGFA increased OCLN mRNA abundance but decreased CGN mRNA abundance (Zhang et al. 2017). CLDN5 protein localizes around granulosa cells in marmoset preantral follicles (Fig. 1D), and in vivo inhibition of VEGFA using soluble recombinant VEGF receptor proteins caused a decrease in theca CLDN5 protein and an increase in granulosa OCLN protein in marmoset antral follicles (Rodewald et al. 2007). Moreover, dexamethasone increased TJP1 and CGN mRNA abundance while EGF decreased and DHT increased abundance of OCLN, TJP1 and CGN mRNA in bovine theca cells (Zhang et al. 2017). Previously, we reported that TC had greater OCLN and TJP1 mRNA than GC, whereas GC had greater CGN mRNA than TC of bovine follicles (Fig. 2A; Zhang et al. 2017). Therefore, we conducted an additional study to determine if abundance of CGN mRNA in GC differed among estrogen-active and estrogen-inactive small, medium and/or large follicles in cattle and found that there were no significant differences in GC CGN mRNA expression among estrogen-inactive follicles of different sizes (Fig. 2B). However, estrogen-active follicles had 3.5-fold and 2.2-fold greater (P<0.05) CGN mRNA abundance than estrogen-inactive follicles in medium and large follicles, respectively (Fig. 2B). Furthermore, estradiol concentrations in FFL and CGN mRNA abundance in GC were positively correlated (r=0.56, P<0.01, n=31) suggesting that estradiol may regulate GC CGN mRNA expression in cattle (Fig. 3), but further study will be required to verify this. We propose a model whereby normal increases in androgens observed during follicle development increases CGN mRNA in GC, and that with increased androgens, both TJP1 and OCLN gene expression in theca cells increase (Fig. 3A). As follicles grow and develop, more androgens are converted to estrogens thereby reducing the positive effect of androgens on TJP1 and OCLN production (Fig. 3B). We further speculate that increased glucocorticoids and cytokines such as TNFα associated with stress and/or infection will prevent the normal decrease observed during development of normal large follicles, thereby increasing TJP1 and OCLN and causing fluid volume to increase leading to the development of cystic follicles. Zhang et al. (2017) proposed that the downregulation of OCLN and other TJ proteins during follicular development could reduce barrier function thereby participating in increasing follicle size by allowing for an increase in the volume of FFL as well as by allowing additional serum factors into the FFL that...
potentially may directly impact GC functions. A model for how these various hormones and growth factors regulate TJ proteins during follicular development in cattle is summarized in Fig. 3.

Of the transmembrane TJ proteins, claudins have been the most studied in the ovary, and evidence is now accumulating to support an important role for claudins in regulating ovarian follicle development.

**Figure 2** Expression of OCLN, TJP1 and CGN in granulosa and theca cells of bovine antral follicles. Panel A: Abundance of OCLN, TJP1 and CGN mRNA in granulosa and theca cells of small and large antral follicles. Data are modified (averaged across small and large follicles) from Zhang et al. (2017) with permission. Within target gene, asterisk (*) indicates theca cell mean differs ($P < 0.05$) from granulosa cell mean. Panel B: CGN mRNA in small (SM)-, medium (MD)- and large (LG)-estrogen-inactive (EI) and estrogen-active (EA) follicles. Granulosa cells (GC) were collected from MD and LG antral follicles individually, whereas GC from several SM antral follicles were pooled. Ovaries from a total of 8 cattle were used. Cells were lysed and extracted for RNA, and FFL was collected for RIA to ascertain estrogen status of follicles (Schütz et al. 2016). Values ($n = 5$–8/group) are normalized to constitutively expressed 18S ribosomal RNA. $a,b$ Means ($±$ s.e.m.) without a common letter differ ($P < 0.05$).

**Figure 3** Schematic model summarizing the interaction among tight junction proteins, hormones and growth factors during small follicle development (Panel A) and during large follicle development (Panel B) in cattle. Tight junction protein 1 (TJP1), occluding (OCLN), claudins (CLDN) and cingulin (CGN) dashed line indicates inhibitory effects; solid line with a $+$, indicates stimulatory effects. As follicles develop (panel A), androgens are stimulatory (solid line) to small antral follicle TJP1 and OCLN production/expression. These small antral follicles have low amounts of free IGF1 and high amounts of FGF9 mRNA stimulating increased amounts of TJP1, OCLN and CLDN. As follicles enlarge (panel B), androgens are converted to estrogens (which have no effect on TJP1, OCLN and CGN) while luteinizing hormone (LH) induces epidermal growth factor (EGF)-like peptide synthesis which subsequently inhibits TJP1 and OCLN production. These large antral follicles have increased amounts of free IGF1 and reduced amounts of FGF9 mRNA, which also contributes to decreased amounts of TJP1 and OCLN. Factors increasing theca CLDN11 and granulosa CGN production are unknown but may include estradiol (E2). The basement membrane is the thick line separating theca cells (TC) from granulosa cells (GC).
CLDN1, 2, 4, 7 and 8 mRNAs were broadly expressed in the ovary of zebrafish (Clelland & Kelly 2010), and CLDN1 and 7 proteins were immunohistochemically localized in human ovarian GC tumors (Soini & Talvensaari-Mattila 2006). CLDN1 was exclusively localized to the plasma membrane of steroidogenic cells and ovarian surface epithelium in the human corpus luteum (Zhu et al. 2004, Groten et al. 2006, Herr et al. 2015). CLDN5 protein and/or CLDN5 mRNA was exclusively localized to the theca vasculature and endothelial compartment in human corpus luteum (Groten et al. 2006, Rodewald et al. 2007, Herr et al. 2015), as well as the endothelial cells within the ovaries (Kitajima et al. 2006). In equine tissues, as determined by the Western blotting, CLDN1, 2, 4 and 5 were identified in uterus, and CLDN5 was strongly expressed in ovary (Lee et al. 2016). Thus, species differences may exist in terms of which CLDN is important for follicular development.

CLDN proteins in endothelial and epithelial cells of ovaries have been reported to be regulated by hormones and altered during ovarian dysfunction. In marmoset monkeys, CLDN5 protein content of theca increases during follicular growth, from the late secondary to the antral and ovulatory stage, and VEGF inhibition decreases CLDN5 protein content of TC (Rodewald et al. 2007) (Table 2). In human GC, hCG administration significantly up-regulated CLDN11 mRNA by 18.6-fold (Wissing et al. 2014), and after treatment with LH and FSH, CLDN3 mRNA was markedly elevated (Rimon et al. 2004). Without FSH-R signaling, CLDN3, 4, and 11 mRNA were selectively upregulated, whereas CLDN1 mRNA decreased in ovarian surface epithelium and tumors in mice (Aravindakshan et al. 2006) suggesting species differences exist in terms of the hormonal regulation of the specific CLDN. Exogenous GDF9 reduced the transcript abundance of CLDN7 in mid-vitellogenic follicles of zebrafish whereas activin A, BMP15, and TGFβ had no effect on CLDN7 mRNA (Clelland & Kelly 2011). Also in zebrafish, CLDN8, 10 and 12 demonstrated stage specific sensitivity to estradiol, whilst in mid-vitellogenic follicles CLDN4 and 7 mRNA abundance increased in response to hCG treatment (Clelland & Kelly 2011). For human ovarian hyperstimulation syndrome (OHSS), endothelial CLDN5 protein content was decreased by hCG alone (Rodewald et al. 2009). In rats, PMSG and hCG administration decreased both CLDN5 protein and CLDN5 mRNA (Kitajima et al. 2006), but CLDN5 protein content was increased by treatment with a VEGF inhibitor (Scotti et al. 2014a). Also, CLDN5 protein content was up-regulated in non-ovarian human endothelial cells incubated for 24 h in the presence of FFL from polycystic ovary syndrome (PCOS) patients vs FFL from control patients (Scotti et al. 2014b). Recently, Hatzirodos et al. (2014) reported that CLDN11 mRNA abundance in TC is greater in large vs small antral follicles of cattle (Table 2), which is opposite to what was observed for another transmembrane TJ protein, OCLN (Zhang et al. 2017) and suggests several TJ proteins may be involved in follicular development in cattle. In mice, CLDN11 (95% homologous to bovine CLDN11) was one of the top estrogen-upregulated genes in whole ovarian extracts (Liew et al. 2011). Clearly, further research is needed to elucidate the hormonal control and role of these various CLDNs in ovarian follicular development.

TJ were also shown to be involved in the function of corpus luteum (CL) in women (Brassart & Maillet 1975, Brassart et al. 1976) and baboons (Khan-Dawood et al. 1996). Specifically, TJP1 immunoreactivity was greatest in the mid-luteal phase than the early and late phases of the baboon (Khan-Dawood et al. 1996). VEGF is thought to be the main FFL factor that caused increased vascular permeability in human ovarian hyperstimulation syndrome because using an in vitro model for cell permeability, human FFL as well as VEGF treatment decreased ZO-1/TJP1 expression and stimulated cell permeability in cultured bovine aortic endothelial cells (Levin et al. 1998). VEGFA is also considered essential to luteal structure and function (Woad & Robinson 2016). VEGF and hCG decreased expression of TJ proteins (i.e., OCLN) during follicular development and CL formation in primates (Rodewald et al. 2007, Herr et al. 2015), and it was suggested that VEGF altered luteal permeability via regulation of CLDN5 (Rodewald et al. 2007). Recently, VEGFA was shown to increase OCLN mRNA and decrease CGN mRNA in theca cells of cattle (Zhang et al. 2017). Therefore, hormones that regulate vascular development may also be regulating TJ proteins.

Ovarian cancer is the leading cause of gynecologic malignancy death (the fifth leading cause of cancer death for women in the United States) with only a 15–30% survival rate for women (Györffy et al. 2008, Liu et al. 2015). Unfortunately, multiple cell types within the ovary are known to transform into ovarian cancer (Murphy 2010) including epithelial cells (van Baal et al. 2017) and germ cells (Nogales et al. 2014). In three studies, differential ovarian tumor ZO-1/TJP1 gene expression was associated with human ovarian cancer therapy response (Györffy et al. 2008). Although ZO-1 (i.e., TJP1) is submembrane protein of TJ, it has a role as a candidate tumor suppressor because of the SH3 domain (Hough et al. 1997) and is important for regulating cell proliferation and differentiation (Pozzi & Zent 2010, Spadaro et al. 2012). ZO-1/TJP1 is downregulated in proliferative cells, different cancer and tumor cells, and several models of epithelial mesenchymal transition (Baldia & Matter 2009).

Table 3  Chronological summary of evidence for a role of tight junction proteins in development of ovarian cancer.

<table>
<thead>
<tr>
<th>References</th>
<th>Species</th>
<th>Protein or mRNA</th>
<th>Over or under expression</th>
<th>Specific cell, tumor or tissue type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hough et al. (2000)</td>
<td>Human</td>
<td>CLDN3 and 4</td>
<td>Overexpressed</td>
<td>Several OVT types</td>
</tr>
<tr>
<td>Rangel et al. (2003a)</td>
<td>Human</td>
<td>CLDN3 and 4</td>
<td>Overexpressed</td>
<td>Several OVT types</td>
</tr>
<tr>
<td>Rangel et al. (2003b)</td>
<td>Human</td>
<td>CLDN16</td>
<td>Overexpressed</td>
<td>Several OVT types</td>
</tr>
<tr>
<td>Santin et al. (2005)</td>
<td>Human</td>
<td>CLDN3 and 4</td>
<td>Overexpressed</td>
<td>Chemotherapy-resistant/recurrent OVT vs chemotherapy-naïve OVT</td>
</tr>
<tr>
<td>Zhu and Sundfeldt (2007)</td>
<td>Human</td>
<td>CLDN3 and 4</td>
<td>Overexpressed</td>
<td>Several OVT types</td>
</tr>
<tr>
<td>Győrfi et al. (2008)</td>
<td>Human</td>
<td>TJP1</td>
<td>Overexpressed</td>
<td>Several OVT types</td>
</tr>
<tr>
<td>Dahiya et al. (2011)</td>
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<td>CLDN7</td>
<td>Underexpressed</td>
<td>SKOV3 tumor size in mice</td>
</tr>
<tr>
<td>Sun et al. (2011)</td>
<td>Human/mouse</td>
<td>CLDN3</td>
<td>Underexpressed</td>
<td>↓ SKOV3 tumor size in mice</td>
</tr>
<tr>
<td>He et al. (2013)</td>
<td>Human/mouse</td>
<td>CLDN3</td>
<td>Underexpressed</td>
<td>↓ SKOV3 tumor size in mice</td>
</tr>
</tbody>
</table>

*CLDN, claudin; OVT, ovarian tumor cell; SKOV3, human ovarian epithelial cell line; ↓, decrease.

(Rangel et al. 2003b) mRNAs are linked to human ovarian tumorigenesis and malignant transformation (Table 3). In particular, CLDN3 (Rangel et al. 2003a) and CLDN7 (Dahiya et al. 2011) are frequently overexpressed in ovarian cancer tumors, and ovarian cancer growth in experimental models has been diminished by inhibiting CLDN3 (Sun et al. 2011, He et al. 2013). Interestingly, Santin et al. (2005) found that chemotherapy-resistant/recurrent ovarian tumors had greater expression of CLDN3 and CLDN4 than chemotherapy-naïve ovarian cancers. Using an immortalized human ovarian cancer cell line, OVCAR2, transepithelial resistance was decreased and permeability was increased with knockdown of CLDN7 (Dahiya et al. 2011). In human endometrial carcinoma, OCLN protein decreases with progression of cancer (Tobioka et al. 2004). Collectively, these previous studies indicate that TJ proteins may be involved in ovarian and reproductive cancers development in addition to normal follicular and luteal development.

Studies summarized here indicate that expression of TJ proteins in follicular cells change during follicular development and are hormonally regulated. Because TJ control paracellular permeability of cell layers as well as cellular activities, the role of TJ in controlling antrum enlargement of ovarian follicles and function of follicular cells in cattle and other species should be further investigated.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

This work was supported in part by: National Natural Science Foundation of China (31501950), Beijing Nova Program (Z1411050018140446), the Oklahoma State University Agricultural Experiment Station (OKL02970), The Endowment of Howard M and Adene R. Harrington Chair in Animal Science (Project 21-58500) and State Scholarship Fund of China (201509110025).

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