Cdh1 Is Essential for Endometrial Differentiation, Gland Development, and Adult Function in the Mouse Uterus[1]

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ABSTRACT

CDH1 is a cell-cell adhesion molecule expressed in the epithelium to coordinate key morphogenetic processes, establish cell polarity, and regulate epithelial differentiation and proliferation. To determine the role of CDH1 in the mouse uterus, Cdh1 was conditionally ablated by crossing Pgr-Cre and Cdh1-flox mice, and the phenotype was characterized. We found that loss of Cdh1 results in a disorganized cellular structure of the epithelium and ablation of endometrial glands in the neonatal uterus. $Cdh1^{d/d}$ mice lost adherens junctions (CTNNB1 and CTNNA1) and tight junctions (claudin, occludin, and ZO-1 proteins) in the neonatal uterus, leading to loss of epithelial cell-cell interaction. Ablation of Cdh1 induced abnormal epithelial proliferation and massive apoptosis, and disrupted Wnt and Hox gene expression in the neonatal uterus. Although the uteri of $Cdh1^{d/d}$ mice did not show any myometrial defects, ablation of Cdh1 inhibited expression of epithelial (cytokeratin 8) and stromal (CD10) markers. $Cdh1^{d/d}$ mice were infertile because of defects during implantation and decidualization. Furthermore, we showed in the model of conditional ablation of both Cdh1 and Trp53 in the uterus that interrupting cell cycle regulation through the loss of Cdh1 leads to abnormal uterine development. The uteri of $Cdh1^{d/d}$ $Trp53^{d/d}$ mice exhibited histological features of endometrial carcinomas with myometrial invasion. Collectively, these findings suggest that CDH1 has an important role in structural and functional development of the uterus as well as adult uterine function. CDH1 has a capacity to control cell fate by altering directional cell proliferation and apoptosis.

Cdh1, endometrial differentiation, gland development, implantation, uterus

INTRODUCTION

Uterine endometrium consists of a simple luminal epithelium (LE) supported by stroma cells that contain coiled endometrial glands [1–3]. Although histogenesis of the uterus is initiated in the fetus, uterine development is not completed until after birth in humans, laboratory rodents, and domestic livestock [2, 4–6]. A major developmental event in the neonatal uterus is adenogenesis, which is defined as the differentiation and development of glands in the endometrium [5, 6].

Histologically, the mouse uterus lacks endometrial glands and consists of a simple LE and relatively undifferentiated mesenchyme at birth [1]. Between birth (Postnatal Day 0 or P0) and P3, the three layers of mesenchyme are distinctly segregated into endometrial stroma and the inner circular and prospective outer longitudinal layers of myometrium. By P5–6, epithelial invaginations appear in the LE that represent the formation of glandular epithelium buds [7]. By P10–12, endometrial glands extend from the LE into the surrounding endometrial stroma, and the outer longitudinal layer of the myometrium is fully organized into bundles of smooth muscle cells [1]. The basic adult histoarchitectural configuration of the mouse uterus is established by P15–20 [8, 9]. Uterine morphogenesis is critical because disruption of endometrial adenogenesis and mesenchymal specification and differentiation can cause permanent fertility problems in the adult [5, 10–13].

Complex Wnt and Hox gene networks between epithelium and stroma regulate developmental processes of the uterus [14–17]. Knockout mice studies have shown that mice lacking Wnt7a, Wnt5a, or Foxa2, a member of the forkhead transcription factor family, lack uterine glands and have disrupted uterine adenogenesis [10, 17–19]. Thus, epithelial differentiation and growth are key processes in uterine morphogenesis and are central to the formation of uterine development.

In epithelial tissues, interactions between neighboring cells are mediated by adherens junctions. Adherens junctions play a role in the establishment of cell polarity and maintenance of the epithelial phenotype, and are essential for epithelial development, including cell differentiation and proliferation [20]. CDH1 (E-cadherin) belongs to the cadherin superfamily of cell adhesion molecules [21] and is a single-span transmembrane glycoprotein consisting of five extracellular cadherin-binding domains and a conserved intracellular domain that associates with members of the catenin family. CTNNB1 (β-catenin) is one of the critical intracellular mediators necessary for the maintenance and function of CDH1 in cell-cell interactions. CTNNB1 binds to the cytoplasmic domain of CDH1 [22, 23], and CTNNA1 (α-catenin) bridges the cadherin-catenin adhesion complex with the actin filament network [24]. The intracellular mechanism of cadherin/catenin adhesive activity is critical to regulate architectural development of epithelial differentiation.

In the present study, we investigated the role of CDH1 in uterine function. We generated a mouse model in which Cdh1 was conditionally ablated in the developing uterus because global mutation of Cdh1 causes early embryonic lethality [25, 26]. The ablation of Cdh1 resulted in defects in epithelial gland formation and adult uterine function as a result of alternation of

uterine cell fate, disruption of epithelial-stromal interaction, and induction of abnormal epithelial differentiation.

MATERIALS AND METHODS

Animals and Tissue Collection

Mice were maintained in the vivarium at Southern Illinois University according to the institutional guidelines for the care and use of laboratory animals. B6.129-$Pgr^{tm2(cre)Lyd}$ (also known as $Pgr^{cre/+}$) mice were provided by Drs. Franco DeMayo and John Lydon [27]. B6.129-$Cdh1^{tmKem2}$/J (also known as $Cdh1^{flox}$, Jax no. 005319) and FVB.129-$Trp53^{tm1Brn}$ (also known as $Tp53^{flox}$, no. 01XC2) were obtained from The Jackson Laboratory and the Mouse Models of Human Cancers Consortium, National Institutes of Health, respectively. To determine the effects of loss of $Cdh1$ or $Trp53$ on neonatal uterine morphogenesis and gene expression, female pups were necropsied on P5, P10, P15, P20, P35, or P60 (n = 5/day). Two hours before they were euthanized, the pups received subcutaneous injections of 100 mg/kg of bromodeoxyuridine (BrdU) (Sigma) to assess cell proliferation.

Pregnancy samples were obtained by the mating of wild-type C57BL/6 mice, and the day that a vaginal plug was observed was considered Day 0.5 of pregnancy. Artificial decidualization was hormonally induced following previously described methods [28]. The uterine decidual response was observed 5 days after stimulation. At collection, uterine tissues were fixed in fresh 4% paraformaldehyde in PBS at room temperature for 8–12 h and embedded in paraffin, or snap-frozen in liquid nitrogen and stored at −80°C.

Immunohistochemistry

Immunolocalization of CDH1, CK8 (cytokeratin 8; official symbol KRT8), CTNNB1, BrdU, claudin, occludin, ZO-1 (official symbol TJP1), CTNNA1, CD10 (official symbol MME), and αSMA (smooth muscle alpha actin; official symbol ACTA2) was performed in cross-sections (5 μm) of paraffin-embedded uterine sections using specific antibodies and a Vectastain Elite ABC kit (Vector Laboratories). The antibodies used in the analyses were anti-CDH1 (2 μg/ml [final concentration], 610181; BD Biosciences), anti-CK8 (1:2000 dilution, MMS-162P; Covance), anti-CTNNB1 (1.5 μg/ml [final concentration], 610513; BD Biosciences), anti-BrdU (1:200 dilution, 11 170 376 001; Roche), anti-claudin (1.25 μg/ml [final concentration], 51-9000; Invitrogen), anti-occludin (2.5 μg/ml [final concentration], 71-1500; Invitrogen), anti-ZO-1 (5 μg/ml [final concentration], ab2272; Abcam), anti-CTNNA1 (1:100 dilution, ab51032; Abcam), anti-CD10 (1:50 dilution, ms 728S0; Thermo Fisher Scientific), and anti-αSMA (1:500 dilution, ab5694; Abcam); negative controls were performed by substituting the same concentration of normal immunoglobulin G (Sigma) for the primary antibody. Antigen retrieval using a boiling citrate buffer was performed as described previously [29].

Quantitative Real-Time RT-PCR

Total RNA was isolated from mouse uterus using the Trizol reagent (Invitrogen) according to the manufacturer's recommendations. The quantity and quality of total RNA was determined by spectrometry and denaturing agarose gel electrophoresis, respectively. The cDNA was synthesized from total RNA (2 μg) using iScript Select cDNA synthesis kit (BioRad). Real-time PCR analysis of mRNA expression was performed using a MyiQ Single-Color real-time PCR detection system (BioRad) with iQ SYBR Green supermix (BioRad) as the detector according to the manufacturer's recommendations. The primers shown in Supplemental Table S1 (all the supplemental data are available online at www.biolreprod.org) were designed to amplify cDNAs of around 100 bp, and all exhibited similar amplification efficiency (95% ± 3%) as assessed by amplification of cDNA dilution series. PCR cycle parameters were 95°C for 15 sec and 60°C for 1 min for 40 cycles. The threshold line was set in the linear region of the plots above the baseline noise, and threshold cycle (C_T) values were determined as the cycle number at which the threshold line crossed the amplification curve. PCR without template or template substituted with total RNA were used as negative controls to verify the experimental results. After amplification, the specificity of the PCR was determined by both melt-curve analysis and gel electrophoresis to verify that only a single product of the correct size was present. Data were normalized against $Gapdh$ and are shown as the average fold increase ± SEM. The fold changes are equivalent to $2^{(x-y)}$ where x is the C_T value of the control and y is the C_T value of ablation of $Cdh1$.

Statistical Analysis

All the experimental data were subjected to one-way ANOVA, and differences between individual means were tested by a Tukey multiple-range test or a Student t-test using Prism 4.0 (GraphPad). Real-time PCR data were corrected for differences in sample loading using the $Gapdh$ data as a covariate. Tests of significance were performed using the appropriate error terms according to the expectation of the mean squares for error. A P value of 0.05 or less was considered significant. Data are presented as least-square means with the SEM.

RESULTS

Impact of Conditional Ablation of Cdh1 in Neonatal Uterus

$Cdh1$-null mice show early embryonic lethality [25, 26]. In order to examine the role of CDH1 in the neonatal uterus, conditional ablation of $Cdh1$ was conducted to circumvent the embryonic lethal phenotype. We utilized the $Pgr^{Cre/+}$ mouse line in which Cre recombinase is under the control of the Pgr promoter [27]. $Pgr^{Cre/+}$ mice were crossed with $Cdh1^{f/f}$ mice [30] to provide a tissue-specific knockout of $Cdh1$ ($Pgr^{Cre/+}$ $Cdh1^{f/f} = Cdh1^{d/d}$) in Pgr-expressing cells. Our previous study confirmed that the $Pgr^{Cre/+}$ mouse line is an excellent model to ablate epithelial genes in the neonatal uterus [8, 18].

Histological analysis of neonatal uteri was conducted in $Cdh1^{f/f}$ and $Cdh1^{d/d}$ mice on P5, P10, P15, and P20 (Fig. 1, A and B). Uterine histology revealed that ablation of $Cdh1$ in the neonatal uterus resulted in a disorganized cellular structure of the epithelium and disrupted endometrial adenogenesis. Although there were no developing endometrial glands in control uteri on P5, LE in the uteri of $Cdh1^{d/d}$ mice already exhibited disorganization. On P10, we did not see a formed, single epithelial cell layer, and the epithelial cells present were extremely abnormal and rounded in shape. The majority of cells had lost cell-cell connection, were detached from the uterus, and gathered in the uterine lumen. On P15 and P20, epithelial cells were distinguishable; however, each cell was disorganized lacking any normal epithelial cellular structure. High-resolution examination of the uteri on P20 using transmission electron microscopy revealed that the uterine epithelium of $Cdh1^{d/d}$ mice had neither columnar epithelium nor cell adhesion between neighbor cells (Fig. 1B). However, we did not see any difference in total uterine size between $Cdh1^{f/f}$ and $Cdh1^{d/d}$ mice on P20 (Fig. 1C).

To validate CDH1 ablation, CDH1 immunoreactivity was determined in the neonatal uterus (Fig. 2). Immunohistochemical analysis confirmed that epithelial CDH1 was ablated by Pgr-driven Cre activity on P10 and P20. CK8, an epithelial cell-specific marker, was decreased but still detectable in the uteri of $Cdh1^{d/d}$ mice on P10; however, it was completely lost on P20, suggesting that disorganized and abnormal cells were losing epithelial characteristics during endometrial adenogenesis. CD10 is a known marker of normal endometrial stromal cells [31]. Because epithelial-stromal interaction is a key component of uterine development, immunoreactivity toward CD10 was also examined. While we observed stromal-specific CD10 in $Cdh1^{f/f}$ mice, CD10 was completely absent in the uteri of $Cdh1^{d/d}$ mice, suggesting that ablation of $Cdh1$ causes abnormal stromal differentiation. In contrast, there was no difference in the localization of αSMA, which is a marker of smooth muscle, in the uteri of $Cdh1^{f/f}$ and $Cdh1^{d/d}$ mice, indicating that the loss of $Cdh1$ in neonatal uterus does not have any gross effect on the myometrium (Fig. 2).

Previous [8] and current results (Fig. 2) show that proliferative cells are present in the endometrial and myometrial layers of the neonatal uterus. Specifically, active cell proliferation is obvious in the developing glands on P10 but ceases by P20 when uterine morphogenesis is structurally completed. In $Cdh1^{d/d}$ mice, we observed that the majority of abnormal epithelial cells were BrdU positive on P10 and P20. Further, TUNEL analysis revealed that detached cells observed
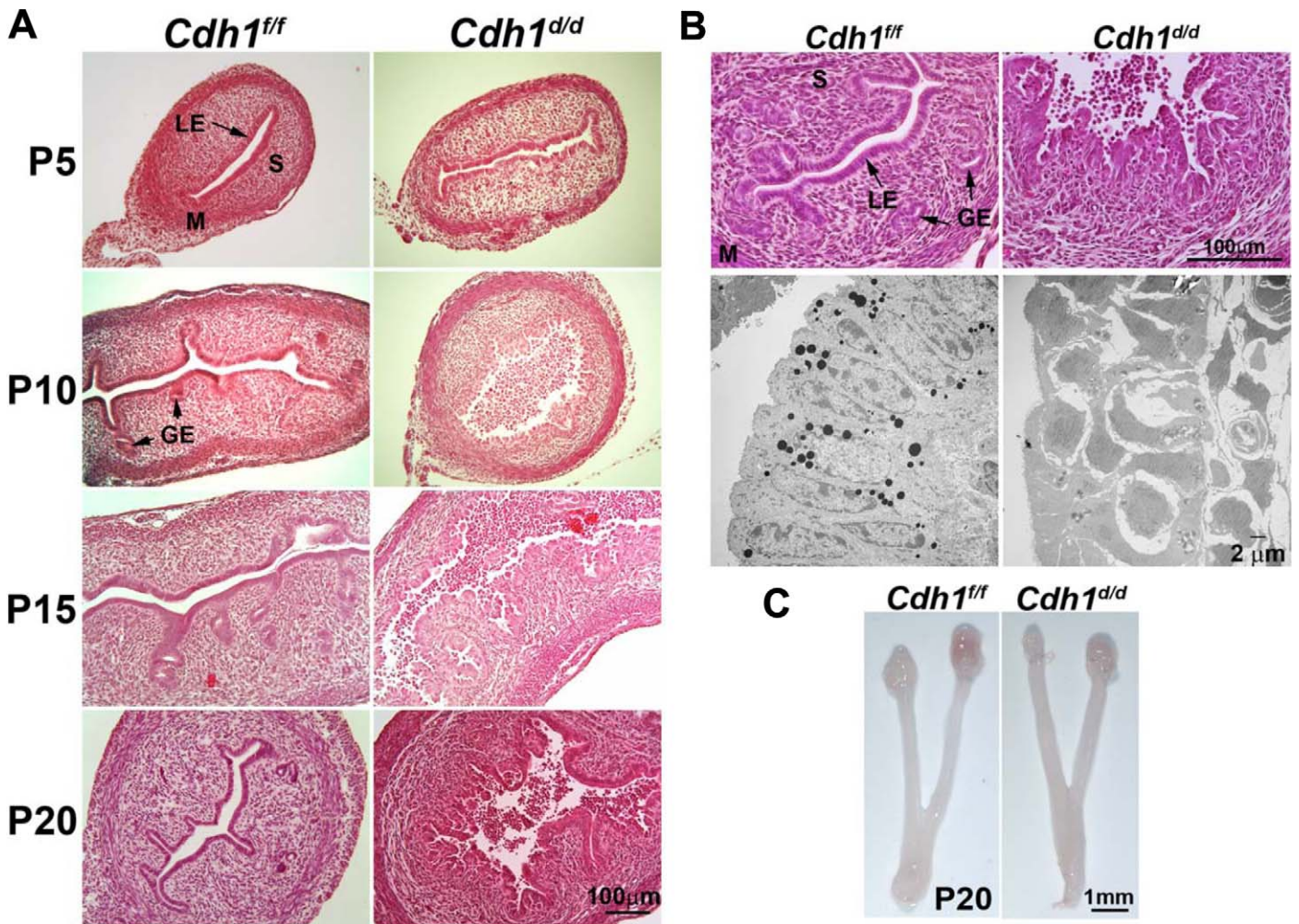
Article 141

FIG. 1. Ablation of *Cdh1* disrupts mouse uterine gland development. **A**) Uterine histology of *Cdh1^f/f* and *Cdh1^d/d* mice. Tissues were stained using hematoxylin and eosin. GE, glandular epithelium; LE, luminal epithelium; M, myometrium; P, postnatal; S, stroma. **B**) Uterine histology and transmission electron microscopy of *Cdh1^f/f* and *Cdh1^d/d* mice on P20. **C**) Gross anatomy of ovary and uterus of *Cdh1^f/f* and *Cdh1^d/d* mice on P20.

in the uterine lumen of *Cdh1^d/d* mice were apoptotic on P10, whereas fewer cells were TUNEL positive in the LE of *Cdh1^f/f* mice. On P20, apoptotic cells were still observed in disorganized epithelium of the uteri of *Cdh1^d/d* mice but not in the uteri of *Cdh1^f/f* mice. Thus, ablation of *Cdh1* affected the critical period of endometrial adenogenesis by changing epithelial cell fate.

Because epithelial cells exhibit loss of cell-cell connection in the uteri of *Cdh1^d/d* mice, we further surveyed epithelial cell adhesion markers (Fig. 3). Upon establishing cell-cell adhesion, cadherins cluster in specialized cell adherens junctions that associate with the actin cytoskeleton [32]. CDH1 creates a transcellular network that enables groups of cells or tissues to coordinate their function [32, 33]. Therefore, we examined the localization of adherens junction molecules (CTNNB1 and CTNNA1) associated with CDH1 in the cytoplasm. The critical intracellular mediator CTNNB1 was detected in most of the uterine cell types in uteri of *Cdh1^f/f* mice but was absent in the epithelium and decreased in the stroma following ablation of *Cdh1* in the uterus. CTNNA1 was detected in endometrial epithelia of *Cdh1^f/f* mice and was inhibited in the epithelium by ablation of *Cdh1*. Next, we studied the tight junction molecules claudin, occludin, and ZO-1 (Fig. 3), which are associated with the adherens junctions and are epithelial-specific molecules that support epithelial structure. Ablation of *Cdh1* inhibited claudin,

occludin, and ZO-1 in the epithelium. The results indicate that abnormal epithelial structure was induced by the loss of adherens junction and tight junction molecules in the uteri of *Cdh1^d/d* mice.

At 5-wk old, examination of the uteri of *Cdh1^f/f* and *Cdh1^d/d* mice clearly showed loss of uterine glands upon ablation of *Cdh1* (Supplemental Fig. S1). We also observed rounded and noncolumnar epithelium in *Cdh1^d/d* mice. The number of uterine glands per uterine section was significantly reduced ($P < 0.001$) in the uteri of *Cdh1^d/d* mice (~6% of controls). This result was supported by decreased levels of *Foxa2*, which is a glandular marker in the uterus. Both the ovary and oviduct of 5-wk-old *Cdh1^d/d* mice appeared normal (Supplemental Fig. S2). Furthermore, we also observed normal vaginal opening and estrous cycle of adult *Cdh1^d/d* mice (data not shown), suggesting that the ovarian function in *Cdh1^d/d* mice is normal.

Gene Expression in the Uteri of Cdh1^d/d Mice

To examine the effect of *Cdh1* ablation on *Wnt* and *Hox* genes, known to be critical for uterine development [8], real-time RT-PCR analysis was conducted (Fig. 4). Ablation of *Cdh1* decreased epithelial *Wnt* genes (*Wnt7a* and *Wnt11*), *Wnt* receptors (*Fzd6* and *Fzd10*), and epithelial and stromal homeobox genes (*Hoxa10, Hoxa11, Msx1,* and *Msx2*) on P10 and/or P20 ($P < 0.05$). The expression of stromal genes,
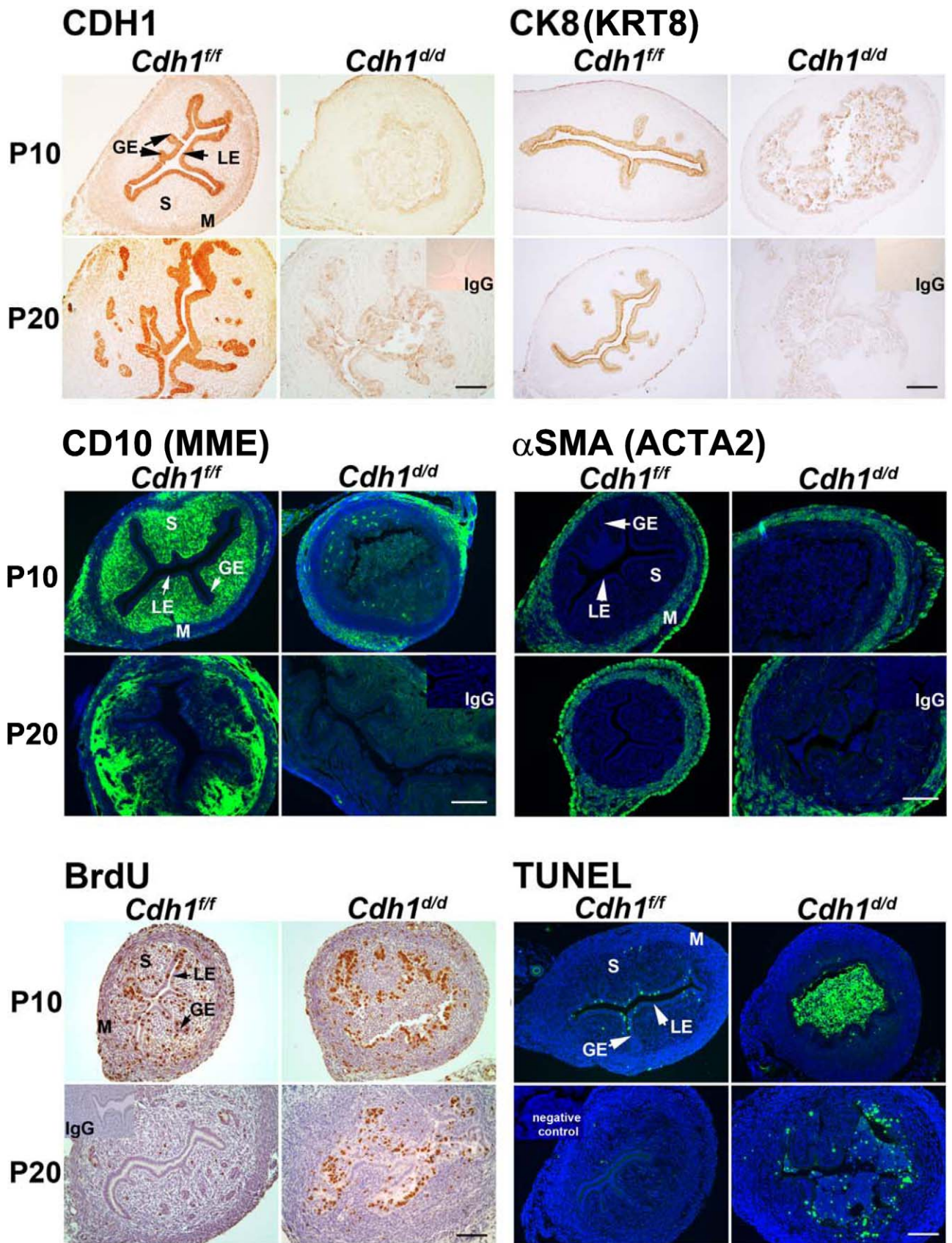
Article 141

FIG. 2. Analysis of conditional ablation of *Cdh1* on markers of epithelium, stroma, myometrium, cell proliferation, and apoptosis in the neonatal mouse uterus. CDH1, cytokeratin 8 (CK8; official symbol KRT8), CD10 (official symbol MME), smooth muscle alpha actin (αSMA; official symbol ACTA2), and incorporated BrdU were detected by immunohistochemistry. Cellular apoptosis was determined by TUNEL analysis. GE, glandular epithelium; LE, luminal epithelium; M, myometrium; P, postnatal; S, stroma. Bars = 100 μm.
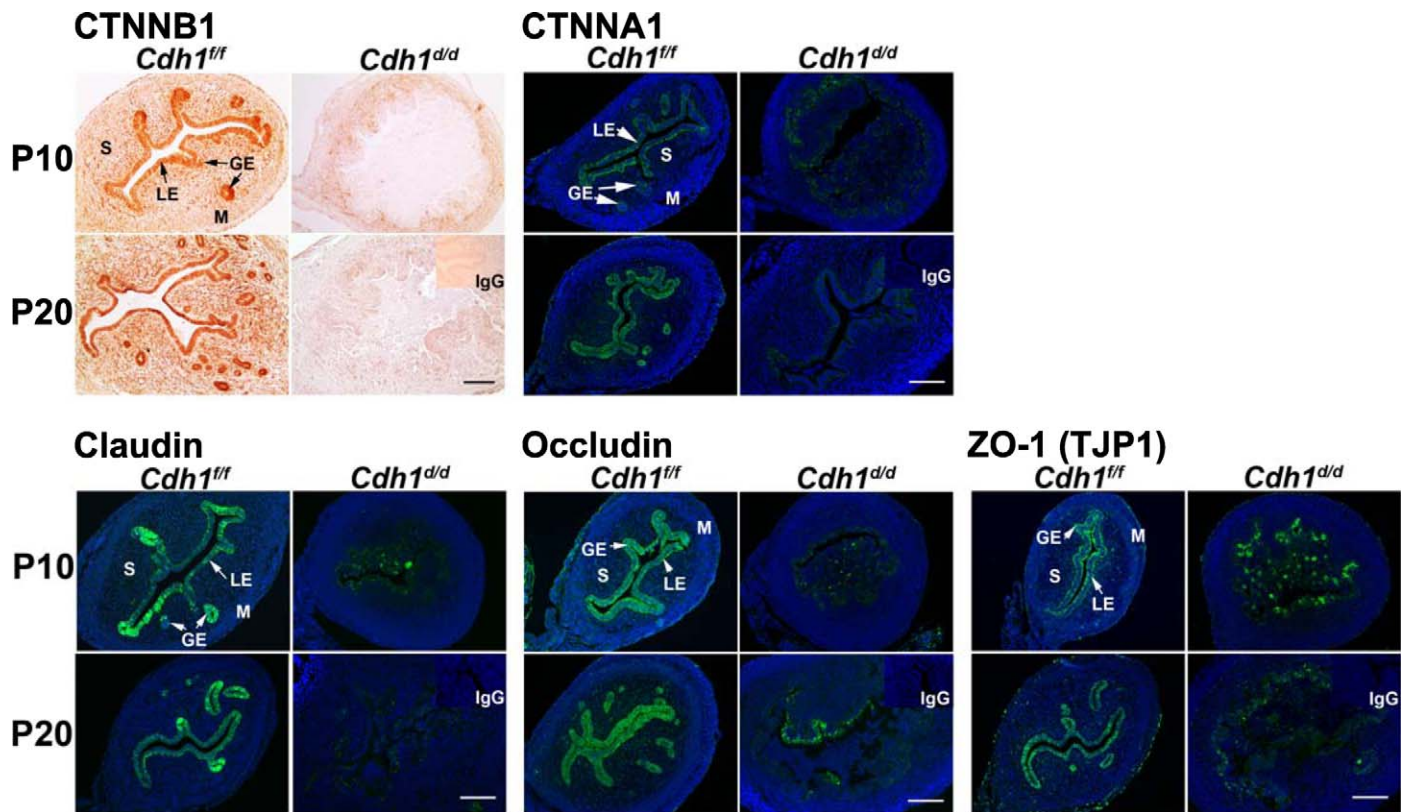
FIG. 3. Analysis of conditional ablation of *Cdh1* on adherens junction and tight junction molecules in the neonatal mouse uterus. Immunoreactivity toward CTNNB1, CTNNA1, claudin, occludin, and ZO-1 (TJP1) was detected. GE, glandular epithelium; LE, luminal epithelium; M, myometrium; P, postnatal; S, stroma. Bars = 100 μm.

Wnt5a and *Wnt16*, was not altered by the loss of *Cdh1* in the neonatal uterus. Interestingly, stromal *Wnt4* and epithelial *Wnt7b* were elevated in the uteri of $Cdh1^{d/d}$ mice ($P < 0.05$). *Vangl2*, which is required for planar cell polarity, was reduced in the uteri of $Cdh1^{d/d}$ mice ($P < 0.05$).

Fertility and Implantation Defect in Cdh1d/d Mice

To determine the role of *Cdh1* in adult uterine function, $Cdh1^{f/f}$ and $Cdh1^{d/d}$ mice were mated to wild-type male mice for 6 mo. $Cdh1^{f/f}$ mice exhibited normal fecundity over the period, whereas $Cdh1^{d/d}$ mice were found to be infertile (Table 1). To determine the cause of the infertility, we examined whether blastocysts were able to undergo successful implantation. On the morning of Day 5.5 of pregnancy, implantation sites were observed in the uteri of $Cdh1^{f/f}$ but not in the uteri of $Cdh1^{d/d}$ mice (Fig. 5A). Histological examination revealed no blastocysts attached to the uterine lumen and no decidual response in the uteri of $Cdh1^{d/d}$ mice. We confirmed that no difference was found in the number and morphology of embryos recovered from the uterus of $Cdh1^{f/f}$ and $Cdh1^{d/d}$ mice when the uterus was flushed on Day 3.5 (Fig. 5B), suggesting that matured embryos are not able to attach to or invade the uterine lumen of $Cdh1^{d/d}$ mice.

Defect of Decidualization in Cdh1d/d Mice

To determine whether ablation of *Cdh1* affects the ability of stromal cells to undergo decidualization, the reaction to hormonally induced artificial decidualization was observed in the uteri of $Cdh1^{f/f}$ and $Cdh1^{d/d}$ mice (Fig. 5C). As we expected, the right uterine horn of $Cdh1^{f/f}$ mice exhibited a robust decidual response 5 days after artificial stimulation. In contrast, $Cdh1^{d/d}$ mice displayed significant reduction in decidual response. These results indicate that the uteri of $Cdh1^{d/d}$ mice do not retain any functions required for the onset of implantation and stromal differentiation to undergo decidualization.

Ablation of Cdh1 Leads to Abnormal Uterine Development with Loss of Trp53

Because our results revealed that *Cdh1* ablation alters epithelial cell fate following abnormal epithelial proliferation and apoptosis, we expected that these cells undergo abnormal cell cycle. Therefore, we interrupted the uterine cell cycle by ablating *Trp53*, which regulates cell cycle arrest, DNA repair, and programmed cell death [34], in addition to ablation of *Cdh1*. Conditional ablation of *Trp53* alone in the uterus using $Pgr^{Cre/+}$ mice ($Pgr^{Cre/+} Trp53^{f/f} = Trp53^{d/d}$) does not show any significant differences of uterine histology and function [35]. In this study, we deleted both *Cdh1* and *Trp53* in the uterus ($Pgr^{Cre/+} Cdh1^{f/f} Trp53^{f/f} = Cdh1^{d/d} Trp53^{d/d}$) (Fig. 6). At 8-wk old, ablation of *Cdh1* still exhibited no endometrial glands, but ablation of *Trp53* did not show any differences in the uterus, as expected. The size of the uteri in $Cdh1^{f/f} Trp53^{f/f}$, $Cdh1^{d/d}$, and $Trp53^{d/d}$ mice were similar. In contrast, the uteri of $Cdh1^{d/d} Trp53^{d/d}$ mice showed abnormal uterine development, including disorganized glandularlike adenosis into stroma and myometrium similar to histological features of neoplastic transformation, which is mixed endometrioid and serous adenocarcinomas with myometrial invasion. The size of the uteri appeared larger in the uteri of $Cdh1^{d/d} Trp53^{d/d}$ mice.
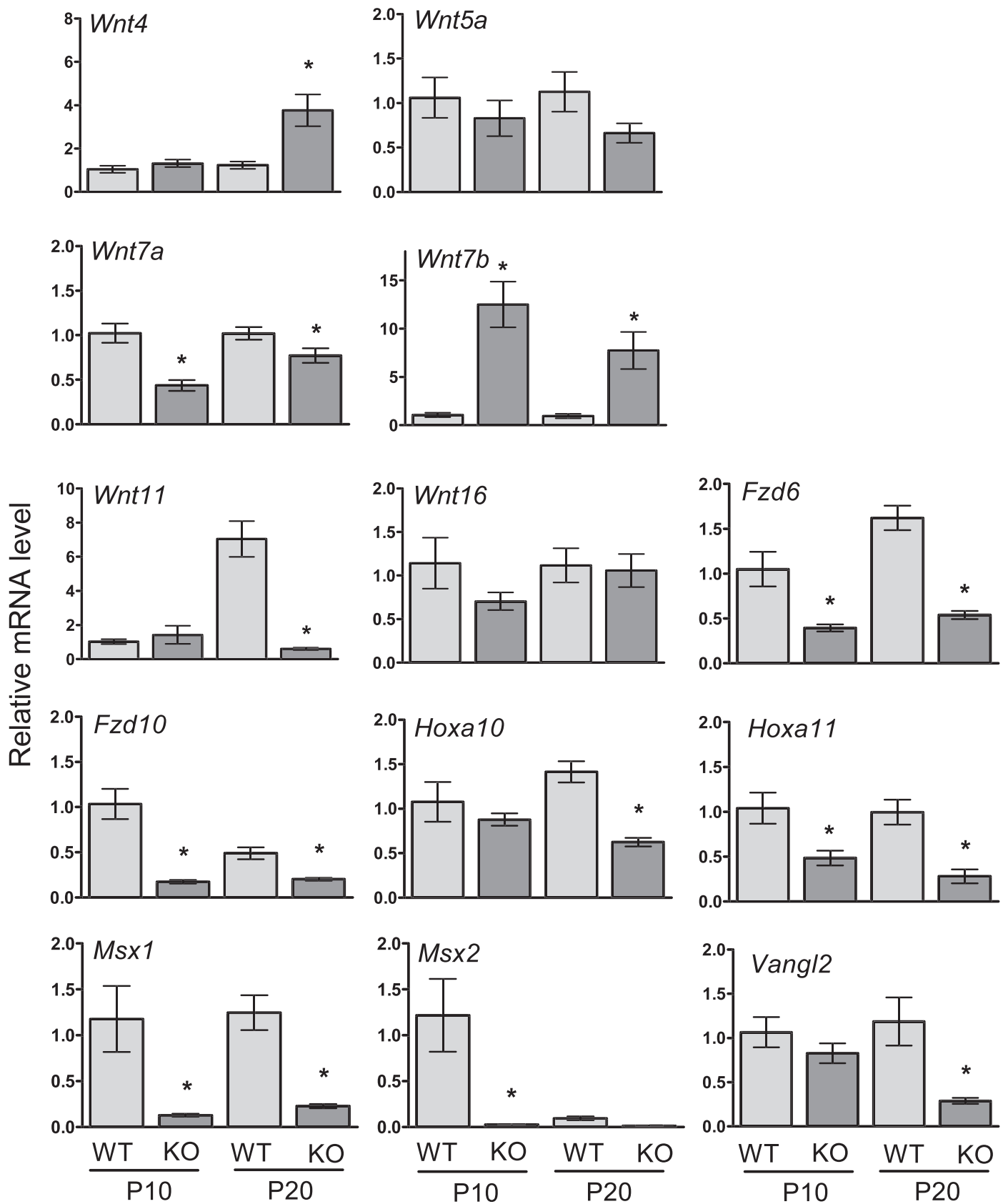
FIG. 4. Analysis of conditional ablation of *Cdh1* in the neonatal mouse uterus. The level of gene expression was measured in the uteri of *Cdh1f/f* (wild type, WT) and *Cdh1d/d* (knockout, KO) mice on Postnatal Day (P) 10 and P20 by real-time RT-PCR. The results represent the mean ± SEM (WT vs. KO, *P < 0.05).

Article 141

TABLE 1. Fertility defect of *Cdh1*$^{d/d}$ mice.

Mouse	No. of females	No. of litters	No. of pups	Average pups/ litter	Average litters/ mouse
Cdh1$^{f/f}$	5	32	240	7.5	6.4
Cdh1$^{d/d}$	6	0	0	0	0

DISCUSSION

CDH1 is critical to regulate architectural development of epithelial differentiation because CDH1 forms cell-cell adhesion structures known as adherens junctions that mediate intercellular adhesion through dynamic interactions with the actin cytoskeleton [36]. In fact, embryos with global mutation of *Cdh1* exhibit severe abnormalities at the transition from compacted morula to blastocyst because of the incorrect establishment of adhesion junctions in the trophectoderm and die around the peri-implantation period [25, 26]. Mice lacking *Cdh1* fail to produce milk proteins as a result of undifferentiated alveolar epithelium [30]. CDH1 also regulates ductal lumen formation during branching morphogenesis of the salivary gland [37]. In the present study, we demonstrate that CDH1 is one of the critical regulators of uterine formation and development. First, we found that loss of *Cdh1* in the neonatal uterus ablates endometrial glands and causes infertility due to defects of implantation and decidualization. Second, we also found that CDH1 has a capacity to control cell fate by altering directional cell proliferation and apoptosis. Third, we showed in the model of conditional ablation of both *Cdh1* and *Trp53* in the uterus that interrupting cell cycle regulation under the loss of *Cdh1* leads to abnormal uterine development. Taken together, these findings suggest that CDH1 has an important role in structural and functional development of the uterus as well as adult uterine function.

Ablation of *Cdh1* disrupted epithelial cellular structure in the developing uterus. The result from immunohistochemical analysis confirmed that uterine epithelium in *Cdh1*$^{d/d}$ mice does not have cell adhesion because of the loss of adherens junction and tight junction molecules. The epithelial marker CK8 disappeared in the epithelium of *Cdh1*$^{d/d}$ mice, resulting in loss of epithelial character in these cells in the developing uterus. Loss of *Cdh1* altered expression of a number of epithelial *Wnt* genes as well as an epithelial cell polarity regulator, *Vangl2*. Thus, our results indicate that a novel mechanism promoting uterine adenogenesis was perturbed by ablation of *Cdh1* in the neonatal uterus, suggesting that the uteri of *Cdh1*$^{d/d}$ mice are structurally and functionally abnormal. It is well known that epithelial-stromal interaction is critical for uterine morphogenesis [6, 8, 16, 17]. Stromal *Wnt* and *Hox* genes, as well as growth factors and receptors, regulate endometrial adenogenesis [6, 8, 16, 17, 38]. While the stromal layer looks mostly intact, ablation of *Cdh1* inhibited the stromal marker CD10 in the uteri of *Cdh1*$^{d/d}$ mice, suggesting that stromal function is potentially abnormal. Currently, we are performing microarray analyses to identify additional genes regulated by loss of *Cdh1* in the stroma.

Cdh1$^{d/d}$ mice lost adult uterine function and were infertile with an inability of their uteri to support embryo attachment and/or invasion and decidualization. The uteri of adult *Cdh1*$^{d/d}$ mice do not contain any endometrial glands. Endometrial glands and their secretions are critical regulators of peri-implantation embryo survival, implantation, and establishment of uterine receptivity and decidualization [12, 39–41]. Ablation of *Wnt7a* and *Foxa2* results in defects of both implantation and decidualization due to absence of endometrial glands [10, 18,
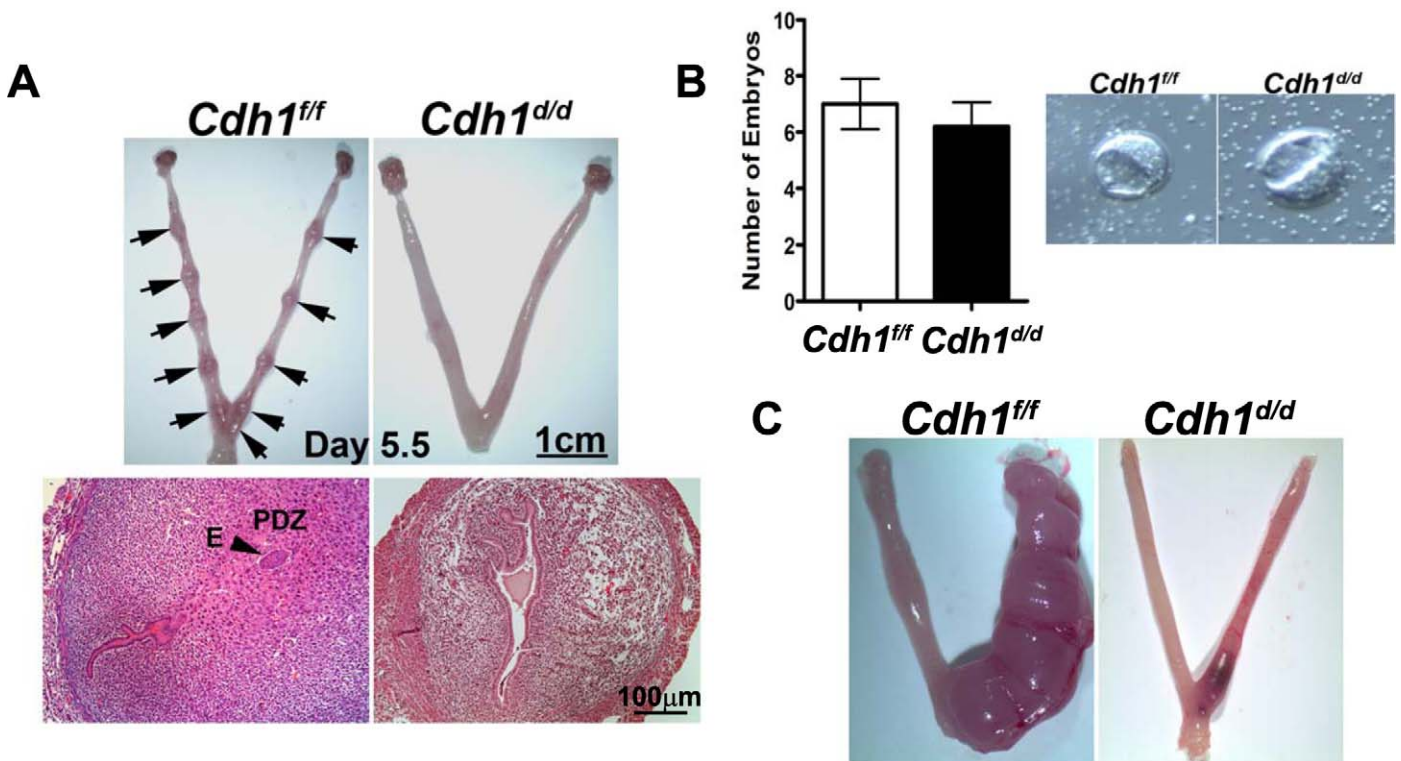

FIG. 5. Implantation and decidualization defect in *Cdh1*$^{d/d}$ mice. **A**) Gross anatomy shows no implantation sites in the uteri of *Cdh1*$^{d/d}$ mice on Day 5.5. Implanted blastocysts (arrows) were observed in the uteri of *Cdh1*$^{f/f}$ mice on Day 5.5 but not in *Cdh1*$^{d/d}$ mice. E, embryo; PZD, primary decidual zone. **B**) Embryos recovered from the uteri of *Cdh1*$^{f/f}$ and *Cdh1*$^{d/d}$ mice on Day 3.5. **C**) Gross anatomy showed a significant decrease in size of the decidual horn in *Cdh1*$^{d/d}$ mice.
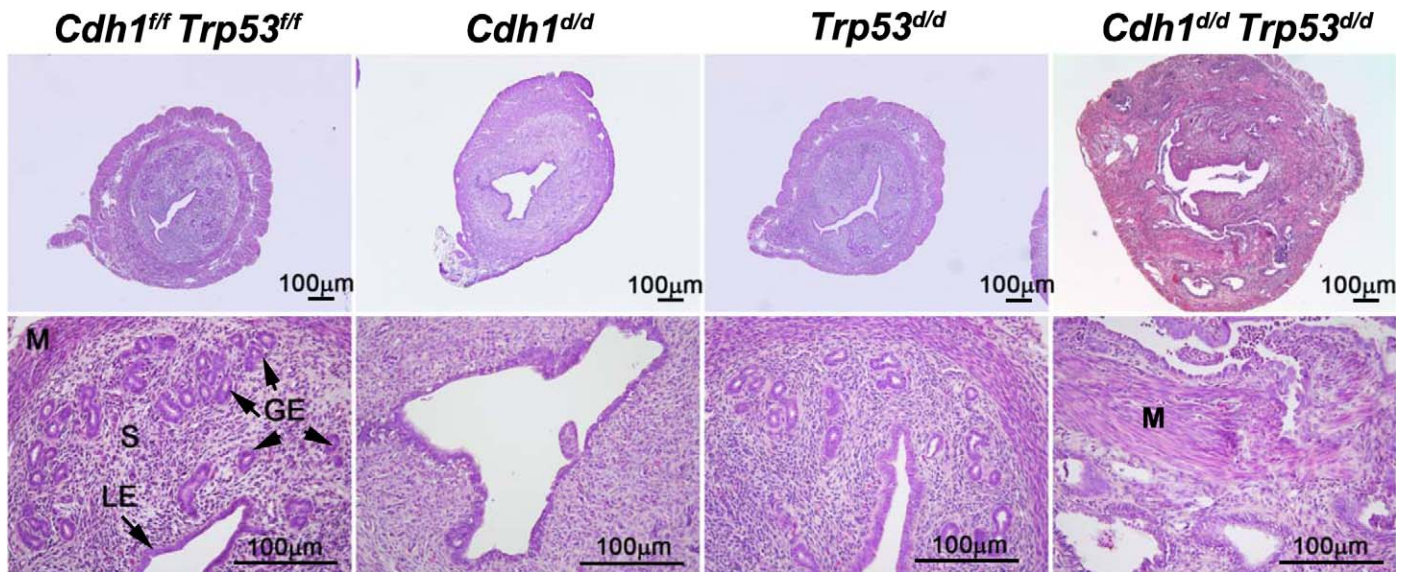
Article 141

FIG. 6. Uterine histology of *Cdh1^{f/f} Trp53^{f/f}*, *Cdh1^{d/d}*, *Trp53^{d/d}*, and *Cdh1^{d/d} Trp53^{d/d}* mice at 8-wk old. Tissues were stained using hematoxylin and eosin. Ablation of *Cdh1* and *Trp53* displayed histological features of endometrial carcinomas with myometrial invasion. GE, glandular epithelium; LE, luminal epithelium; M, myometrium; S, stroma.

42]. *Lif* mutant mice also fail at blastocyst implantation and induction of decidualization because LIF is an implantation critical factor secreted from endometrial glands [43]. Therefore, the lack of uterine glands in *Cdh1^{d/d}* mice leads to the key defect that underlies their infertility and defect of implantation. In addition, we have confirmed that infertility was caused by the uterine defect because no histological and functional defects of the ovary, oviduct, or pituitary were observed in *Cdh1^{d/d}* mice.

The process of implantation involves a series of cell-cell communications between the two epithelial tissues, trophectoderm derived from the embryo and maternal LE. Prior to implantation, they are two separate entities and are polarized with a continuous seal of junctional complexes and cell adhesion molecules. With the onset of embryo attachment, the trophectoderm and maternal LE make first contact at their apical borders, at which point the trophoblast cells become invasive [44, 45]. The trophoblast cells permeate the LE to anchor the implanting embryo to the decidualizing stroma, a process achieved by a series of membrane-mediated events occurring between embryonic and maternal cells. CDH1 is abundantly expressed in the LE prior to implantation [46, 47], while CDH1 is lost from the LE before invasion of the blastocyst into the stroma [48]. This downregulation is controlled by calcitonin, which is a critical regulator of implantation, and suggests that remodeling the adherens junctions between epithelial cells is a critical event during implantation of the embryo [48]. In the present study, our maternal LE does not express any CDH1 in *Cdh1^{d/d}* mice. Epithelium of adult uteri in *Cdh1^{d/d}* mice shows abnormal columnar structure, indicating that adult epithelial cells are not able to remodel the adherens junctions for implantation even though the embryonic trophectoderm is normal. Thus, these results suggest that the embryo is not able to attach to and invade the uterine lumen as a result of not only the absence of endometrial glands but abnormally differentiated epithelium in *Cdh1^{d/d}* mice as well.

Abnormal epithelial differentiation occurred in the neonatal uterus of *Cdh1^{d/d}* mice. We observed the majority of proliferative cells in the epithelium of *Cdh1^{d/d}* mice. In the developing uterus, cell fate is regulated by the interaction of epithelial and stromal factors (i.e., *Wnt* and *Hox* genes). Proliferating cells are primarily localized to the tip of the endometrial glands and stroma on P10 and are absent on P20 when the uterus is structurally completed. Apoptotic cells are also observed in the epithelium on P10 as a result of active cell cycling in the developing uterus. Thus, the present results indicate that the ablation of *Cdh1* alters uterine cell fate, especially epithelial cells, leading to unregulated or uncontrolled cell types. Abnormal *Wnt* and *Hox* gene expression is probably one of the reasons. Interestingly, the ablation or inhibition of *Cdh1* in the mammary gland [30] and salivary gland [37] induces massive apoptosis in the epithelium. We assume that abnormal cells are not able to keep cell-cell interaction due to loss of cell adhesion, supporting the result of massive apoptotic cells in the uterine lumen.

In support of the role of CDH1 in cell differentiation, we found a difference in ablation of *Cdh1* compared to loss of *Wnt7a* in the developing uterus. Conditional ablation of *Wnt7a* in the neonatal uterus disrupts endometrial gland development but does not affect cell proliferation in any uterine cell types [18]. Thus, *Wnt7a* is a critical factor for gland development, but loss of *Wnt7a* does not lead to further abnormal cell differentiation. To further examine alternation of epithelial cell fate by *Cdh1* ablation, we interrupted the uterine cell cycle by ablating *Trp53*. TRP53 mutation and loss of CDH1 occur in 90% of type II endometrial carcinomas [49, 50]. In the present study, we found that loss of the cell cycle regulator *Trp53* in addition to the ablation of *Cdh1* induces unexpected abnormal uterine development. Conditional ablation of *Trp53* does not show any defect in the uterus [35], and loss of *Cdh1* causes loss of endometrial glands. However, ablation of both *Trp53* and *Cdh1* induces histological features of endometrial carcinomas with myometrial invasion. Thus, these results suggest that cell fate in the uteri of *Cdh1^{d/d}* mice has been altered, and these cells are able to differentiate abnormally because of an interruption of cell cycle regulation, leading to neoplastic transformation. However, the abnormal uterine phenotype due to loss of *Trp53* and *Cdh1* in these mice needs to be further characterized.

Collectively, the results of the present study indicate that CDH1 regulates endometrial differentiation, gland development, and adult uterine function. Although ablation of epithelial *Cdh1* causes abnormal stromal differentiation, we are unsure of the mechanism of this disruption. Further investigation of epithelial-mesenchymal interaction and/or transition between epithelium and stroma, and characterization of loss of *Trp53* and *Cdh1* in the uterus are warranted. Nevertheless, our study shows the functional and structural importance of CDH1 in the uterus.

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