Essential roles of mesenchyme-derived beta-catenin in mouse Müllerian duct morphogenesis

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Abstract

Members of the Wnt family of genes such as Wnt4, Wnt5a, and Wnt7a have been implicated in the formation and morphogenesis of the Müllerian duct into various parts of the female reproductive tract. These WNT ligands elicit their action via either the canonical WNT/beta-catenin or the non-canonical WNT/calcium pathway and could possibly function redundantly in Müllerian duct differentiation. By using the Müllerian duct-specific anti-Müllerian hormone receptor 2 cre (Amhr2-cre) mouse line, we established a conditional knockout model that removed beta-catenin specifically in the mesenchyme of the Müllerian duct. At birth, loss of beta-catenin in the Müllerian duct mesenchyme disrupted the normal coiling of the oviduct in the knockout embryo, resembling the phenotype of the Wnt7a knockout. The overall development of the female reproductive tract was stunted at birth with a decrease in proliferation in the mesenchyme and epithelium. We also discovered that Wnt5a and Wnt7a expression remained normal, excluding the possibility that the phenotypes resulted from a loss of these WNT ligands. We examined the expression of Frizzled (Fzd), the receptors for WNT, and found that Fzd1 is one receptor present in the Müllerian duct mesenchyme and could be the putative receptor for beta-catenin activation in the Müllerian duct. In summary, our findings suggest that mesenchymal beta-catenin is a downstream effector of Wnt7a that mediates the patterning of the oviduct and proper differentiation of the uterus.

Keywords: Anti-Müllerian hormone receptor 2 (Amhr2); Beta-catenin; Female reproductive tract; Frizzled; Müllerian duct; Oviduct; Wnt

Introduction

The Müllerian duct is the embryonic precursor of the female reproductive tract. In the mouse, the Müllerian duct begins to form as an invagination of the surface epithelium of the mesonephros at embryonic day 11.5 or E11.5 and continues to elongate until it reaches the cloaca at E13.5 in both male and female embryos (Kobayashi and Behringer, 2003). The transformation of the Müllerian duct into various parts of the female reproductive tract does not occur until later in embryonic development. By 2 weeks after birth in the mouse, the Müllerian duct has developed rostrally into the oviducts, uterus, cervix, and upper portion of the vagina (Yin and Ma, 2005). Formation of the Müllerian duct results from a collective action of transcription factors and signaling molecules including Pax2, Lim1, Emx2, and Wnt4 (Torres et al., 1995; Kobayashi et al., 2003; Miyamoto et al., 1997; Vainio et al., 1999). In embryos lacking either Pax2, Lim1, or Emx2, which are transcription factors expressed in the Müllerian duct epithelium, the Müllerian duct failed to form leading to a complete absence of the female reproductive tract (Torres et al., 1995; Kobayashi et al., 2003; Miyamoto et al., 1997). Wnt4, a signaling molecule produced by the Müllerian duct mesenchyme, is also essential for the initial formation of the Müllerian duct (Vainio et al., 1999).

The transformation of the Müllerian duct into distinct structures of the female reproductive tract also requires a coordinate interaction among transcription factors and local signaling molecules. Expression of the homeobox genes such as Hoxa9, Hoxa10, Hoxa11, and Hoxa13, are restricted to specific regions of the female reproductive tract after birth and null mutations of these homeobox genes lead to various defects in Müllerian duct patterning. A Hoxa10 mutation resulted in homeotic transformation; the anterior segment of the uterus transformed into the oviduct (Benson et al., 1996). In Hoxa10<sup>−/−</sup>; Hoxa11<sup>−/−</sup> embryos, abnormal uterotubal junctions and a narrow uterus arose (Branford et al., 2000). Furthermore, Hoxa13 female mutants were infertile, which was proposed to be due to hypoplasia of the vaginal cavity and the

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Fig. 1. Amhr2-Cre Activity (A & B) and phenotypic differences in the female reproductive tract between Amhr2<sup>cre/+; Catnb<sup>floxed/+</sup> (C–H) and Amhr2<sup>cre/+; Catnb<sup>floxed/−</sup> (I–N) animals at birth. (A and B) Amhr2-Cre activity indicated as EGFP fluorescent staining in the mesonephros of E15.5 Z/EG reporter female embryos. Panel B (20×) is a zoom-in picture of the white rectangle area in panel A (4×). Light microscopic images of the female reproductive tract were obtained at birth or P0 (C, F, I, L) and at 2 months of age or P60 (G and M). Panels D, E, J, and K are H&E staining of cross-sections with the same magnification (20×) from utero-oviductal junctions and middle uterine horns (indicated by the brackets) at birth, respectively. Panels H and N are cross-sections of upper uterine horns at birth immunostained for Ki67. Brown nuclear deposits indicate specific staining and blue nuclear staining is a hematoxylin counter stain (magnifications are 10× for the original and 100× for the rectangle amplification). O=ovary; OD=oviduct; U=uterine horns. The sample size was N=6 for each genotype.
cervix (Post and Innis, 1999). Overall, mutations in this set of Hox genes disrupt the regional specification of the female reproductive tract.

In addition to homeobox genes, signaling molecules such as Wnt5a and Wnt7a have been shown to be critical for the patterning of the Müllerian duct. Wnt5a is expressed in the mesenchyme of the uterus, cervix and vagina (Miller et al., 1998a,b). In the absence of Wnt5a, the uterus did not develop ureteric glands and the anterior/posterior structures such the cervix and vagina did not form properly (Mericckay et al., 2004). In contrast to the mesenchyme expression of Wnt4 and Wnt5a, Wnt7a is produced specifically in the luminal epithelium (Miller et al., 1998a,b). Without Wnt7a, the oviducts failed to coi1 and the uterine wall was thinner and less muscular in the mutants as compared with wild-type mice. Wnt7a mutants also lacked ureteric glands and developed shallow vaginal fornices (Carta and Sassoon, 2004; Miller et al., 1998a,b).

WNT proteins are known to elicit their function through two pathways in a ligand- and receptor-dependent manner: the canonical WNT/beta-catenin pathway and the non-canonical WNT/Ca2+ pathway (Miller, 2001). The concurrent presence of Wnt4, Wnt5a, and Wnt7a in the Müllerian duct suggests a certain degree of redundancy among these WNTs in Müllerian duct patterning. In this study, we investigated the overall role of the canonical WNT pathway by removing beta-catenin specifically in the mesenchyme of the Müllerian duct. By comparing the Müllerian duct phenotypes of the mesenchyme-specific beta-catenin knockout with other Wnt knockouts, we were able to establish a connection between these WNT ligands and the canonical WNT pathway during female reproductive tract differentiation.

Materials and methods

Animals, breeding scheme, and mating

Amhr2cre/+ or Amhr2-Cre mice were generously provided by Dr. Richard Behringer (Jamin et al., 2002). B6.Cg-Tg(ACTB-Bgeo/GFP)21Lbe (or Z/EG, Jax #004178), B6.FVB-TgN(EIIa-Cre)C5379Lmgd (or EIIa-Cre, Jax #003314) and B6.129-Catnbfloxed/floxed1 (or Catnb_floxed/floxed, Jax #004152) were obtained from the Jackson Laboratory. To increase the efficiency in the production of live offspring from the Jackson Laboratory. To increase the efficiency in the production of live offspring.

Whole mount in situ hybridization

Tissues were fixed overnight with 4% paraformaldehyde in PBS at 4 °C and processed according to the standard non-radioisotopic procedure using digoxigenin-labeled RNA probes. The sources of RNA probes and their optimal hybridization temperature are as follows: Frizzled1, Frizzled2, and Frizzled7 (Dr. Sam Pleasure; 70 °C) and Wnt5a and 7a (Dr. Andrew McMahon; 70 °C).

Reverse transcriptase polymerase chain reaction (RT-PCR)

To detect the presence of mRNA for Frizzled1 to 8 in the mesonephros, total RNA from 20 E14.5 female mesonephroi was isolated using Trizol reagent. Before the reverse transcriptase (RT) reaction, 1 μl of RNA was used to synthesize cDNA. RNA from 10 E14.5 female mesonephroi was isolated using Trizol reagent. 1 μl of cDNA was mixed and subjected for PCR cycles (30 cycles for 94 °C 1 min, 60 °C 2 min, and 72 °C 3 min). PCR reactions using 306b primers were performed on the RT, RT free, and no template samples to ensure proper reverse transcription occurred. RNA from adult ovaries was used as a positive control for various Fads. Following the PCR reaction, samples were run on a 1.5% TAE gel.
**Imaging**

Light microscopy pictures for whole mount in situ hybridization and macroscopic pictures of tissues were taken with the Leica DFC320 camera with a Zeiss Stemi SV11 microscope using ImagePro Discovery Software. Fluorescent pictures were taken with the Axiocam MRm Zeiss microscope using the X-Cite 120 fluorescence illumination system. Histological pictures were taken with the Olympus BX51 microscope using ProgRes C14 Olympus camera and software. All images were subsequently refined in Adobe Photoshop.

**Results**

**Phenotypes in the female reproductive tract of Amhr2cre/+;Catnb−/− embryos**

We utilized the Amhr2-Cre mouse line to target Cre recombinase in the mesenchyme of the Müllerian duct (Jamin et al., 2002). Previous reports indicated that the activity of the Cre recombinase was detected in the Müllerian duct mesenchyme as early as E12.5 using the ROSA reporter mice (Jamin et al., 2002). To determine the efficiency of Cre recombination in our hands, we crossed the Amhr2-Cre line to the Cre reporter line Z/EG (or B6.Cg-Tg(CTB-Bgeo/GFP)21Lbe, Novak et al., 2000), which expresses Enhanced Green Fluorescent Protein (EGFP) upon Cre recombination. We found that EGFP began to appear in the Müllerian duct mesenchyme at E15.5 (Figs. 1A and B), about 72 h later than originally reported. This delayed detection of Amhr2-Cre activity could result from the time required for proper folding of EGFP or the accessibility of the reporter construct to the Cre recombinase. Based on our results and previous findings, it is certain that by E15.5, Amhr2-Cre has become active and most importantly not all cells in the mesenchyme were positive for Cre activity at this stage (Fig. 1B).

To increase the efficiency in the production of homozygous beta-catenin (Catnb) null alleles and to decrease the incidence of mosaic deletion, we generated Amhr2cre/+;Catnbflxed/flxed embryos by crossing Amhr2cre/+;Catnb−/+ mice to Amhr2+/+; Catnbflxed/flxed mice. Reproductive tracts of the female Catnb conditional knockout and control litter mates (Amhr2cre/+; Catnbflxed/flxed) were first examined at birth. The uterine horns of the knockout newborns (Fig. 1I, N=6 for all experiments) were hypotrophic as compared with the control (Fig. 1C). The oviducts of the knockout newborn lacked the characteristic coiling which was apparent in the control (Figs. 1F and L). The lack of oviduct coiling was distinctly apparent in the knockout newborns (Fig. 1I, N=6) as compared with the control at birth (Figs. 1H and N, N=6). TUNEL assay revealed no differences in apoptosis in either the mesenchyme or the epithelium between the control and knockout samples (data not shown). In conclusion, the absence of Catnb in the Müllerian duct mesenchyme in embryos led to a decrease in proliferation in both the epithelium and the mesenchyme of the female reproductive tract but had no effect on cell survival at birth.

**Expression of Wnt5a and Wnt7a in the Amhr2cre/+;Catnbflxed/flxed Müllerian duct**

The lack of oviduct coiling in the Catnb conditional knockout female closely resembled the phenotypes in the Wnt7a knockout (Parr and McMahon, 1998). This suggested that the oviduct phenotypes in our Catnb conditional knockout could result from the loss of Wnt7a expression. To test this possibility, we examined the expression of Wnt7a as well as Wnt5a, another Wnt important in female reproductive tract development. Whole mount in situ hybridization on E15.5 and E19.5 mesonephroi revealed that expression patterns of Wnt5a and Wnt7a were not altered in the absence of mesenchymal Catnb (Fig. 2). These results indicated that the phenotypes in the Catnb conditional knockout resulted from a loss of Catnb, not the loss of Wnt5a or Wnt7a.

**Expression of Frizzled receptors in the normal female mesonephros**

In both the canonical and non-canonical WNT signaling pathways, the WNT ligand binds to its receptor Frizzled (FZD),...
At present, ten Fzd receptors have been found in the mouse, and their expression during Müllerian duct differentiation has yet to be described. To identify the Fzd(s) that may be responsible for activating the canonical or WNT/beta-catenin pathway, we performed RT-PCR for Fzd receptors 1–8 on RNA isolated from E14.5 female mesonephroi. Fzd9 and Fzd10 were not examined as their expression patterns have been characterized in the mesonephros (Nunnally and Parr, 2004; Wang et al., 1999) and no apparent phenotypes were observed in the individual knockout (Fzd9: MGI Direct Data Submission MGI:3609573; Zhao et al., 2005; Ranheim et al., 2005; Fzd 10: MGI Direct Data Submission MGI:3604450). E14.5 was

Fig. 2. Whole mount in situ hybridization for Wnt5a (A) and Wnt7a (B) on Amhr2<sup>cre</sup><sup>/+</sup>;Catnb<sup>boxed<sup>+</sup></sup> (Control) and Amhr2<sup>cre</sup><sup>/+</sup>;Catnb<sup>boxed<sup>+</sup></sup> (Knockout) mesonephroi at E15.5 and E19.5. The entire mesonephros was shown for E15.5 samples whereas only a small segment of the mesonephros was shown for the E19.5 samples. Dark purple/brownish deposits represent specific staining. White arrows point to the Müllerian duct. White scale bars represent 100 μm. The sample size was N=5 for each genotype.
chosen because this is the point when the Müllerian duct is fully formed in the female. We found that mRNA for \(Fzd1\), \(Fzd2\), and \(Fzd7\) were present in the female mesonephroi at E14.5 (Fig. 3A, \(N=3\)). RNA from adult ovaries was used as positive controls for each \(Fzd\) to demonstrate the specificity and correct amplification of the PCR products (Hsieh et al., 2002).

To identify the cellular compartments of \(Fzd\) expression in the mesonephros, we performed whole mount (Fig. 3B) and section (Fig. 3C) in situ hybridization for \(Fzd1\), \(Fzd2\), and \(Fzd7\). In the E14.5 mesonephros, \(Fzd1\) mRNA was detected in both the epithelium and the mesenchyme of the Müllerian duct (Figs. 3B and C arrow and arrowhead, respectively). On the other hand, \(Fzd2\) and \(Fzd7\) expression was not detected in either the Müllerian duct epithelium or mesenchyme (Fig. 3B). Arrows for \(Fzd2\) and \(Fzd7\) in Fig. 3B indicate the outline of the Müllerian duct due to the artifact of lighting. No staining was found in the duct or mesenchyme (Fig. 3C).

**Phenotypes in female tracts of adult Amh2\(^{cre/+}\);Catnb\(^{floxed/-}\) animals**

Previous observations using the \(Amh2^{cre/+}\);\(Catnb^{floxed/floxed}\) model revealed that the uterine smooth muscle cells underwent a myogenesis to adipogenesis transformation postnatally (Arango et al., 2005). We found this similar phenotype in the \(Amh2^{cre/+}\);\(Catnb^{floxed/-}\) model macroscopically (Figs. 4A and E) and microscopically (Figs. 4B, C, F, and G). The longitudinal myometrium in the knockout was lost with the infiltration of adipose tissue. However, part of the transverse myometrium and the uterine glands were still present (Figs. 4C and G). In addition to this phenotype, we observed a unique defect in which the ovary of the \(Catnb\) conditional knockout was enclosed in a liquid-filled sac (Figs. 4E and H, \(n=6\)). We speculate that the sac was the derivative of ovarian bursa and the fluid was the accumulation of follicular fluids after ovulation.
Fig. 4. Phenotypes in the adult female reproductive tracts of Amhr2$	extsuperscript{cre+/+};$Catnb$	extsuperscript{floxed/+}$ (A–D) and Amhr2$	extsuperscript{cre+/+};$Catnb$	extsuperscript{floxed/-}$ (E–H) animals at 2 months of age. Panels A and E are light microscopic images (1.6×) of whole mount female reproductive tracts. White arrows indicate the nodule structure with fat accumulation in the knockout sample. Asterisks indicate the fluid-filled sac surrounding the ovary in the knockout animal. FP= fat pad; O= ovary; OD= oviduct; U= uterine horns. Panels B and F are H&E staining on section of the uterus (magnification=8×). Panels C and G are higher magnification (20×) of the rectangle regions of panels B and F, respectively. The brackets outline the two layers of the myometrium (transverse and longitudinal). Black arrowheads indicate the uterine glands. Ad= adipose tissues; En= endometrium; L= longitudinal myometrium; T= transverse myometrium. Panels D and H are H&E staining on sections of ovaries (magnification=8×). Asterisks indicate the liquid-filled sac. The sample size was $N=6$ for each genotype.
Discussion

The transformation of the Müllerian duct into the various parts of the female reproductive tract requires intricate interactions among transcription factors and signaling molecules. Without the proper differentiation of the Müllerian duct in the female and its regression in the male, reproductive tract anomalies and infertility can occur. In the mouse, several Wnts including Wnt4, Wnt5a, and Wnt7a are expressed in a compartment-specific manner in the Müllerian duct and play unique roles in regional specific development of the duct. Wnt4 and Wnt5a are expressed in the mesenchyme of the Müllerian duct while Wnt7a is expressed in the epithelium of the Müllerian duct (Miller et al., 1998a,b). These WNTs signal through the canonical beta-catenin pathway, the non-canonical WNT pathway, or a combination of both. Wnt4 has been found to activate both the canonical and the non-canonical WNT signaling pathways in vertebrate tissues such as the kidney, the Müllerian duct, and the mammary gland (Stark et al., 1994; Vainio et al., 1999; Briskan et al., 2000). Wnt5a mainly signals through the non-canonical pathway in vertebrate species in the limb, the lung, chondrocytes, and the female reproductive tract (Yamaguchi et al., 1999; Li et al., 2006; Yang et al., 2003; Mericskay et al., 2004). On the other hand, Wnt7a elicits its action through the canonical WNT pathway in vertebrates in the limb, the female reproductive tract, and the cerebellum (Parr and McMahon, 1995, 1998; Miller et al., 1998a,b; Hall et al., 2000). To understand the overall role of the canonical WNT pathway in Müllerian duct development, we removed beta-catenin specifically in the Amhr2-expressing mesenchyme of the Müllerian duct and investigated how the WNT/beta-catenin pathway connects to various WNT molecules in this developmental process.

The oviduct phenotype in the Catnb conditional knockout resembles Wnt7a null phenotypes

One of the most intriguing phenotypes in our model is the loss of oviduct coiling. Similar phenotypes were also observed in Wnt7a null mice (Mericskay et al., 2004; Parr and McMahon, 1998). These results suggest that Wnt7a mediates the transformation of anterior Müllerian duct into characteristic oviduct coiling through beta-catenin (Fig. 5). The phenotypes of our model did not resemble the phenotypes in the Wnt5a knockout, indicating that Wnt5a may not signal via beta-catenin in the Müllerian duct mesenchyme. The similar phenotypes between Wnt7a mutants and our Catnb conditional knockout model raise the possibility that the Wnt7a pathway could be affected in the absence of Catnb. However, we did not detect any changes in the expression pattern of Wnt5a and Wnt7a. Therefore, we conclude that expression of Wnt5a and Wnt7a does not require the presence of mesenchymal Catnb. Furthermore, the oviduct phenotypes in our Catnb conditional knockouts did not result from the loss of Wnt5a and Wnt7a.

Due to the fact that the female reproductive tract still formed in the absence of mesenchymal beta-catenin, one would speculate that beta-catenin may not be involved in the pathway induced by Wnt4, which is essential for Müllerian duct formation (Vainio et al., 1999). However, in our conditional knockout system, removal of beta-catenin occurred relatively late at E15.5, a time that is almost 72 h after the formation of the Müllerian duct. Therefore, our model cannot address the question of whether beta-catenin is involved in early Müllerian duct formation. The connection between Wnt4 and beta-catenin in Müllerian duct development remains to be determined.

Fig. 5. A hypothetical model for the role of beta-catenin on Müllerian duct morphogenesis. Based on the similar and different defects in Wnt7a−/− and Amhr2cre+; Catnb floxed−/− newborns, we propose that Wnt7a, which is produced by the Müllerian duct epithelium, acts on the Amhr2-positive mesenchyme cells and activates the canonical WNT/beta-catenin pathway, probably via the Fzd1 receptor, in a regional specific manner. In the anterior Müllerian duct, the future oviduct, activation of the canonical WNT/beta-catenin pathway via Wnt7a leads to the proliferation and proper differentiation of the Müllerian duct mesenchyme. The WNT/beta-catenin pathway could also stimulate the production of unknown factors from the mesenchyme, which in turn regulates the proliferation of the Müllerian duct epithelium and oviduct coiling. In the middle Müllerian duct, the future uterus, WNT7a maintains proper myogenesis of the uterine myometrium via catenin. However, effects of Wnt7a on uterine gland formation probably does not involve beta-catenin in the mesenchyme due to the fact that uterine glands still form when beta-catenin is inactive in the mesenchyme. MD=Müllerian duct.
In addition to Wnts, Hox genes are also important patterning genes in the female reproductive tract, particularly in the differentiation of the oviducts and uterus. A possible interaction between these homeobox genes and WNTs has been proposed. In the Wnt7a knockout female reproductive tract, Hoxa10 and Hoxa11 expression was maintained at birth (Miller et al., 1998a, b). However, at 3 months of age, Hoxa10 and Hoxa11 transcripts are no longer detected in the mutant Wnt7a uterus (Miller et al., 1998a, b). Thus, it was concluded that Wnt7a is required to maintain normal Hox gene expression in the adult female reproductive tract (Miller et al., 1998a, b). However, in the embryo, WNT7a probably does not regulate Hoxa10 and Hoxa11 expression and their effects on Müllerian duct patterning. This was supported by the fact that these Hox genes were still expressed properly in Wnt7a null embryos at birth (Miller et al., 1998a, b). This result indicated that the loss of oviduct coiling in the Wnt7a null (or our Catnb conditional knockout) newborn was not caused by the alteration of Hox gene expression. Instead, Wnt7a and beta-catenin probably directly control the patterning of the female reproductive tract independent of the Hox genes.

Presence of Catnb in the Müllerian duct mesenchyme is essential for proper proliferation in both mesenchymal and epithelial compartments

At birth, the female reproductive tract in the Catnb conditional knockout embryos was hypotrophic and less proliferation was found in both the mesenchyme and the epithelium as compared to the control. The defect in epithelial proliferation is intriguing because Catnb was removed exclusively in the mesenchyme. The defect on proliferation in both compartments suggests that there is a cross-talk between the epithelium and mesenchyme, and this cross-talk requires the presence of Catnb in the Müllerian duct mesenchyme. WNT signaling has been linked to the control of cellular proliferation in the vascular system (Escalante-Acalde and Hernandez, 2003). We propose that the signaling pathway downstream of beta-catenin in the mesenchyme causes the production of unknown proliferation-inducing factor(s), which act as a paracrine signal to regulate epithelium expansion and morphogenesis (Fig. 5). The nature of the proliferation-inducing factor remains to be determined.

Studies in several organisms have revealed that WNT signaling may control apoptosis through different mechanisms including the WNT-BMP signaling loop (Golden et al., 1999), secreted Frizzled-related protein-2 (Ellies et al., 2000), and most importantly the WNT/beta-catenin signaling pathway (Galceran et al., 2000, Brault et al., 2001). However, we did not find any increase in apoptosis in the absence of Catnb, suggesting that the anti-apoptosis mechanism of beta-catenin does not appear to be involved in Müllerian duct differentiation.

Frizzled receptors 1, 2, and 7 are present in the developing mesonephros

To further understand the mechanism of how the canonical WNTs activate the beta-catenin pathway, we searched for the presence of specific Fzd receptors in the mesonephros. We discovered that Fzd1, Fzd2, and Fzd7 are all expressed at the time of Müllerian duct differentiation by RT-PCR. However, whole mount and section in situ hybridization revealed that only Fzd1 was expressed in both the epithelium and the mesenchyme of the Müllerian duct while Fzd2 and Fzd7 were not. Previous experiments have demonstrated that Fzd1 associates with WNT family members in the canonical WNT pathway (Logan and Nusse, 2004). Transfection studies using Wnt7b and Fzd1 have shown that Fzd1 can induce the signal transduction of the WNT/beta-catenin pathway (Wang et al., 2005). Based on these experiments, as well as the mesenchyme expression of Fzd1, we speculate that Fzd1 could be involved in the canonical WNT/beta-catenin pathway in the Müllerian duct. Further examination of Fzd1 knockouts is needed to confirm its role on Müllerian duct patterning.

Unique phenotypes in adult Catnb conditional knockout females

A dramatic increase in adipose tissue deposition on the uterine horns in adulthood was evident in the Catnb conditional knockout female. This phenotype was consistent with the previous findings using a similar Catnb conditional knockout model (Arango et al., 2005). The major difference between our model and the previous model is that we introduced a null allele to the process of generating the conditional allele. Arango et al. examined the Amhr2<sup>Cre<sup>+/−</sup> ; Catnb<sup>flox/flox</sup> animals which yielded a true knockout only when both floxed alleles were removed. The introduction of the null allele in our experiments should theoretically enhance the efficiency in the production of homozygous null alleles because the Cre recombinase only has to remove one floxed allele. We have generated Catnb conditional knockout mice using these two different models and found that the phenotypes were less severe in Amhr2<sup>Cre<sup>+/−</sup> ; Catnb<sup>flox/flox</sup> as compared to Amhr2<sup>Cre<sup>+/−</sup> ; Catnb<sup>flox/flox</sup> . The hypotrophy in the uterine horns was not as severe and the oviduct coiling was not affected. Introduction of one null allele in our model revealed a novel role of Catnb on Müllerian duct patterning, which was not evident in the study by Arango et al.

In conclusion, this study highlights an important role of beta-catenin on morphogenesis of the female reproductive tract, particularly the coiling of the oviduct. We propose that the presence of beta-catenin in the Amhr2-expressing cells in the anterior Müllerian duct mesenchyme is essential for transducing the canonical beta-catenin pathway induced by WNT7a (Fig. 5). The absence of beta-catenin not only adversely affects the oviduct patterning, but also continues this effect throughout adulthood by causing adipose tissue deposition in the uterine wall. However, the uterine glands were still present in the absence of beta-catenin, a phenotype different from the Wnt7a knockout where the uterine glands were lost. These results together demonstrate a unique connection between WNT7a and beta-catenin in the establishment of the oviduct coiling in the anterior Müllerian duct. On the other hand, beta-catenin in the Amhr2-negative cells in mesenchyme of the middle Müllerian
duct is probably not responsible for transducing WNT7a signal for the formation of uterine glands.

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