Wnt signaling is crucial for functioning of the endometrium.

**The Role of Wnt Signaling in Uterus Development (I) and in Homeostasis (II) and Malignancy (III) of the Uterine Endometrium**

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**The Wnt/β-catenin signaling pathway**

- **Wnt off**
  - Absence of ligand
  - Degradation and degradation
  - Stemness, Proliferation

- **Wnt on**
  - Presence of ligand
  - Wnt signaling
  - Differentiation

Hypothesis: Estradiol activates Wnt signaling which induces proliferation, while progesterone inhibits Wnt signaling which induces differentiation.

**The physiological role of the Wnt/β-catenin signaling pathway in the endometrium**

Hypothesis: Estradiol activates Wnt signaling which induces proliferation, while progesterone inhibits Wnt signaling which induces differentiation.
Knock-out experiments showed:

Wnt 4 is required for Mullerian duct initiation (Vainio et al., 1999).
Wnt 7a for subsequent differentiation (Miller and Sassoon, 1998)
Wnt 5a for posterior outgrowth of female reproductive tract (Mericskay et al., 2004)

Aim of current experiments (I):
Analyse the effect of activation of Wnt signaling in epithelial and/or mesenchymal cells of the Mullerian duct.

Overexpression experiments:

1. Overexpression of Wnt signaling in epithelial cells of the Mullerian duct (dpc 9.5-15)
   KSP1.3-Cre; Apclox/lox: Only embryonic detection is possible, so far no phenotype

2. Overexpression of Wnt signaling in mesenchymal cells of the Mullerian duct (dpc 12.5)
   AmhR2Cre/+; Apclox/lox: Abberations in the myometrium. AmhR2Cre/+ (Jamin .. Behringer, 2002)

In the presence of Cre (Cre recombinase), Apclox/lox mice will become ApcFlox/Flox Æ Apc-/-, Wnt signaling activation
AmhR2-LacZ in embryo and adult

The Role of Wnt Signaling in Uterus Development

AmhR2Cre/+; Rosa26LacZlox in embryo and adult (Jamin et al. 2002)

Both inner and outer myometrial layer express Cre driven by the AmhR2 promoter.

In the muscle layer of the uterus, Cre is expressed and recombination of Apclox/+ to Apclox/lox (Apc-/-) was shown.
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**Phenotype** | **Numbers** | **Age**
--- | --- | ---
Apc Flox mothers died during delivery | 2/4 | 103/127
severe myometrial problems | 2 | 178
minor myometrial problems | 3 | 55/132/216
not analyzed yet | 6 | 
control animals no problems | 6/6 | 49/178

In conclusion (I):
Overexpression of Wnt signaling in mesenchymal cells of the Mullerian duct (dpc 12.5) results in malformations in the adult myometrium. Due to these malformations normal delivery is impaired (50%).

These mice will be analyzed further.
The Role of Wnt Signaling in Uterus Development and in Homeostasis (II) and Malignancy of the Uterine Endometrium

Aim of the current investigations (II):
To reveal the molecular mechanism of progesterone induced inhibition of estrogen signaling

Hormonal regulation of proliferation in the human endometrium

Postmenopausal women were treated for 21 days (Klaassens et al., 2006):
• Controls (8)
• E2 treated (7)
• E2+MPA treated (6)
Pure endometrium, RNA isolation
Microarrays (Affymetrix U133plus2)
SAM analysis (Hanifi-Moghaddam et al., 2007)

Progestrone effectively counterbalances E2-induced proliferation

Hormonal regulation of gene expression in the human endometrium

Postmenopausal women were treated for 21 days (Klaassens et al., 2006):
• Controls (8)
• E2 treated (7)
• E2+MPA treated (6)
Pure endometrium, RNA isolation
Microarrays (Affymetrix U133plus2)
SAM analysis (Hanifi-Moghaddam et al., 2007)

4500 differentially expressed genes
Estradiol treatment has a profound effect on endometrial gene expression
Postmenopausal women were treated for 21 days (Klaassens et al., 2006):

- Controls (8)
- E2 treated (7)
- E2+MPA treated (6)

Pure endometrium, RNA isolation
Microarrays (Affymetrix U133plus2)
SAM analysis (Hanifi-Moghaddam et al., 2007)

4500 differentially expressed genes
438 significantly E2 regulated
More than 3-fold over control

Progesterone effectively counterbalances (compensates) E2 activities at the level of gene expression

Our data were combined with the data of Talbi et al., 2006

- Microarray data (Affymetrix U133plus2) at different stages of the menstrual cycle
  - Proliferative endometrium (PE, n = 4)
  - Early secretory endometrium (ESE, n = 3)
  - Mid secretory endometrium (MSE, n = 8)
  - Late secretory endometrium (LSE, n = 6)
- “Raw data” have been made available at Geo Dataset in PubMed
- Combined to our data
  - E2 treated endometrium (E, n = 7)
  - E2+MPA treated endometrium (E+MPA, n = 6)
- Identified 9000 differentially expressed genes
- Cluster analysis

9000 Genes
Hormonal regulation of gene expression in the human endometrium

Hormonal regulation of Wnt targets and components in the human endometrium

Hypothesis: Estradiol activates Wnt signaling which induces proliferation, while progesterone inhibits Wnt signaling which induces differentiation (inhibits proliferation).

• DKK1, an established inhibitor of Wnt signaling, is induced by progestagens
• FOXO1, a suspected inhibitor of Wnt signaling, is induced by progestagens

DKK1 and FOXO1 are both regulated by progestagens.
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (Ishikawa)

Ishikawa cells were transfected with
• PRA, PRB or PRA/PRB
• TOP/FOP reporters (Wnt activation status)
• Cultured in the absence/presence of MPA
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (IK) ishikawa cells were transfected with:
- TOP/FOP reporters (Wnt activation status)
- Cultured in the absence/presence of MPA
- DKK1 and/or FOXO1 expression vectors

In Ishikawa cells, progesterone, DKK1 and FOXO1 inhibit Wnt signaling.

- MPA induces the expression of DKK1 and FOXO1
- When DKK1 and FOXO1 expression is inhibited, MPA does no longer inhibit Wnt signaling

RNA interference:
Ishikawa cells were infected with:
- Short hairpin RNAs against DKK1
- Short hairpin RNAs against FOXO1

Short hairpin RNA directed against DKK1 will inhibit the expression of DKK1
Short hairpin RNA directed against FOXO1 will inhibit the expression of FOXO1.
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (IK) Ishikawa cells (short-hairpin infected) are transfected with:
• TOP/FOP reporters (Wnt activation status)
• Cultured in the absence/presence of MPA

Inhibition of DKK1 and FOXO1 partly circumvents progesterone inhibition of Wnt signaling

In conclusion (II):
Progesterone can effectively inhibit Wnt signaling in the endometrial Ishikawa cell line.
DKK1 and FOXO1 seem to play a role in progesterone induced Wnt inhibition

The Role of Wnt Signaling in Uterus Development and in Homeostasis and Malignancy (III) of the Uterine Endometrium

Estradiol activates Wnt signaling which induces proliferation, while progesterone inhibits Wnt signaling which induces differentiation.
• 30–80% of endometrial cancers are Wnt-activated (nuclear β-catenin)
• Since overweight increases, incidence of endometrial cancer also increases
The Role of Wnt Signaling in Uterus Development and in Homeostasis and Malignancy (III) of the Uterine Endometrium

Nuclear β-catenin is a good marker for high levels of Wnt activation; for low levels of activation it does not work.

Hypothesis:
Estradiol activates Wnt signaling (CD44 positive) which induces proliferation, while progesterone inhibits Wnt signaling (CD44 negative) which induces differentiation.

Progesterone inhibition of Wnt signaling
In vivo results.

Hypothesis: Estradiol activates Wnt signaling (CD44 positive) which induces proliferation, while progesterone inhibits Wnt signaling (CD44 negative) which induces differentiation.

Endometrial hyperplasia → → MPA treatment
Overview

- CD44
- PRAB

Conclusions

- (I) Wnt activation in the myometrium results in malformation and impaired function.
- (II) Progesterone inhibits Wnt signaling in the human endometrium.
- (II) Progesterone inhibits Wnt signaling in endometrial homeostasis by inducing DKK1 and FOXO1 expression.
- (III) In well-differentiated endometrial cancer / hyperplasia, progesterone signaling is intact. In this case, progesterone can inhibit Wnt signaling, which will inhibit tumor growth; in less differentiated endometrial cancer, progesterone receptor may be lost and progesterone can no longer inhibit Wnt signaling and can no longer inhibit tumor growth.
- “Wnt signaling is crucial for functioning of the endometrium.”
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