Erasmus MC
University Medical Center Rotterdam

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

Leen J Blok
Erasmus University Medical Center Rotterdam

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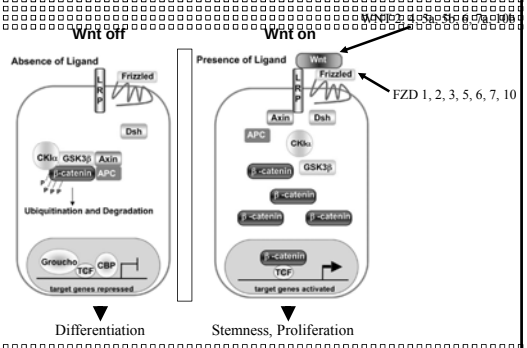
The Wnt/βcatenin signaling pathway



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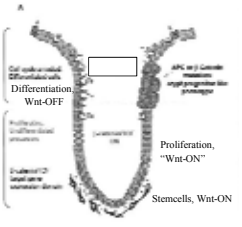
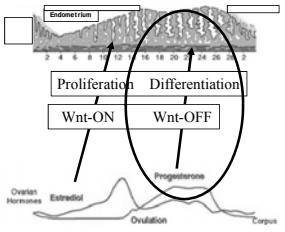
The physiological role of the Wnt/βcatenin signaling pathway in the endometrium



Van Wetering et al., 2002



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

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

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.

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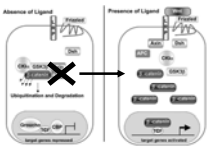
The Role of Wnt Signaling in Uterus Development Mouse models

Overexpression experiments:

1. Overexpression of Wnt signaling in **epithelial** cells of the Mullerian duct (dpc 9.5-15)
KSP1.3-Cre; $Apc^{lox/lox}$: Only embryonic detection is possible, sofar no phenotype

2. Overexpression of Wnt signaling in **mesenchymal** cells of the Mullerian duct (dpc 12.5)
$AmhR2^{Cre/+}$; $Apc^{lox/lox}$: Abberations in the myometrium.
$AmhR2^{Cre/+}$ (Jamin .. Behringer, 2002)



Wnt-Off → Wnt-On

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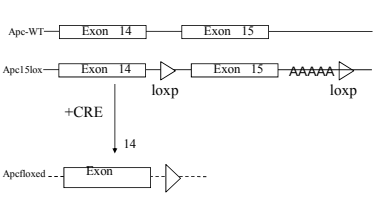
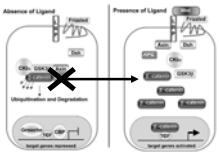
The Role of Wnt Signaling in Uterus Development $Apc^{lox/lox}$; $AmhR2^{Cre/+}$ mice





Wnt-Off → Wnt-On

In the presence of Cre (C-recombinase),
$Apc^{lox/lox}$ mice will become $Apc^{Flox/Flox}$ → $Apc^{-/-}$, Wnt signaling activation

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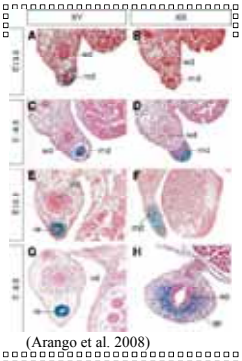
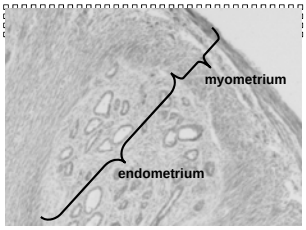
The Role of Wnt Signaling in Uterus Development

AmhR2-LacZ in embryo and adult



(Arango et al. 2008)



AmhR2-LacZ mice, (A.P.N. Themmen)

AmhR2 is expressed in cells surrounding the Mullerian duct

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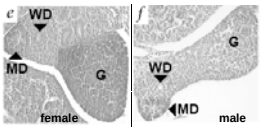
The Role of Wnt Signaling in Uterus Development

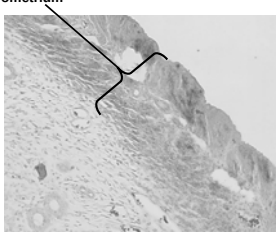
AmhR2$^{Cre/+}$; Rosa26LacZ^{lox} in embryo and adult



(Jamin et al. 2002)

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

myometrium



Cre is expressed in cells surrounding the Mullerian duct

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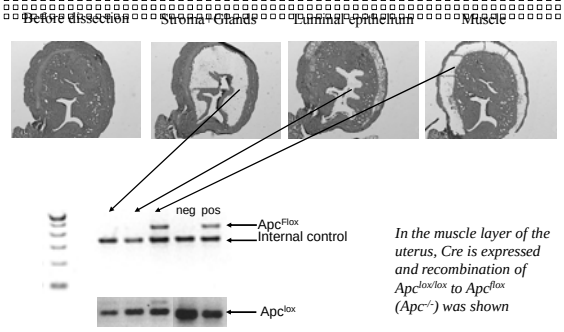
The Role of Wnt Signaling in Uterus Development

Apc$^{lox/lox}$; AmhR2$^{Cre/+}$ mice → Apc$^{Flox/Flox}$



In the muscle layer of the uterus, Cre is expressed and recombination of Apc$^{lox/lox}$ to Apc^{flox} (Apc$^{-/-}$) was shown

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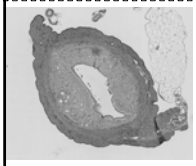
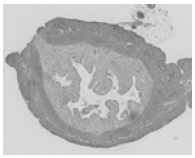
The Role of Wnt Signaling in Uterus Development

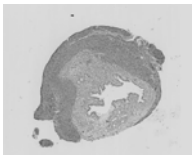
$Apc^{lox/lox}$; $AmhR2^{Cre/+}$ mice

WT

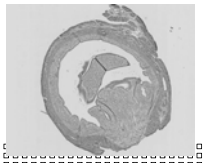
$Apc^{lox/lox}$; $AmhR2^{Cre/+}$







Staining for SMA



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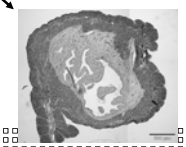
The Role of Wnt Signaling in Uterus Development (I)

$Apc^{lox/lox}$; $AmhR2^{Cre/+}$ mice

$Apc^{lox/lox}$; $AmhR2^{cre/+}$ mice:

Phenotype	Numbers	Age
Apc Flox mothers died during delivery	2/4	123/127
severe myometrial problems	2	178
minor myometrial problems	3	55/132/216
not analyzed yet	6	
control animals no problems	6/6	49-178



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The Role of Wnt Signaling in Uterus Development (I)

$Apc^{lox/lox}$; $AmhR2^{Cre/+}$ mice

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

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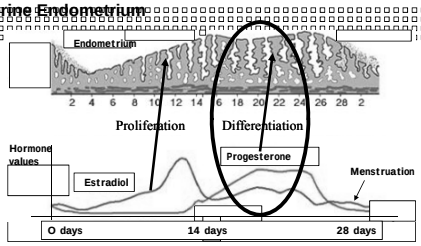
The Role of Wnt Signaling in Uterus Development and in Homeostasis (II) and Malignancy of the Uterine Endometrium



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

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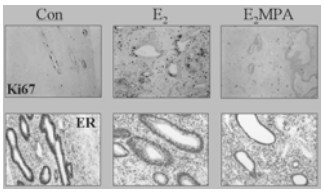
Hormonal regulation of proliferation in the human endometrium

Postmenopausal women were treated for 21 days (Klaassens et al., 2006):
- Controls (8)
- E_2 treated (7)
- E_2+MPA treated (6)

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



Progesterone effectively counterbalances E_2-induced proliferation

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Hormonal regulation of gene expression in the human endometrium

Postmenopausal women were treated for 21 days (Klaassens et al., 2006):
- Controls (8)
- E_2 treated (7)
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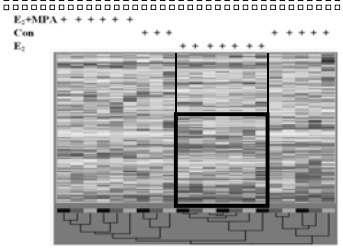
Pure endometrium, RNA isolation
Microarrays (Affymetrix U133plus2)
SAM analysis (Hanifi-Moghaddam et al., 2007)

4500 differentially expressed genes



Estradiol treatment has a profound effect on endometrial gene expression

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Hormonal regulation of gene expression in the human endometrium

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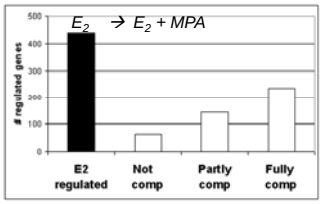

4500 differentially expressed genes
438 significantly E_2 regulated
More than 3-fold over control

Progesterone effectively counterbalances (compensates) E_2-activities at the level of gene expression

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Hormonal regulation of gene expression in the human endometrium

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
  - E_2 treated endometrium (E, n = 7)
  - E_2+MPA treated endometrium (E+MPA, n = 6)
- Identified 9000 differentially expressed genes
- Cluster analysis

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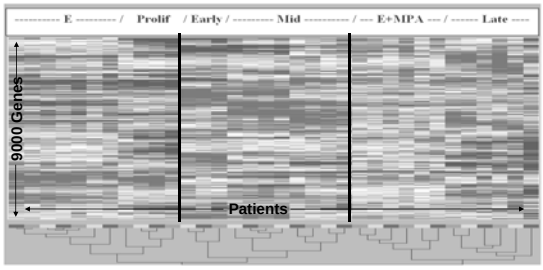
Hormonal regulation of gene expression in the human endometrium

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

- Microarray data at different stages of the menstrual cycle



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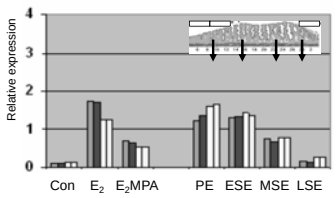
Hormonal regulation of gene expression in the human endometrium

IGF1



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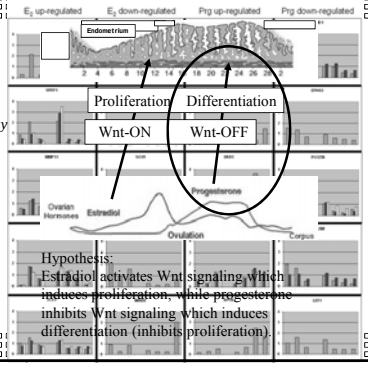
Hormonal regulation of Wnt targets and components in the human endometrium

228 Wnt targets and components were identified to be significantly regulated by E_2 or E_2MPA

Wnt signaling has a role to play in the hormonal regulation of the human endometrium



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

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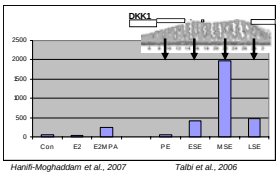
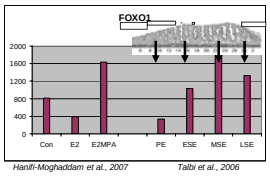
Progesterone inhibition of Wnt signaling

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



Hanifi-Moghaddam et al., 2007 *Talbi et al., 2006*



Hanifi-Moghaddam et al., 2007 *Talbi et al., 2006*

DKK1 and FOXO1 are both regulated by progesterone

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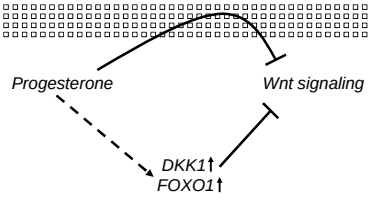

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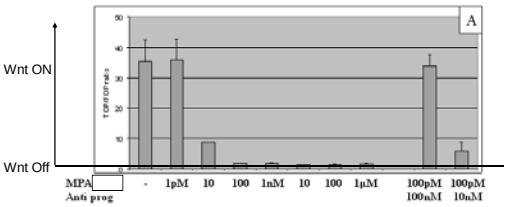
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (Ishikawa)

Ishikawa cells were transfected with

- PRA + PRB
- TOP/FOP reporters (Wnt activation status)
- Cultured in the absence/presence of MPA



In Ishikawa cells, progesterone inhibits Wnt signaling through the PR

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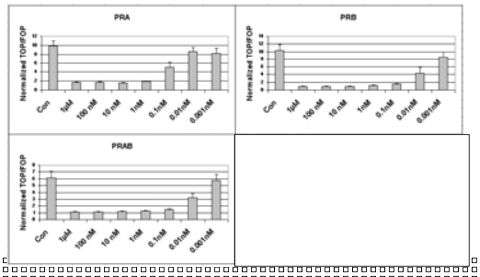
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (Ishikawa)

Ishikawa cells were transfected with

- PRA, PRB or PRA/PRB
- TOP/FOP reporters (Wnt activation status)
- Cultured in the absence/presence of MPA



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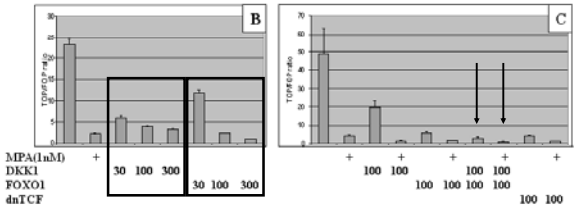
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (IK)

Ishikawa cells were transfected with
- PRA + PRB
- 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

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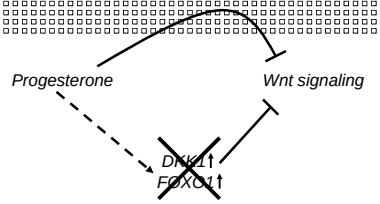
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (IK)



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

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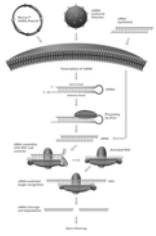
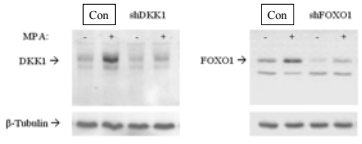
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (IK)





Short hairpin RNA directed against DKK1 will inhibit the expression of DKK1
Short hairpin RNA directed against FOXO1 will inhibit the expression of FOXO1

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

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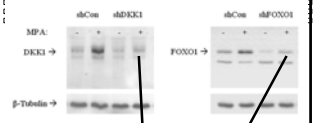
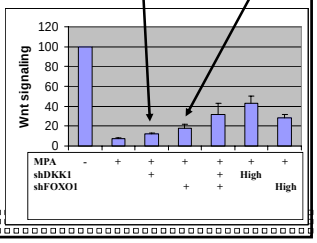
Progesterone inhibition of Wnt signaling in a human endometrial cancer cell line (IK)

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





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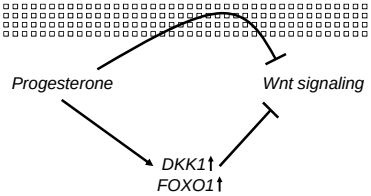
Progesterone inhibition of Wnt signaling



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

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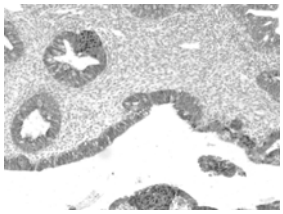
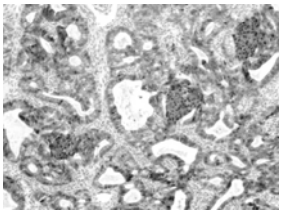
The Role of Wnt Signaling in Uterus Development and in Homeostasis and Malignancy (III) of the Uterine Endometrium

Hypothesis:

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





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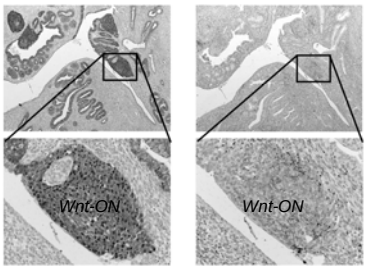
The Role of Wnt Signaling in Uterus Development and in Homeostasis and Malignancy (III) of the Uterine Endometrium

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



nuclear β-catenin CD44

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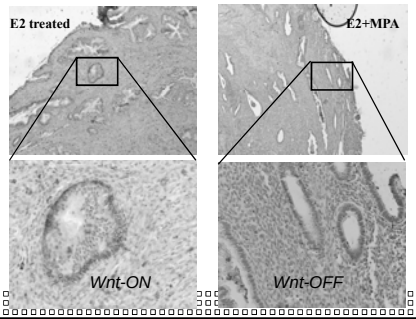
Progesterone inhibition of Wnt signaling In vivo results.

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



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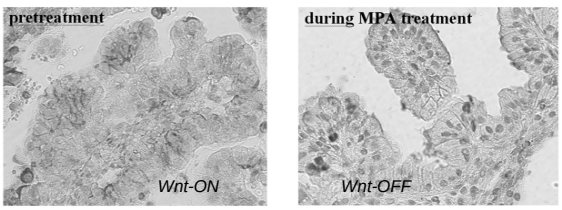
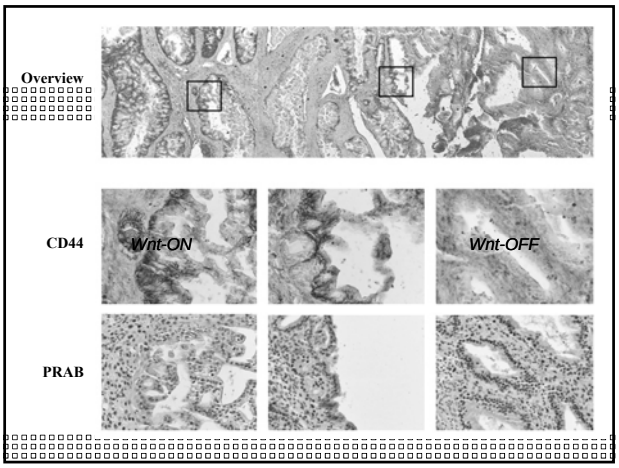
hypothesis:
Estradiol activates Wnt signaling (CD44 positive) which induces proliferation, while progesterone inhibits Wnt signaling (CD44 negative) which induces differentiation.

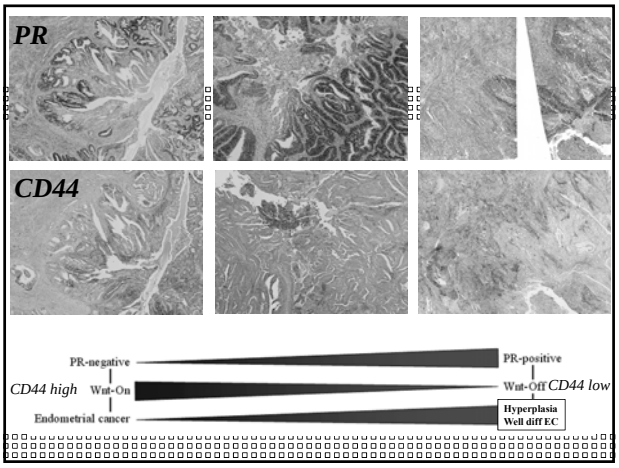


Endometrial hyperplasia → → → MPA treatment

↓

Foci of Well differentiated AdenoCA





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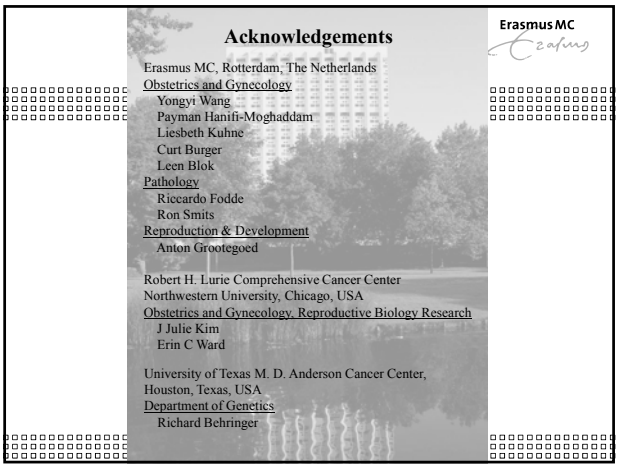
Conclusions

- (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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