Stem Cells as Targets that lead to Increased Cancer Susceptibility

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Is the risk of Prostate Cancer influenced by fetal or perinatal estrogen exposures?

- Maternal
- Pharmaceutical (DES)
- EDCs
Inappropriate estrogenic exposures (dosing, timing, type) can reprogram the prostate gland and increase disease risk with aging.
Prostate Epithelial Cell Hierarchy

- Sox2
- ABCG2
- CD 44, 117, 133
- CD49fhi, Trop2

**Stem Cell**
- self-renewal

**Bipotent Progenitor**
- transit amplification

**Unipotent Basal Progenitor**
- symmetric commitment

- **Basal**
  - CK5/15+
  - P63+

- **Luminal**
  - CK8/18+
  - AR+
  - Nkx3.1+
  - Hoxb13+
  - PSA

- **Neuroendocrine**
  - Chromogranin A
  - Synaptophysin

**E2, EDCs**

**CD49f**
- Symmetric renewal
- Asymmetric division
- Symmetric commitment
Primary culture of normal (i.e. disease-free) prostate epithelial cells (PrEC)

< 0.2 % form prostaspheres (PS) through self-renewal

3-D culture in Matrigel

Prostate stem cell markers

Human Prostaspheres express ERs

Estradiol stimulates stem-progenitor cell self-renewal

Day 7 PS

E$_2$ ↑ prostasphere # and size

40-80µm

>80µm

PS number (fold changes)

0.0 0.5 1.0 1.5 2.0 2.5 3.0

C 1 10 100 1000

E2 (nM)

C 1 10 100 1000

E2 (nM)

*  † **

C

E2

(nM)

1 10 100 1000

CD49f-APC

control

1 nM E2

Trop2 - AF488

Trop2 - AF488

Trop2 - AF488

Fold change

0.0 0.4 0.8 1.2 1.6

C 1 10 100

E2 (nM)
Estradiol maintains prostasphere stemness

Day 7 PS

E$_2$ ↑ PS stemness genes

*P<0.05 vs control
N=4

Fold changes
Bisphenol A stimulates prostate stem-progenitor cells

Day 7 PS

BPA ↑ PS # and size

BPA ↑ PS stemness genes

Low-dose BPA phenocopies most of E2 effects on prostate stem-progenitor cells
E₂ signaling pathways in human prostaspheres

**Classic genomic signaling:**
Transient ERE-tk-luciferase reporter in **Day 5 PS**
+16 hr E₂ or BPA

**Rapid Non-genomic signaling:**

Day 7 PS:

- **Bisphenol A**
  - 10 nM
  - Control, 15 min, 30 min, 60 min, 6 hrs

- **Estradiol**
  - 10 nM
  - Control, 15 min, 30 min, 60 min, 6 hrs

- **pAKT**
  - ser473

- **AKT**

- **pERK**
  - thr202/tyr204

- **ERK**

**Graphs:**

- Relative luciferase activity
- Log(10⁻³ pERK / total ER)

**Authors:** Cheryl Walker
Estrogens act through both nuclear and membrane-initiated receptor signaling pathways in prostate stem-progenitor cells.
In vivo Chimeric Model of Normal Humanized Prostate Tissue

- embryonic rat
- UG mesenchyme

+ 1 month

PSA, DAPI
Estrogen-induced prostate carcinogenesis

1 month

at 1 month:
\( T+E_2 \) pellets
- 25 mg T
- 2.5 mg \( E_2 \)

1-4 months

embryonic rat
UG mesenchyme

Open biopsy
Estradiol Drives Adenocarcinoma in Human Prostate Epithelium

1 month T+E<sub>2</sub>  
Hyperplasia

2 month T+E<sub>2</sub>  
HG-PIN

2 - 4 month T+E<sub>2</sub>  
PCa

PIN Incidence by 4 mo: 31%

Prostate Cancer Incidence: 11%

Hu et al, Endocrinology 152 :2150, 2011
Developmental BPA Exposure and PCa Susceptibility

**BPA (200 nM)**
- 0-7 days
- 1x/day
- 0-2 wks

**Low-dose: (100 or 250 µg/kg BW)**

<table>
<thead>
<tr>
<th>Treatment (µg BPA/kg BW)</th>
<th>Free BPA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>BLD</td>
</tr>
<tr>
<td>100</td>
<td>0.39 ± 0.17</td>
</tr>
<tr>
<td>250</td>
<td>1.35 ± 0.29</td>
</tr>
</tbody>
</table>

Embryonic rat UG mesenchyme +

Day 7 Serum BPA levels

T+E pellets 1-4 mo

Blinded Pathology Dx

Developmental BPA Increases Human PCa Susceptibility

Dx at 2-4 months T+E

<table>
<thead>
<tr>
<th></th>
<th>Oil</th>
<th>BPA in vivo 100 µg/kg</th>
<th>BPA in vivo 250 µg/kg</th>
<th>BPA in vitro + in vivo 200 nM, 250 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>38</td>
<td>36</td>
<td>27</td>
<td>42</td>
</tr>
<tr>
<td>Normal</td>
<td>10 (26%)</td>
<td>4 (11%)</td>
<td>0 (0%)*</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Abnormal: Benign Hyperplasia, SQM</td>
<td>28 (74%)</td>
<td>32 (89%)</td>
<td>27 (100%)*</td>
<td>38 (90%)**</td>
</tr>
<tr>
<td>Abnormal: Cancerous HG-PIN &amp; PCa</td>
<td>5 (13%)</td>
<td>12 (36%) *</td>
<td>9 (33%)**</td>
<td>19 (45%)**</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01 vs oil;  Note: Some specimens contain multiple diagnoses.

HG-PIN

PCa

Prins et al, Endocrinology 155:805, 2014
Conclusions: Bisphenol A and Human Prostate Cancer

Direct *in vivo* evidence that developmental exposure to bisphenol A (BPA) at levels found in humans *increases* cancer susceptibility in the *human prostate* epithelium.

Lobaccaro JM, Trousson A. *Endocrinology* 155, 2014
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