Prostate Stem Cells as EDC Targets that Increase Cancer Susceptibility

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Developmental Origins of Adult Disease

The risk of Prostate Cancer is likely influenced by fetal or perinatal **estrogen exposures**.

- Maternal (*Henderson, Ross, '88; Ekbom, '96, 2000*)
- Pharmaceutical (DES) (*Aria & Bern*, '78; *Prins* '97, '01)
- EDCs (*Cooke*, '90; *Peterson*, '97; *Nagel*, '97; *Prins* et al, '07)

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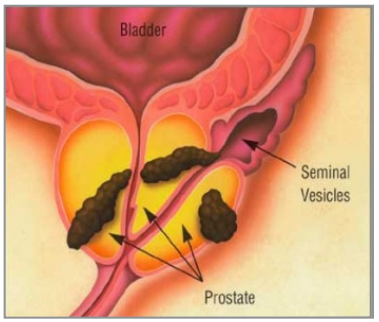


ENDOCRINE DISRUPTING CHEMICALS

ER agonists AR antagonist T synthesis inhibitor TH inhibitor

HERBICIDES

2,4,-D
2,4,5,-T
Alachlor
Amitrole
Atrazine
Linuron
Metribuzin
Nitrofen
Trifluralin

FUNGICIDES

Benomyl
Ethylene thiourea
Fenarimol
Hexachlorobenzene
Mancozeb
Maneb
Metiram - complex
Tri-butyl-tin
Vinclozolin
Zineb

INSECTICIDES

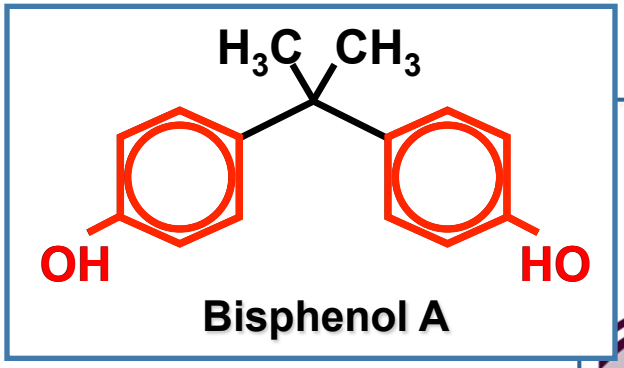
Aldicarb
beta-HCH
Carbaryl
Chlordane
Chlordecone
DBCP
Dicofol
Dieldrin
DDT and metabolites
Endosulfan
Heptachlor / H-epoxide
Lindane (gamma-HCH)
Malathion
Methomyl
Methoxychlor
Oxychlordane
Parathion
Synthetic pyrethroids
Transnonachlor
Toxaphene

INDUSTRIAL CHEMICALS

Bisphenol - A
Polycarbonate
Butylhydroxyanisole (BHA)
Cadmium
Chloro- & Bromo-diphenyl ether
Dioxin (2,3,7,8-TCDD)
Furans
Lead
Manganese
Methyl mercury
Nonylphenol
Octylphenol
PBDEs
PCBs
Pentachlorophenol
Penta- to Nonylphenols
p-tert-Pentylphenol
Phthalates
Styrene

Bisphenol A (BPA): Estrogenic EDC

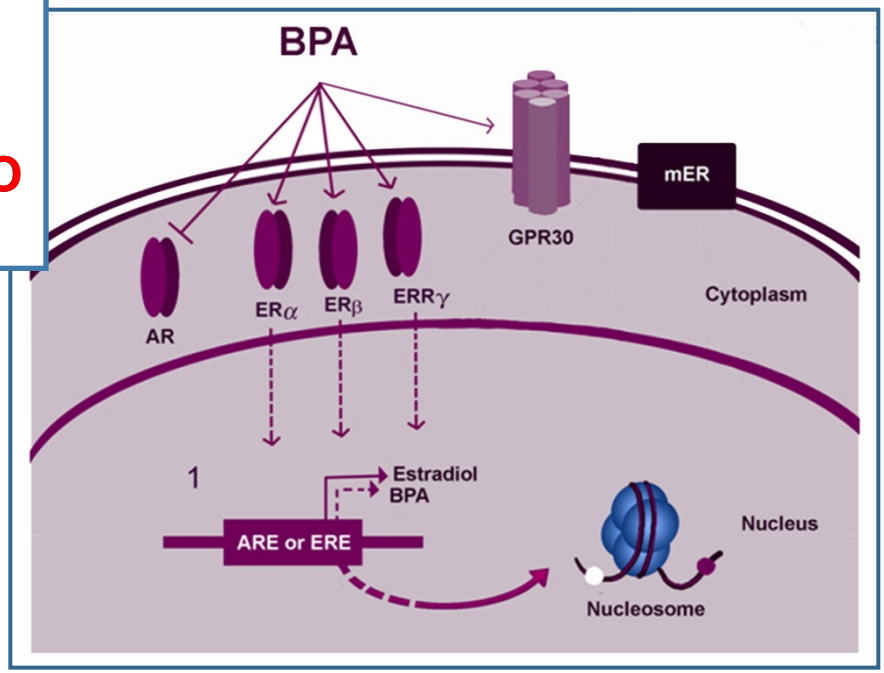
Epoxy resins: can linings



Bisphenol A

Polycarbonate plastics

Carbonless paper receipts



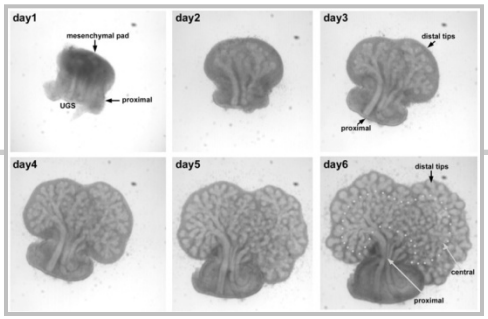
- Most humans are chronically exposed.
  - 93% human urines BPA+ (CDC assay)
- The *human fetus* and *neonate* is <u>chronically</u> exposed to BPA:

Early-life BPA Increases Prostate Cancer Susceptibility

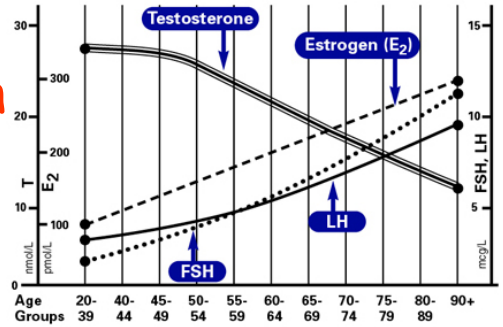
BPA (10 μg/kg BW)

Birth **Puberty** No PCa

Adult Estrogen ↑ PCa incidence



↑ E/T in aging men

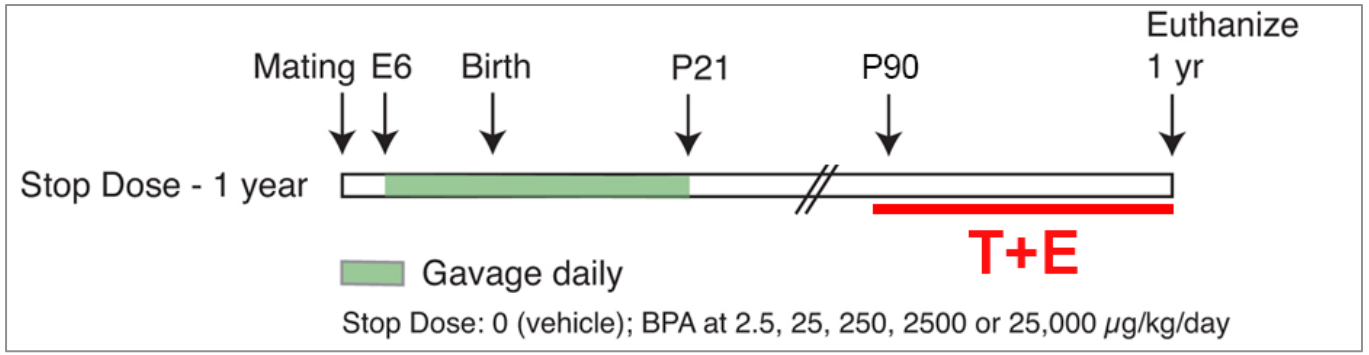


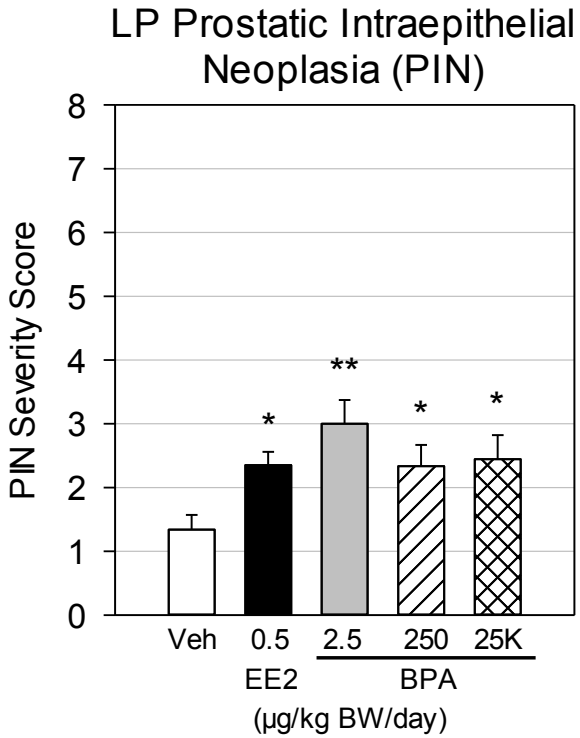
UIC

Ho, et al, *Cancer Res*, 2006; Prins, et al, *Repro Tox*, 2011; Prins, et al. *EHP* 2017

CLARITY Study: FDA-NIEHS Investigator Consortium

Consortium Linking Academic and Regulatory Insights on BPA Toxicity





Lateral Prostate Lobe:

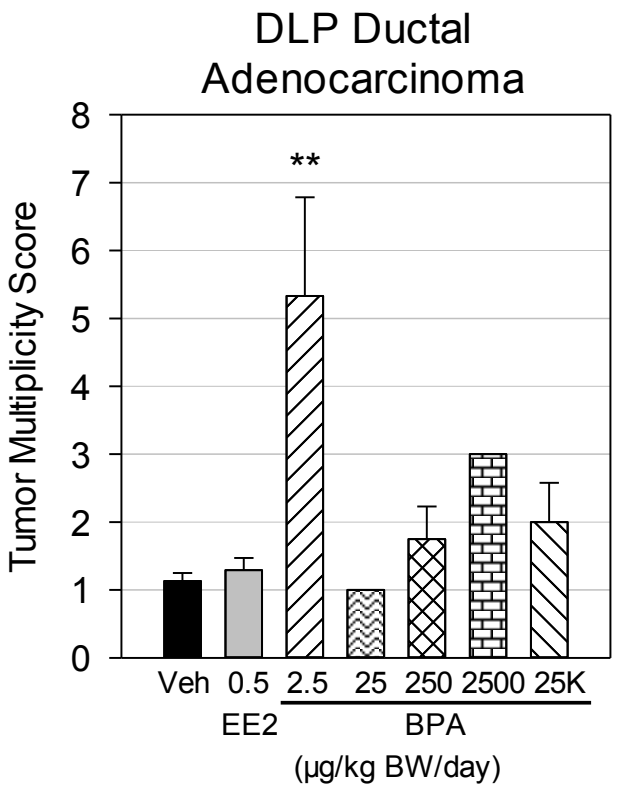
- **No difference in PIN incidence**
- **Increased *severity*** of **LP PIN**

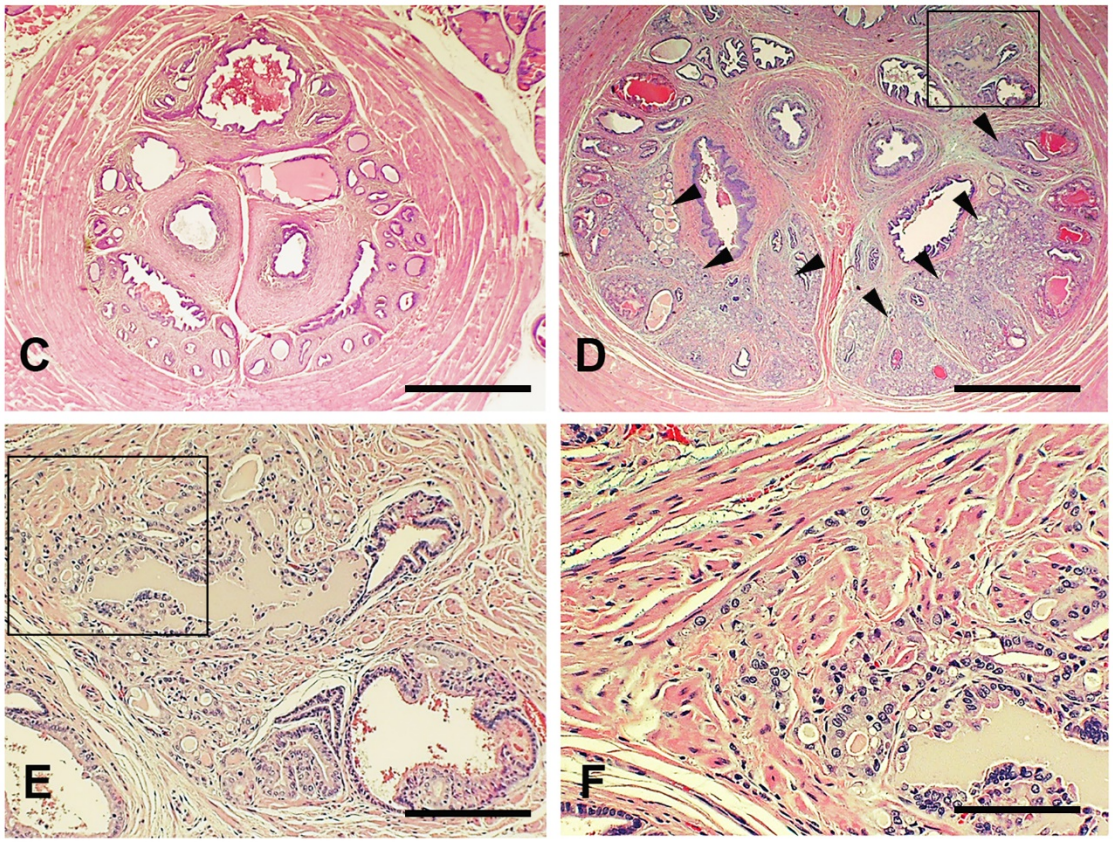
in EE and 2.5, 250 and 25,000 μg/kg BPA

(**P<0.01, * P<0.05 vs Vehicle)

Prins et al, *Environ Health Perspectives* Nov;126(11):117001, 2018

Low-dose BPA increased E2-induced adenocarcinoma multiplicity



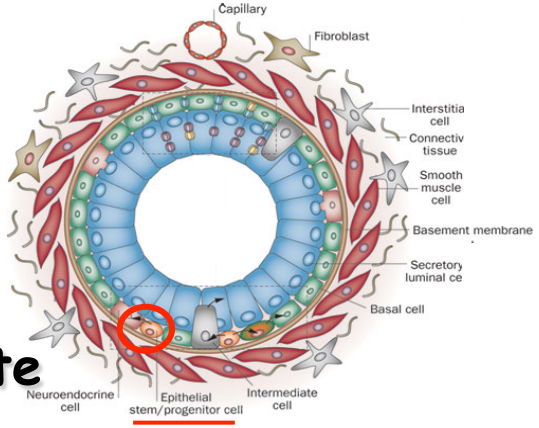


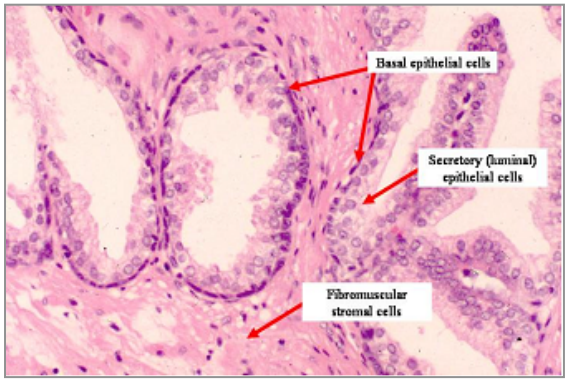
DLP Ducts:

- *Incidence* of adenocarcinoma (25-50%) not modified by EE/BPA.
- **Increased *multiplicity*** of ductal **adenocarcinoma** in 2.5µg BPA
  (P<0.01 vs Vehicle). Trending for higher doses; borderline significance in parametric analysis.

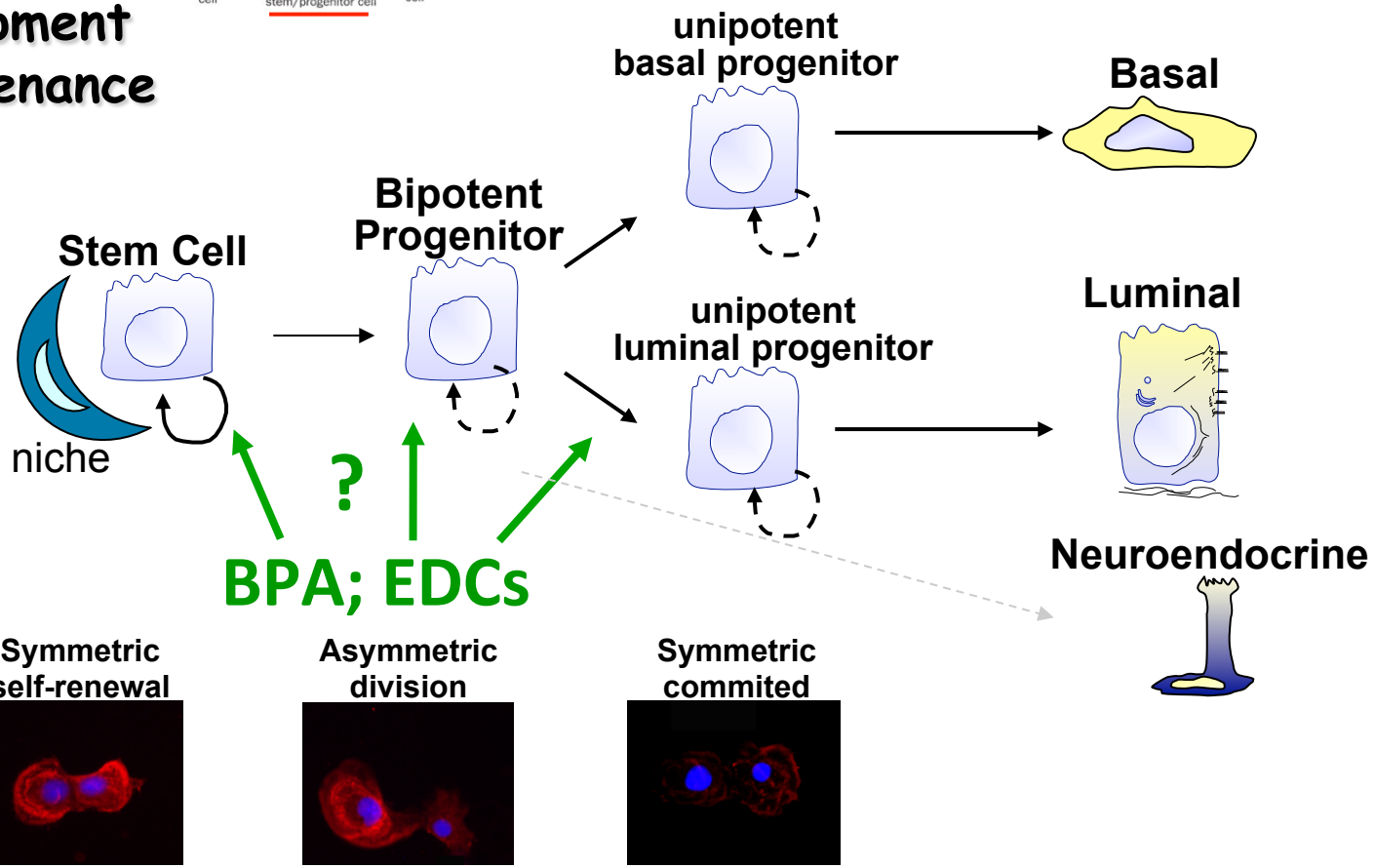
Prins GS et al, *Environ Health Perspectives* Nov;126(11):117001, 2018

Prostate Epithelial Cell Hierarchy





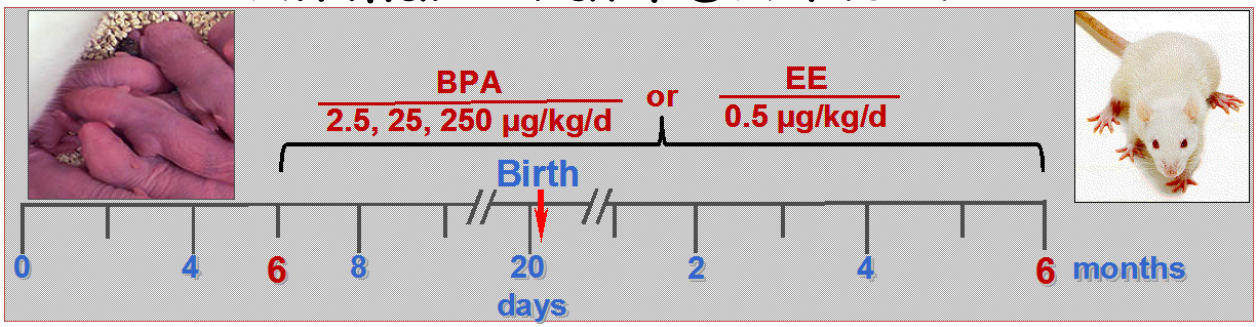
Normal Prostate Development & Maintenance



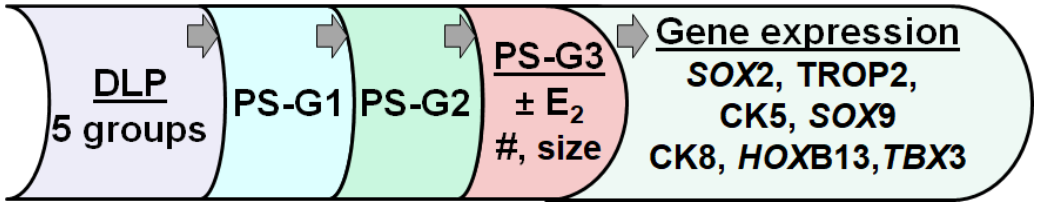
CLARITY Study: FDA-NIEHS Investigator Consortium

Examine the stem and progenitor cells from BPA-exposed rat prostates

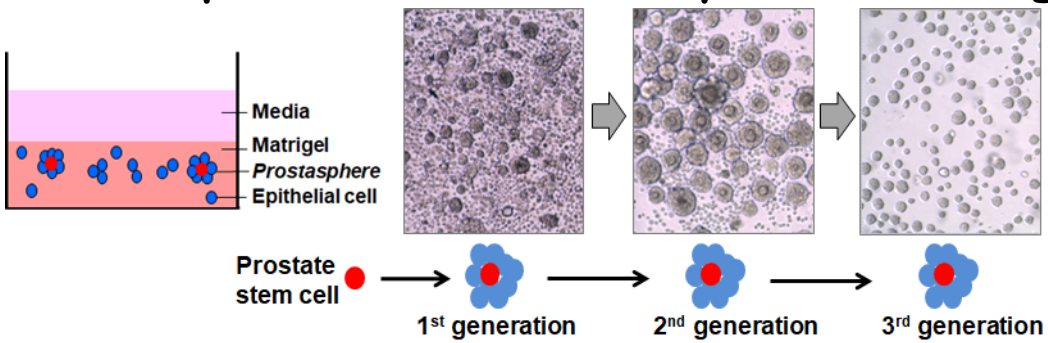
Animal Tx at FDA-NCTR



Workflow at UIC



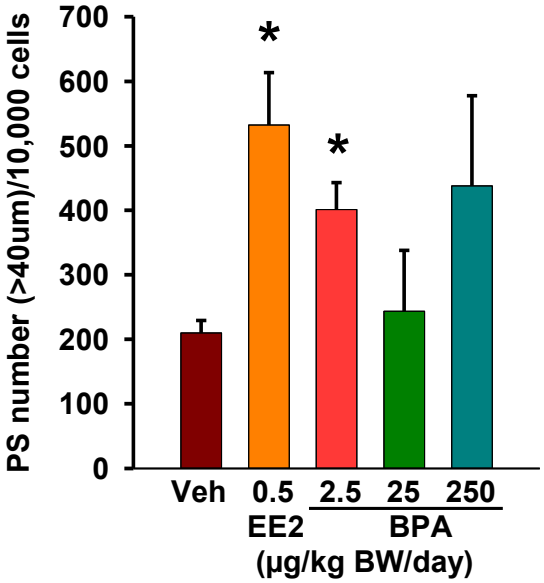
Prostasphere (PS) Assay and Passage



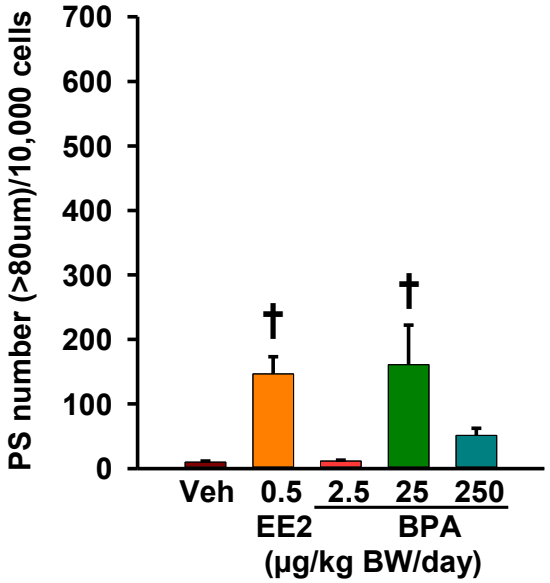
Prins GS et al, *Environ Health Perspectives* Nov;126(11):117001, 2018

Chronic exposure to low-dose EE or BPA increased prostate stem cell # and progenitor cell proliferation

Passage 3 – Total PS

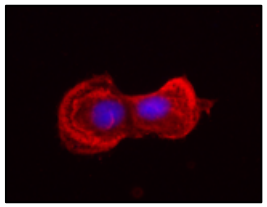


Passage 3 – PS > 80 µM



Representative of stem cell number

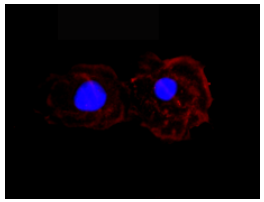
↓



Symmetric Self-renewal

Representative of progenitor cell proliferation

↓

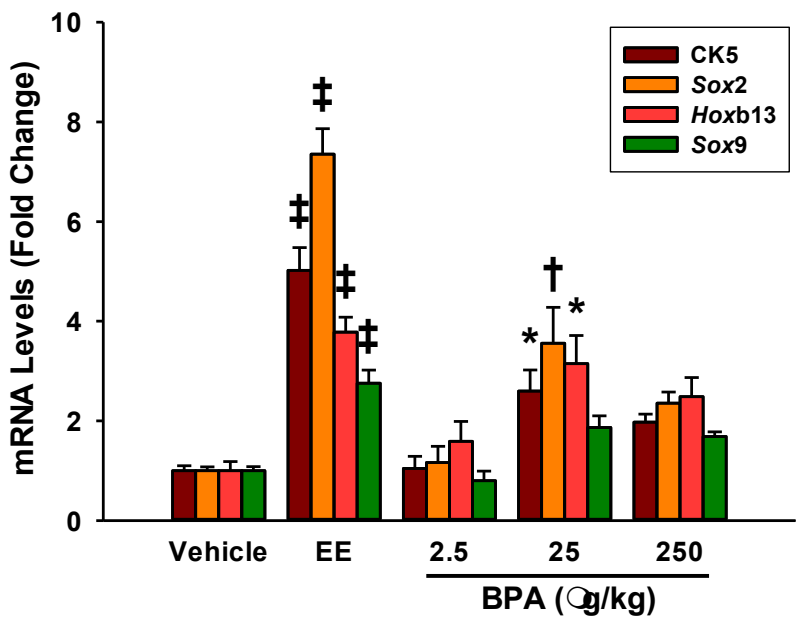


Symmetric Committed division

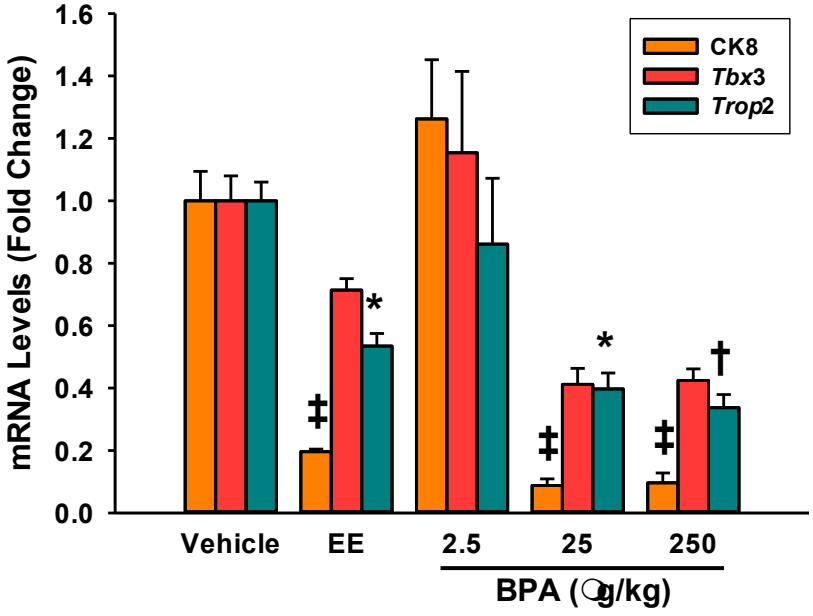
Prins GS et al, *Environ Health Perspectives* Nov;126(11):117001, 2018

Chronic low-dose EE and BPA (25 and 250 μg/kg) exposures alter progenitor cell lineage commitment

Basal Progenitor Pattern



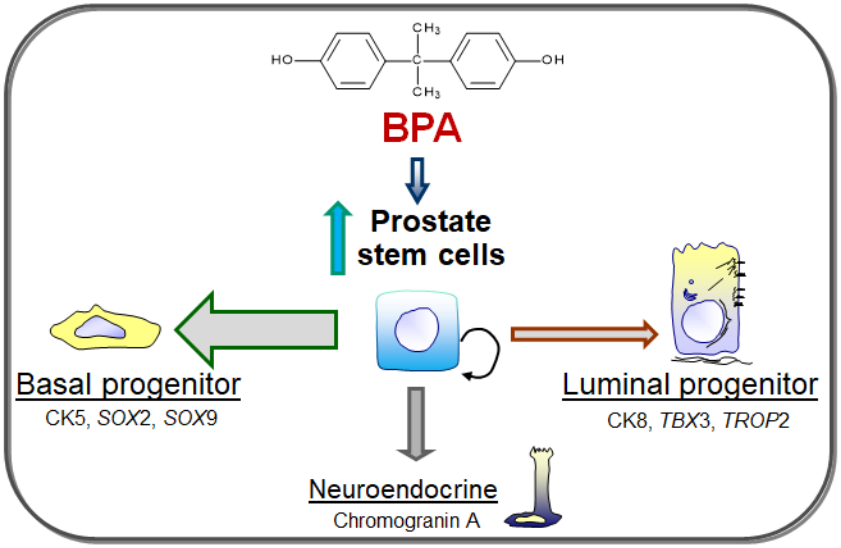
Luminal Progenitor Pattern



See a shift towards increased basal progenitor lineage at the expense of decreased luminal progenitor lineage

Prins GS et al, *Environ Health Perspectives* Nov;126(11):117001, 2018

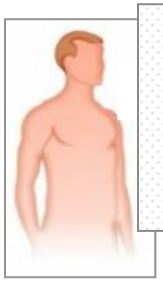
CLARITY Study: Summary Model



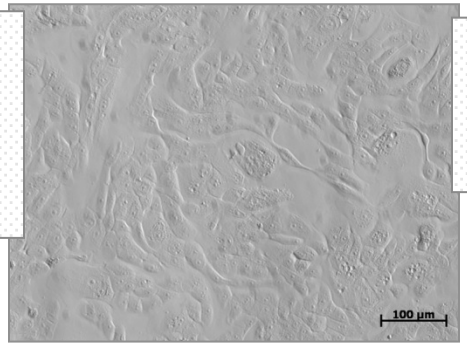
- How might the stem cell changes influence PCa susceptibility?
  - Cancer risk is highly correlated to # normal stem cell divisions in most tissues, *including prostate* (Tomasetti & Vogelstein, *Science*, 2015, 2017)
  - Tumor initiating cells for human PCa are largely localized to basal cell population (Goldstein et al, Science, 2010)

- *Propose*: Chronic *in vivo* low-dose BPA exposures ↑ prostate stem cell numbers and altered lineage commitment underpin increased carcinogenic risk with aging

Human Prostate: Growth & Analysis of Stem-Progenitor Cells

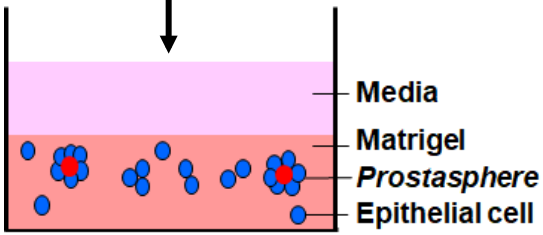


Primary culture of <u>normal</u> (i.e. disease-free) prostate epithelial cells (PrEC)

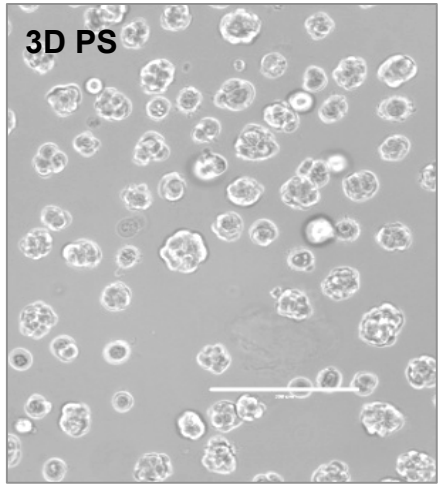


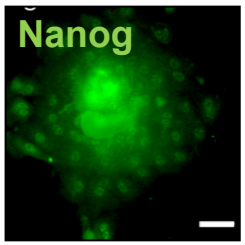
scRNA-seq:
minor population of stem cells:
KRT13+ PSCA+ LY6D+

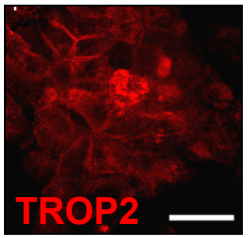
3-D culture in Matrigel

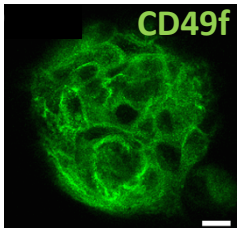


< 1 % form Prostaspheres (PS) through self-renewal



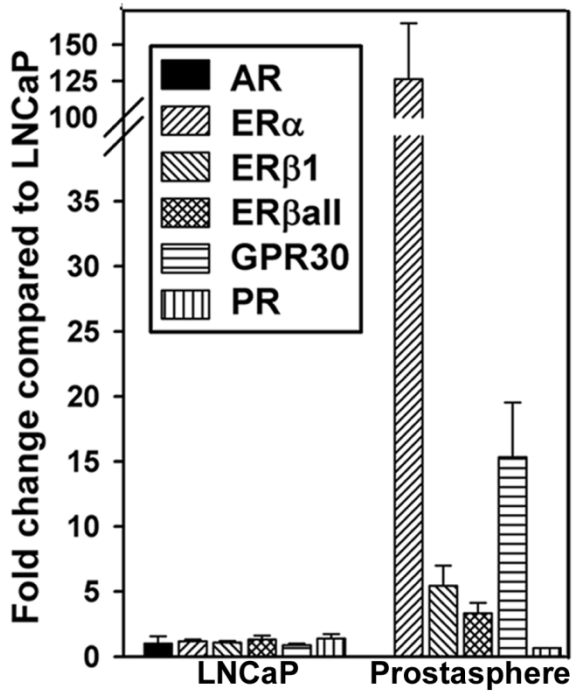


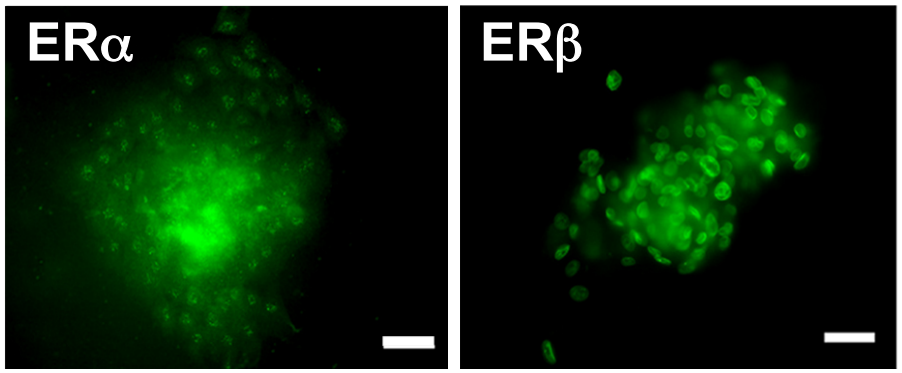




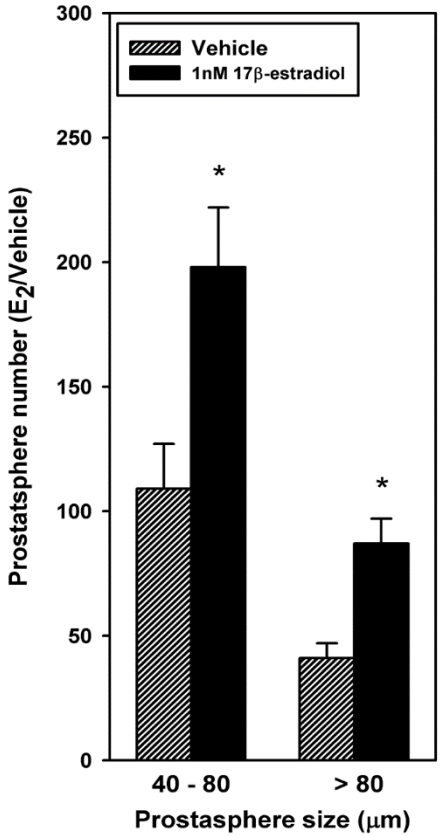
Hu et al, *Endocrinology* 152 (6):2150, 2011

Human Prostaspheres express Estrogen Receptors





E_2 ↑ PS # and size

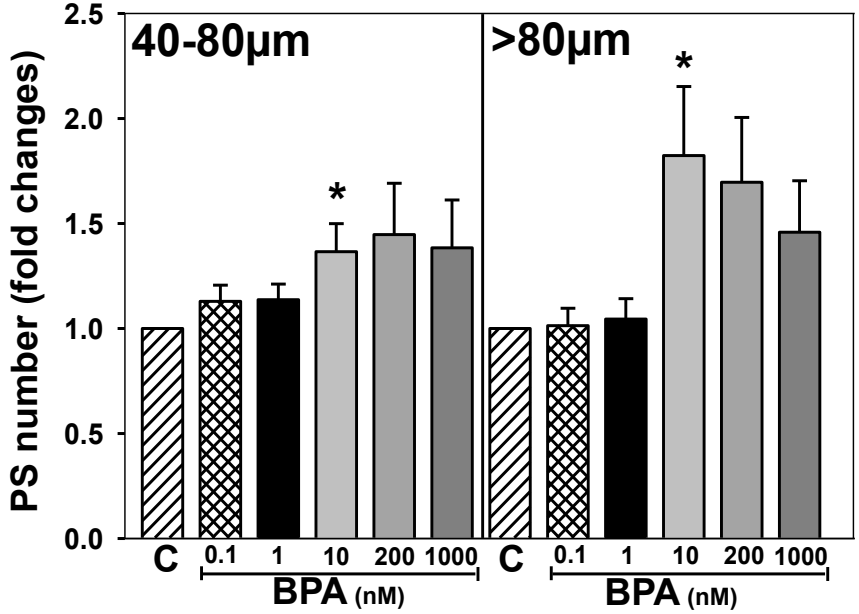


Hu et al, *Endocrinology* 152 (6):2150, 2011

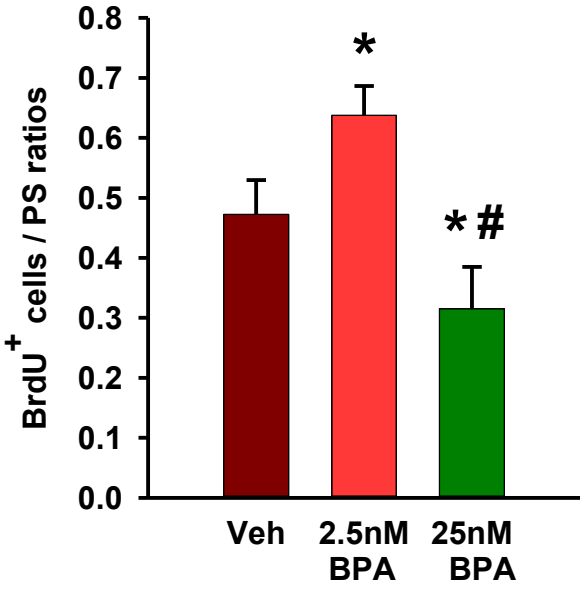
Bisphenol A stimulates prostate stem-progenitor cells

Day 7 PS

BPA ↑ PS # and size



BPA ↑ PS stem cell #s at low-dose

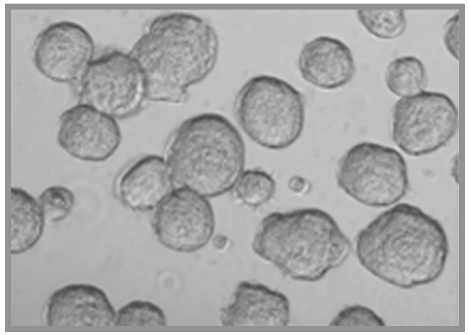


Low-dose BPA phenocopies most of E2 effects on prostate stem-progenitor cells

Prins et al, *Endocrinology* 155: 805, 2014

? *Can BPA increase prostate cancer susceptibility in human prostate epithelium?*

In vivo Chimeric Model of Normal Humanized Prostate Tissue



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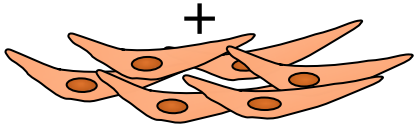
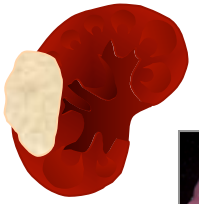
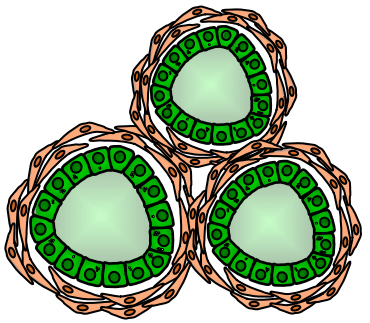
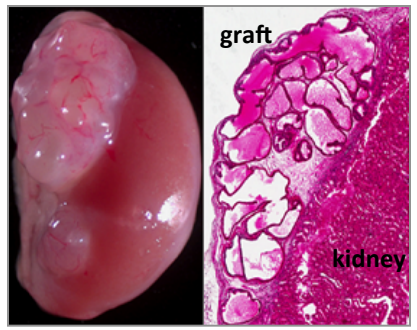


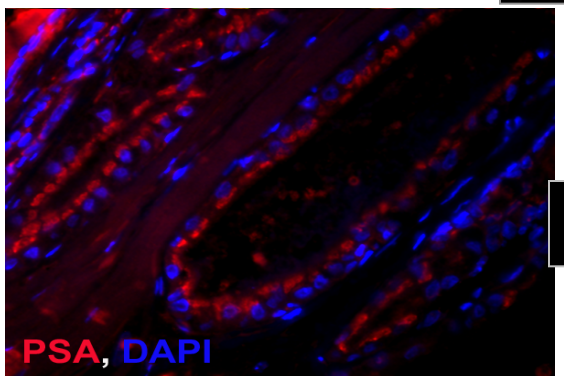
embryonic rat
UG mesenchyme

1 month



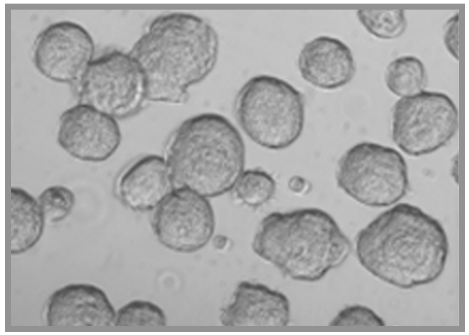






PSA

Estrogen-induced prostate carcinogenesis



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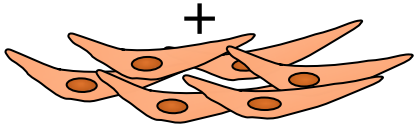

embryonic rat
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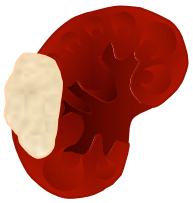
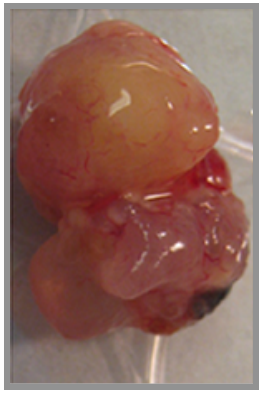


at 1 month:
T+E_2 pellets
- 25 mg T
- 2.5 mg E_2

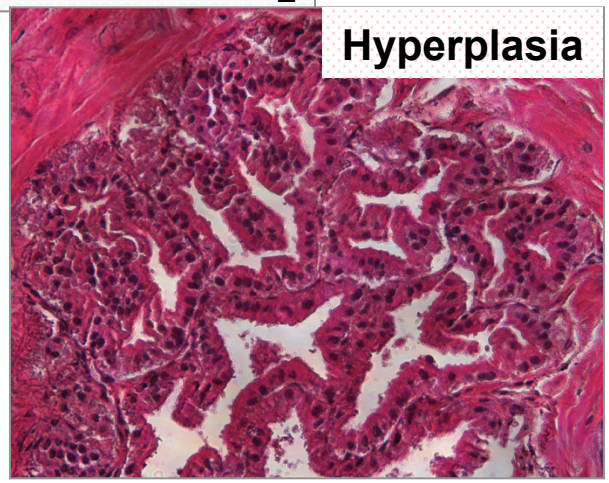
1-4 months

Open biopsy

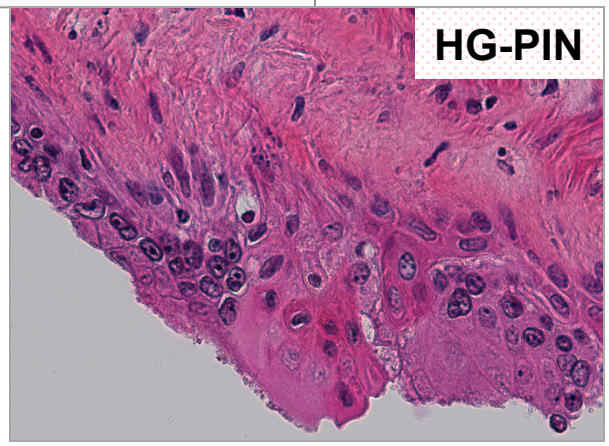


Estradiol Drives Adenocarcinoma in Human Prostate Epithelium

1 month T+E_2

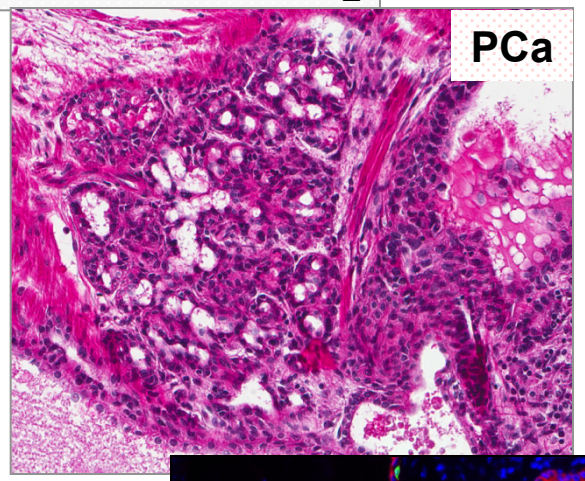


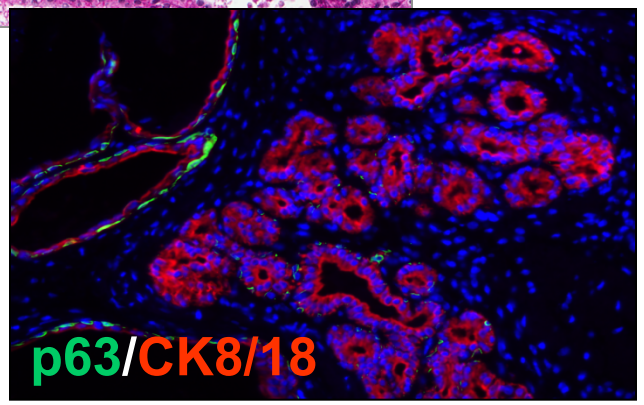
2 month T+E_2



PIN Incidence by 4 mo: 31%

2 - 4 month T+E_2





Prostate Cancer Incidence: 11%

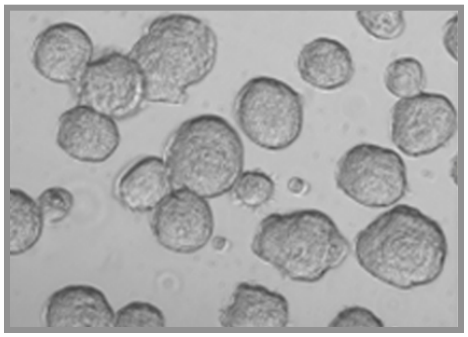
Hu et al, *Endocrinology* 152 :2150, 2011

Developmental BPA Exposure and PCa Susceptibility

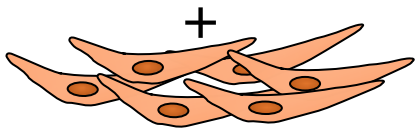
Low-dose: (100 or 250 μg/kg BW)

BPA (200 nM)

0-7 days



\+

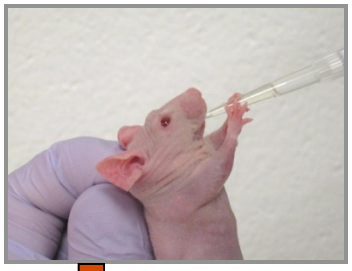


embryonic rat
UG mesenchyme

BPA

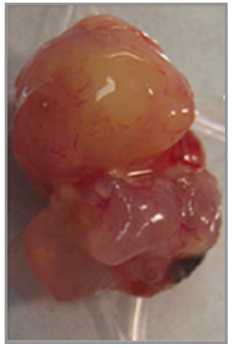
1x/day

0-2 wks



T+E pellets

1-4 mo



Day 7 Serum BPA levels

Treatment (μg BPA/kg BW)	**Free BPA** (ng/mL)
vehicle	BLD
100	0.39 ± 0.17
250	1.35 ± 0.29

Blinded Pathology Dx

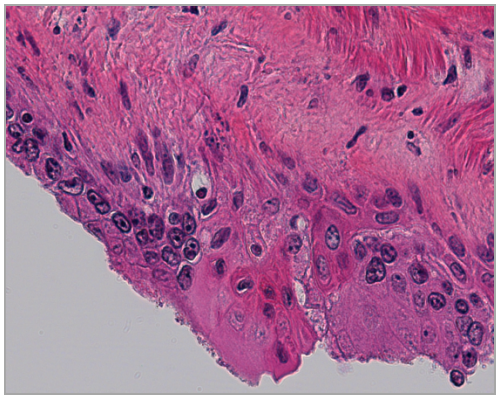
Prins et al, *Endocrinology* 155:805, 2014

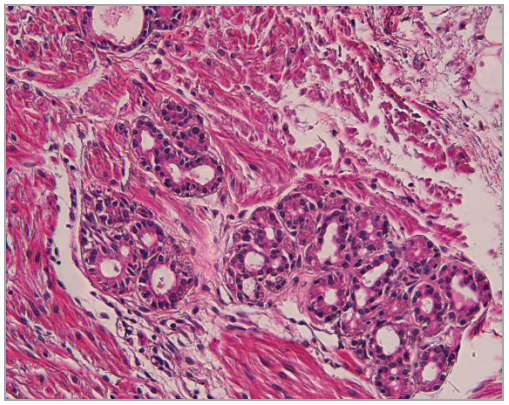
Developmental BPA Increases Human PCa Susceptibility

Dx at 2-4 months T+E

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

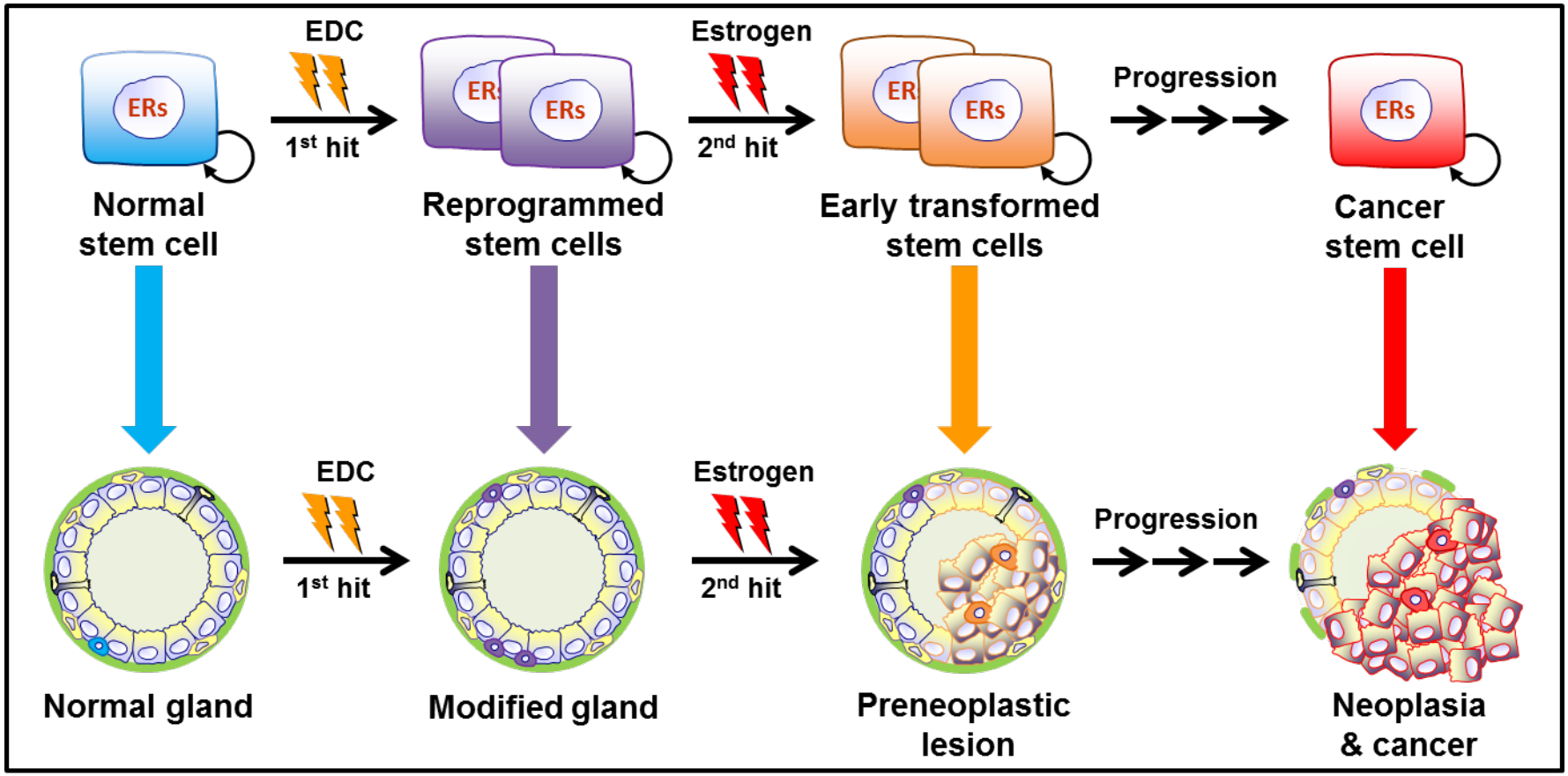
***P*<0.05, ****P*<0.01 *vs* oil**; *Note*: Some specimens contain multiple diagnoses.

HG-PIN 

 PCa

Prins et al, *Endocrinology* 155:805, 2014

Developmental BPA exposures reprograms prostate stem-progenitor cells resulting in increased carcinogenic susceptibility



Prins et al, *Endocrinology*, 156:3451, 2015

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