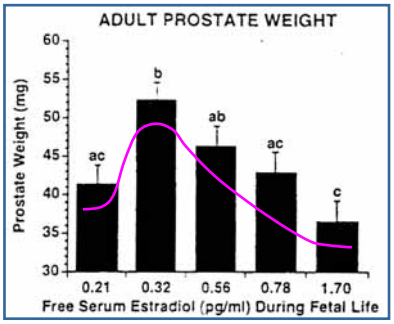
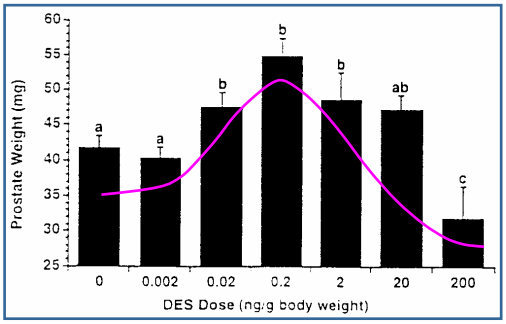
Dose-response profiles





Vom Saal et al. (1997)

Low-dose non-monotonic dose-response of prostate to estradiol and DES, EDCs

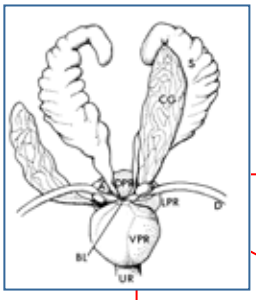
- **mice**
- **fetal exposure**

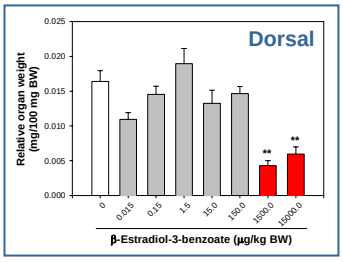
- **Does low-dose effect *really* exist?**
- **Reproducibility?**
- **Species or even strain differences?**
- **Does it drive prostate pathology?**

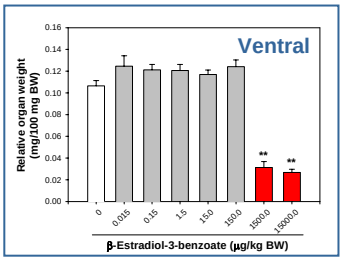
Dose-response profiles

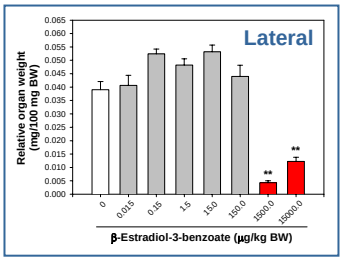
0.015 to 1500 estradiol μg/kg BW

Relative prostate weights in SD rats (PND 90)



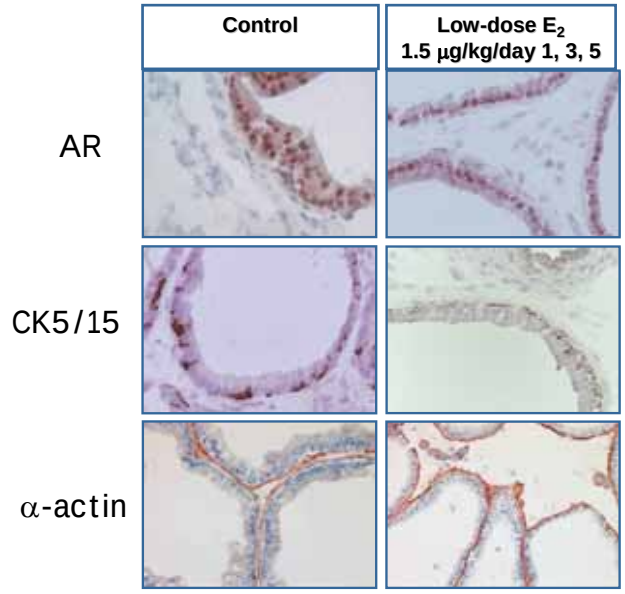






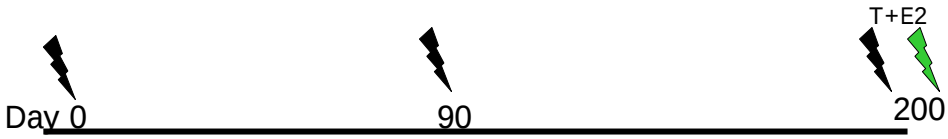
Low-dose neonatal estrogen exposure: SD rats

Effects of cellular differentiation marker on adult ventral prostate



Putz, et al
Biol Repro
65:1496, 2001

Animal model (Sprague-Dawley rats)

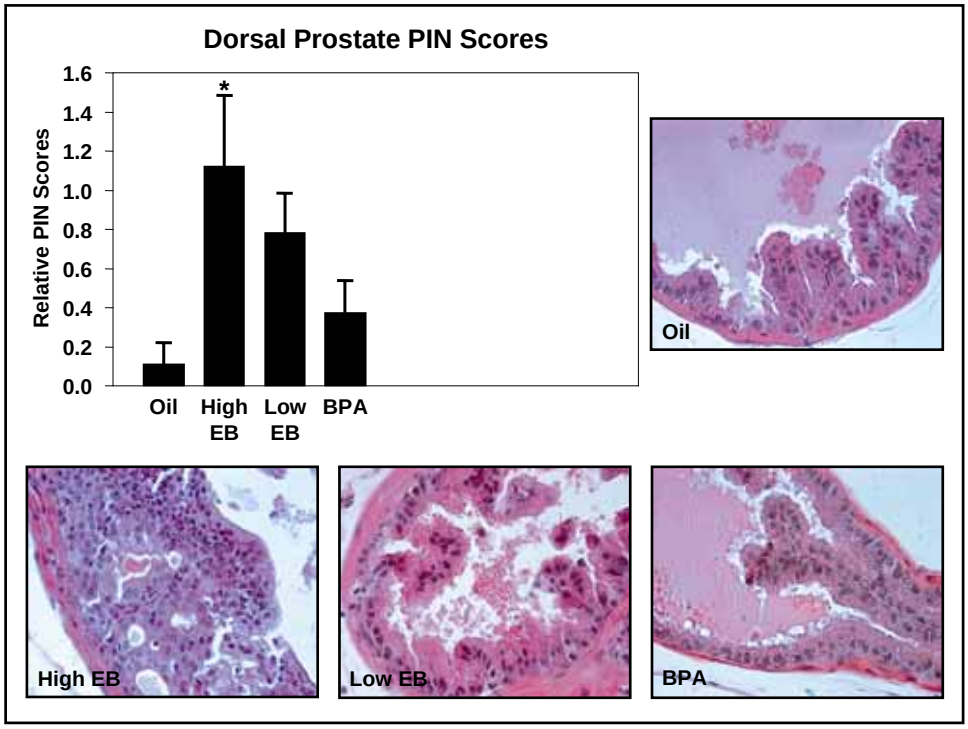


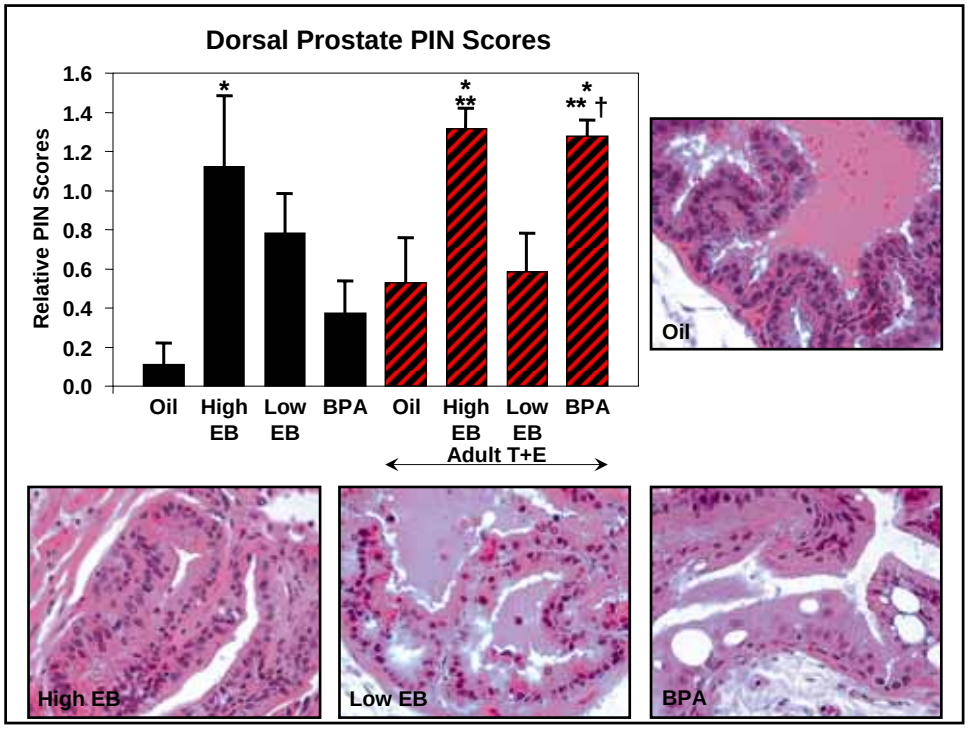
Treatment:

Treatment: Empty capsules
Capsule of T + E, 16 weeks

1. Oil (acts as control)
2. Estradiol-benzonate: high dose, 25 μg (2500 μg/kg BW)
3. Estradiol-benzonate: low dose, 0.001 μg (0.1 μg/kgBW)
4. Bisphenol A: low-dose, 0.1 μg (10 μg/kg BW)

Day 200: Histologic analysis (blinded)
-PIN scores (grade 0-3)
-PIN incidence
-basal marker (p63) for estrogenized phenotype
-proliferation & apoptosis


Dorsal Prostate PIN Scores
Relative PIN Scores
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Oil
High EB
Low EB
BPA
Oil
High EB
Low EB
BPA


Dorsal Prostate PIN Scores
Relative PIN Scores
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
**
*
** †
Oil
High EB
Low EB
BPA
Oil
High EB
Low EB
BPA
Adult T+E
Oil
High EB
Low EB
BPA

Low dose Estradiol/BPA Summary:

Ho, Wang, Belmonte and Prins, *Cancer Research* 66:5624, 2006

- Low dose estradiol may predispose to PIN with aging.

- Neonatal exposure to environmentally relevant doses of Bisphenol A may increase susceptibility of the prostate gland to carcinogenesis following additional adult insults.

?

How does a brief low-dose exposure permanently alter the "memory" of prostate cells long after hormone withdrawl?

Question:

Is estrogen imprinting of the prostate mediated, in part, by epigenetic alterations in developmental methylation patterns of prostatic genes?

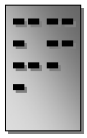
- McLachlan demonstrated abnormal demethylation of CpG/-464 in lactoferrin promoter of mouse uteri following neonatal DES (Li et al, Can Research 57:4356, 1997)
- Lubahn demonstrated hypermethylation of 5 candidate genes in mouse uteri exposed fetally to DES (Day et al, Endo Soc abstract, 2001)

Either *hypomethylation* or *hypermethylation*
→ chromosomal instability, inappropriate transcriptional activities
→ alter developmental timelines and disease development.

Experimental Work-Flow

Genomic DNA extracted from tissues

MSRF
(Methylation Sensitive Restriction Fingerprinting)



DNA extracted from gel and re-amplified by PCR

TA cloning (pCR2.1vector)

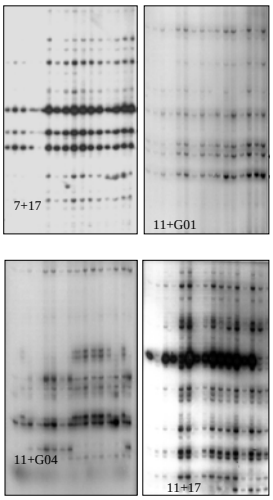
Sequencing / BLAST / BLAT search

Data confirmation

Real-Time PCR
In vitro demethylation

Bisulfite Sequencing
Methylation-specific PCR

Candidate clones identified from MSRF

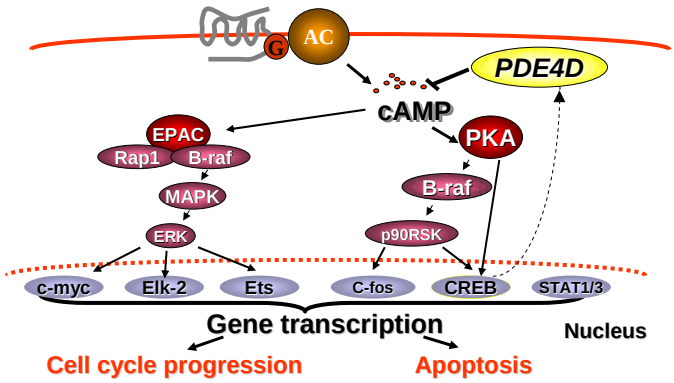


Clone Name	Primer 1	Primer 2	Hypermethylation	Chromosome band	Gene homology	Location	Related pathways
2p717	7	17	LE,HE, BPA (10,90,200)	1q22	CAR-X1	5' end	
3p717	7	17	LE (200)	1q22	CAR-X1	5' end	
1p11G1	11	G01	LE, BPA(200)	1q43	PLC beta 3	EX19	PKC, Ips
3p11G1	11	G01	LE, BPA(200)	7q12	N/A		
4p11G1	11	G01	LE, BPA(90,200)	18q12.1	SLC12A2	EX17	Na-K-2Cl cotransport
5p11G1	11	G01	LE, BPA(200)	18q12.1	SLC12A2	EX17	Na-K-2Cl cotransport
6p11G1	11	G01	LE, BPA(200)	18q12.1	SLC12A2	EX17	Na-K-2Cl cotransport
8p11G1	11	G01	C (10,90)	6q32	N/A		
9p11G1	11	G01	LE, BPA(200)	6q16	HPCAL1	IN1	cAMP signaling
10p11G1	11	G01	LE, BPA(200)	18p12	N/A		
11p11G1	11	G01	C (10,90)	2q14	N/A		
12p11G1	11	G01	LE(10,90,200)	6q32	N/A		
3p11G4	11	G04	LE,HE,BPA (90,200)	7q34	N/A		
5p11G4	11	G04	LE,HE,BPA (90,200)	6q24	N/A		
6p11G4	11	G04	LE,HE,BPA (90,200)	18q12.1	SLC12A2	EX17	Na-K-2Cl cotransport
7p11G4	11	G04	LE,HE,BPA (90,200)	8q22	N/A		
8p11G4	11	G04	LE,HE,BPA (90,200)	2q45	CARK	5' end	
9p11G4	11	G04	LE (90,200)	4q31	N/A		
10p11G4	11	G04	LE,BPA (90,200)	4q31	N/A		
11p11G4	11	G04	LE,BPA (90,200)	4q31	N/A		
14p11G4	11	G04	LE(90,200)	4q31	N/A		
15p11G4	11	G04	LE(90,200)	19q12	N/A		
17p11G4	11	G04	LE(90,200)	8q32	GPCR14	5' end	
18p11G4	**11**	**G04**	**C (10,90,200)**	**2q14**	**PDE4D4**	**5'flanking**	**cAMP signaling**
2p1117	11	17	LE, HE, BPA (200T+EZ)	20q13	N/A		
3p1117	11	17	BPA (10)	7q11	N/A		
4p1117	11	17	LE, HE, BPA (10)	17p12	N/A		
5p1117	11	17	C(10)	14p11	PDGFR alpha	IN4	MAPK/ERK

CAR-X1: Carbonic anhydrase-related X1 protein
PLCbeta3: Phospholipase C beta-3
SLC12A2: Solute carrier family 12 member 2
HPCAL: Neural visinin-like Ca2+ binding protein type 3

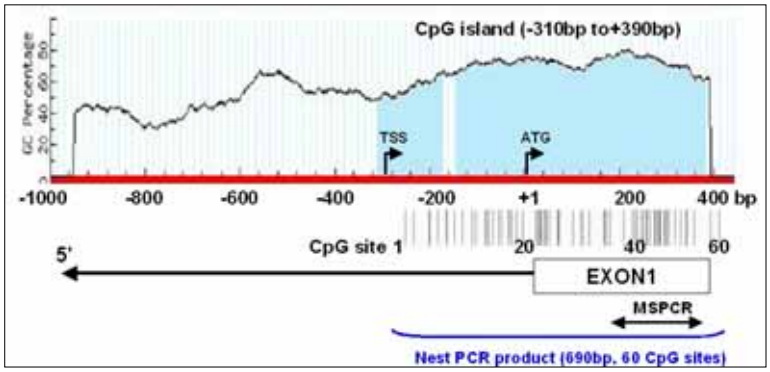
CARK: Cardiac ankyrin repeat kinase
GPCR14: G-protein coupled receptor 14
PDE4D4: Phosphodiesterase type 4 variant 4
PDGFRα: platelet-derived growth factor receptor

Phosphodiesterase type IV variant 4 (PDE4D4)



- Functions in cAMP degradation
- Maintains cAMP in a narrow concentration range that is critical for growth and differentiation
- Involved in tumor growth suppression by inhibiting cAMP activity in glioma, osteosarcoma and lymphocytic leukemia cells (Chen et al., 2002; Narita et al., 2003; Lerner et al., 2000)

5' flanking region of phosphodiesterase type IV variant 4 (PDE4D4)



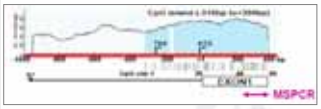
- Large CpG island (GC% >60%) observed at the 5' flanking region of rat PDE4D4.
- By nested PCR, an entire region of 690 bp at promoter/exon1 of PDE4D4 was amplified and included 60 CG dinucleotides.

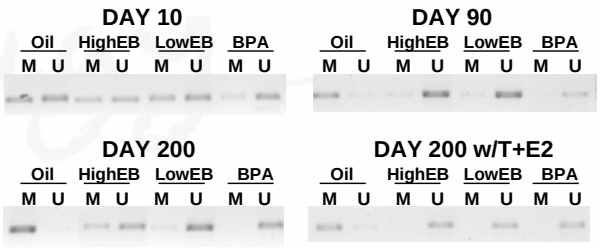
Ho, Wang, Belmonte and Prins, *Cancer Research* 66:5624, 2006

PDE4D4 gene: *Hyper*-methylated and silenced with aging

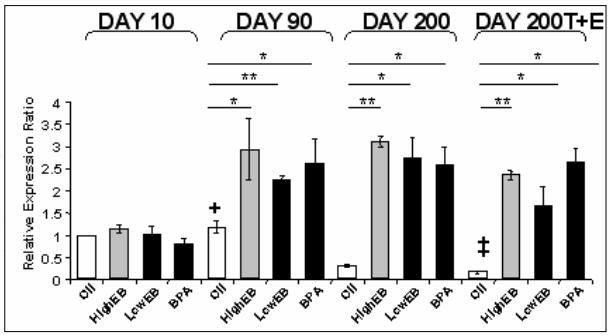
Hypo-methylated and up-regulated with neonatal E_2/BPA

Methylation-specific PCR





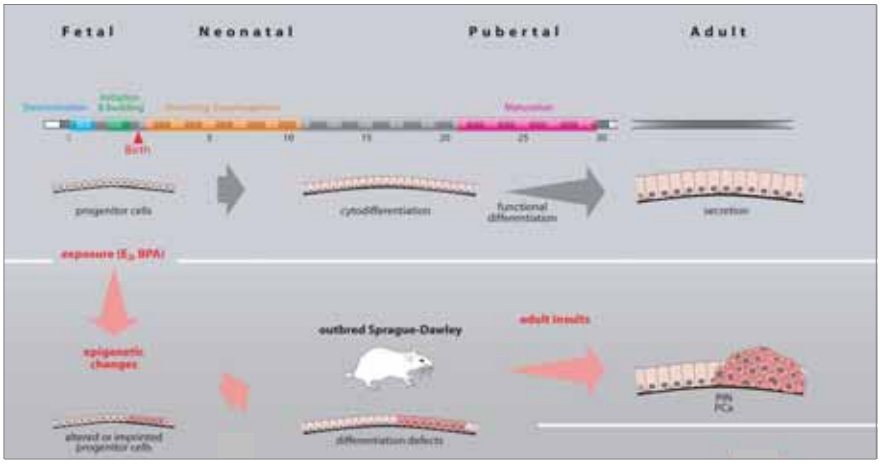
Real-time RT- PCR



Summary and Conclusions

- Blockade of PDE4D4 DNA methylation at specific CG sites during cellular or tissue differentiation alters gene expression.
- Prostatic *PDE4D4 dysregulation*, via hypomethylation initiated by early E_2/BPA exposure, may induce persistent gene expression changes. These may, in turn, alter signaling transduction pathways that contribute to prostatic carcinogenesis.
- By MSRF, more than 50 candidate clones were identified and found to be *differentially methylated* between oil-treated controls and tissues exposed neonatally to estradiol or bisphenol A.
- *Specific chromatin structures* are altered by early life exposure to estradiol or bisphenol A.

Working Model: Epigenetic basis for developmental estrogenization of the prostate gland



ACKNOWLEDGMENTS

PRINS LAB

- Jessica Belmonte, BS
- Lynn Birch, MS
- Oliver Putz, PhD
- Yong-Bing Pu, MD, PhD
- Liwei Huang, MD, PhD

Ho LAB University of Cincinnati

- Winnie Tang, Ph.D.
- Irwin Leav, M.D.
- Robert Cheng, D.V.M.