Developmental Programming: How Early Life Exposure Influences the Occurrence of Adult Leiomyoma

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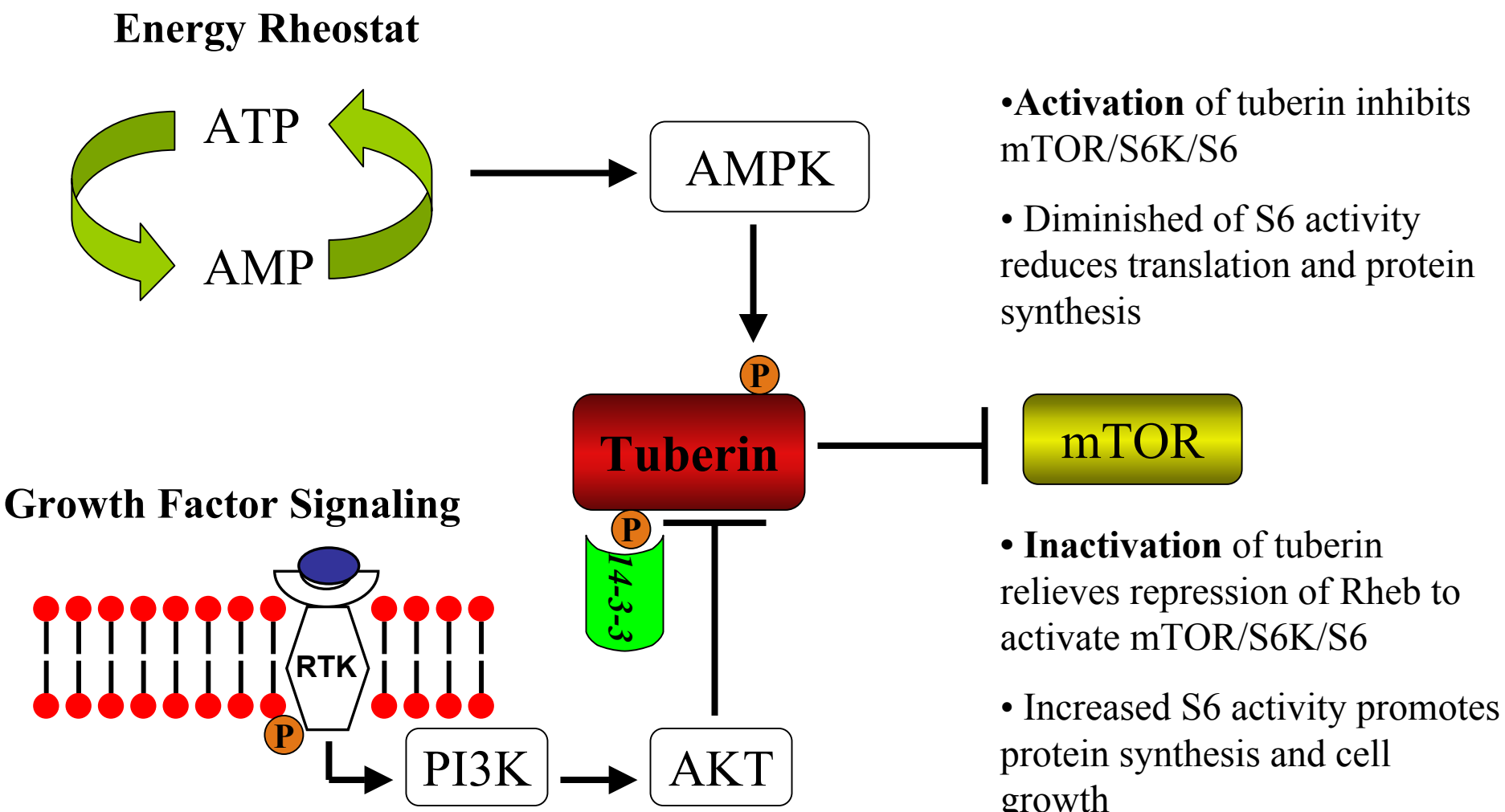
Tuberous Sclerosis Complex Tumor Suppressor Genes

- Hereditary tumor syndrome caused by the TSC1 or TSC2 tumor suppressor genes located on chromosomes 9 or 16 respectively
- Alterations in TSC-1 or TSC-2 predispose to characteristic brain lesions (tubers) and benign and neoplastic lesions in other organs
- TSC1 protein, hamartin, and TSC2 gene product, tuberin, interact as a heterodimer *in vivo* to form a functional tumor suppressor

Tuberin Integrates Cellular Energy and Growth Factor Signaling



•**Activation** of tuberin inhibits mTOR/S6K/S6

• Diminished of S6 activity reduces translation and protein synthesis

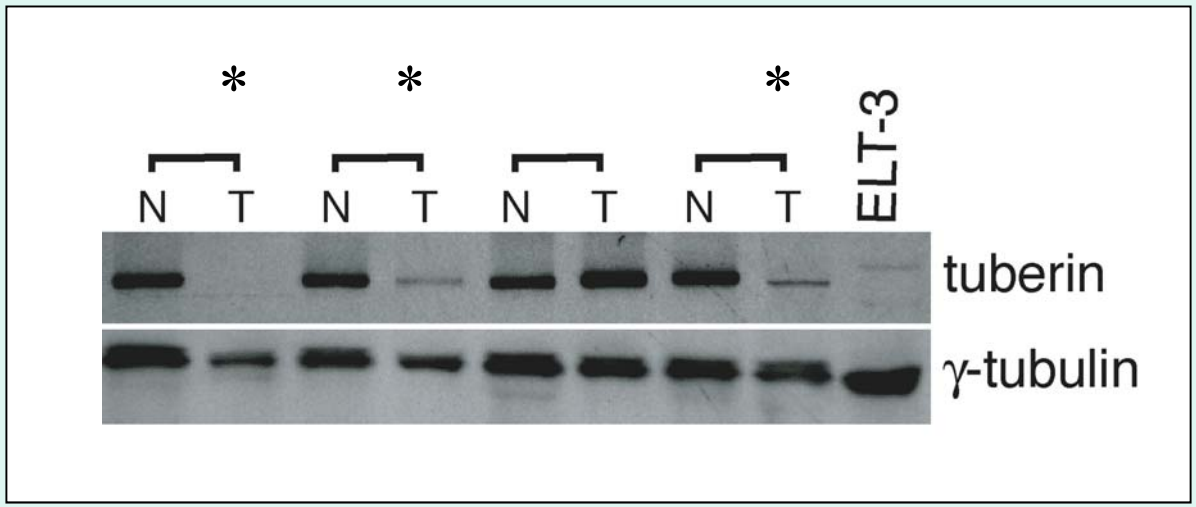
• **Inactivation** of tuberin relieves repression of Rheb to activate mTOR/S6K/S6

• Increased S6 activity promotes protein synthesis and cell growth

Genetic Linkage of Leiomyoma and RCC

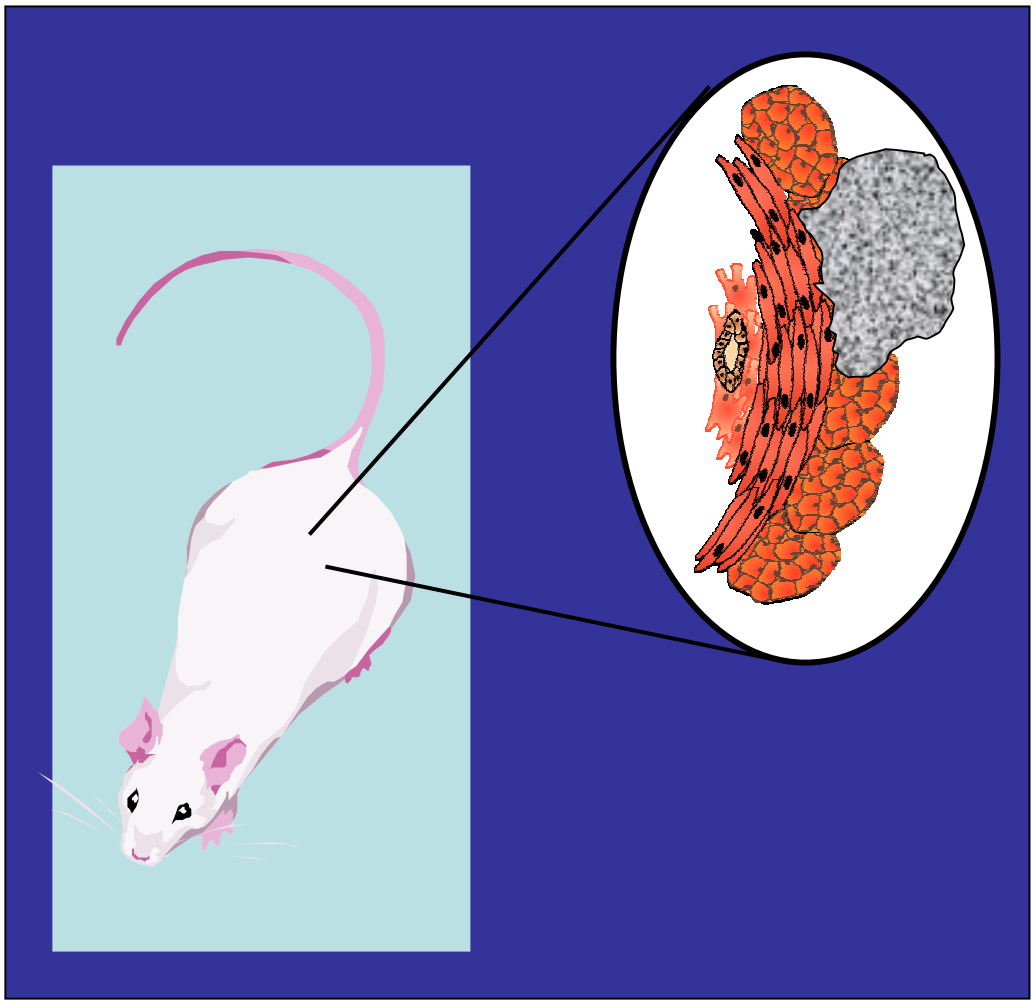
Gene	RCC Phenotype	Associated Tumors	
VHL	Typical clear cell	Hemangioblastoma (Vascular)	
TSC-2	Angiomyolipoma	Hemangiosarcoma	
	Clear cell RCC	(Smooth muscle)	Leiomyoma
FH	Papillary (Type II)		Leiomyoma
c-Met	Papillary (Type I)		
BHD	Chromophobe		Leiomyoma
	Oncocytoma		

Diminished Tuberin Expression in Human Leiomyoma

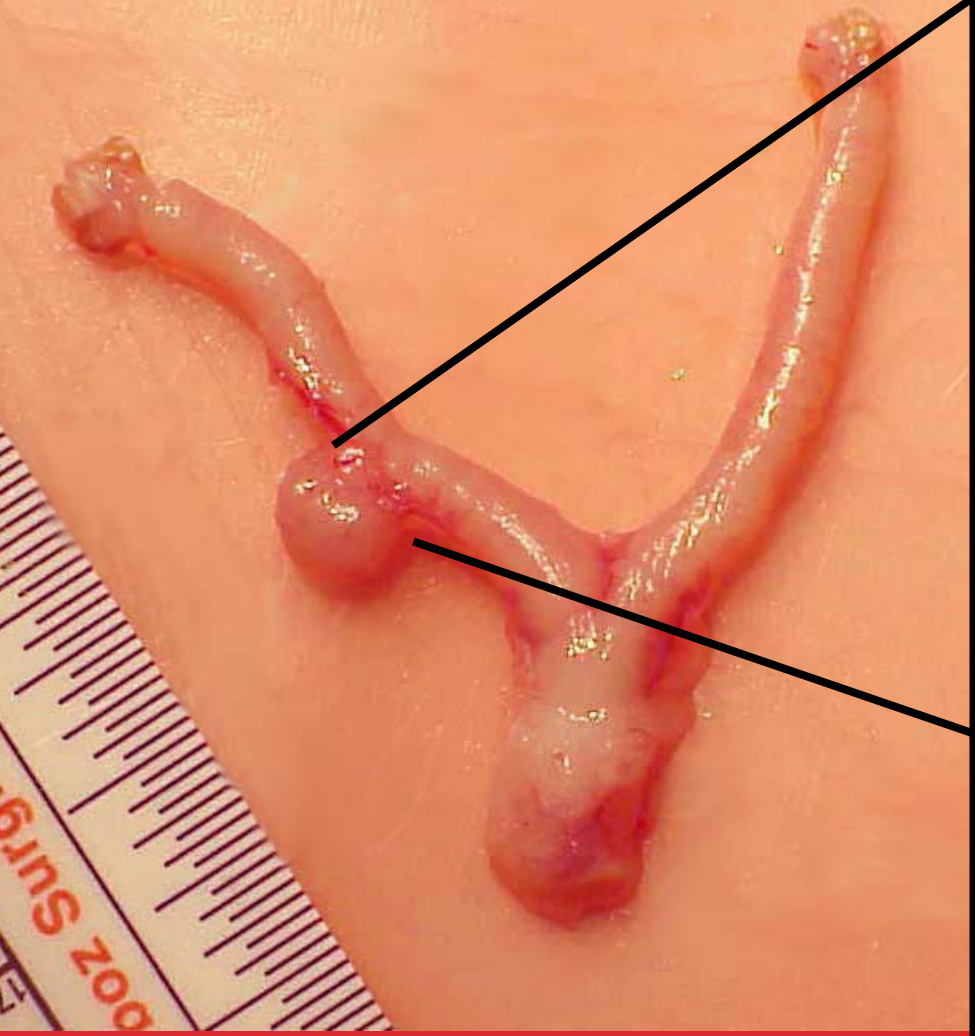


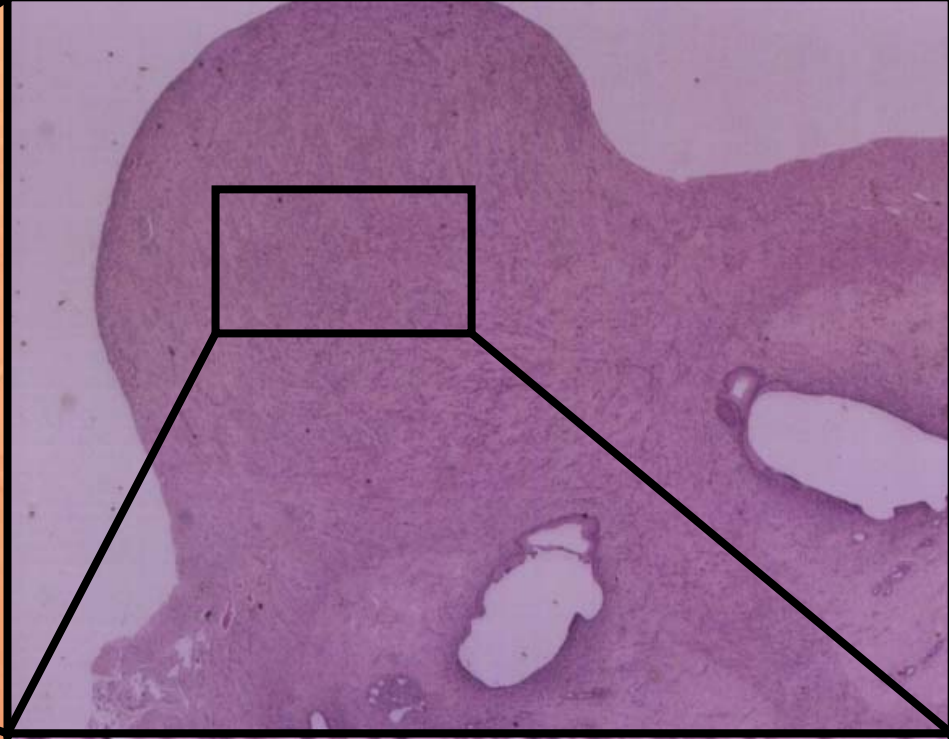
Tuberin expression is lost/diminished in 10/40 (25%) of human leiomyomas

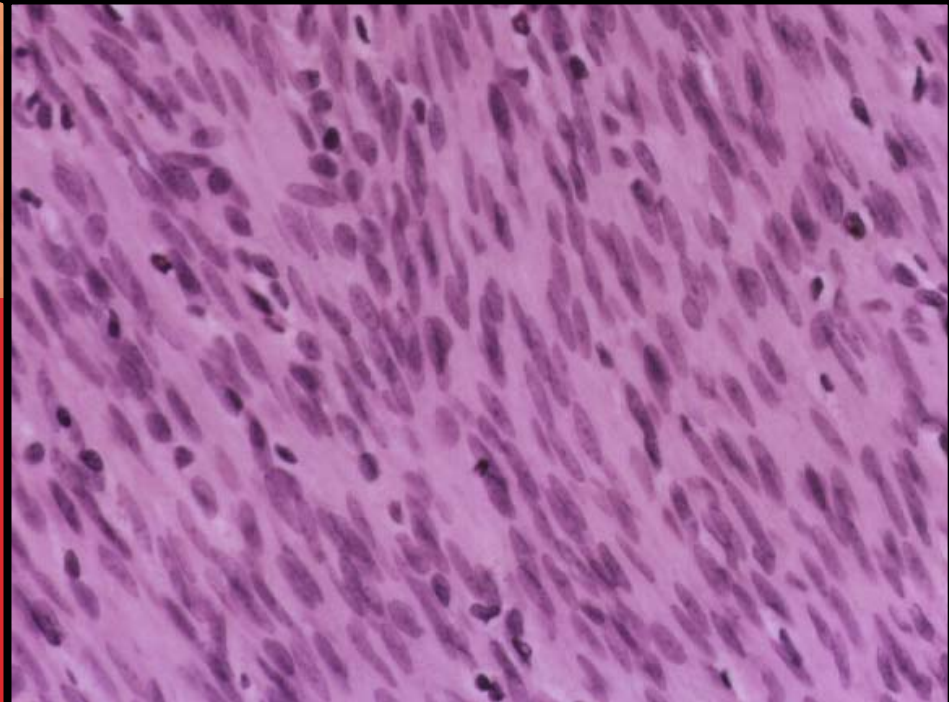
Eker Rat Model for Uterine Leiomyoma



- Eker rats carry a germline defect in the Tsc-2 tumor suppressor gene
- 65% of female carriers (Tsc-2 $^{Ek/+}$) develop uterine leiomyoma by 16 months of age
- Tumors are phenotypically similar to the human disease







Eker Rat Uterine Leiomyoma

Our Environment Includes:

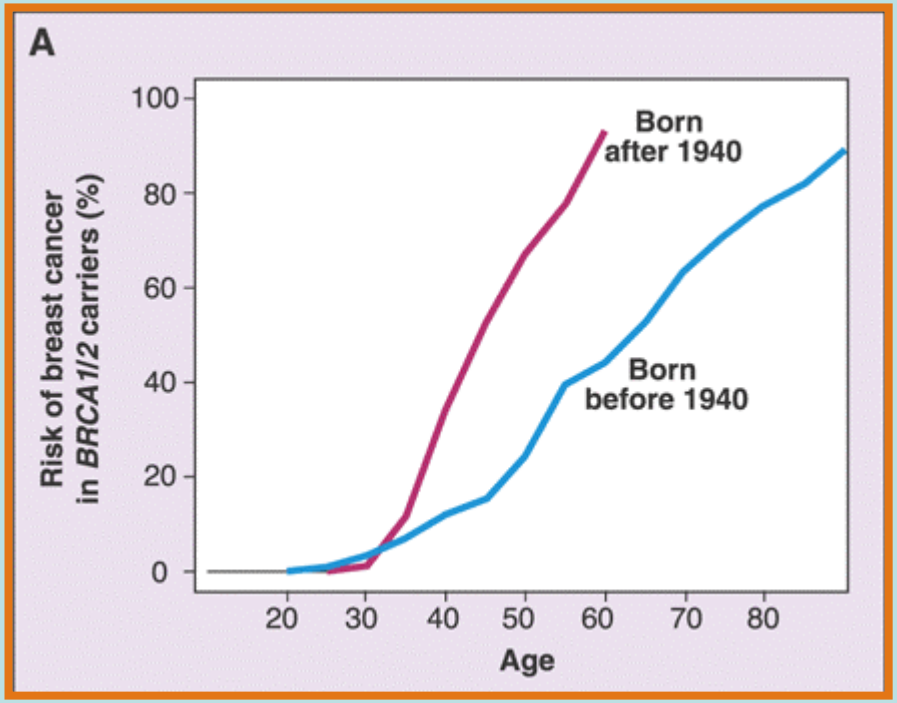
- Industrial chemicals
- Agricultural chemicals
- By-products of combustion and industrial processes (e.g. dioxin)
- Physical agents (e.g. heat, radiation)
- Biological agents (e.g. viruses)
- Foods and nutrients
- Prescription drugs
- Lifestyle choices and substance abuse
- Social and economic factors

Developmental Programming

- Gene-environment interaction traditionally interpreted as facilitating acquisition of multiple somatic alterations required for tumorigenesis
- Developmental programming hypothesis has been proposed for adult metabolic disorders (diabetes) and cardiovascular disease
- Adverse environment *in utero* (eg maternal starvation) "reprograms" normal metabolic processes in the fetus, predisposing to adult disease

Developmental Programming and Genetic Susceptibility to Hormone-responsive cancers

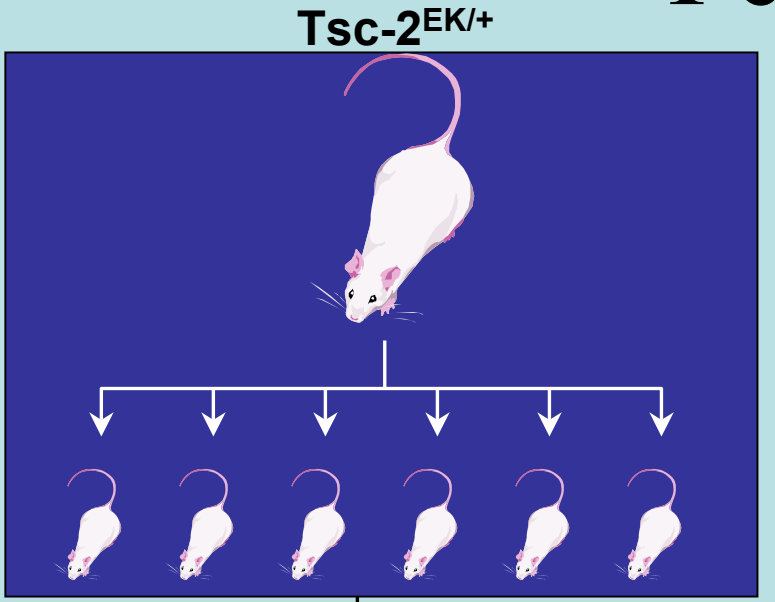
- Increased use of environmental estrogens
  - Phytoestrogens
  - Oral contraceptives
  - Pesticides
  - plasticizers
- Endometrial cancer (PTEN mutations)
- Breast cancer (BRCA1/2 mutations)



Importance of Environmental Factors on Cancer Risk in BRCA1/2 Ashkenazi Jew mutation carriers

***Science*, October 2003**

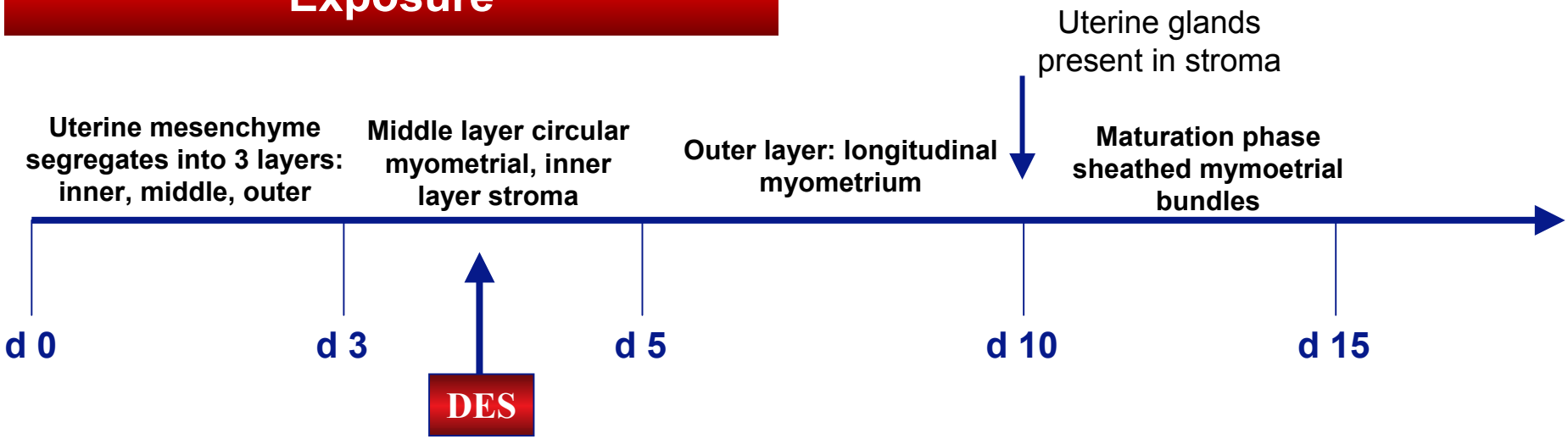
Impact of Environmental Exposure on TSC-2 Tumor Suppressor Gene Penetrance

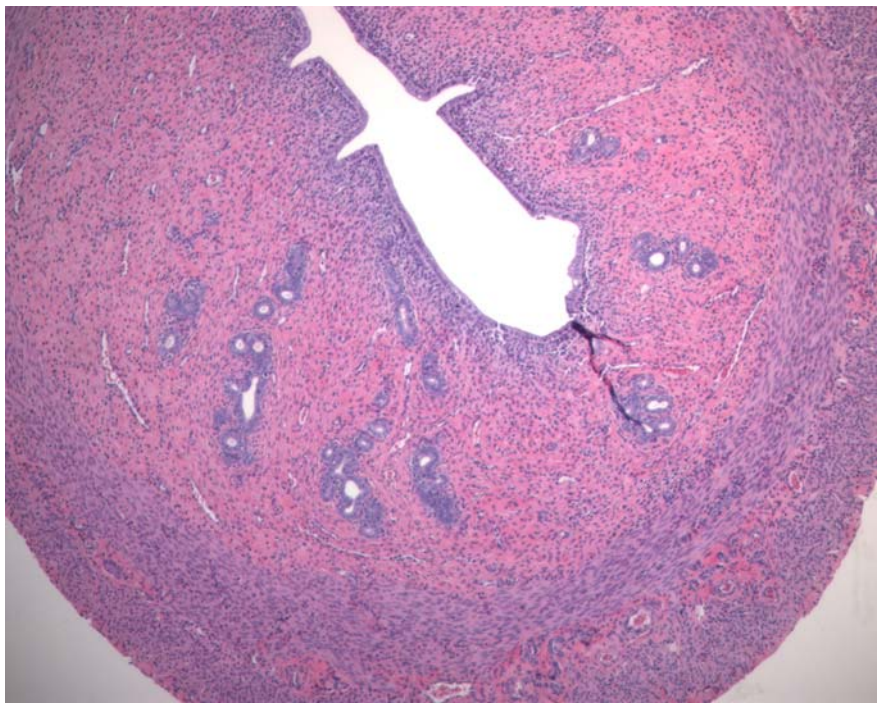


♀ Inject with 10µg DES or vehicle Days 3, 4, 5 → Sacrifice 16 mo.

	n=
Carrier (Tsc-2$^{EK/+}$) + DES	24
Carrier (Tsc-2$^{EK/+}$) + Vehicle	28
Wildtype (Tsc-2$^{+/+}$) + DES	34
Wildtype (Tsc-2$^{+/+}$) + Vehicle	33

Developmental Xenoestrogen Exposure



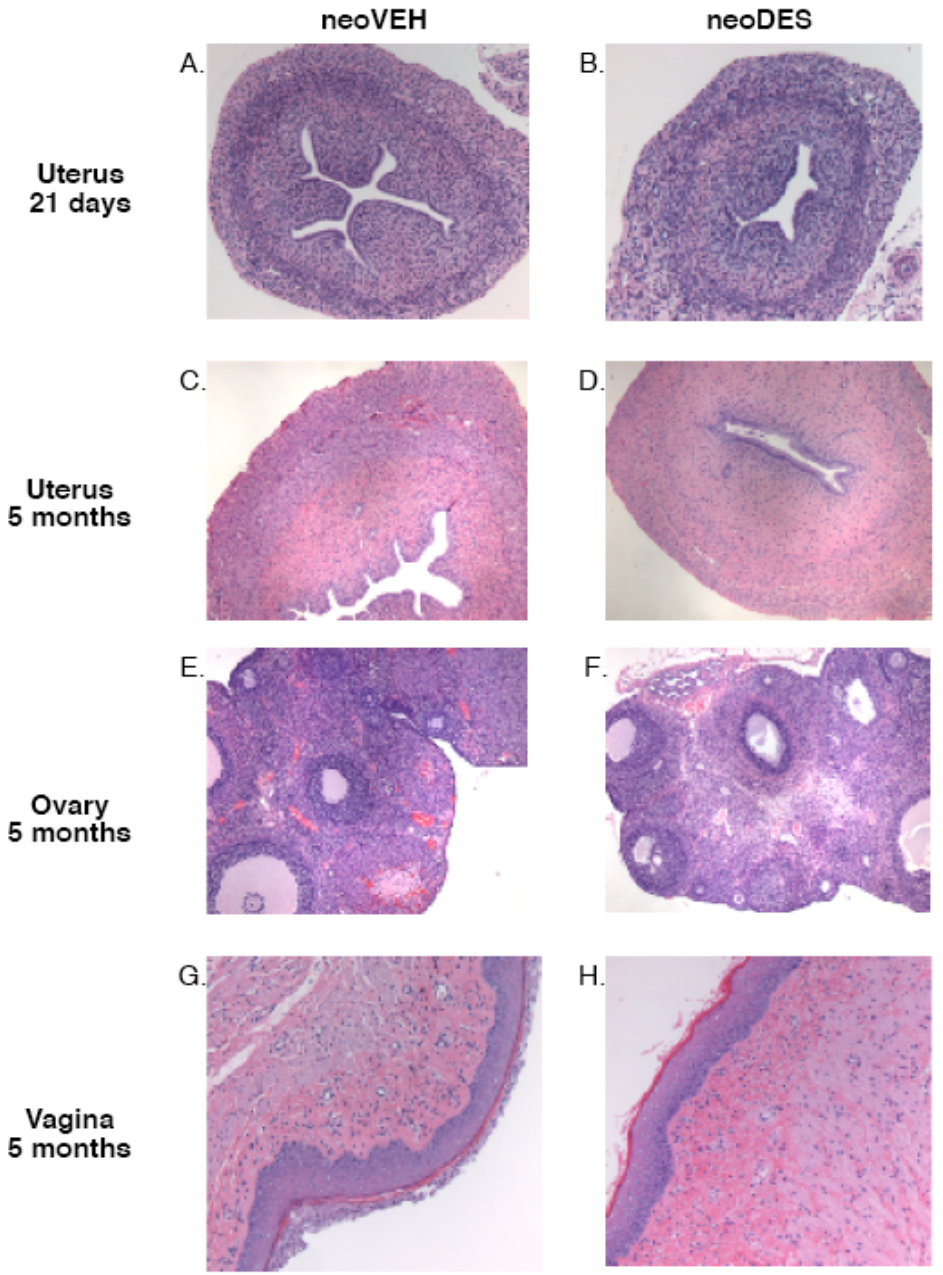


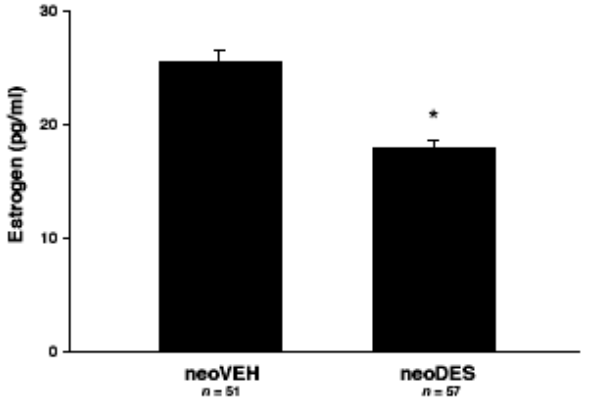
-**ER present as early as E.D. 14**

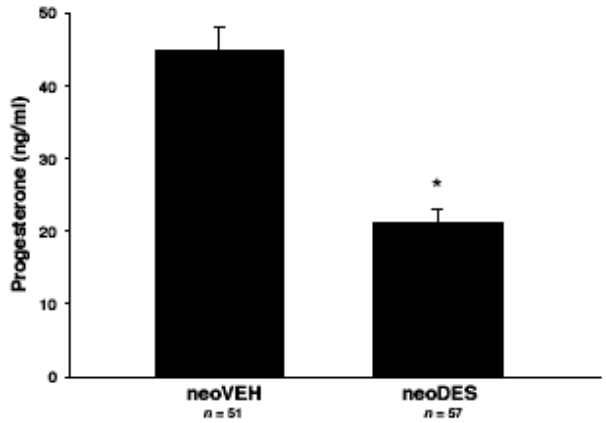
-**Estrogen is sequestered by hormone binding proteins**

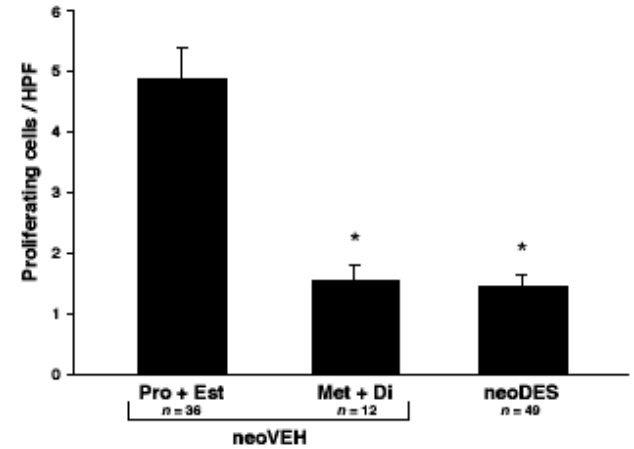
Adapted from Brody and Cunha, American Journal of Anatomy, 1989

Developmental Programming of the Reproductive Tract









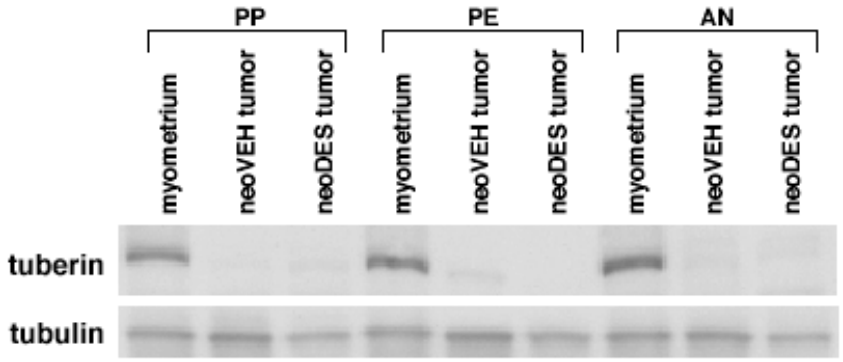
Diminished Circulating Hormone Levels and Reduced Proliferation in neoDES-exposed Females

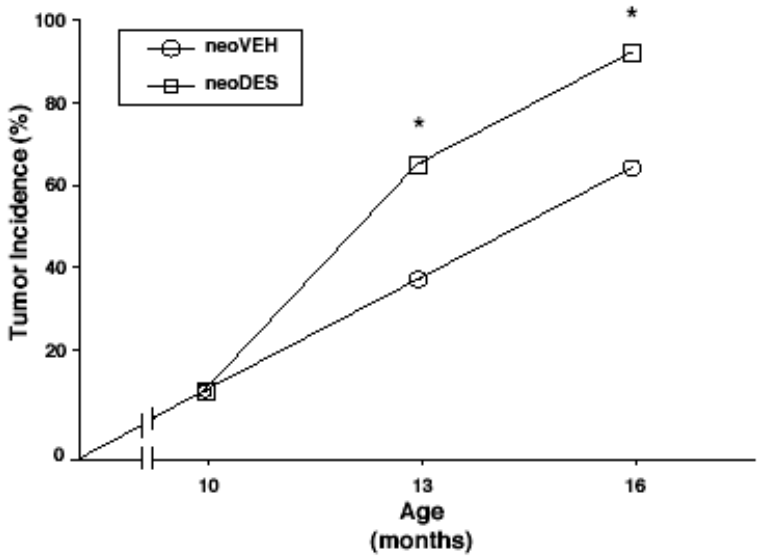
- Compromised ovarian function and reduced circulating estrogen and progesterone
- Uteri of exposed animals smaller, hypoplastic
- Myometrial cells quiescent

Developmental Xenoestrogen Exposure Increases Tsc-2 Tumor Suppressor Gene Penetrance

Genotype	Treatment	N of rats	% Tumor Incidence	Multiplicity (mean no. of tumors/rat)	Size (cm^3) Mean ± S.E.M.
Tsc-2$^{Ek/+}$	vehicle	28	64	0.82	2.3 ± 1.1
	DES	24	92*	1.33*	10.5 ± 2.7*
Tsc-2$^{+/+}$	vehicle	34	0	N/A	N/A
	DES	34	0	N/A	N/A

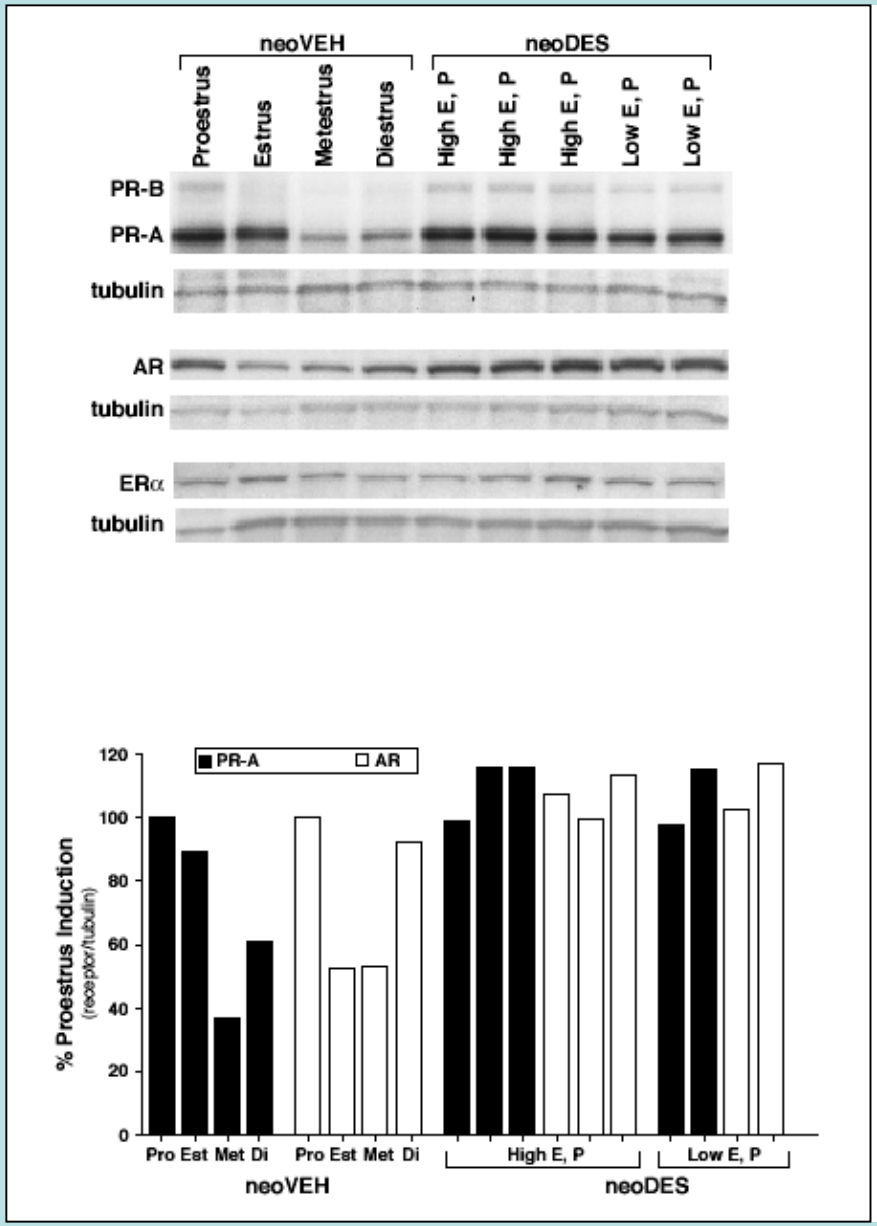
Susceptible (*Tsc-2* $^{Ek/+}$) and wild-type (*Tsc-2* $^{+/+}$) neoDES and neoVeh Eker rats were killed at 16 months of age, and the number and size of gross and microscopic tumors counted and measured. Clonally distinct, multiple tumors within the same uterus were identified by either gross examination or by differences in LOH at the *Tsc-2* locus detected by dHPLC. *$P < 0.02$ (determined by the chi-square test for tumor incidence and Student's t-test for multiplicity and tumor size).





Loss of Tuberin Remains Rate-Limiting for Tumorigenesis

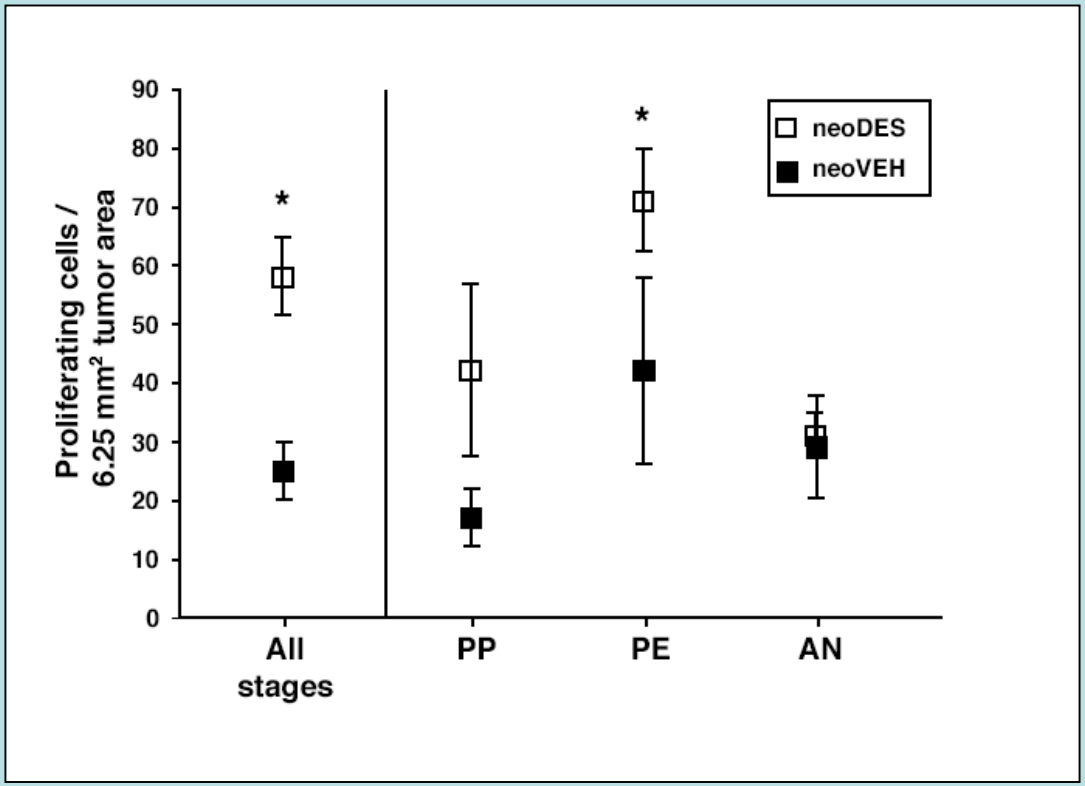
- Tuberin lost in >95% of tumors
- Frequency of LOH the same in neoDES and neoVEH tumors
- No difference in tumor latency



Developmental Re-programming of Estrogen Responsiveness in neoDES Females

- Target myometrial cells in neoDES animals hyper-responsive to (low) estrogen levels
- Not observed in liver, which is fully developed in neonates
- Estrogen receptor levels unchanged

Enhanced Proliferative Response to Hormones in Tuberin-null Leiomyomas Arising in neoDES Females

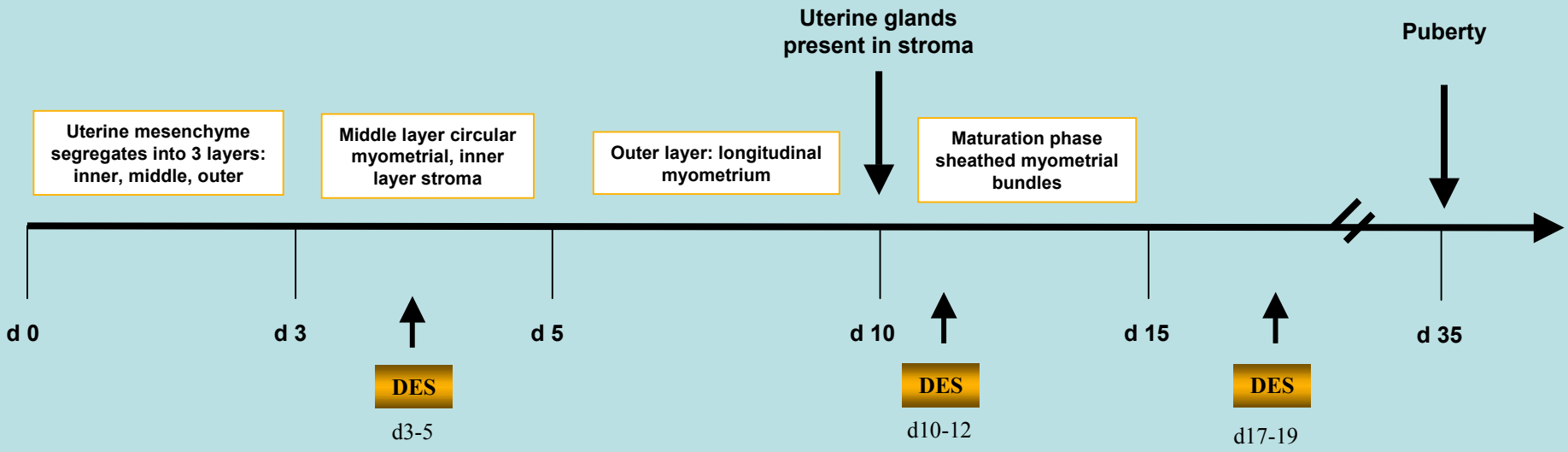


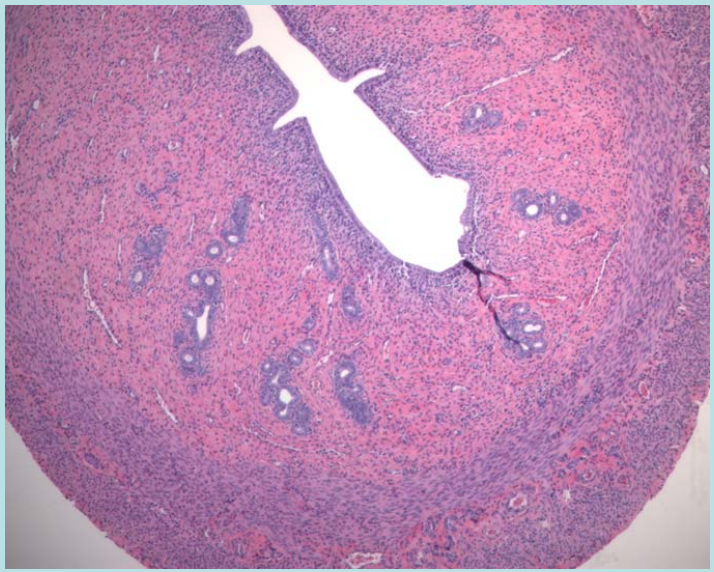
Developmental Programming of Tumor Suppressor Gene Penetrance: A New Paradigm for Gene-Environment Interactions

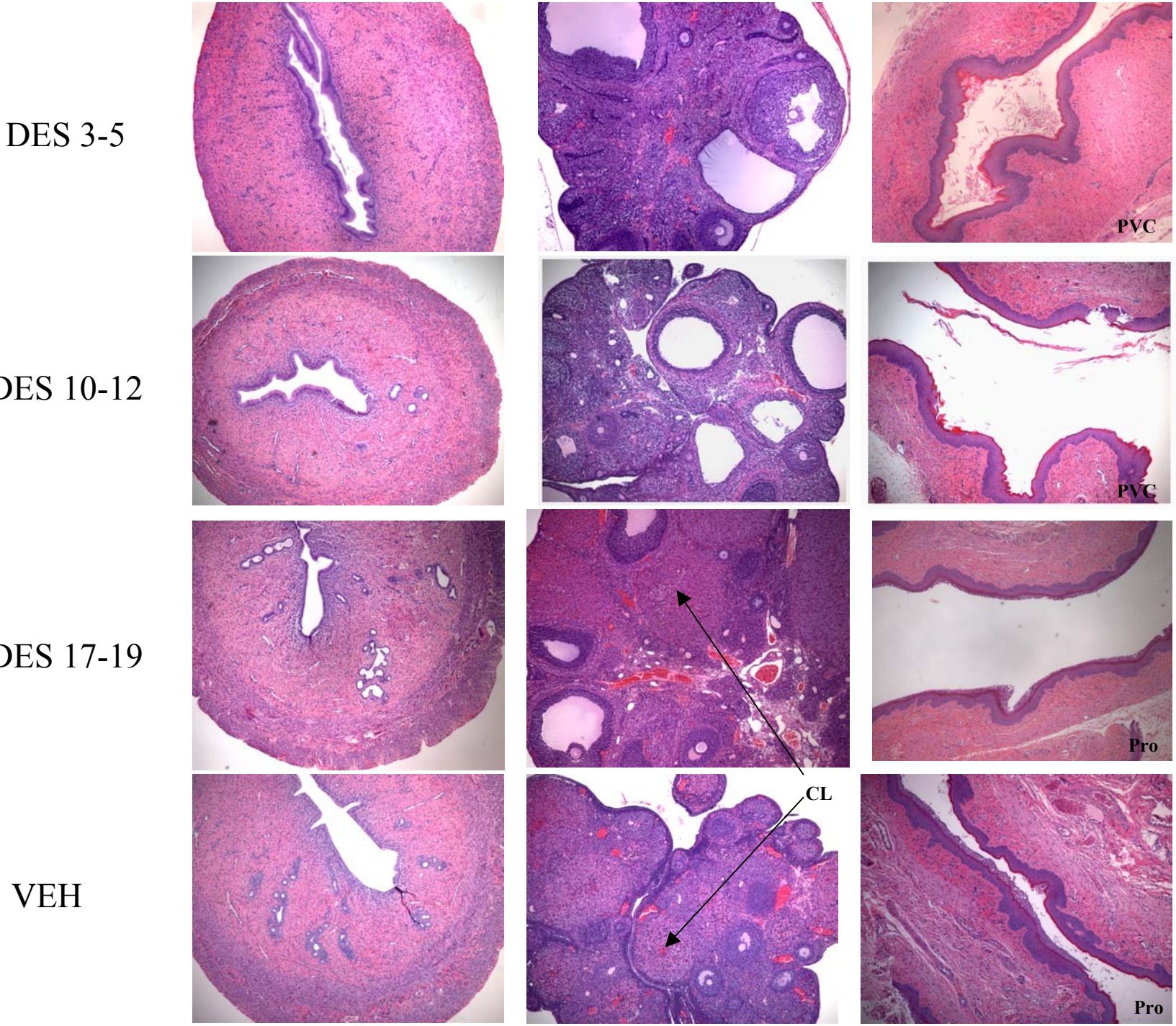
- Brief early life exposure to an environmental estrogen significantly increased TSC-2 penetrance
- Environmental exposure during development "re-programmed" the normal hormonal response of the target tissue
- Although loss of Tsc-2 function was still required, **increased hormone responsiveness of the target tissue** combined with loss of tuberin promoted tumor development

Neonatal Myometrial Maturation

Adapted from Brody and Cunha, American Journal of Anatomy, 1989






DES 3-5
DES 10-12
DES 17-19
VEH
PVC
PVC
CL
Pro
Pro

Window of Susceptibility for Early Life Environmental Exposure

Exposure	Treatment	# of Rats	% Tumor Incidence	Multiplicity (tumors/rat ± SEM)	Size (cm^3 ± SEM)
Days 3-5 (study 1)	Vehicle	28	64	0.82 ± 0.15	2.3 ± 1.10
	DES	24	94*	1.33 ± 0.17*	10.5 ± 2.7*
Days 3-5 (study 2)	Vehicle	32	63	0.75 ± 0.12	7.3 ± 4.0
	DES	20	95*	1.10 ± 0.09*	11.2 ± 4.6
Days 10-12	Vehicle	32	63	0.75 ± 0.12	7.3 ± 4.0
	DES	24	100*	1.33 ± 0.10*	17.1 ± 4.8
Days 17-19	Vehicle	32	63	0.75 ± 0.12	7.3 ± 4.0
	DES	26	85	1.10 ± 0.12	14.4 ± 5.3

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