Association of Early-Life Arsenic Exposure and Cancer in Adulthood

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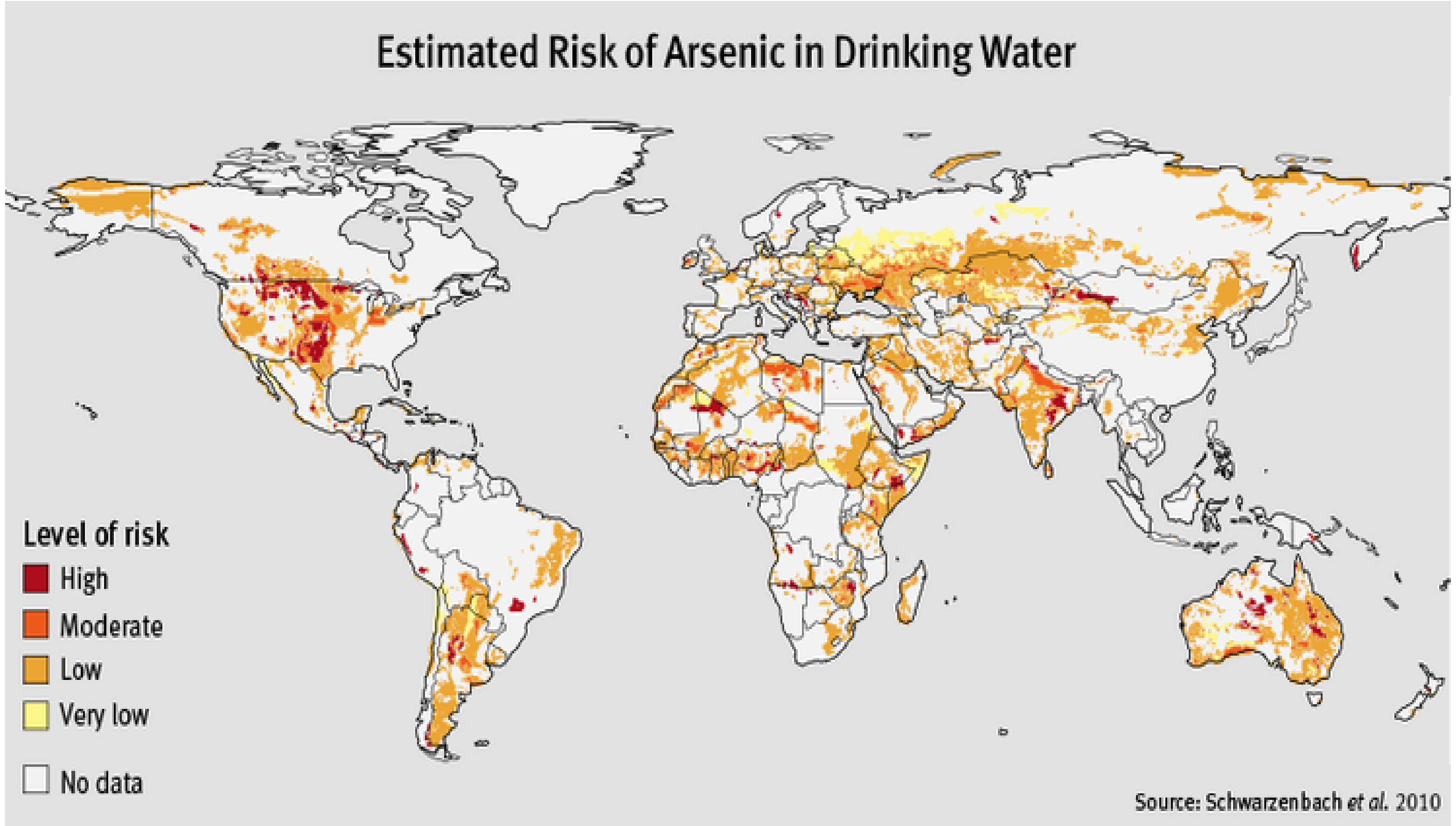
Outline

- Background
  - Arsenic and cancer
  - Stem cells and cancer stem cells
- *In vivo* and *in vitro* work
  - Animal models
  - Arsenic transformation and cancer stem cell overabundance
- Microenvironment
  - Stem cell “recruitment”
  - Extracellular vesicles and cargo
- Conclusions

Exposure to Inorganic Arsenic

- Millions of people worldwide:
  - Water, foods, inhaled
- Multi-site human carcinogen
  - Skin, lung, bladder, liver, kidney, prostate
- Linked to many other adverse health effects
  - CVD, diabetes, obesity, neurotoxicity, immunotoxicity, etc.



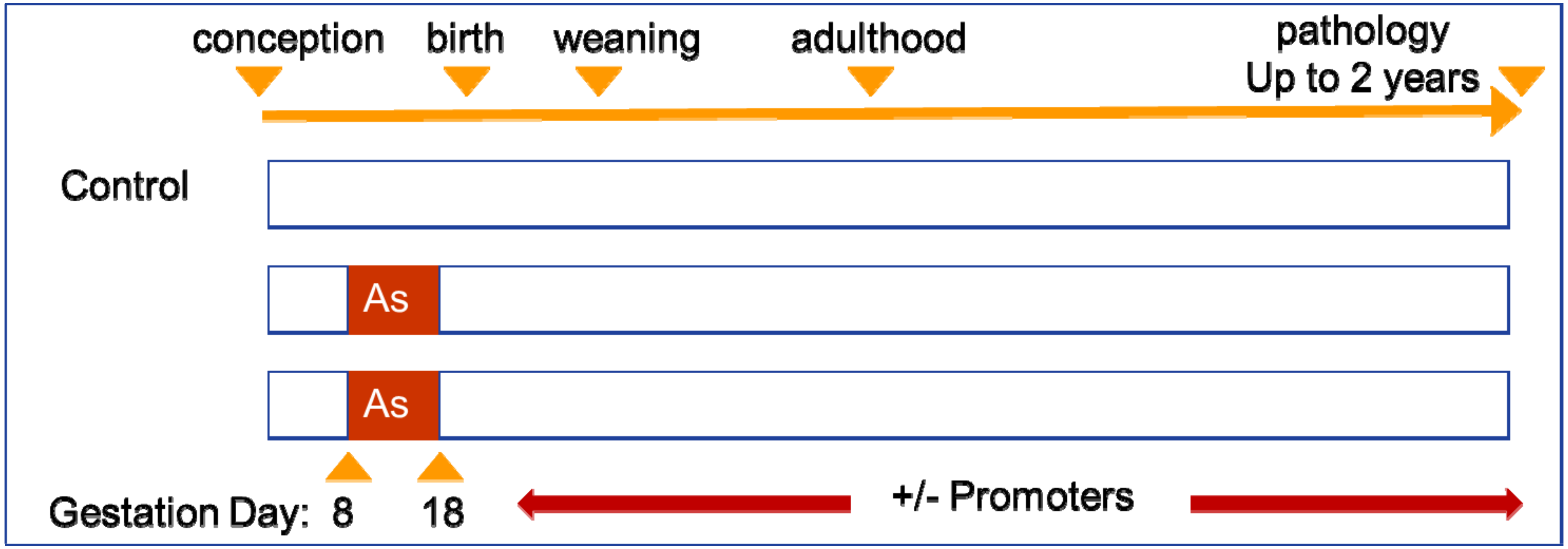
A Paradoxical Toxicant

- Effective chemotherapeutic
  - Cures certain fatal leukemias
  - “Resetting” leukemic stem cells (SCs)
- Strong human data but limited rodent data
  - Known human carcinogen since 1880s
  - Several animal studies all with negative results
    - Animals treated as adults

Our Hypothesis

- Knowing this about arsenic, we hypothesized:
  - Ability to alter SC phenotype may indicate affinity for SCs
  - To be carcinogenic in rodents may require exposure at periods of high sensitivity
    - Perinatal, early-life
    - Periods with abundant SC numbers and activity

Transplacental (TPL) *In Vivo* Rodent Models



- Arsenic given in maternal drinking water
- Done in several strains (C3H, CD1, Tg.AC)
- Tumors or neoplasia in both female and male offspring


Mike Waalkes

› Toxicol Appl Pharmacol. 2003 Jan 1;186(1):7-17. doi: 10.1016/s0041-008x(02)00022-4.

Transplacental Carcinogenicity of Inorganic Arsenic in the Drinking Water: Induction of Hepatic, Ovarian, Pulmonary, and Adrenal Tumors in Mice

Michael P Waalkes [1], Jerrold M Ward, Jie Liu, Bhalchandra A Diwan

› Carcinogenesis. 2004 Jan;25(1):133-41. doi: 10.1093/carcin/bgg181. Epub 2003 Sep 26.

Induction of Tumors of the Liver, Lung, Ovary and Adrenal in Adult Mice After Brief Maternal Gestational Exposure to Inorganic Arsenic: Promotional Effects of Postnatal Phorbol Ester Exposure on Hepatic and Pulmonary, but Not Dermal Cancers

Michael P Waalkes [1], Jerrold M Ward, Bhalchandra A Diwan

› Toxicol Appl Pharmacol. 2006 Jun 15;213(3):216-23. doi: 10.1016/j.taap.2005.10.010. Epub 2005 Dec 20.

Transplacental Arsenic Plus Postnatal 12-O-teradecanoyl phorbol-13-acetate Exposures Associated With Hepatocarcinogenesis Induce Similar Aberrant Gene Expression Patterns in Male and Female Mouse Liver

Jie Liu [1], Yaxior
Diwan, Daniel L

› Toxicol Appl Pharmacol. 2006 Sep 15;215(3):295-305. doi: 10.1016/j.taap.2006.03.010. Epub 2006 May 18.

Enhanced Urinary Bladder and Liver Carcinogenesis in Male CD1 Mice Exposed to Transplacental Inorganic Arsenic and Postnatal Diethylstilbestrol or Tamoxifen

Michael P Waalkes [1], Jie Liu, Jerrold M Ward, Bhalchandra A Diwan

› Cancer Res. 2006 Feb 1;66(3):1337-45. doi: 10.1158/0008-5472.CAN-05-3530.

Urogenital Carcinogenesis in Female CD1 Mice Induced by in Utero Arsenic Exposure Is Exacerbated by Postnatal Diethylstilbestrol Treatment

Michael P Waalkes [1], Jie Liu, Jerrold M Ward, Douglas A Powell, Bhalchandra A Diwan

› Int J Toxicol. May-Jun 2010;29(3):291-6. doi: 10.1177/1091581810362804.

Arsenic Exposure in Utero and Nonepidermal Proliferative Response in Adulthood in Tg.AC Mice

Erik J Tokar [1], Bhalchandra A Diwan, Michael P Waalkes

› Toxicol Lett. 2012 Mar 7;209(2):179-85. doi: 10.1016/j.toxlet.2011.12.016. Epub 2011 Dec 31.

Renal, Hepatic, Pulmonary and Adrenal Tumors Induced by Prenatal Inorganic Arsenic Followed by Dimethylarsinic Acid in Adulthood in CD1 Mice

Erik J Tokar [1], Bhalchandra A Diwan, Michael P Waalkes

› Arch Toxicol. 2012 Jun;86(6):975-82. doi: 10.1007/s00204-012-0820-8. Epub 2012 Mar 8.

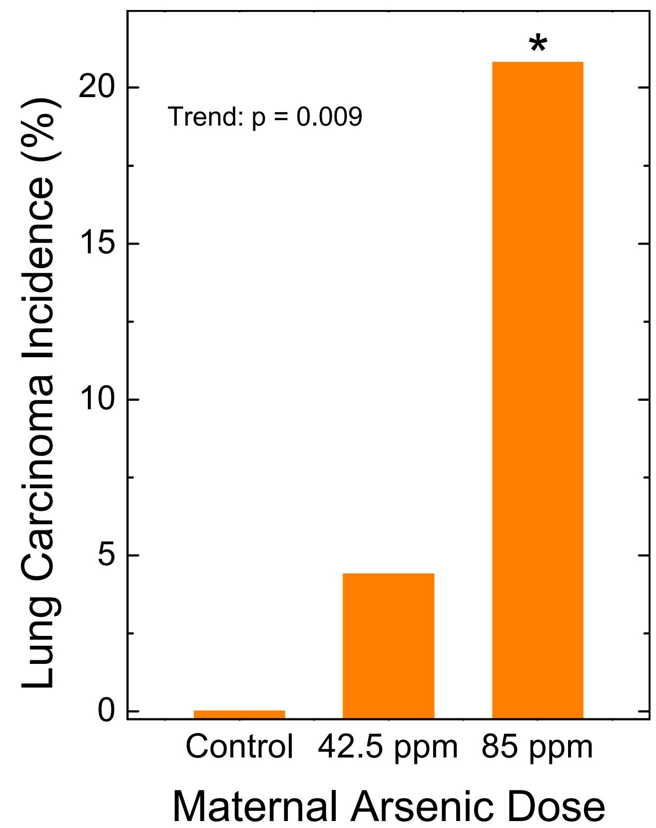
Tumors and Proliferative Lesions in Adult Offspring After Maternal Exposure to Methylarsonous Acid During Gestation in CD1 Mice

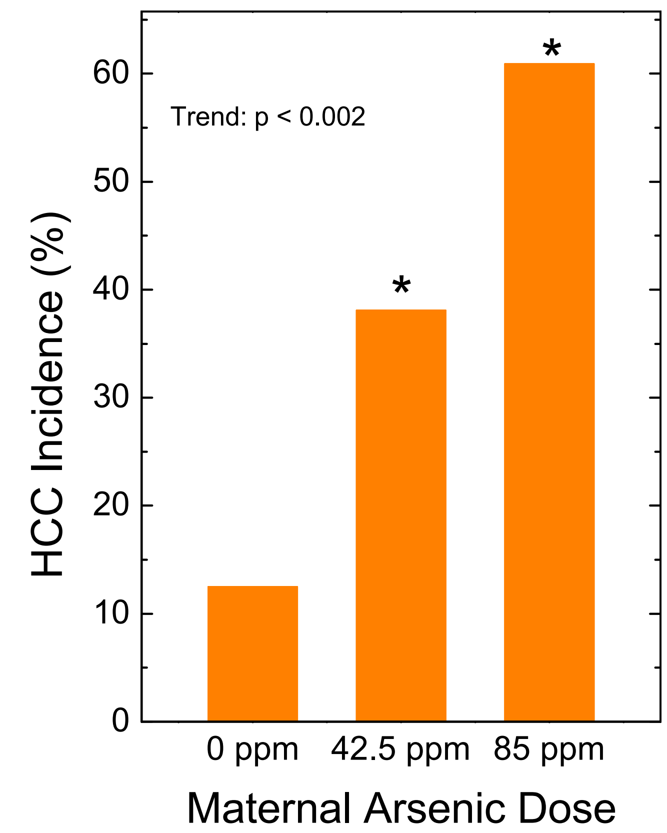
Erik J Tokar [1], Bhalchandra A Diwan, David J Thomas, Michael P Waalkes

Ex: Adult Female and Male C3H Offspring

Arsenic is a TPL carcinogen

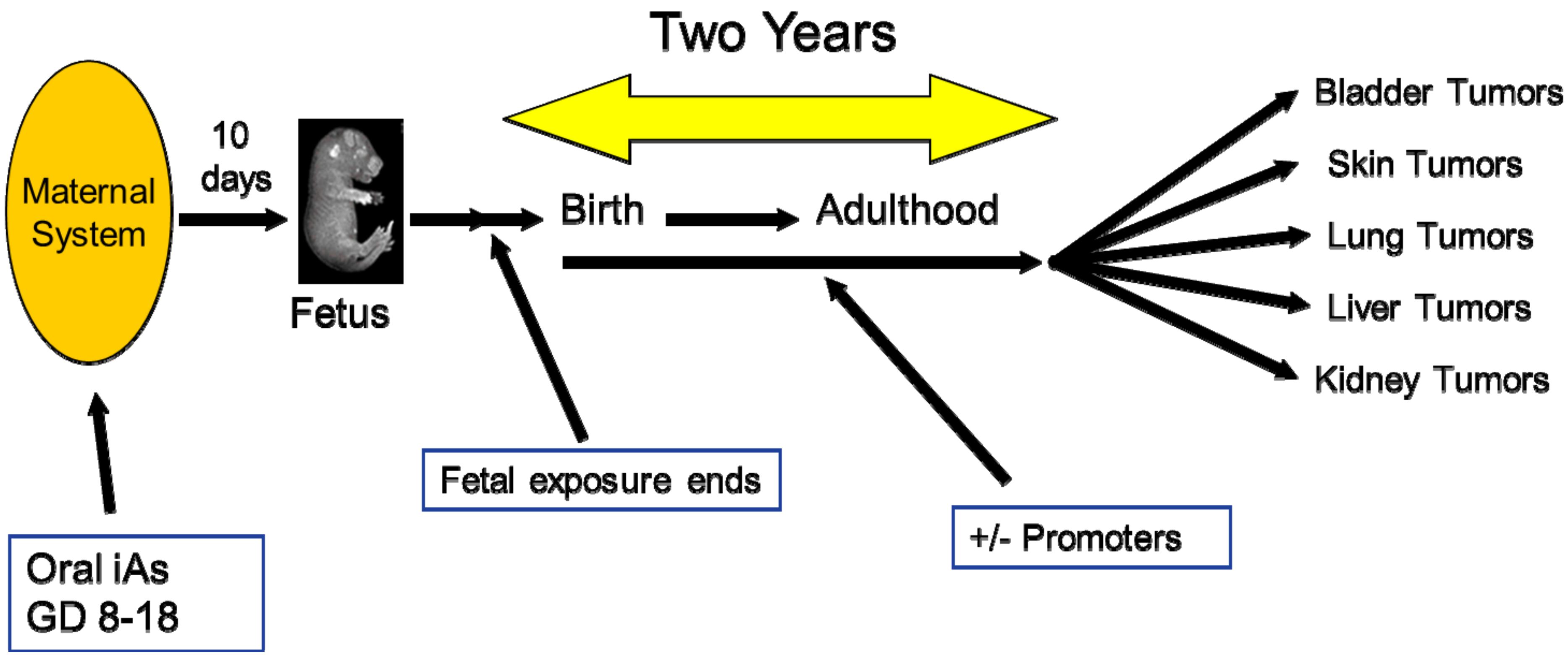
- Female
  - Lung carcinoma (left)
  - Liver, UB, adrenal, ovary, uterus, oviduct, etc.
- Male:
  - Liver (HCC; right)
  - Lung, adrenal, UB, etc.
- Similar results in other strains





Modified from: Waalkes *et al*. (2003) *TAAP* 186:7

Summary of TPL Mouse Models



- Near perfect concordance with human target sites (except prostate)
- Tumor formation long after arsenic exposure ends
  - Points to long-lived target cell (SC?)

Early-life Exposures in Human Populations

Ex: As-contaminated Baby Formula in Japan

Unusual Cancer Excess After Neonatal Arsenic Exposure From Contaminated Milk Powder FREE

Takashi Yorifuji, Toshihide Tsuda, Philippe Grandjean

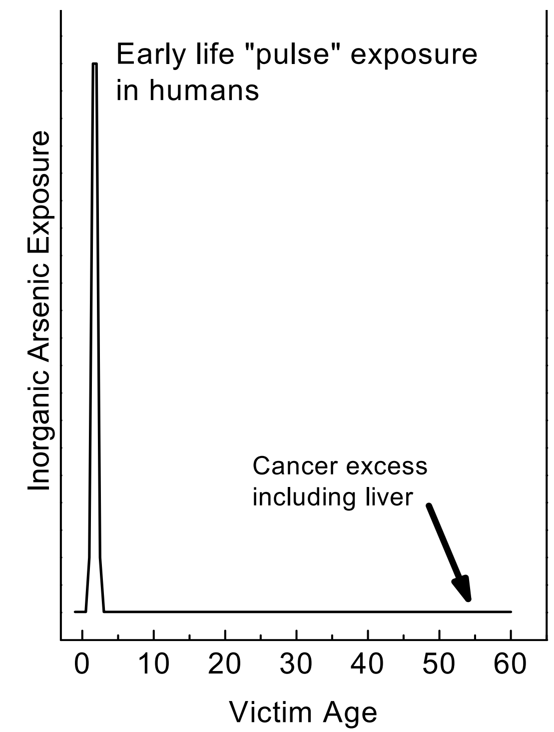
JNCI: Journal of the National Cancer Institute, Volume 102, Issue 5, 3 March 2010, Pages 360–361, https://doi.org/10.1093/jnci/djp536

Published: 03 March 2010

> Environ Health Prev Med. 2011 May;16(3):164-70. doi: 10.1007/s12199-010-0182-x. Epub 2010 Sep 29.

Cancer Excess After Arsenic Exposure From Contaminated Milk Powder

Takashi Yorifuji [1], Toshihide Tsuda, Hiroyuki Doi, Philippe Grandjean



- Similar to Chilean population studied by Steinmaus and Smith

Issues with Mouse TPL Model

In Utero

- People are exposed during all periods of their lives.
- We only tested the fetal life stage in mice.
- Testing at any one stage is not “environmental”

Childhood → Adolescence → Adulthood

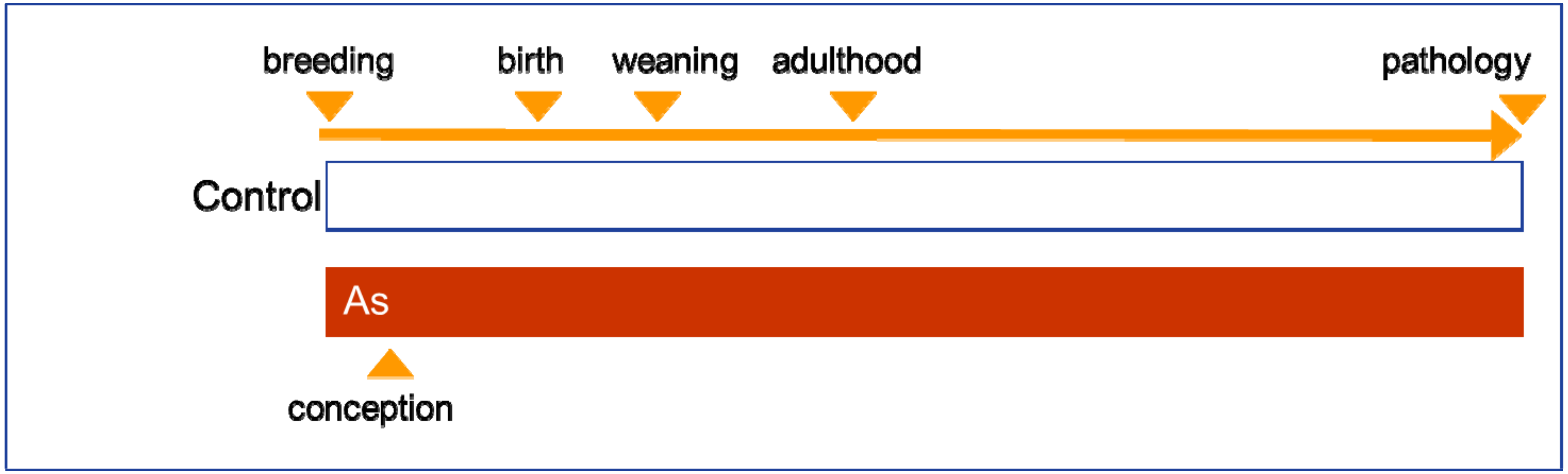
Tested here: sensitivity high in mice

Sensitivity unknown

Sensitivity unknown

Negative in rodents: but not fully “environmental”

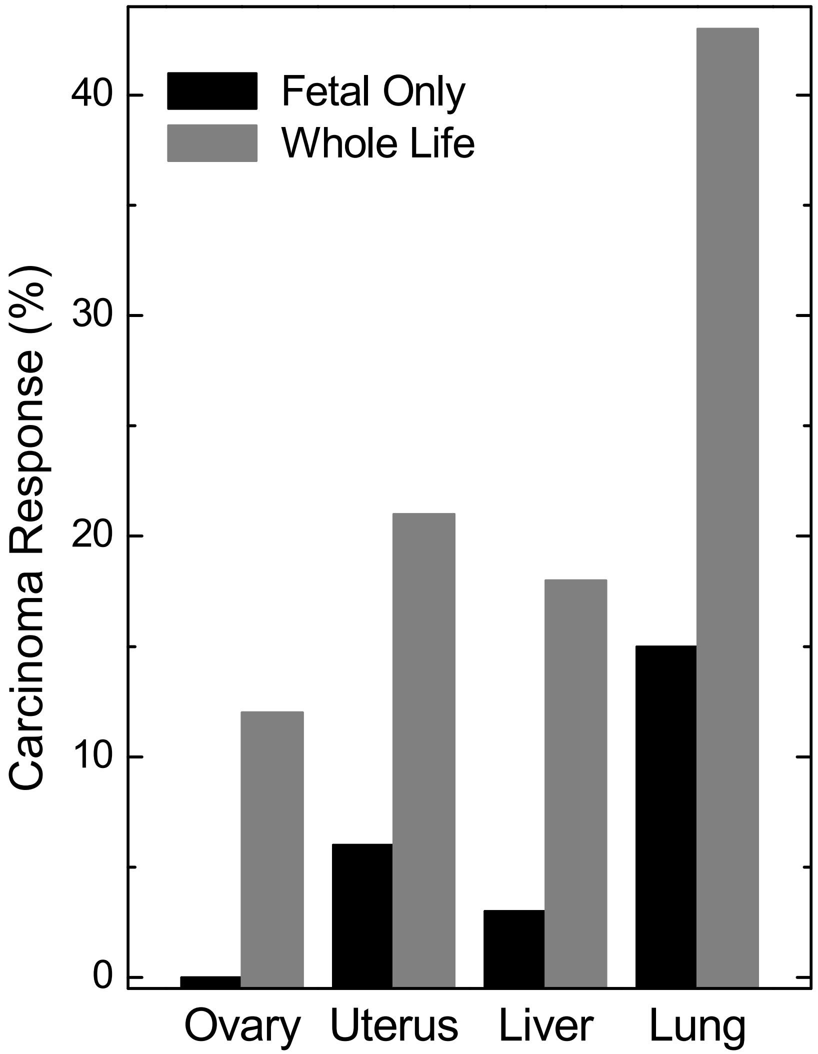
"Whole Life" (WL) Rodent Models



- Arsenic given in drinking water
- Offspring mice observed for up to 2 years
- Doses approaching human exposure levels

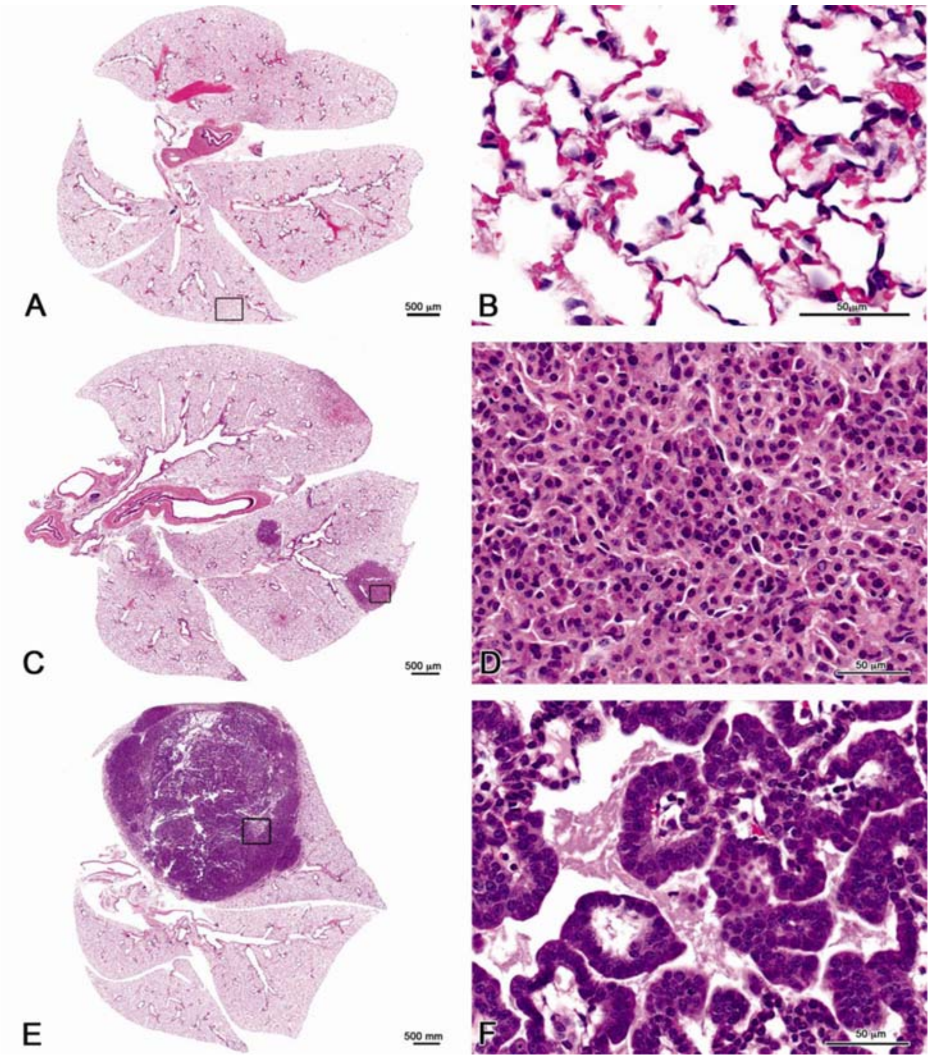
Arsenic is a TPL and WL Carcinogen

E.g.: Carcinoma in female mice



Data from: Tokar et al. *Toxicol Sci.* 119(1):73.

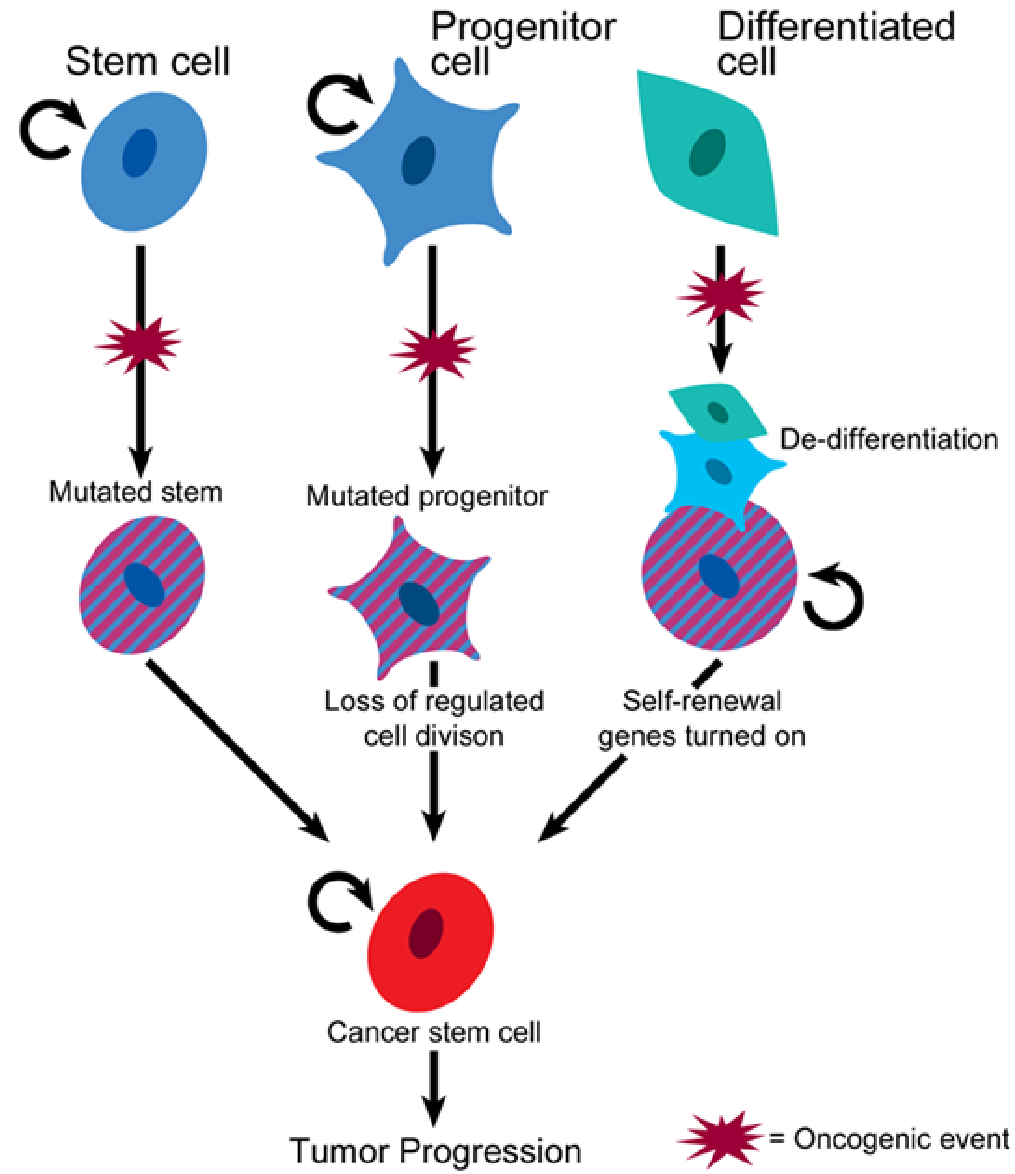
Lung tumors at human-relevant doses (50 and 500 ppb)



Waalkes et al. *Arch Toxicol* 88(8):1619-1629.

Stem Cells and Cancer Stem Cells

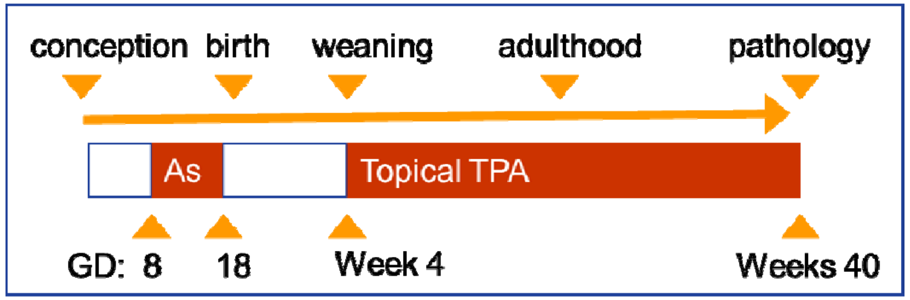
- Share several fundamental characteristics
- Cancer stem cell (CSC) hypothesis
  - SCs drive tumorigenic process?
- Secondary questions:
  - Cell of origin?
  - # of CSCs/tumor?
    - Carcinogen and/or tissue dependent?



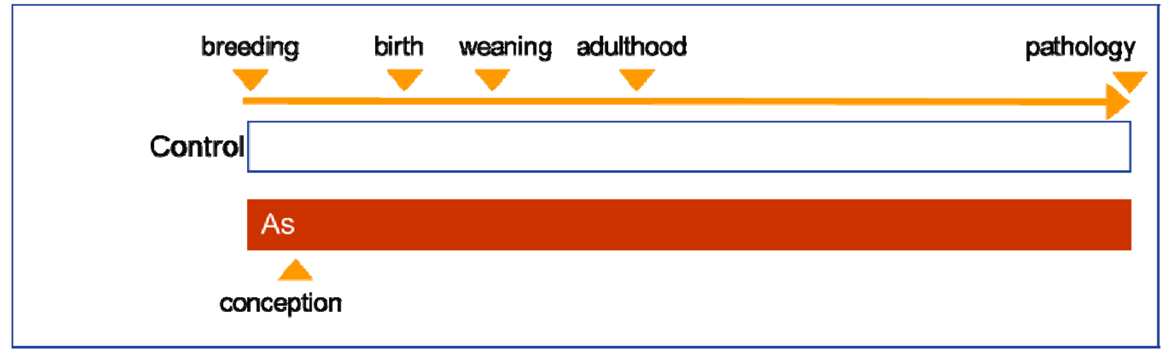
Cancer Stem Cell (CSC) Overabundance

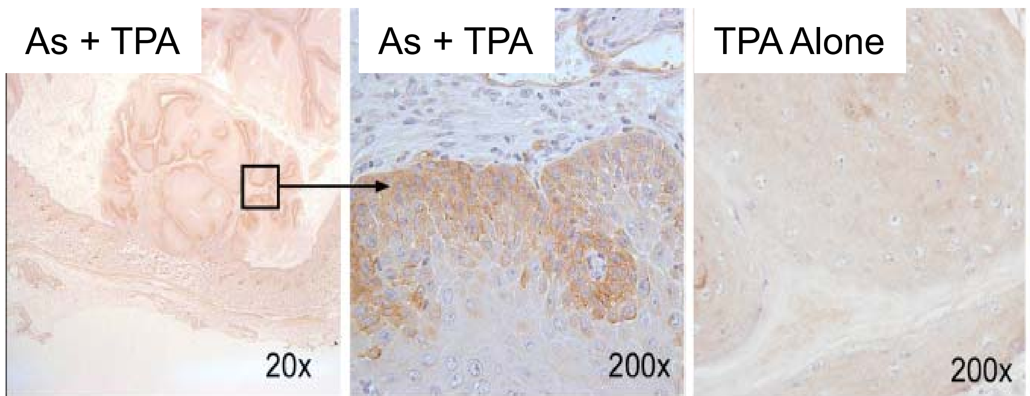
In Vivo Models

Transplacental

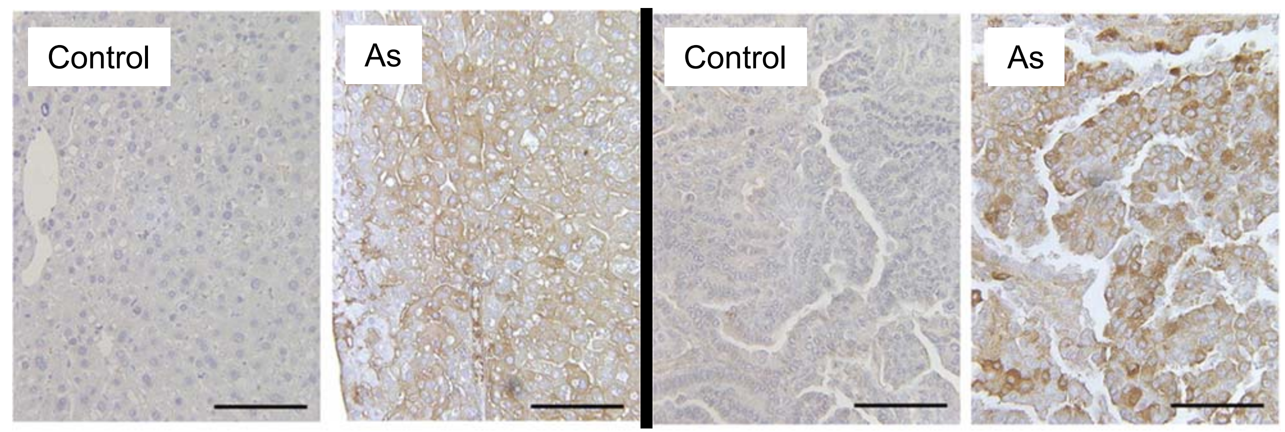


Whole Life





Squamous cell carcinomas stained with CD34 (skin SC/CSC marker)



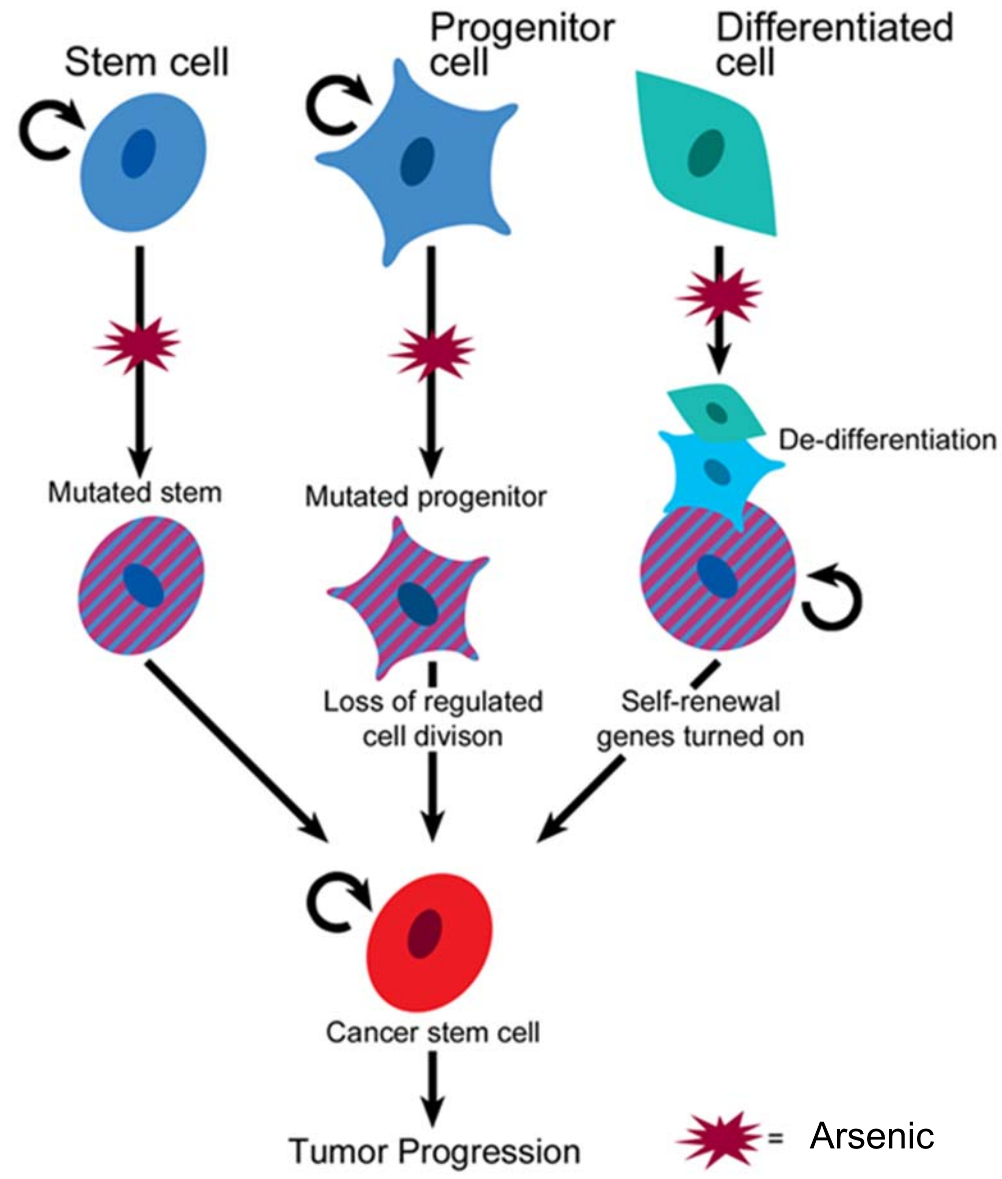
Liver adenocarcinomas (ALDH1A stained) Lung adenocarcinomas (ALDH1A stain)

Waalkes et al. *Cancer Res*. 68:8278.

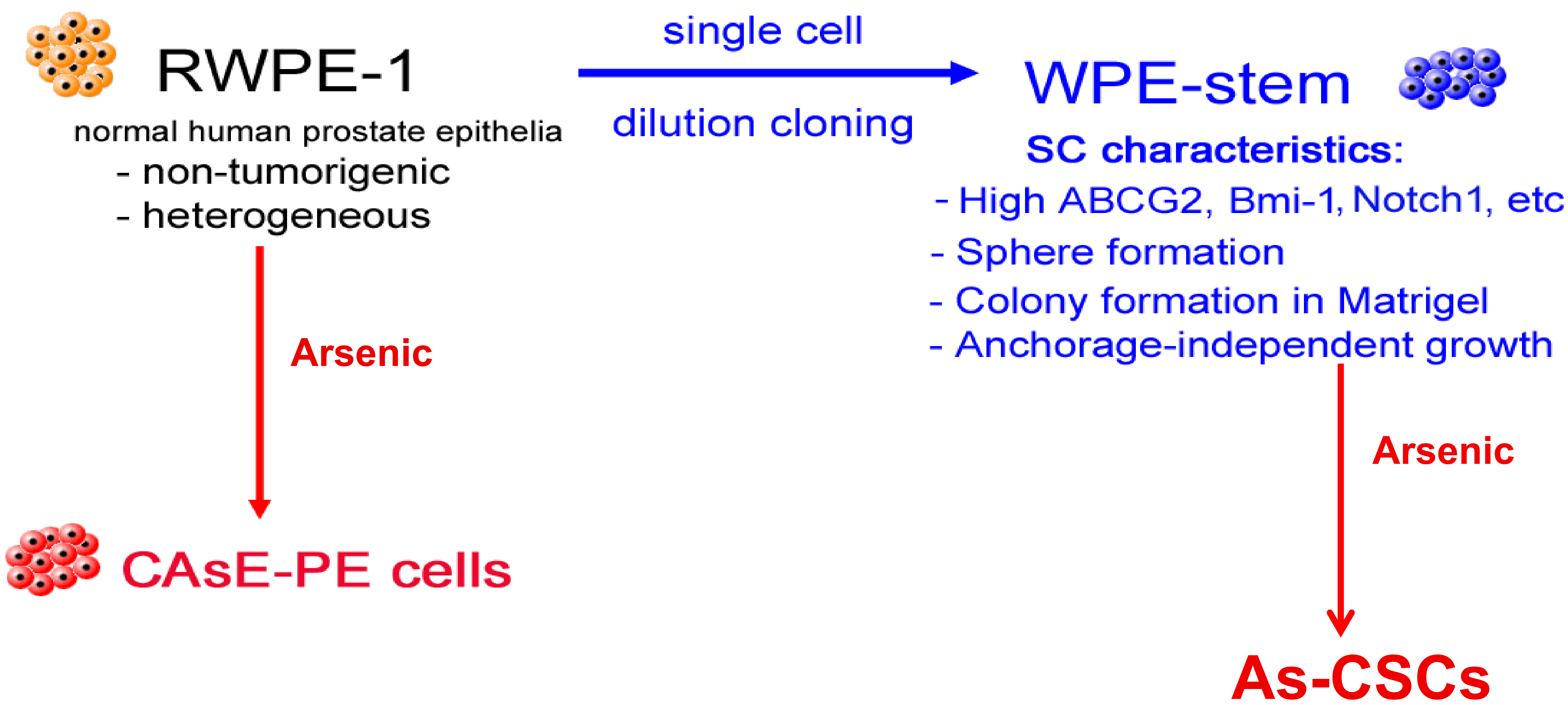
Modified from: Tokar et al. *Tox. Sci*. 119:73.

In Vitro Hypothesis Testing

- Hypothesis:
  - Arsenic directly attacks SCs
    - Formation and overabundance of CSCs
    - Increases SC number during transformation



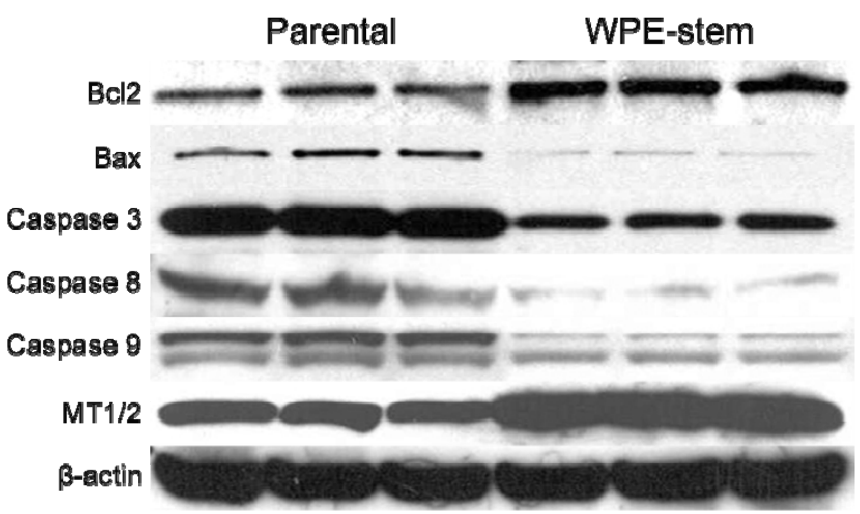
Isogenic Human Cell Models



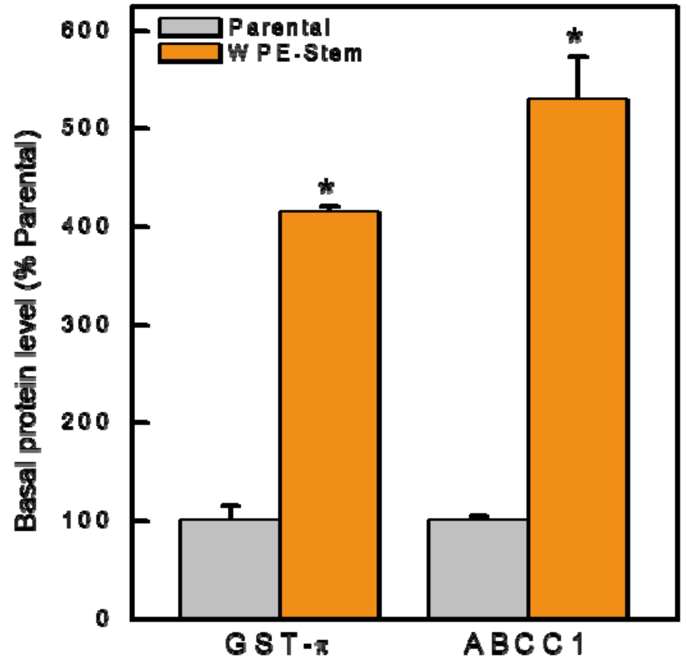
- Similar models for lung, skin, kidney, breast, liver, pancreas

Apoptotic Resistance and Hyper-Adaptability in SCs

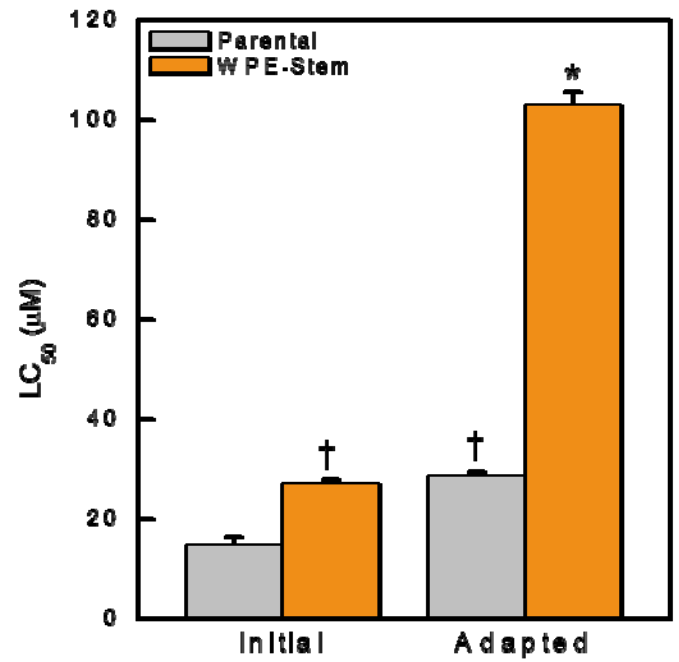
Apoptosis factors



As Efflux/Resistance Factors



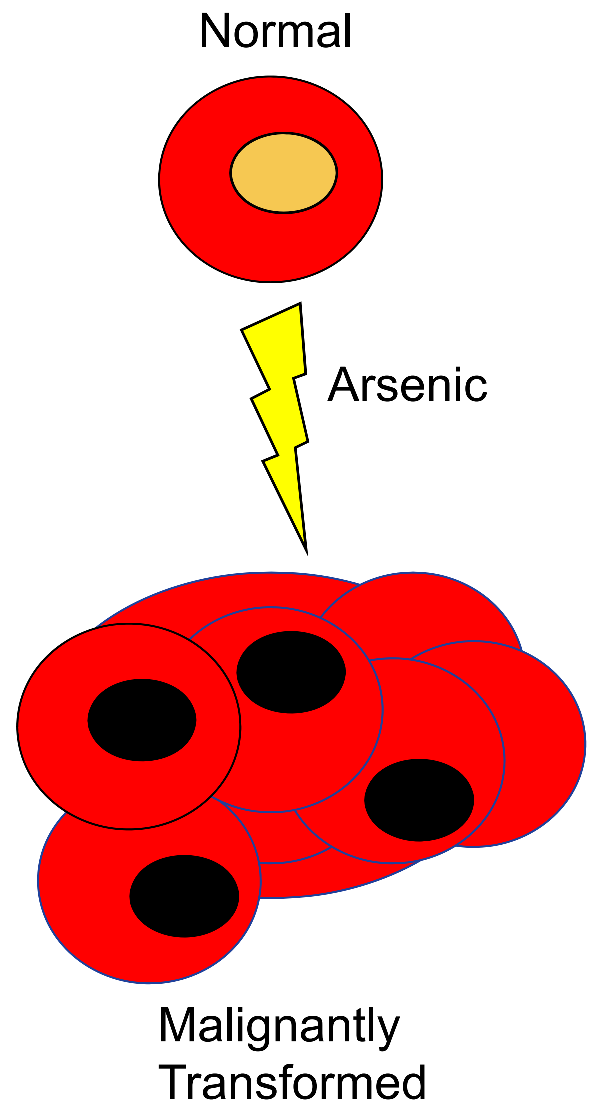
Hyper-adaptability



Modified from Tokar et al. *J Natl Cancer Inst*. 102:638.

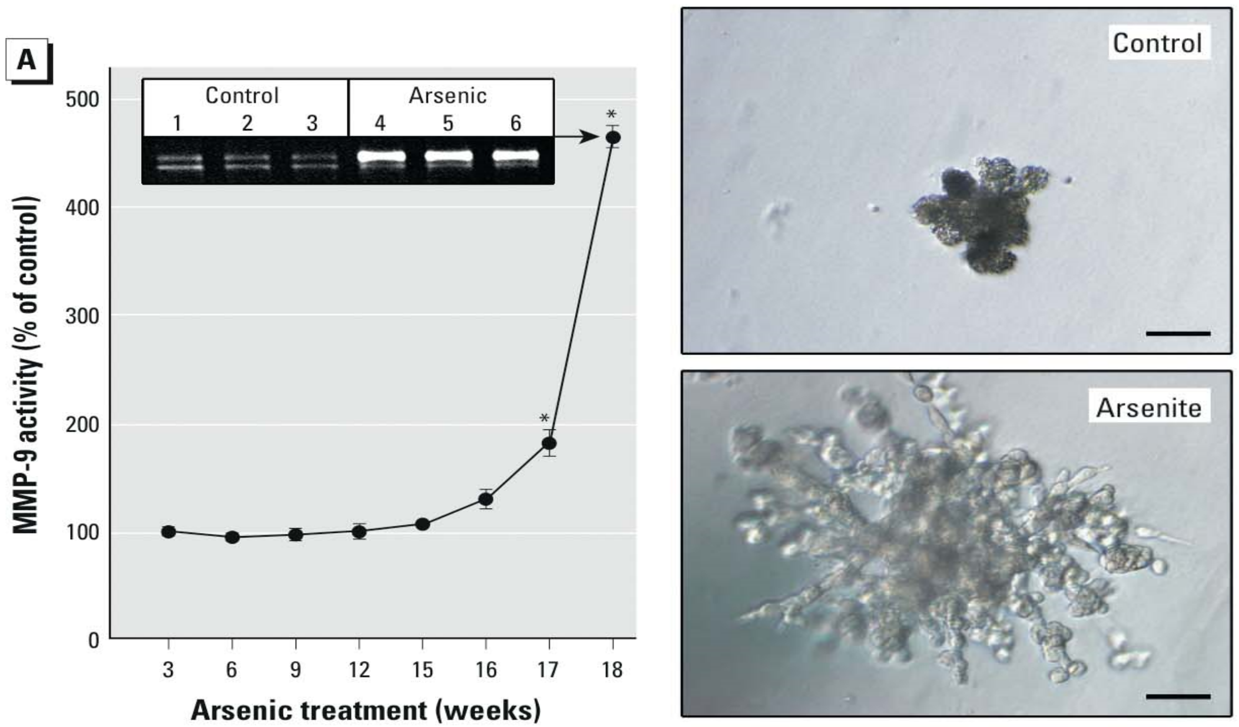
Arsenic Transformation of SCs

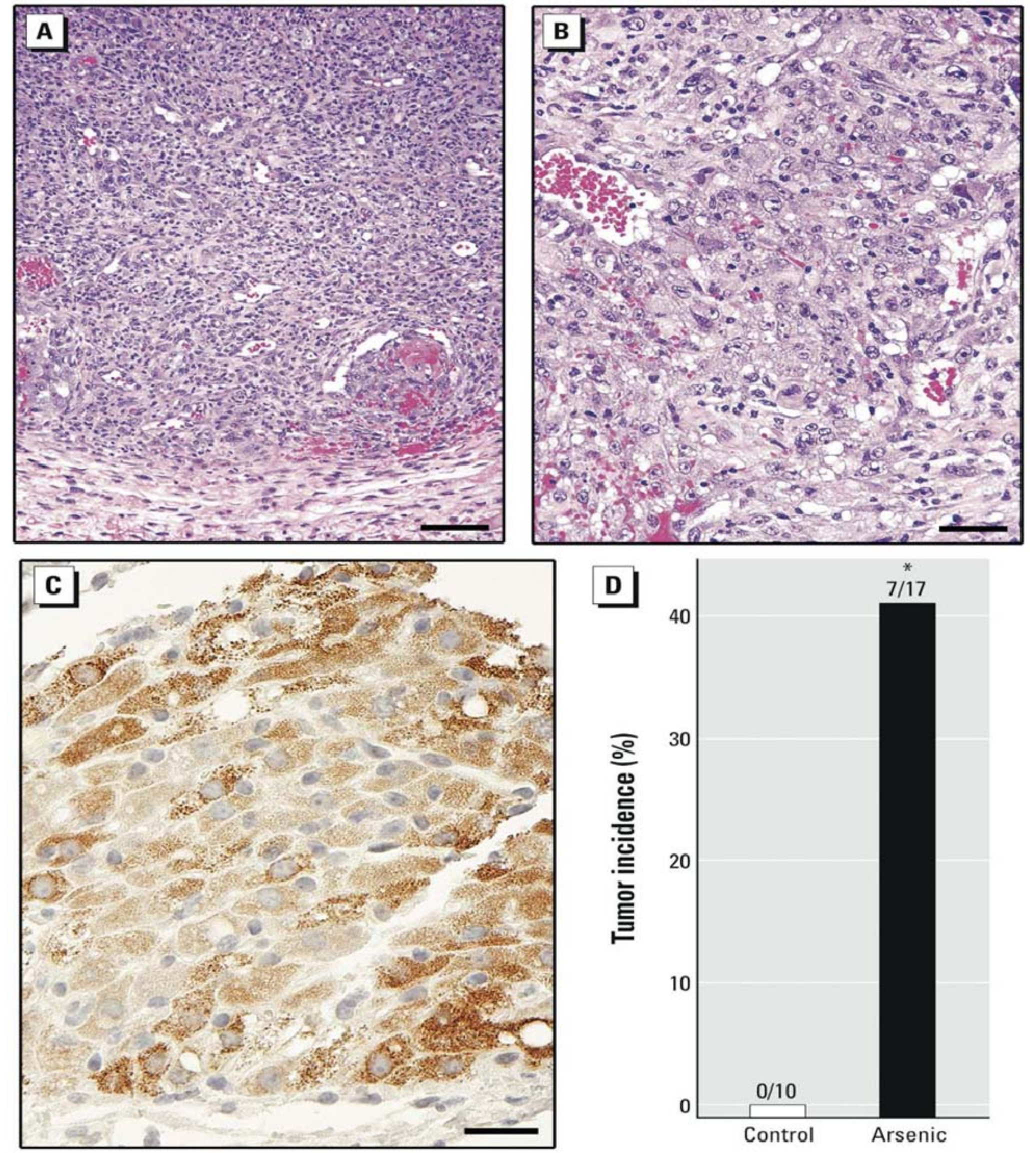
- SCs show survival selection but
  - Can arsenic induce a malignant phenotype
- Continuous arsenic exposure
  - Environmentally relevant level
    - Periodically assess
      - Markers of malignant phenotype
        - MMP-9, invasion, colony formation
      - Xenograft studies when transformation likely



MMP = Matrix Metalloproteinase, a common tumor cell marker

SCs Rapidly Transformed, Form Aggressive Pleiomorphic Tumors

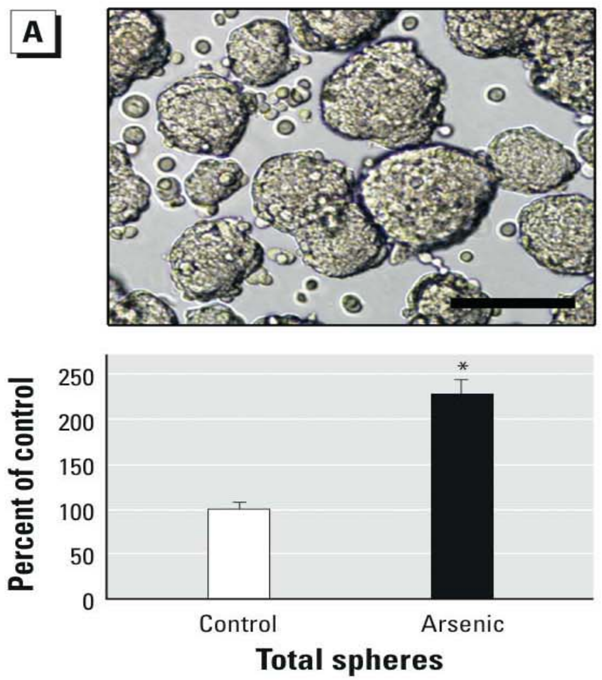




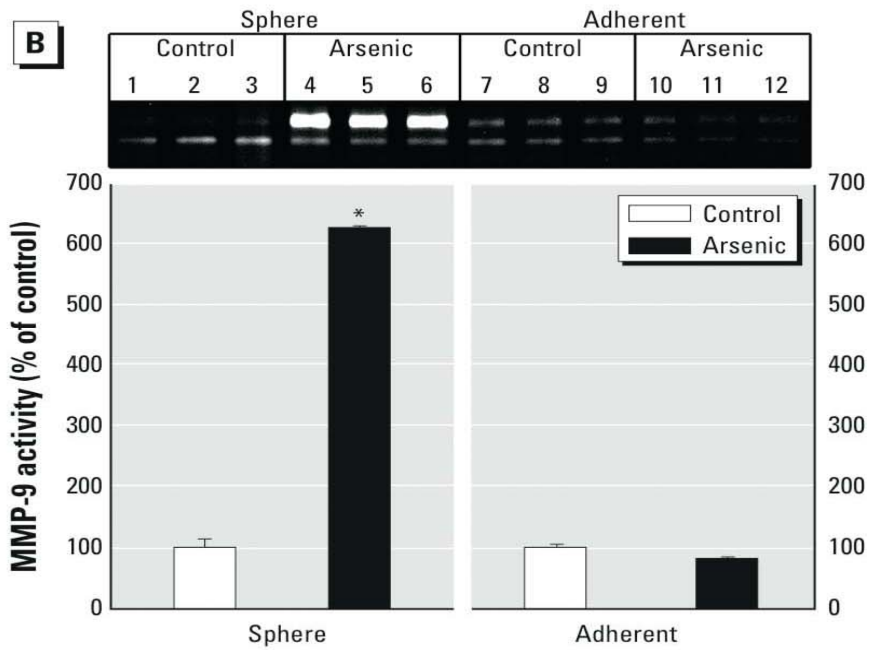
Modified from Tokar et al. *Environ Health Perspect*. 118:108.

Arsenic Increased CSC Characteristics

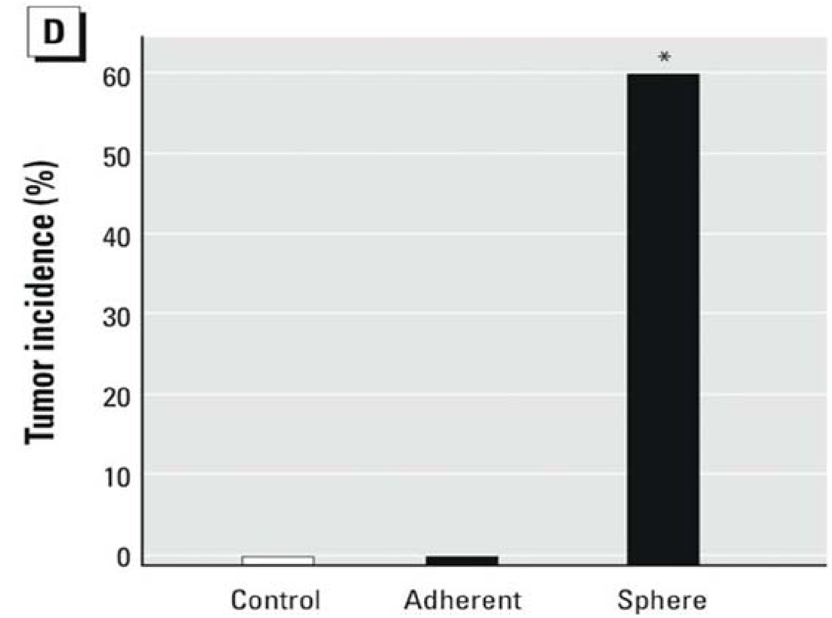
3D Spheres



MMP Activity



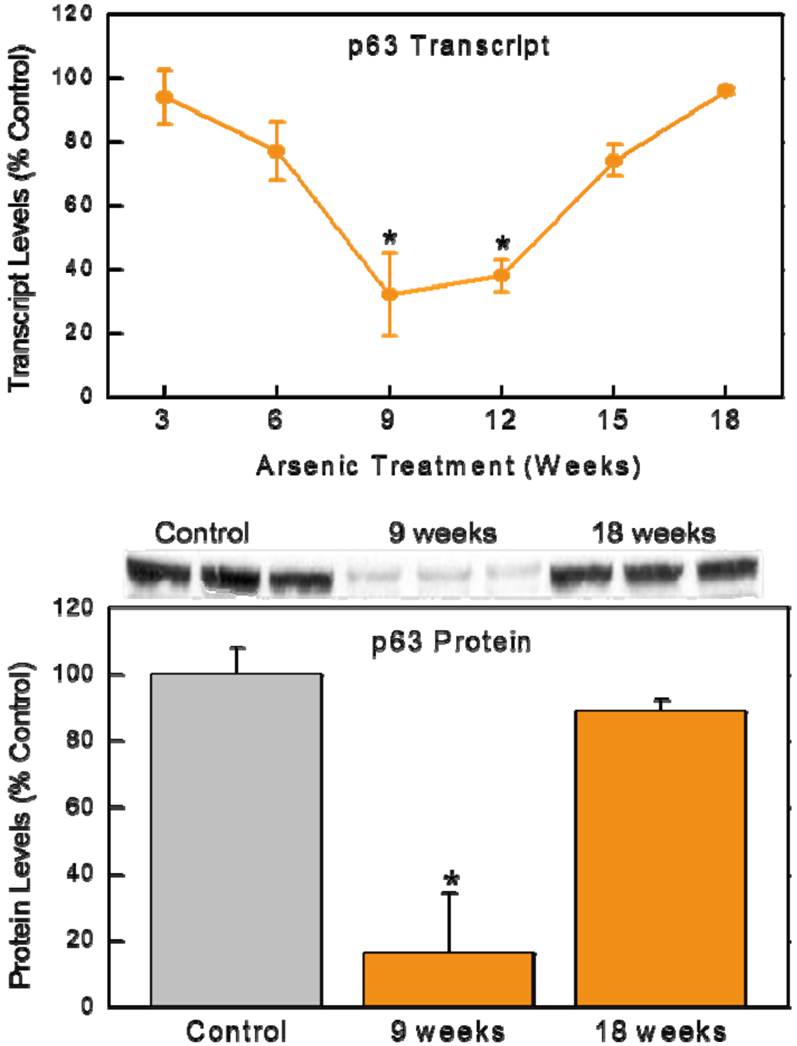
Tumor Incidence

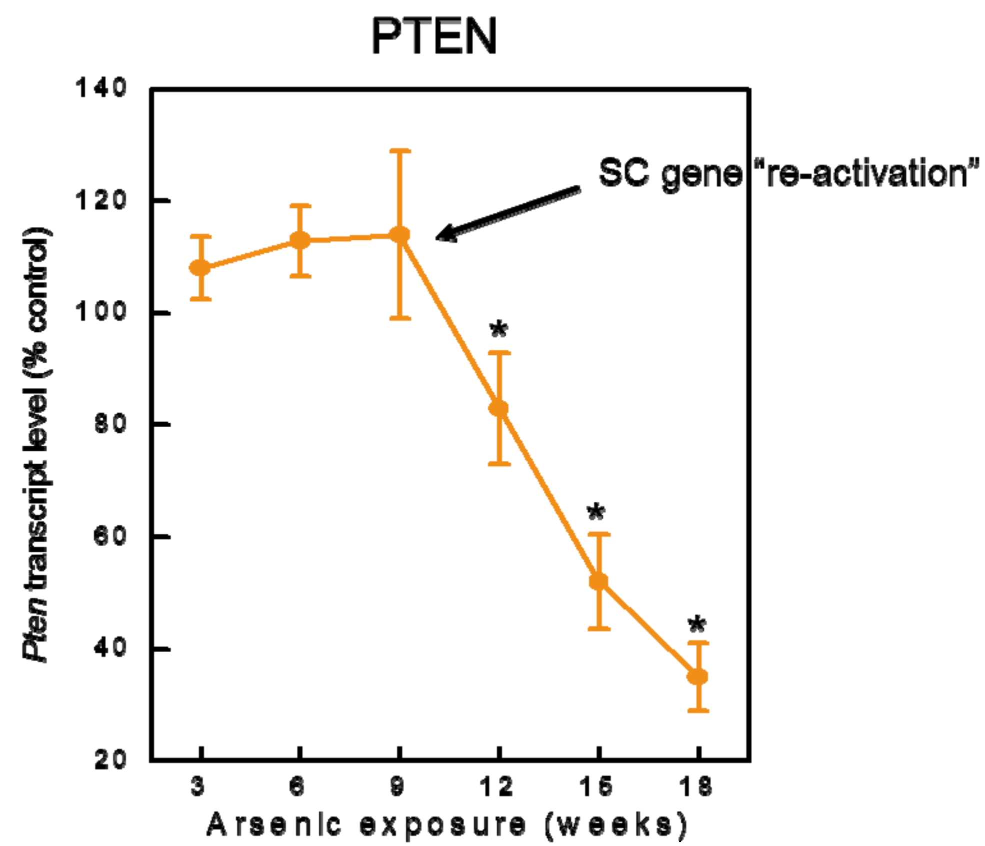


- Similar results in renal, skin, lung, liver, pancreas models

Tokar et al. *Environ Health Perspect*. 118:108.

Aberrant Differentiation, Decreased PTEN



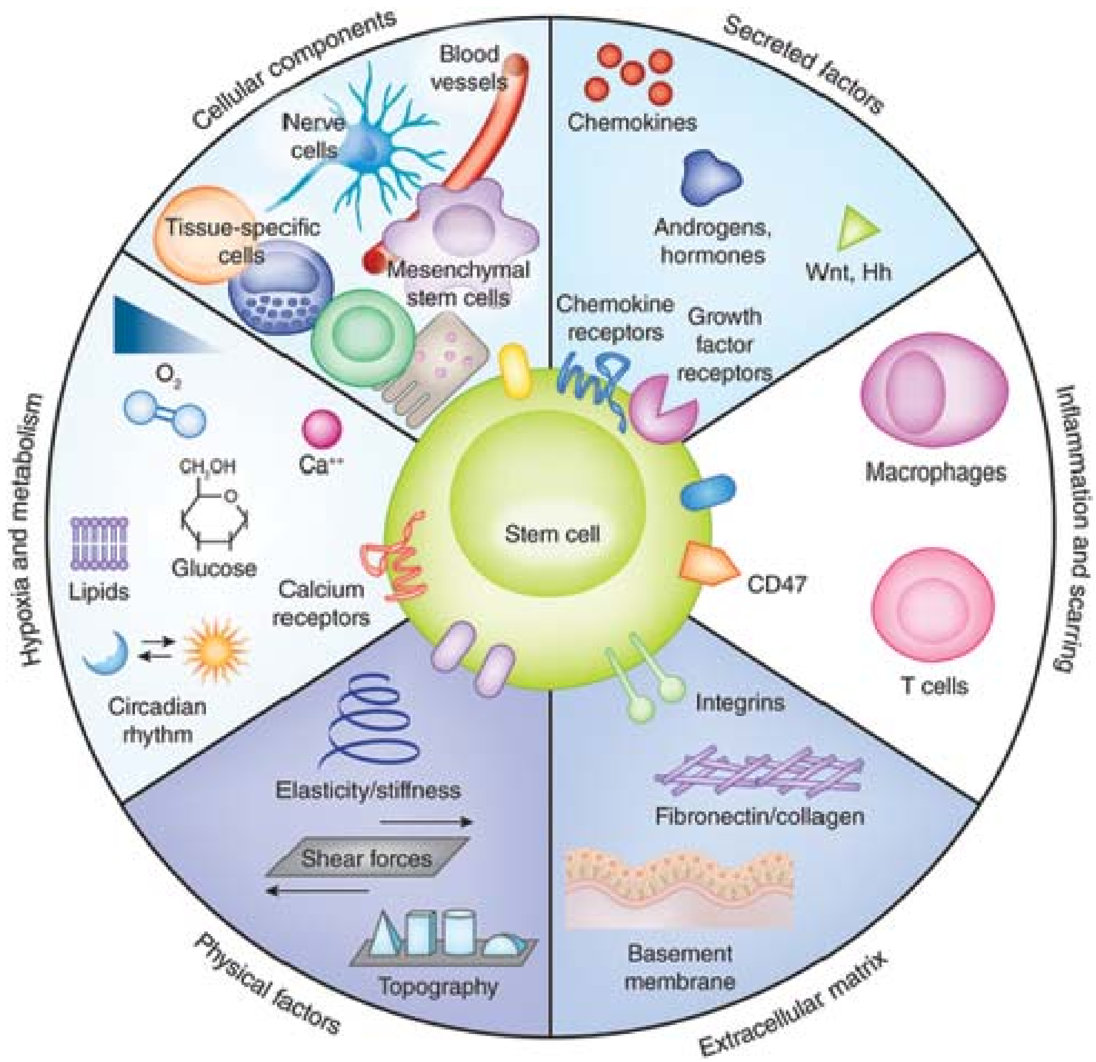


- Similar trend with BMI-1, NOTCH1, ABCG2, OCT4, SHH, WT-1, K5

Modified from Tokar et al. *Environ Health Perspect*. 118:108.

The Microenvironment

- Highly specialized, dynamic, cell type-specific niche
- Provides chemical, mechanical and topographical cues facilitating SC renewal and controlling SC fate
  - ECM, growth modulating signals, location
- Aberrantly altered can:
  - Facilitate tumor formation/progression
- Play a role in CSC overabundance seen with As?



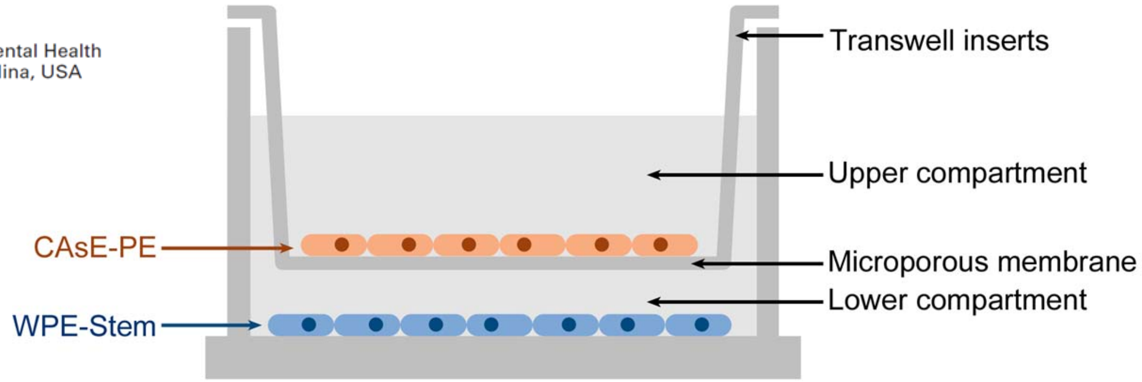
Co-culture Method

Yuanyuan Xu

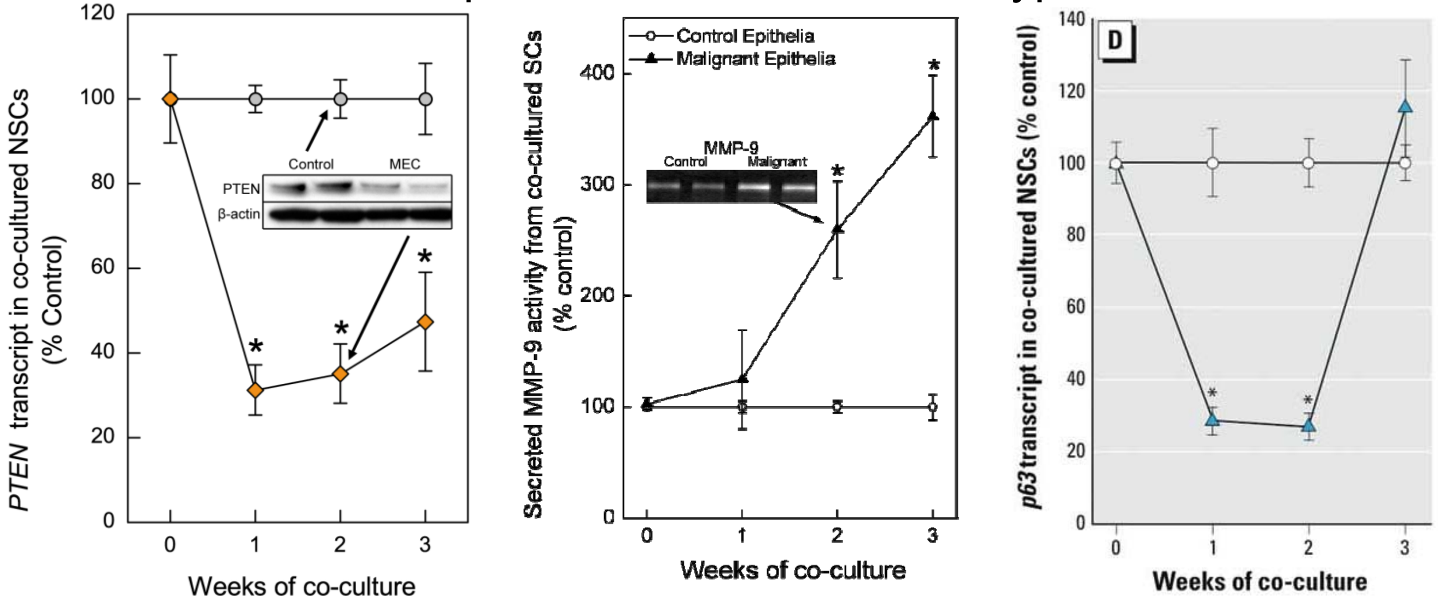
Arsenic-Transformed Malignant Prostate Epithelia Can Convert Noncontiguous Normal Stem Cells into an Oncogenic Phenotype

Yuanyuan Xu, Erik J. Tokar, Yang Sun, and Michael P. Waalkes

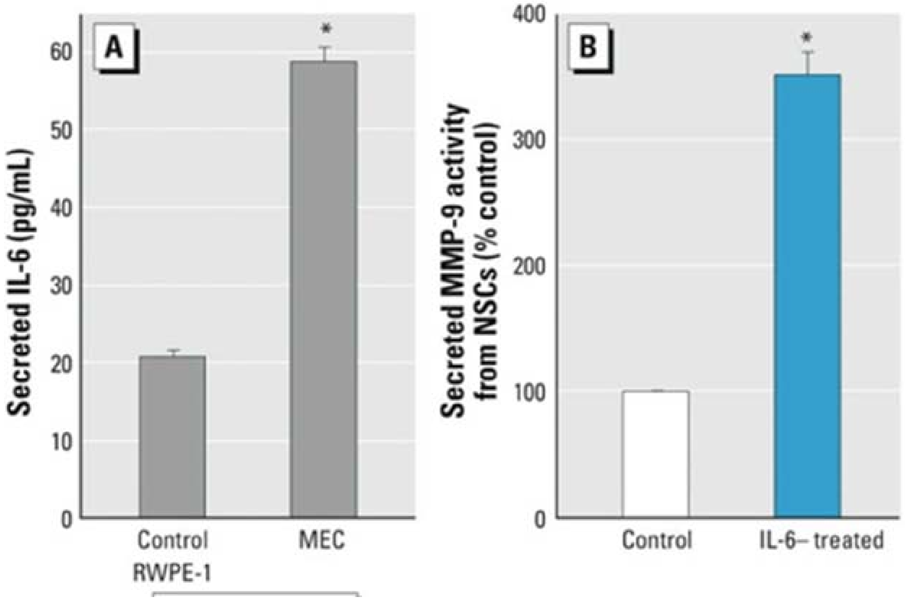
National Toxicology Program Laboratory, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA



Acquisition of Cancer Phenotype



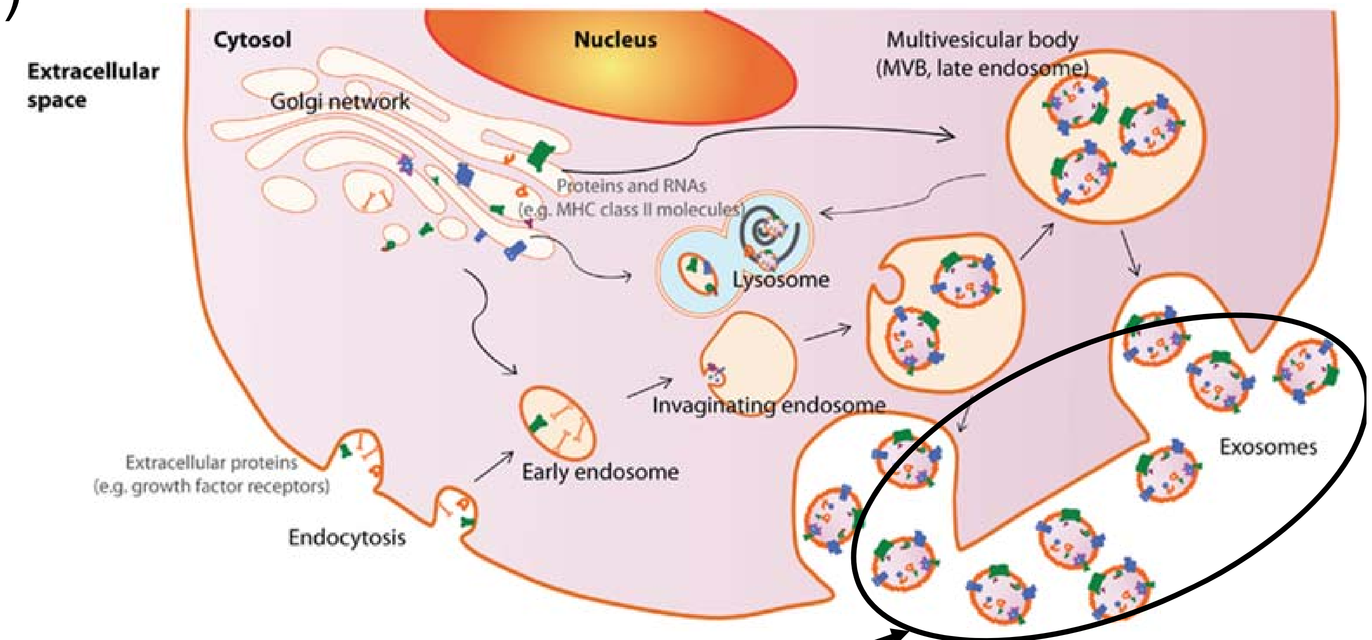
Interleukin-6



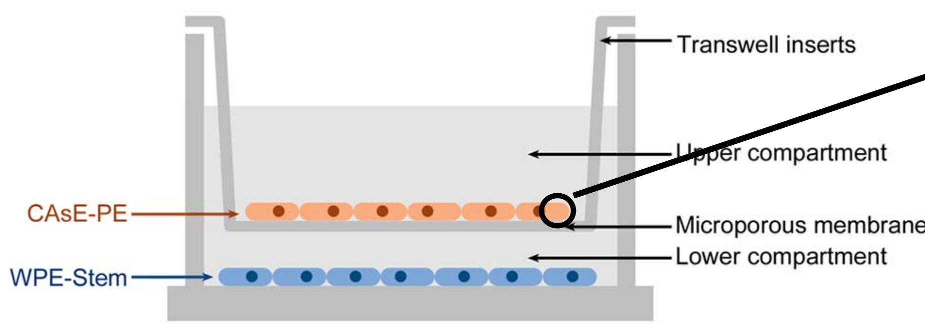
Are Extracellular Vesicles Involved in SC Recruitment?

Exosomes

- Extracellular vesicles (EVs; ~20-120 nm)
- Released by most cells, found in all biofluids
- Biological "cargo"
  - RNA, protein, ncRNAs
- Mediate:
  - Carcinogenesis
  - Cell:cell communication
  - Immune system function



Zhang et al (2014) *Front Immunol* 5:518.



- Isolated by ultracentrifugation
  - From RWPE-1 and CAsE-PE
- RNA, protein collected

EVs Recruit SCs to Oncogenic Phenotype

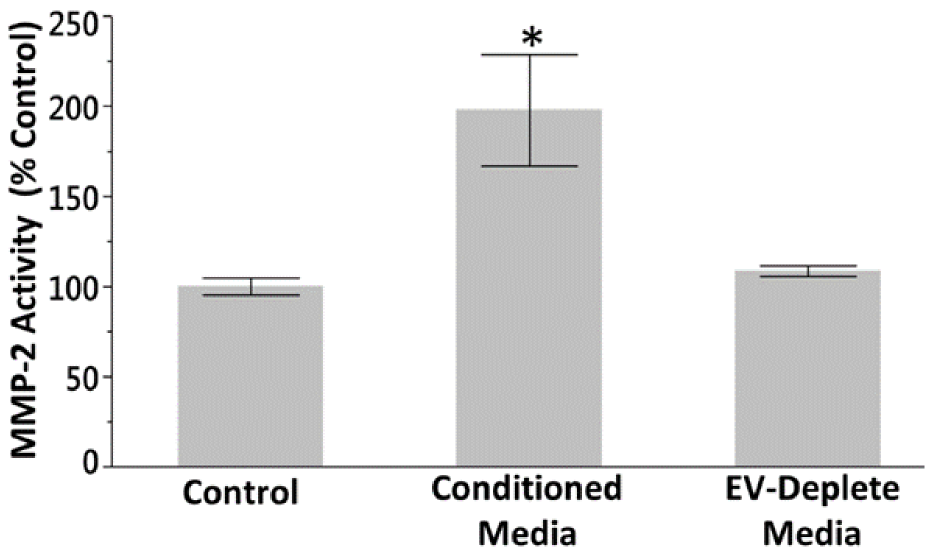
Ntube Ngalame

Tony Luz

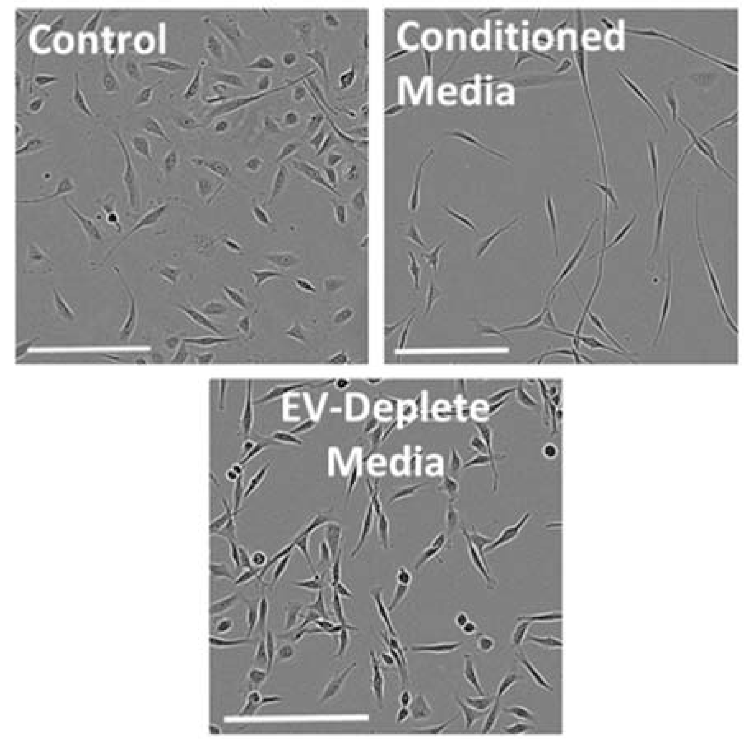
Arsenic Alters Exosome Quantity and Cargo to Mediate Stem Cell Recruitment Into a Cancer Stem Cell-Like Phenotype

Ntube N O Ngalame [1], Anthony L Luz [1], Ngome Makia [1], Erik J Tokar [1]

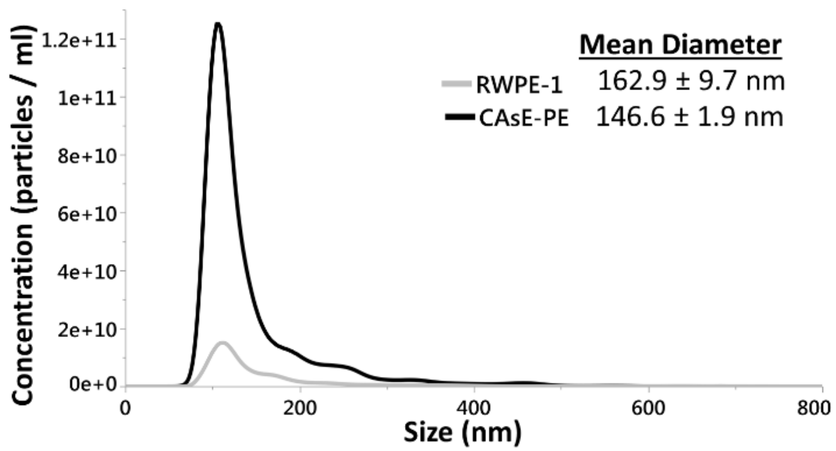
Matrix metalloproteinase activity

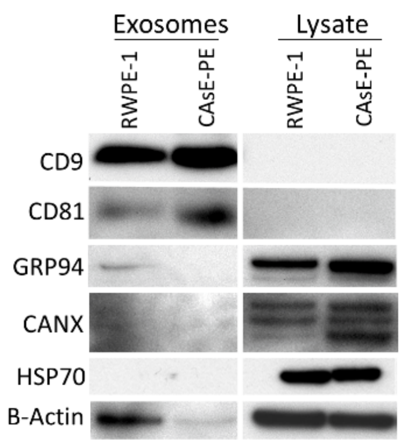


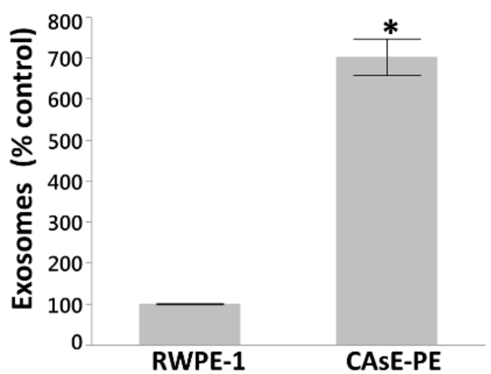
EMT

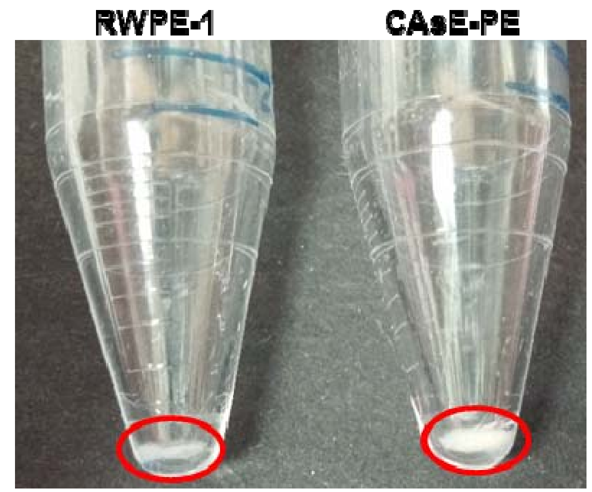


Exosome Isolation and Quantification









Exosomes	RWPE-1	CAsE-PE	% Control
Total Particle Number	5.8e+11	4.1e+12	700%
Total Protein	11 ug	70 ug	636%
Total RNA	0.5 ug	2.1 ug	420%

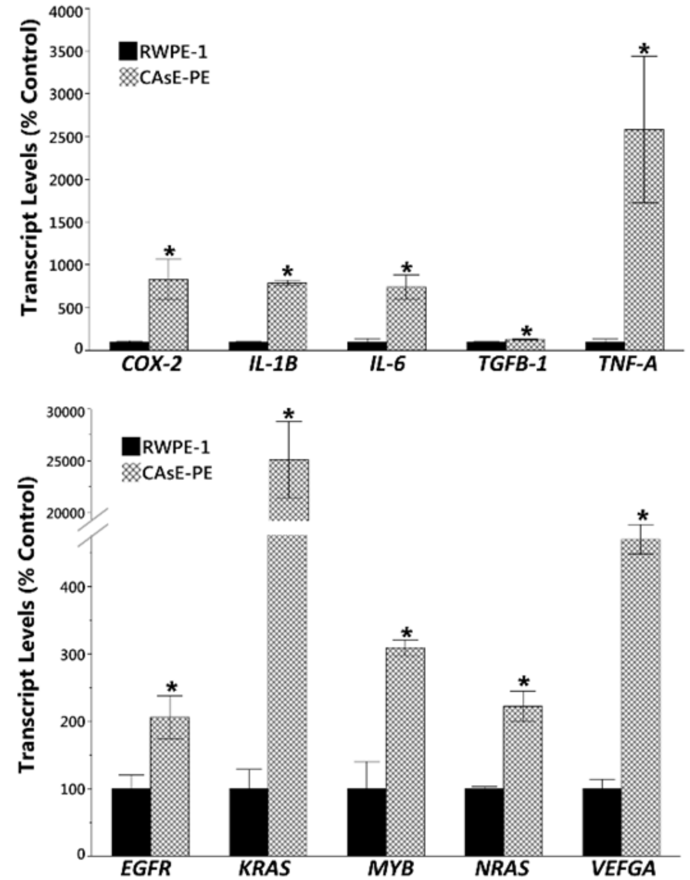
Ngalame et al. *Tox Sci* 165(1):40-49.

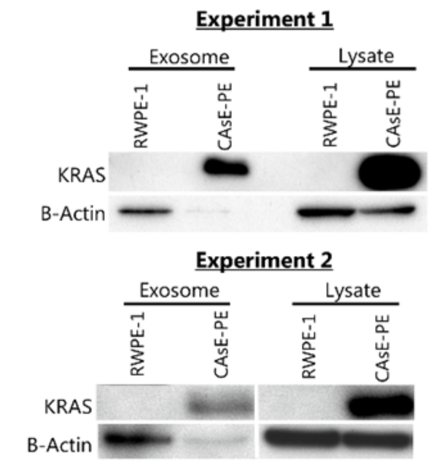
*All data normalized to 50 x 10^6 cells/cell line

Cancer-associated Exosome Cargo

	Fold regulation[1]			Fold regulation[1]	
microRNA	**Exosomal**	**Cellular**	**microRNA**	**Exosomal**	**Cellular**
miR-9-5p	7.2 up	7.4 up	miR-19a-3p	3.6 down	No Change
let-7f-5p	2.7 up	No Change	miR-17-5p	3.5 down	No Change
miR-183-5p	2.1 up	2.0 up	let-7b-5p	3.4 down	2.6 down
miR-10a-5p	1.8 up	No Change	miR-146a-5p	3.3 down	No Change
miR-27b-3p	1.3 up	No Change	miR-150-5p	3.3 down	No Change
miR-34c-5p	42.3 down	5.9 down	miR-27a-3p	3.2 down	No Change
miR-138-5p	30.5 down	4.2 down	miR-124-3p	3.1 down	No Change
miR-146b-5p	14.6 down	5.7 down	miR-16-5p	3.1 down	No Change
miR-135b-5p	14.1 down	5.4 down	miR-132-3p	3.0 down	No Change
miR-193a-5p	13.8 down	No Change	miR-25-3p	3.0 down	No Change
miR-143-3p	13.3 down	No Change	miR-128-3p	3.0 down	No Change
miR-155-5p	12.5 down	4.4 down	miR-18a-5p	2.9 down	No Change
miR-205-5p	8.7 down	4.1 down	miR-191-5p	2.9 down	No Change
miR-7-5p	8.6 down	No Change	miR-10b-5p	2.9 down	2.9 down
miR-222-3p	7.8 down	5.0 down	miR-20b-5p	2.7 down	No Change
miR-181d-5p	7.6 down	2.8 down	miR-15a-5p	2.7 down	No Change
miR-181b-5p	5.9 down	2.6 down	miR-127-5p	2.7 down	13.2 down
miR-130a-3p	5.4 down	No Change	miR-372-3p	2.5 down	No Change
miR-181a-5p	5.2 down	2.0 down	miR-378a-3p	2.4 down	No Change
miR-184	4.9 down	No Change	miR-193b-3p	2.4 down	No Change
miR-144-3p	4.9 down	No Change	miR-15b-5p	2.1 down	No Change
miR-142-5p	4.7 down	No Change	let-7g-5p	1.9 down	No Change
miR-34a-5p	4.7 down	2.0 down	miR-29b-3p	1.9 down	No Change
miR-20a-5p	4.7 down	No Change	miR-32-5p	1.8 down	No Change
miR-215-5p	4.5 down	No Change	miR-30c-5p	1.6 down	No Change
miR-149-5p	4.4 down	No Change	miR-210-3p	1.5 down	No Change
miR-203a-3p	4.4 down	No Change	miR-373-3p	No Change	10.6 down
miR-133b	3.9 down	No Change	miR-218	No Change	2.7 down
miR-125b-5p	3.9 down	1.8 down	miR-96-5p	No Change	2.7 up
miR-125a-5p	3.8 down	2.6 down	miR-98-5p	No Change	2.4 down
miR-214-3p	3.8 down	No Change	miR-196a-5p	No Change	2.0 down
miR-134-5p	3.8 down	14.6 down	miR-181c-5p	No Change	1.9 down
let-7i-5p	3.7 down	2.1 down	let-7e-5p	No Change	1.9 down
miR-335-5p	3.6 down	No Change	let-7c-5p	No Change	1.8 down
miR-100-5p	3.6 down	No Change	miR-126-3p	No Change	1.6 down

[1]Fold regulation is compared to microRNA expression in RWPE-1 exosomes or cell lysate, and are significantly different ($p < 0.05$).





Ngalame et al. *Tox Sci* 165(1):40-49.

Conclusions

- Arsenic carcinogenesis:
  - TPL and WL carcinogen
  - Results in a CSC overabundance both *in vivo* and *in vitro*
  - Alters several key SC-associated signaling pathways
  - Decrease in PTEN
  - Altered miRNA levels → Increase in KRAS
- Arsenic impacts microenvironment
  - “Recruits” SC into CSC-like phenotype
  - Alters quantity and cancer-favoring cargo of exosomes

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Questions?