Association of Early-Life Arsenic Exposure and Cancer in Adulthood

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June 8th, 2020
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• 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.
• 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
• 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
• Arsenic given in maternal drinking water
• Done in several strains (C3H, CD1, Tg.AC)
• Tumors or neoplasia in both female and male offspring
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, Bhachandra A Diwan


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, Bhachandra A Diwan


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, Xavior Diwan, Daniel L


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, Bhachandra A Diwan


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, Bhachandra A Diwan


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

Erik J Tokar 1, Bhachandra A Diwan, Michael P Waalkes


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

Erik J Tokar 1, Bhachandra A Diwan, Michael P Waalkes


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

Erik J Tokar 1, Bhachandra A Diwan, David J Thomas, Michael P Waalkes

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
• 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

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

Cancer Excess After Arsenic Exposure From Contaminated Milk Powder

Takashi Yorifuji, Toshihide Tsuda, Hiroyuki Doi, Philippe Grandjean

• Similar to Chilean population studied by Steinmaus and Smith
Issues with Mouse TPL Model

- 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”

In Utero

Tested here: sensitivity high in mice

Childhood

Adolescence

Adulthood

Sensitivity unknown

Sensitivity unknown

Negative in rodents: but not fully “environmental”
• 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


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

• 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**

- conception
- birth
- weaning
- adulthood
- pathology

- GD: 8
- 18
- Week 4
- Weeks 40

- As
- Topical TPA

**Whole Life**

- breeding
- birth
- weaning
- adulthood
- pathology

- Control

- As

- conception

As + TPA

TPA Alone

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

Liver adenocarcinomas (ALDH1A stained)

Lung adenocarcinomas (ALDH1A stain)


In Vitro Hypothesis Testing

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

- RWPE-1: normal human prostate epithelia
  - non-tumorigenic
  - heterogeneous

- Arsenic treatment:

- CAsE-PE cells

- Single cell dilution cloning:

- WPE-stem
  - SC characteristics:
    - High ABCG2, Bmi-1, Notch1, etc
    - Sphere formation
    - Colony formation in Matrigel
    - Anchorage-independent growth

- Arsenic treatment:

- As-CSCs

- Similar models for lung, skin, kidney, breast, liver, pancreas
Apoptotic Resistance and Hyper-Adaptability in SCs

Apoptosis factors

As Efflux/Resistance Factors

Hyper-adaptability

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

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

Aberrant Differentiation, Decreased PTEN

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

• 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?
Yuanyuan Xu

**Co-culture Method**

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**

![Graphs showing changes in PTEN transcript, secreted MMP-9 activity, and p63 transcript over weeks of co-culture.](image)

**Interleukin-6**

![Bar graphs showing interleukin-6 secretion from RWPE-1 and MEC, both with and without IL-6 treatment.](image)
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

Are Extracellular Vesicles Involved in SC Recruitment?


- Isolated by ultracentrifugation
  - From RWPE-1 and CAsE-PE
- RNA, protein collected
EVs Recruit SCs to Oncogenic Phenotype

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

![Graph showing MMP-2 activity with Control, Conditioned Media, and EV-Deplete Media conditions]

*Significant difference

EMT
Exosome Isolation and Quantification

### Exosome Data

<table>
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<tr>
<th></th>
<th>RWPE-1</th>
<th>CAsE-PE</th>
<th>% Control</th>
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<td>Total Particle Number</td>
<td>5.8e+11</td>
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<tr>
<td>Total Protein</td>
<td>11 ug</td>
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<td>Total RNA</td>
<td>0.5 ug</td>
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*All data normalized to 50 x 10^6 cells/cell line

Cancer-associated Exosome Cargo

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

• Arsenic carcinogenesis:
  – TPL and WL carcinogen
  – Results in a CSC overabundance both \textit{in vivo} and \textit{in vitro}
  – Alters several key SC-associated signaling pathways
  – Decrease in PTEN
  – Altered miRNA levels $\rightarrow$ Increase in KRAS

• Arsenic impacts microenvironment
  – “Recruits” SC into CSC-like phenotype
  – Alters quantity and cancer-favoring cargo of exosomes
Acknowledgements

• Stem Cells Toxicology Group
  – Xian Wu, PhD
  – Yichang Chen, PhD
  – Anthony Luz, PhD
  – Ntube Ngalame, PhD
  – Ngome Makia, PhD
  – Yuanyuan Xu, PhD
  – Matt Bell

• NTPL, NTP, NIEHS
  – Alex Merrick, PhD

• Mike Waalkes, PhD (ret)
Questions?