Welcome to the CLU-IN Internet Seminar

Early-life Exposures - Long-term Health Consequences: Session 2, Metals and Metal Mixtures

Sponsored by: NIEHS Superfund Research Program

Delivered: March 28, 2012, 1:00 PM - 3:00 PM, EDT (17:00-19:00 GMT)

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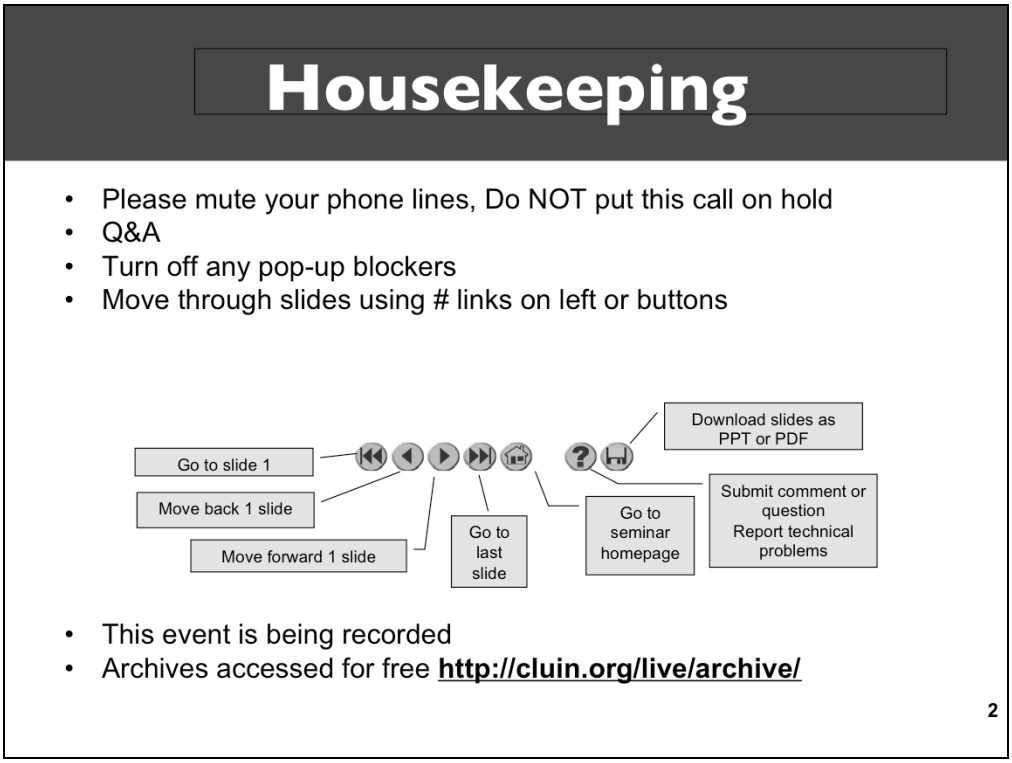
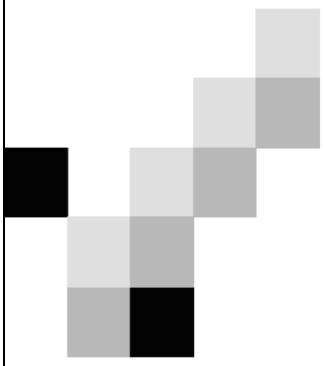


Although I'm sure that some of you have these rules memorized from previous CLU-IN events, let's run through them quickly for our new participants.

Please mute your phone lines during the seminar to minimize disruption and background noise. If you do not have a mute button, press *6 to mute #6 to unmute your lines at anytime. Also, please do NOT put this call on hold as this may bring delightful, but unwanted background music over the lines and interupt the seminar.

You should note that throughout the seminar, we will ask for your feedback. You do not need to wait for Q&A breaks to ask questions or provide comments. To submit comments/questions and report technical problems, please use the ? Icon at the top of your screen. You can move forward/backward in the slides by using the single arrow buttons (left moves back 1 slide, right moves advances 1 slide). The double arrowed buttons will take you to 1st and last slides respectively. You may also advance to any slide using the numbered links that appear on the left side of your screen. The button with a house icon will take you back to main seminar page which displays our agenda, speaker information, links to the slides and additional resources. Lastly, the button with a computer disc can be used to download and save today's presentation materials.

With that, please move to slide 3.



Chemical Mixtures and Neurodevelopment

Robert O. Wright MD MPH
Director, Harvard SRP
Associate Professor of Pediatrics

Why should we study Mixtures?

- Real life exposure scenario
- Most Superfund sites are mixtures
  - Can guide which chemicals to assess

Why should we study Mixtures?

- Mixed Exposures can be thought of as an extension of the “2-hit” hypothesis
  - 1st hit leaves brain in vulnerable state
  - 2nd hit needed to produce toxicity
    - Fits with developmental theories of plasticity

Mixtures may be most relevant to the general population

- High vs low doses of chemicals
- Mixtures may be irrelevant at “high” doses
  - If blood lead is >100 ug/dL, can a low dose of Mn make any difference?
  - If blood Pb is 10 ug/dL perhaps a second hit by Mn then becomes relevant

Chemical Mixtures and Brain Development

- Metals
  - Pb, Mn, As, Hg, methyl Hg,
- Organic chemicals
  - PCBs, DDT,
  - Solvents
- Pesticides
  - Organophosphates, Carbamates, pyrethroids
- Drugs of abuse
  - GHB, cocaine, benzodiazepine, ketamine

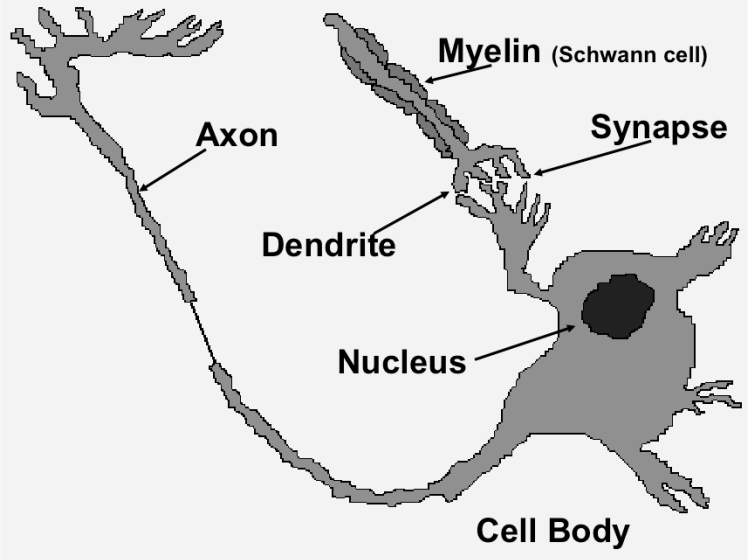
Neurodevelopment-Review

- How do chemicals produce neurotoxicity in the developing brain?
  - High dose
    - Neurodegeneration, damage, cell death
  - Low dose
    - May be no signs of damage
    - Interferes with network formation

Developmental Neurotoxicology

- Vulnerable periods
  - Childhood
    - Neurodevelopment
  - Elderly
    - Neurodegeneration
- Critical Developmental Windows
  - Developmental life stages at which processes occur (i.e. gene expression) which may not occur at other life stages.

Neuronal Cells

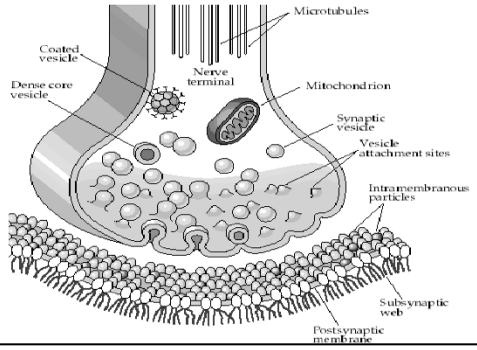


Biological Vulnerability-Neurodevelopment

- Construction of the central nervous system (CNS) begins in utero,
- Continues throughout childhood and involves the production of 100 billion nerve cells and 1 trillion glial cells.
- Cell migrate, differentiate, and form synapses

Synapses

- Transmit signals between neurons
  - Environmental stimuli will cause neurons to fire
  - Neuronal/synaptic firing is a signaling process to mold the synaptic architecture of the brain



How does the Brain Build this Network?

- Some of it is stochastic
  - Synapses are made by the billions, and in some respects randomly, between neurons.
  - We make a net gain in synapses from fetal life till about age 2 years
  - Then the number of synapses in our brain starts to decrease
    - Why?

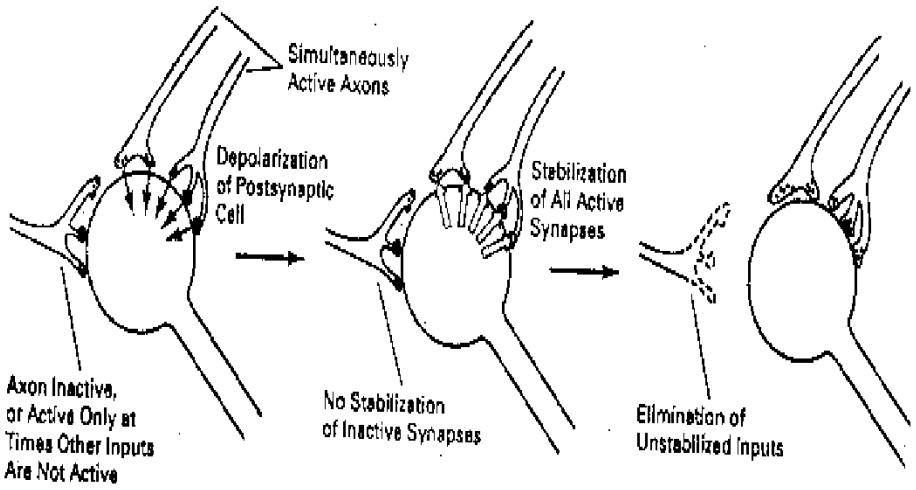
Synaptic Network

- Environmental Stimuli cause nerves to fire:
- When they fire, neurotransmitters are released into synaptic junctions
  - This releases growth factors
  - Signals that this is an important neuronal connection (i.e. it gets used)

Synaptic Pruning

- Environmental stimuli mold the CNS.
  - Synapses that produce function are repeatedly fired and kept
  - Synapses that are dormant are deleted
- In other words there is a “natural selection” process
  - Functional synapses release growth factors
  - Nonfunctional synapses do not release the growth factors

Hebb Synapses



Weisel and Hubel

- Newborn kittens
  - Patch one eye for one month
  - Retinal development (specifically the development of neuronal connections) in the patched eye would not occur.
- Patch Adult cat eye for one month
  - Compare neuronal networks between patched and unpatched eye
    - No difference than comparing unpatched cats

Implication

- Natural Selection is not just a process by which genetic variants are selected.
- Neuronal Cells and synaptic networks may also undergo a process of natural selection

So how do Chemicals affect Development?

- Lead as a “paradigm” toxicant
- At “low” doses (blood lead around 5-10 ug/dL)
  - Lead will interact with Protein Kinase C
    - Stimulate neurotransmitter release
    - Neurons fire in the absence of an appropriate environmental stimuli
  - Lead mimics calcium
    - Calcium is critical to nerve signal transmission
    - Calcium enters neurons during depolarization
    - Lead blocks calcium channels

Lead and the Brain

- Net effect
  - Lead stimulates nerves to fire in a more stochastic fashion
  - Lead also inhibits neurotransmission (both appropriate neurotransmission and inappropriate neurotransmission)
- Makes it hard to think/concentrate
- Changes the underlying synaptic architecture, making it less efficient

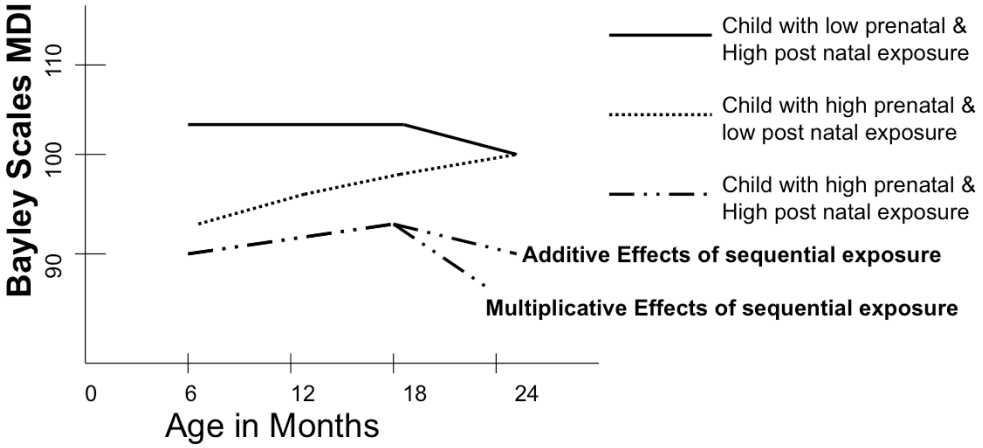
Childhood Lead Poisoning

- Lead exposure introduces noise to the process of synaptic pruning
  - Which synapses are chosen for survival and which regress becomes more random
- Net effect if prolonged- is that the underlying neuronal networks are less efficient.
- Structurally no damage is evident
- Functionally, deficits are measurable.

Plasticity

- The brain's capacity to diminish the effects of toxic insults through structural/functional changes
  - This occurs through the same processes as synaptic selection
  - In other words plasticity allows for new connections to be made which improve function following an insult
- Maladaptive vs adaptive plasticity

Effects of Sequential Toxic Metal Exposure on Neurodevelopment



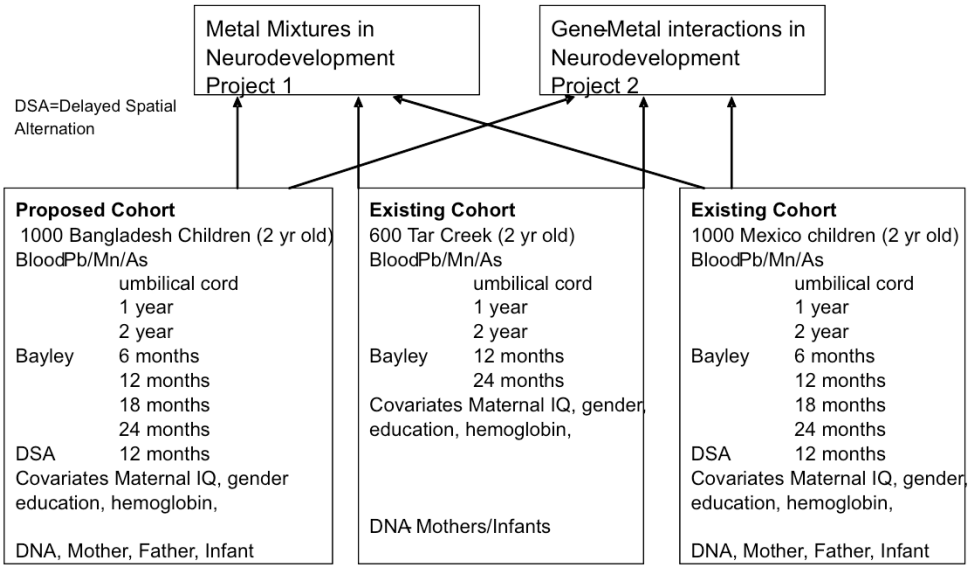
Harvard SRP Project 1: ***Epidemiology of Developmental Windows, Metal Mixtures and Neurodevelopment***

- Uses existing infrastructure/data in 2 ongoing cohorts of neurodevelopment and metals
  - Mexico City, Tar Creek
  - Measure As, Mn, Pb
- Use existing infrastructure on a 3rd cohort designed to assess reproductive health study in Bangladesh on Arsenic
  - Add follow-up and neurodevelopment measures
    - Add Pb and Manganese measure

Design

- Prenatal exposure biomarkers in mother
  - 2nd, 3rd trimester, delivery
- Post natal exposure biomarkers in child
  - 1 and 2 years of age
- Bayley Scales of infant development
  - 1 and 2 years of age
- Either Pooled across cohorts
  - Or as a meta-regression

Outline of Cohort Resource Utilization For this Program



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Plots of MDI scores vs Blood Lead by Quintiles of Blood Mn

Current work

- Finishing Year 2
  - Pooling vs meta-regression
    - Meta data issues
  - Biomarkers
    - All blood
    - Avoids issues that come up if using biomarkers from different matrices
      - Urine vs blood vs hair
        - Different half lives

Other complexities

- Different doses in different cohorts
  - Bangladesh>Mexico>Tar Creek
- Which developmental windows are important for mixtures?
  - Repeated measures of exposure at different life stages
- Interactions may occur across time
  - Prenatal may modify 1 year blood Metal

Summary

- Low level chemical exposures may be more relevant in children
- Low level chemical mixed exposures may also be more relevant in children
- Our program is designed to test 2 and 3 way interactions among Pb, Mn and As
  - 2 way Mn-Pb interactions already demonstrated

Summary

- Chemical mixtures reflect real life
- While complex, understanding the variance in dose response curves requires understanding mixed exposures
- Ignoring mixed exposures will lead to biased effect estimates

Summary

- Like understanding G X E interactions mixtures research requires
  - Large sample sizes
  - Validation in multiple populations
  - Complex analytical approaches

HEALTH IMPLICATIONS OF EARLY LIFE EXPOSURE TO TOXIC METALS

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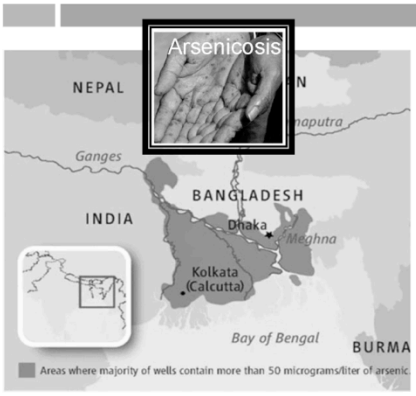
iAs is classified as a Group 1 Carcinogen



SCIENCE VOL 315 23 MARCH 2007
A Sluggish Response to Humanity's Biggest Mass Poisoning

Classified as Group 1 Carcinogen by the International Agency for Research on Cancer (IARC): Chronic exposure results in many cancers: **skin, bladder, lung, liver, prostate and kidney**

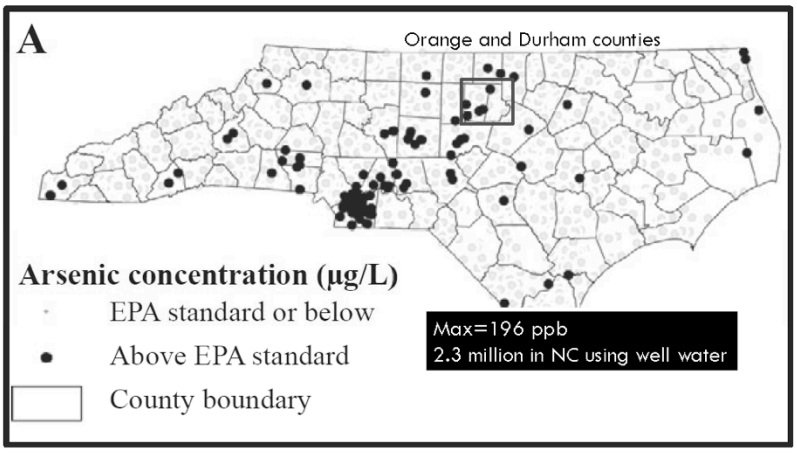
Exposure is associated with non-cancer endpoints: neurological disorders, reproductive effects, cardiovascular disease, diabetes

Highest Priority Contaminant of the ATSDR Agency for Toxic Substances and Disease Registry

iAs continues to poison the drinking water of millions of people around the world

Global nature-almost every continent
40 million exposed to >5 times the WHO limit in South East Asia alone

Arsenic in North Carolina: Public Health Implications



Sanders et al. 2011
Environment International 38 (2011) 10–16

OPEN ACCESS Freely available online

PLoS one

Towards Prenatal Biomonitoring in North Carolina: Assessing Arsenic, Cadmium, Mercury, and Lead Levels in Pregnant Women

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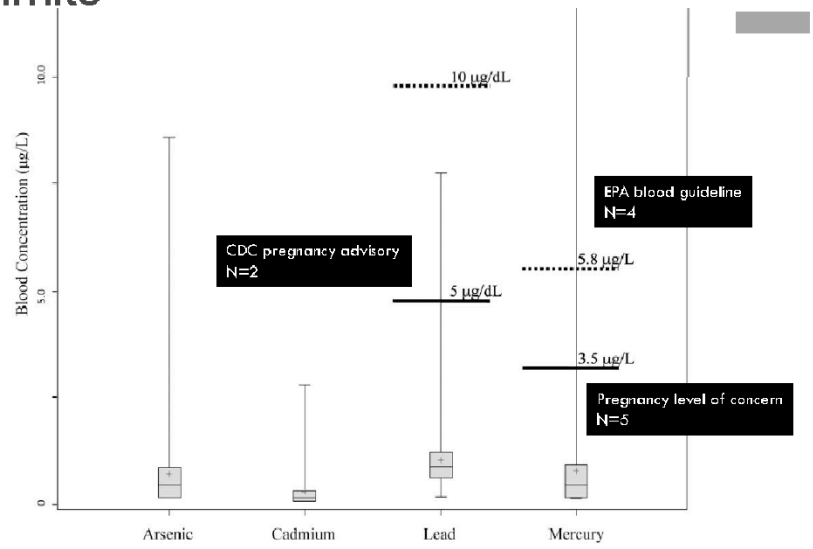
Toxic metals were detectable in pregnant women in North Carolina

Table 2. Detectable levels and geometric averages of the four toxic metals in women.

Blood Metals	n	% Detected	Geometric Mean[a] (range)
Arsenic	210	65.7	0.445 (<0.23–8.58) µg/L
Cadmium	211	57.3	0.181 (<0.11–2.79) µg/L
Mercury	210	63.8	0.453 (<0.23–11.78) µg/L
Lead	211	100	0.890 (0.19–7.72) µg/dL

Some samples exceed acceptable limits



County of residence and race were associated with metals levels

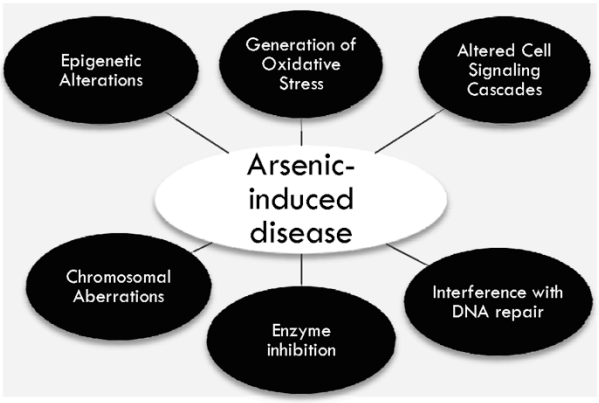
Table 4. Linear regression of age-adjusted maternal race on blood metal levels (Beta coefficient and 95% CI).

	Asian	Hispanic	NHB	NHW (ref)
As	0.51 (0.14–0.88)*	−0.13 (−0.35–0.08)	0.12 (0.02–0.23)*	–
Cd	0.39 (0.01–0.77)*	0.20 (−0.02–0.41)	0.25 (0.15–0.36)**	–
Hg	0.64 (0.23–1.05)*	−0.07 (−0.31–0.16)	0.01 (−0.11–0.12)	–
Pb	−0.01 (−0.24–0.22)	−0.08 (−0.21–0.05)	−0.07 (−0.13–0.00)*	–

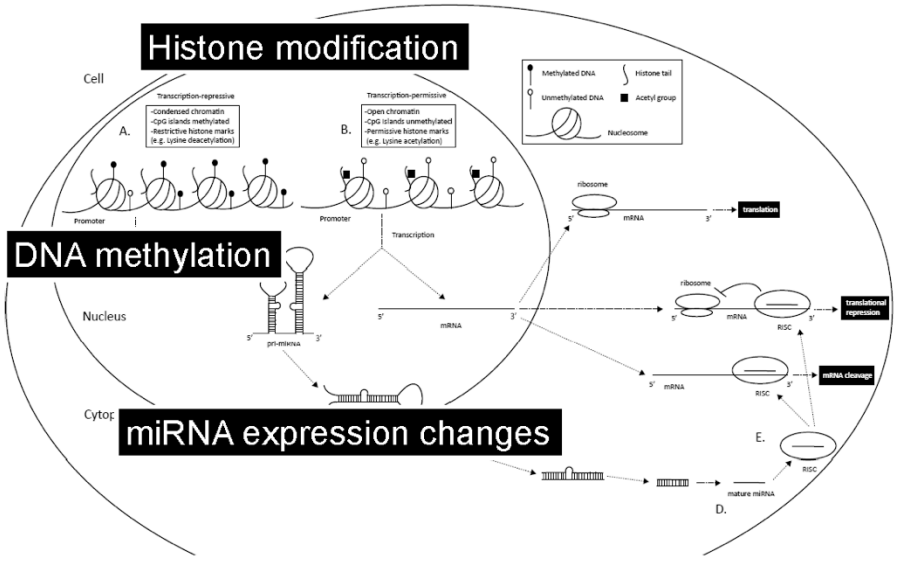
NHW served as the referent group.
*p<0.05;
**P<0.001;
Metal levels were log-transformed.

Complications in associating arsenic with cancer endpoints

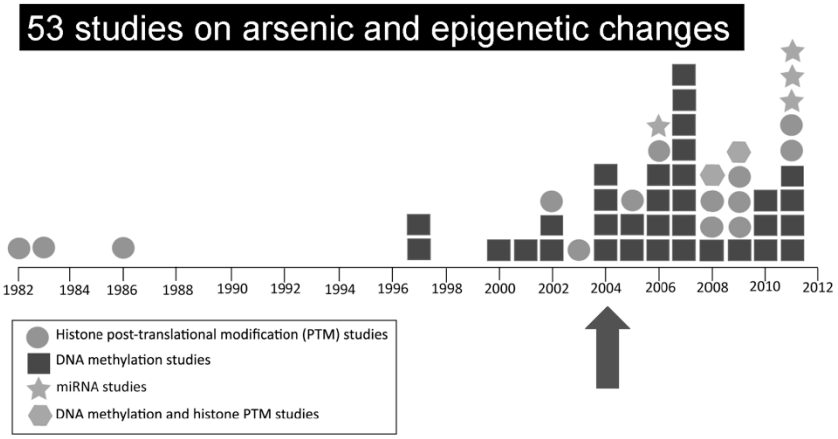
- iAs is not a point mutagen
- iAs is generally negative in standard animal carcinogenesis studies
  - Research supports complex mode of action



Three primary epigenetic modifications-influencing gene regulation

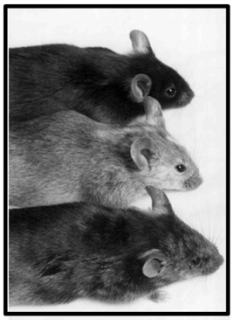


Increasing efforts to identify the role of epigenetic modifications in iAs-induced disease



in utero exposure to iAs in rodents- alarming findings

In utero exposure is associated with adult onset disease



exposure to arsenic during gestation results in 5-fold increase in hepatocellular carcinomas

Gene expression changes in livers of offspring exposed to arsenic *in utero* when reach adulthood

DNA methylation changes in target tissues-ER-α showed hypomethylation

Waalkes, M. P. et al *Toxicol Appl Pharmacol*, **198**. 377-384 (2004b).
Waalkes, M. P., et al, *Journal of the National Cancer Institute*, **96**. 466-474 (2004a).
Xie, Y., et al, *Toxicology*, **236**. 7-15 (2007).

Prenatal iAs exposure in humans: alarming findings

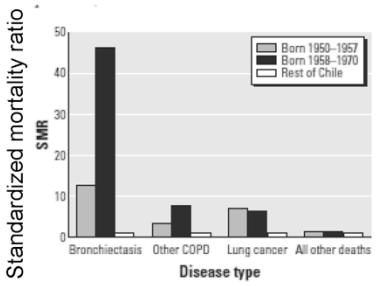


Figure 2. COPD SMRs for Antofagasta/Mejillones for individuals 30–49 years of age, pooled.

Long term health effects

Increased mortality from **liver** and **lung** cancer from prenatal and early childhood arsenic exposures

(Liaw et al., 2008; Smith et al., 2006).

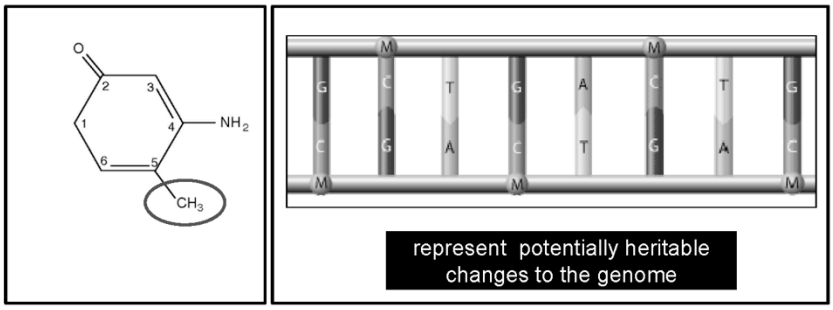
Prenatal exposure in humans and adult disease

Adult disease from early life exposure

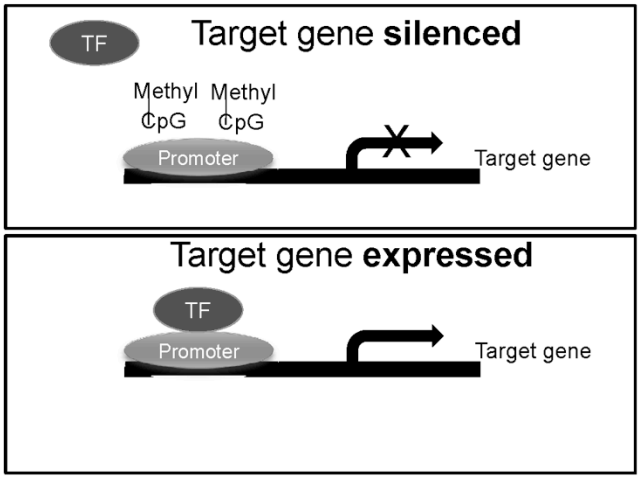
increased evidence for a role of
epigenetic dysregulation
in arsenic-induced disease

Epigenetic modification of interest: DNA methylation of cytosines

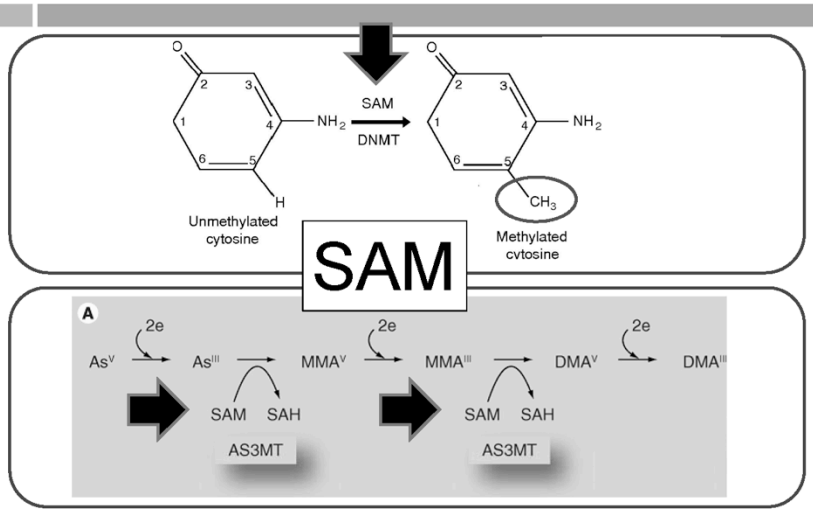
Methylation of Cytosine tends to occur at CpG sites
CpG sites are enriched in islands



DNA methylation at promoter regions can impede target gene expression

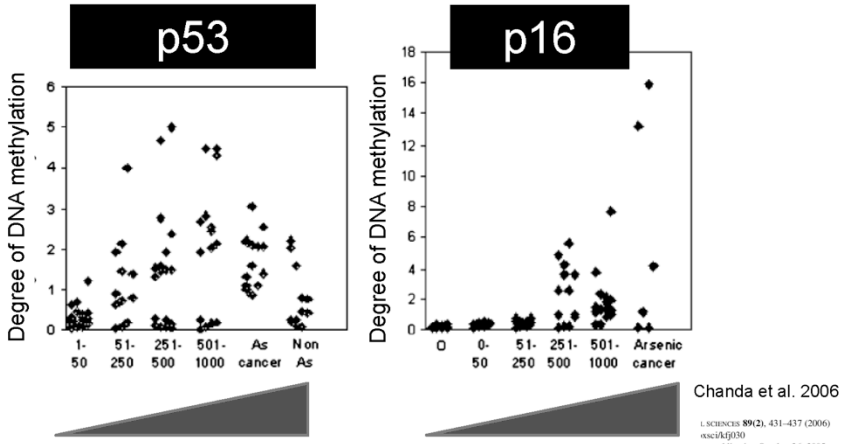


Putative mechanism of arsenic-induced changes to DNA methylation



Global methylation versus gene specific methylation-tumor suppressors

Tumor suppressors p53 and p16 show increased methylation in humans exposed to arsenic



Chanda et al. 2006

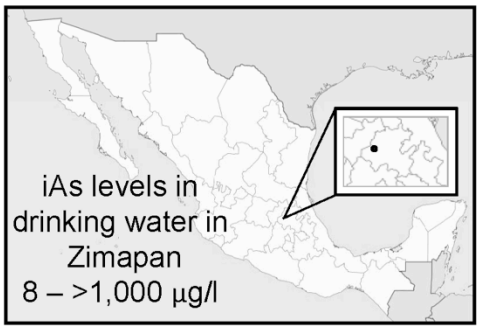
l. SCIENCES **89(2)**, 431–437 (2006)
oxsci/kfj030
SS publication October 26, 2005

How different are the

methylated genomes

of healthy individuals versus individuals with signs of arsenicosis??

Study Site in Zimapan, Mexico
Established high levels of iAs in water



Sites of Funded Studies of Dr. Styblo

Zimapan, Mexico is an area of endemic arsenic with **established high levels** in drinking water affecting the study site (Valenzuela et al. 2005, Del Razo).

Individuals in Zimapan, Mexico show signs of arsenicosis

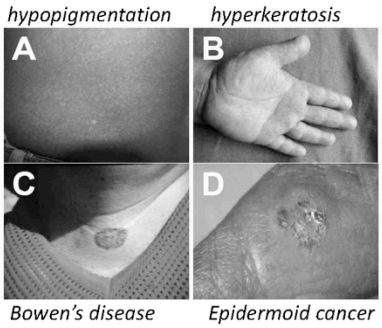


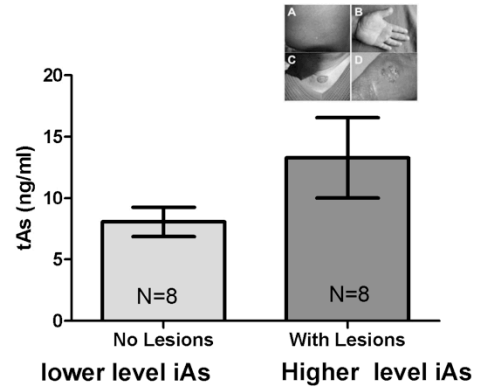
Photo courtesy of M. Styblo

The association of **inorganic arsenic exposure and skin lesions** is established in Zimapan Mexico (Valenzuela et al. 2009).

Study Population characteristics in Zimapan Mexico

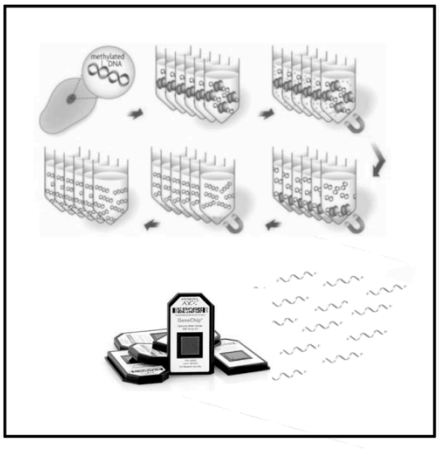
	N (mean)	% (SD)
Population	147	
Female	109	74.2
Age	(29)	(16.3)
Water consumption (L/day)	(1.8)	(0.8)
Skin lesions	50	34.0

A subset of females selected for epigenomic analysis



- Blood was drawn for lymphocyte DNA extraction
  Apply methylation technology to DNA

Using a genome-wide DNA methylation technology to assess epigenetic alterations



Methylated CpG Island Recovery-Chip assay

Methyl binding complex MBD2b/MBD3L1

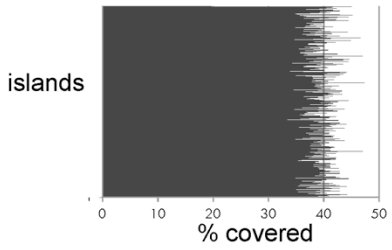
- Affymetrix Human Promoter 1.0R arrays
- 4.6 million probes representing over 14, 000 human promoter regions with islands
- translating to ~59 percent of annotated CpG islands

Comprehensive assessment of CpG islands

4.6 million sites

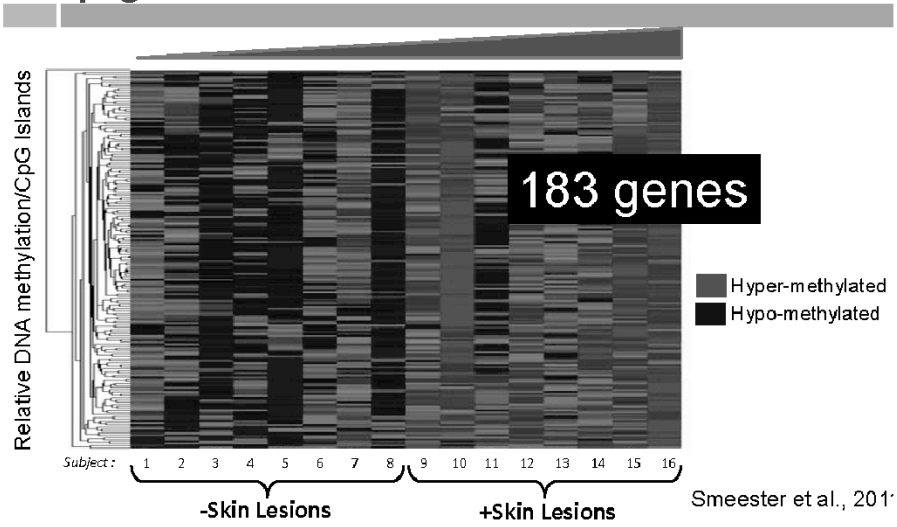
Human Genome Assembly 18

14, 362 CpG islands



Comparative analysis
between individuals with
lower level exposure (no skin lesions)
and higher level exposure (skin lesions)

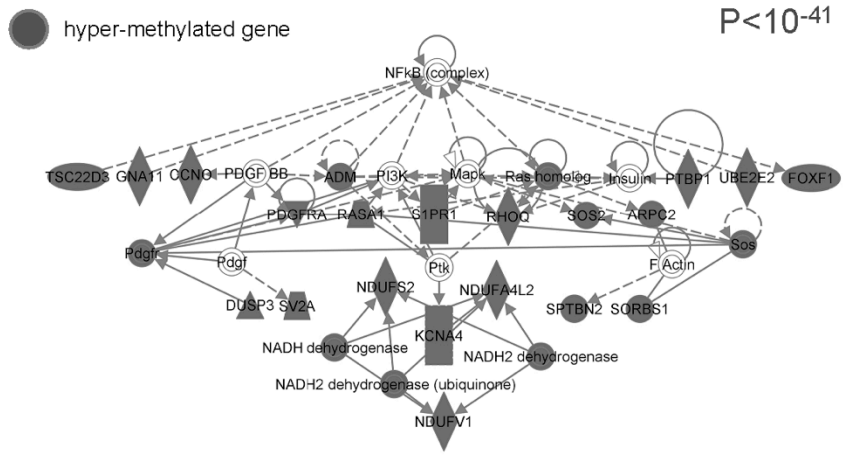
Arsenic-induced changes to the epigenome



Smeester et al., 201

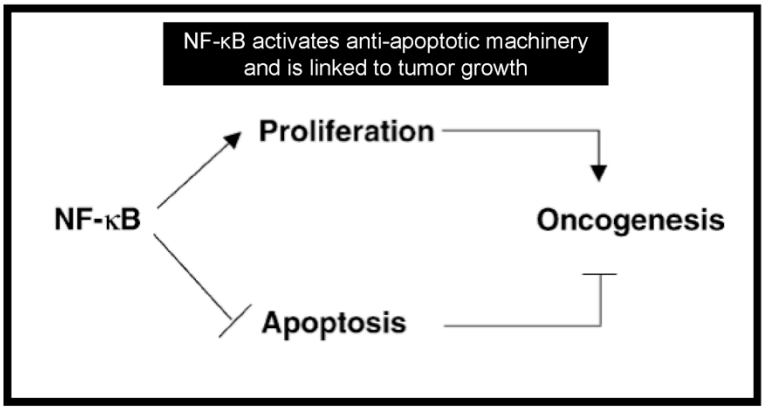
Is there an enrichment for
biological pathways
that are
epigenetically altered ?

Epigenetic changes of members of the Nuclear Factor-kappa Beta pathway



Smeester et al., 2011

NF-κB is a key regulator of oncogenesis



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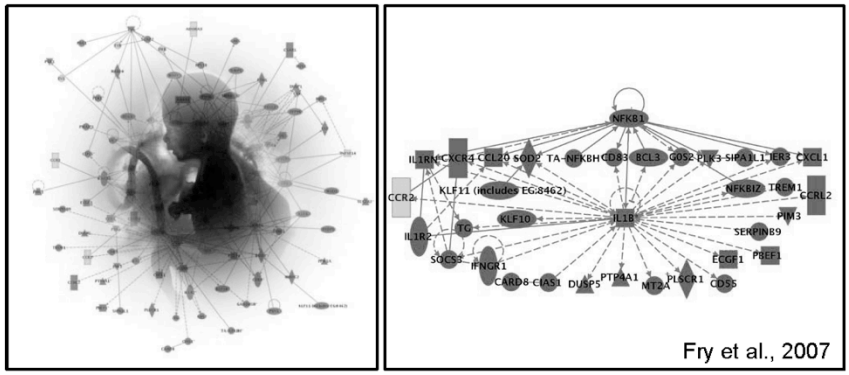
In utero exposure to arsenic and genomic dysregulation of the NF-KB pathway



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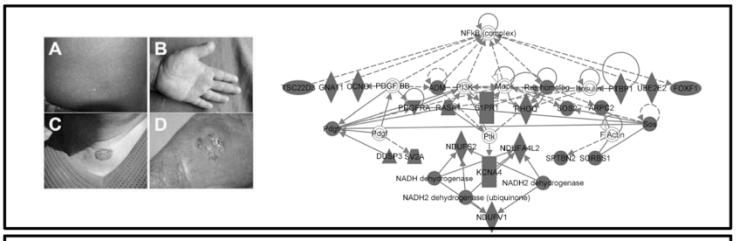
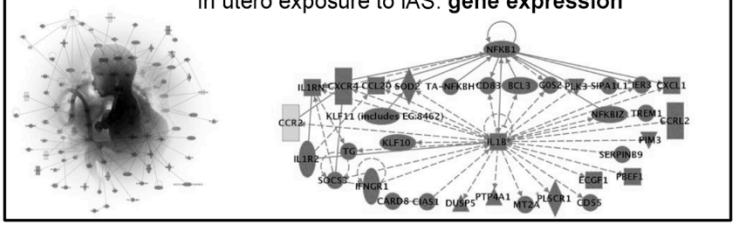
Common pathway perturbation by iAs

Adult disease associated with iAS exposure: **DNA methylation**



In utero exposure to iAS: **gene expression**



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Are there tumor suppressors that have altered promoter methylation?

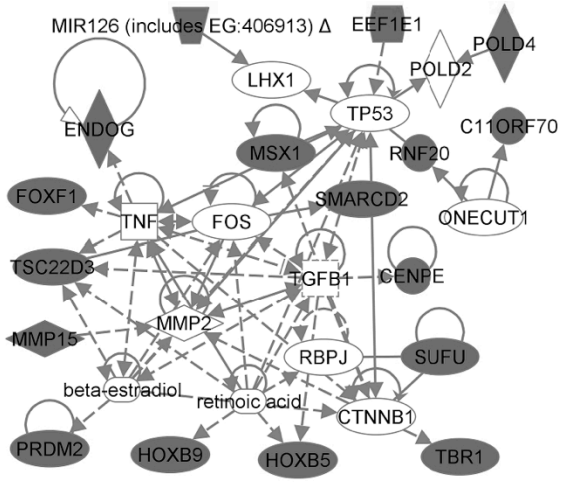
64

Gene Symbol	Entrez Gene Name	Associated Disease/ altered pathway
C11orf70	chromosome 11 open reading frame 70	testicular cancer[1]
CENPE	centromere protein E, 312kDa	hepatocellular carcinoma[2], type 2 diabetes[3]
EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	p53 inactivation[4]
ENDOG	endonuclease G	hepatocellular carcinoma[5]
FOXF1	forkhead box F1	breast cancer cell lines and invasive ductal carcinomas[6]
HOXB5	homeobox B5	lung cancer cells[7]
HOXB9	hor	
MIR126	mic	rs[8]
MMP15	ma	
MSX1	msh	ncer
POLD4	pol	
PRDM2	PR	
RNF20	ring	proto-oncogene suppression[13]
SMARCD2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	prostate cancer[14]
SUFU	suppressor of fused homolog	altered hedgehog signaling[15]
TBR1	T-box, brain, 1	breast cancer[16]
TSC22D3	TSC22 domain family, member 3	antiproliferative role in lymphocyte regulation[17]

17 tumor suppressors
Silencing associated with
tumor development

65

Significant interaction of 17 tumor suppressors

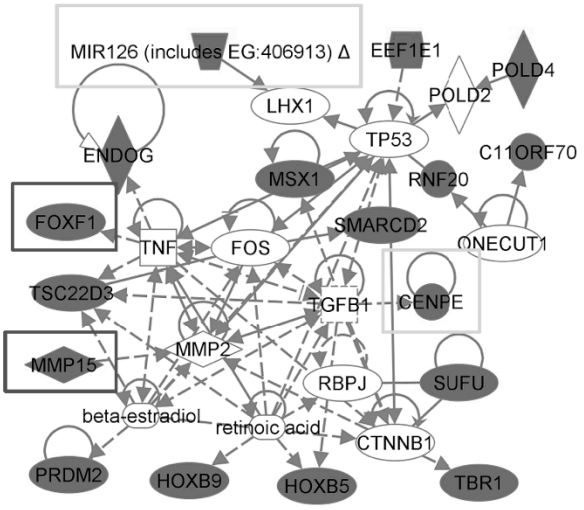


$P<10^{-15}$

Smeester et al., 2011

66

Significant interaction of 17 tumor suppressors



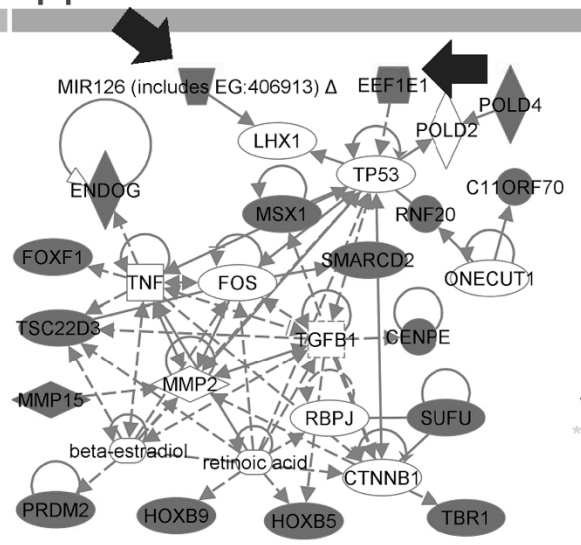
$P<10^{-15}$

* **Cardiovascular disease**

** **Diabetes**

Smeester et al., 2011

Significant interaction of 17 tumor suppressors

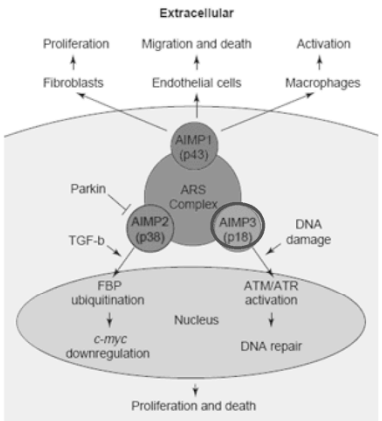


$P<10^{-15}$

* **Cardiovascular disease**
** **Diabetes**

Smeester et al., 2011

EEF1E1/AIMP3 controls p53

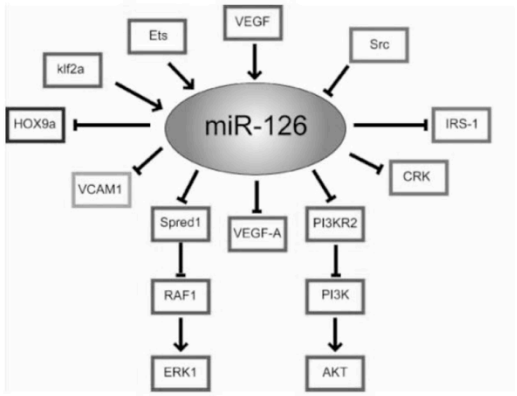


aminoacyl-tRNA synthetase (*ARS*) *complex*

- Silencing of AIMP3 impairs activity of ATM/ATR to activate p53
- We have identified increased promoter methylation of AIMP3 in individuals with arsenic poisoning

miR126: A New Player in Cancer

Mini-Review Meister and Schmidt
TheScientificWorldJOURNAL (2010) 10, 2090–2100
ISSN 1537-744X; DOI 10.1100/tsw.2010.198



- Angiogenesis and vascular integrity
- Cell proliferation, migration and invasion
- Inflammation
- Cell differentiation

miR126: A New Player in Cancer

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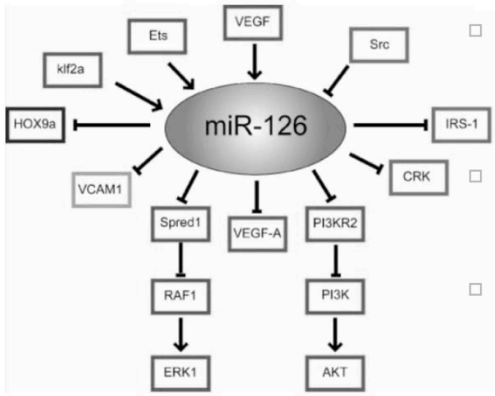

- Silencing of miR-126 is associated with numerous cancers:
- lung, stomach, cervix, bladder, prostate, colon
- We have identified increased promoter methylation of miR126 in individuals with arsenic poisoning

Systems Biology Applied to Samples from iAs-Exposed Humans

- Toxic metals are poisoning individuals around the globe, including populations in North Carolina
- Many genes (182) are **hypermethylated** in individuals exposed to arsenic and with signs of arsenicosis
- **NF-kB pathway** is epigenetically altered in individuals with arsenicosis and altered in newborns exposed to arsenic
- These are enriched for a **tumor suppressor complex** hypermethylated in individuals with arsenicosis (new targets identified including miR-126)

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