Pulmonary Biomarkers Based on Alterations in Protein Expression Following Exposure to Arsenic

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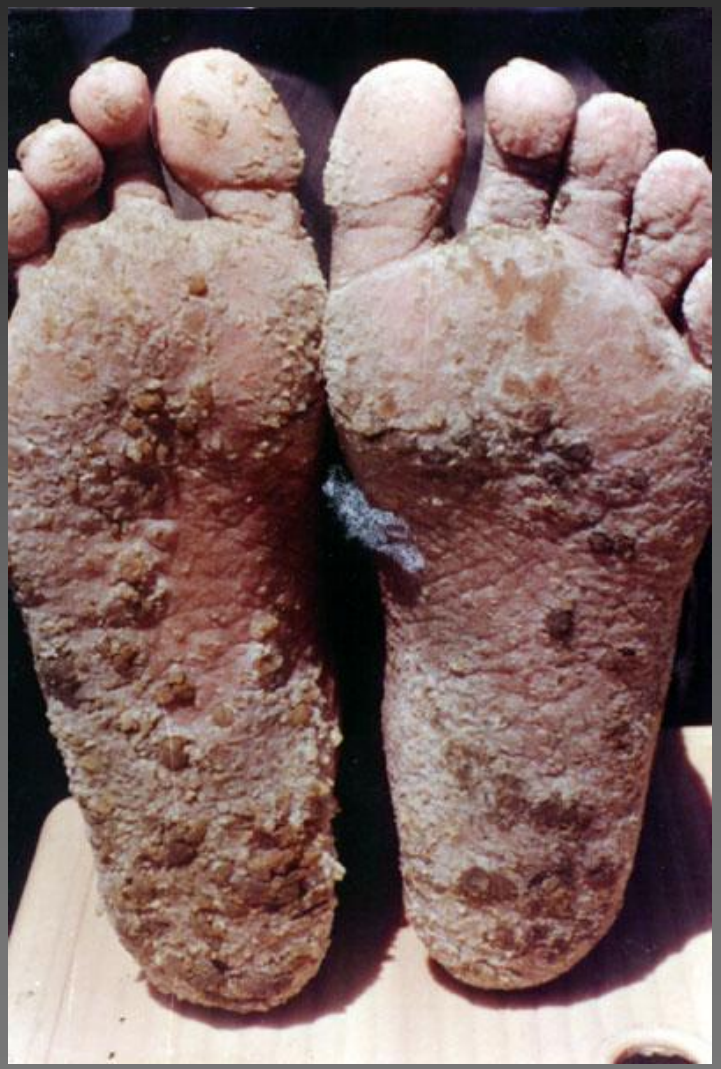
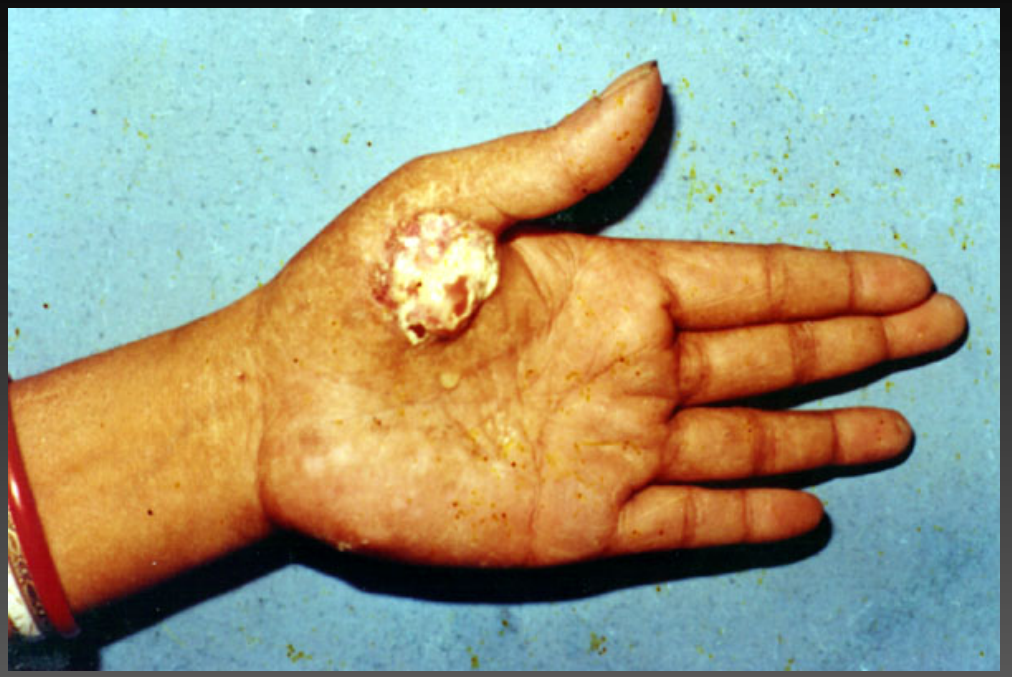
Introduction

- Arsenic is widespread in the environment
  - Inhalation
  - Water

Epidemiological studies implicate arsenic as a bladder, skin and lung carcinogen.





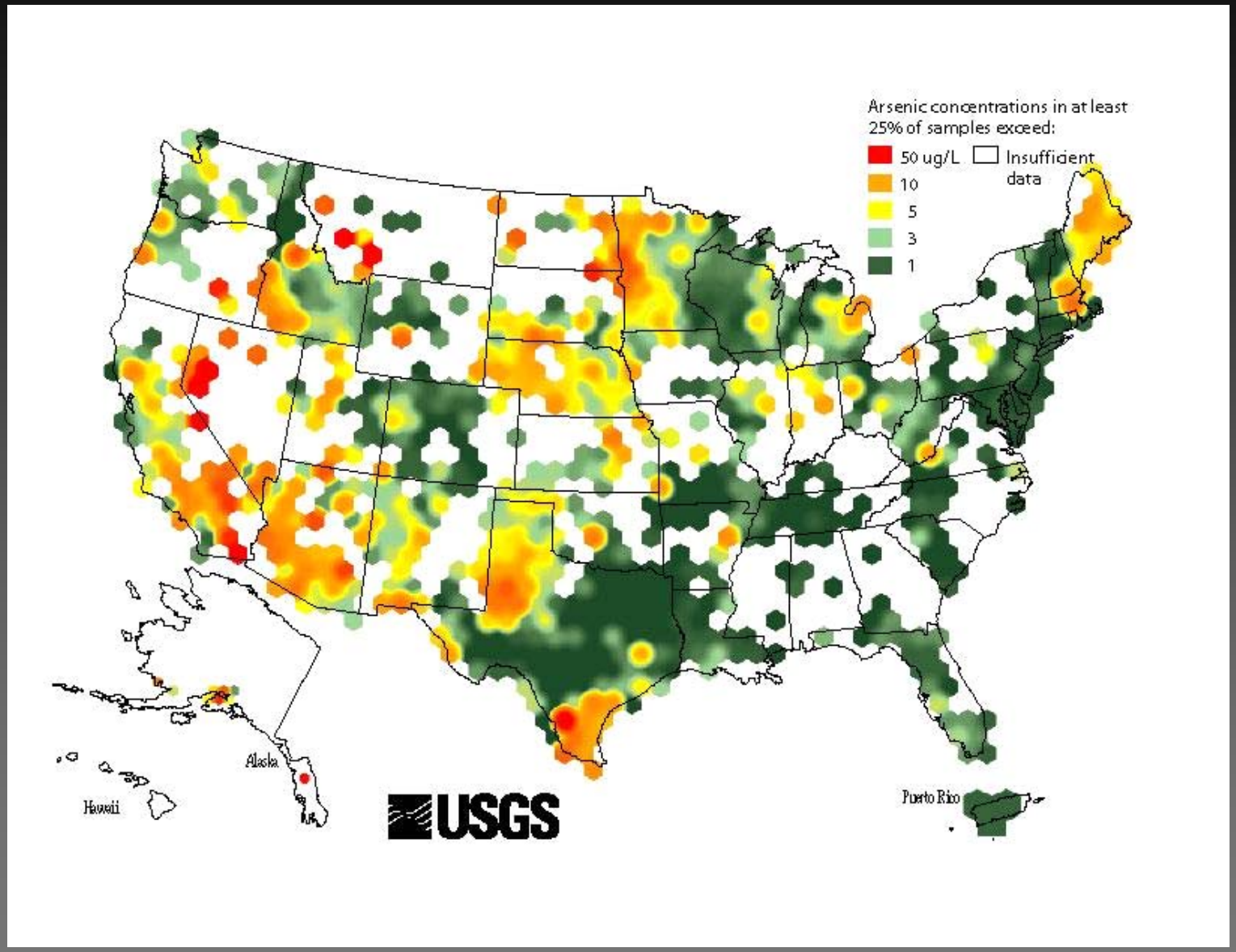
Other health effects include:

- hyperkeratosis
- atherosclerosis
- diabetes
- COPD

Chronic ingestion of arsenic

- Majority of the exposure is from ingestion
- Lung is a target for ingested arsenic
  - Cancer
  - Chronic bronchitis
  - Bronchiectasis
- Examine chronic exposure to environmentally relevant doses of arsenic in drinking water

Drinking water exposure is the major route of exposure.

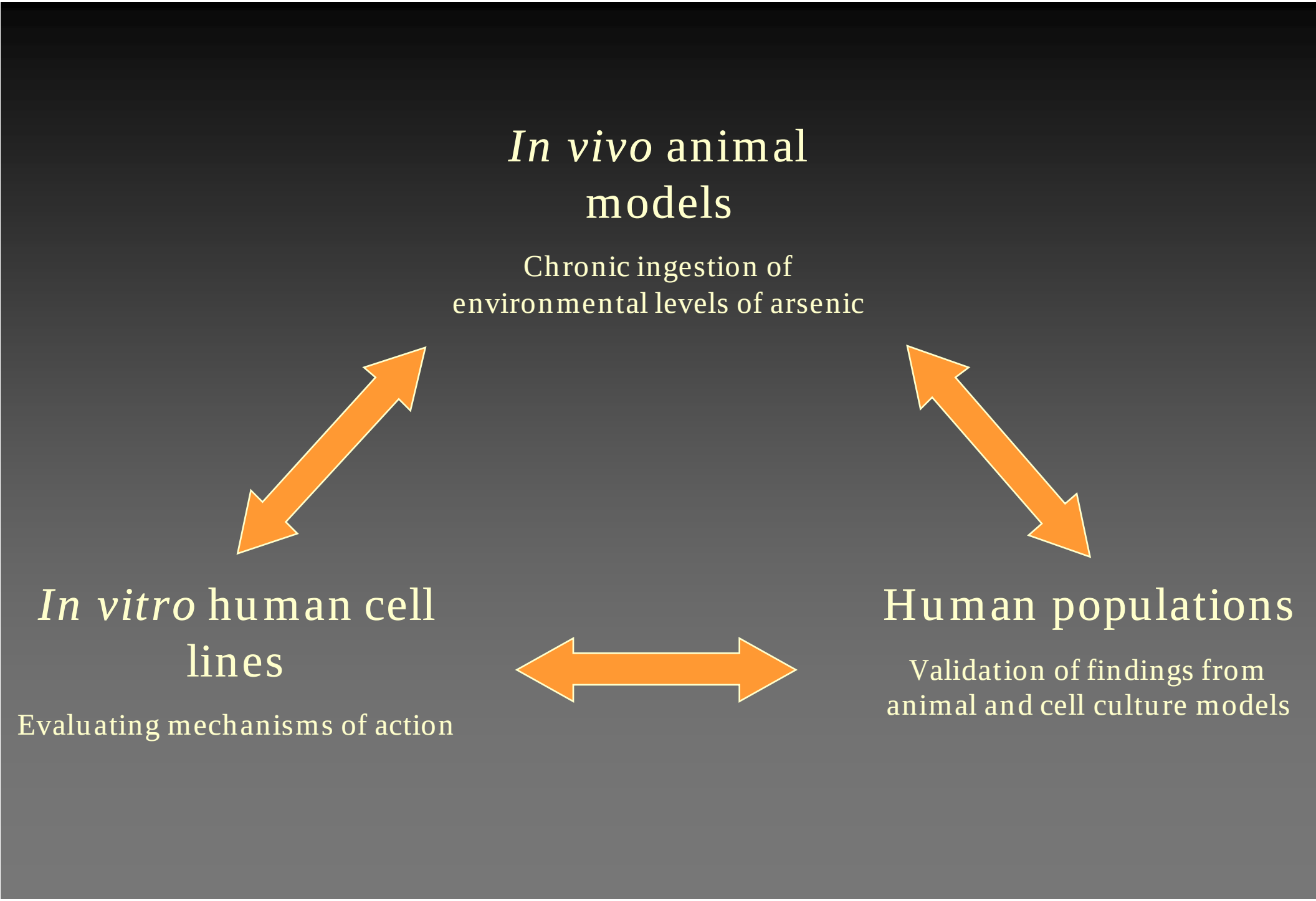


OVERALL OBJECTIVES

- To determine the modes and sites of action following chronic exposure to environmentally relevant levels of arsenicals
- Develop biomarkers of effect and exposure that can be used to study human populations

Background and Objectives

- Identify protein biomarkers that are related to the effects of the toxicant
  - Use a proteomics approach
  - Difficult in humans because of variability and low abundance
- Biomarkers should be obtainable through minimally invasive techniques
  - Blood, urine, other body fluids
- Biomarkers should be organ specific
  - Lung lining fluid (induced sputum)



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Arsenic-induced alterations in protein expression

Arsenic ingestion

- Adult male mice were given arsenic in the drinking water (0, 10 or 50 ppb)
- Animals were exposed for up to 8 weeks
- Whole lung analysis
  - BD Powerblot (Westerns)
- Lung lining fluid
  - 2-D gel and MS protein identification
- Airway epithelial cells
  - Selective digestion and collection of epithelial proteins
  - Evaluated by Westerns or by MS proteomics approaches

Whole lung protein results

Protein isolated from whole lungs of mice that were exposed to 50 ppb for 8 weeks.

Protein was analyzed by BD Powerblot (~1000 validated antibodies)

Curated data mining program

- Disease
  - Cancer
  - Wound healing
- Process
  - Cell motility

Protein	Change in expression
Acetylcholinesterase	0.30
Hic-5 (TGF-β1 induced transport)	0.28
Rac1	5.82
Syntaxin 8	present in cont - absent in As
CapZ α	0.36
Stat3	0.52
Calretinin	0.20
Caspase-3	0.23
Melusin	0.10
β-Catenin	0.66
4.1N	2.65
DMPK	0.39
Gelsolin	0.52
IKKγ/NEMO	0.58
Nucleoporin p62	0.32
OPA1	0.32
Rab4	2.23
RCC1	2.65

Proteomics analysis of soluble BALF proteins

DOWN REGULATED

RAGE (receptor for advanced glycation end products) (associated with wound healing and MMP-9 expression)

GST omega 1-1 (arsenic metabolizing enzyme)

Alpha-1 antitrypsin (important in development of emphysema)

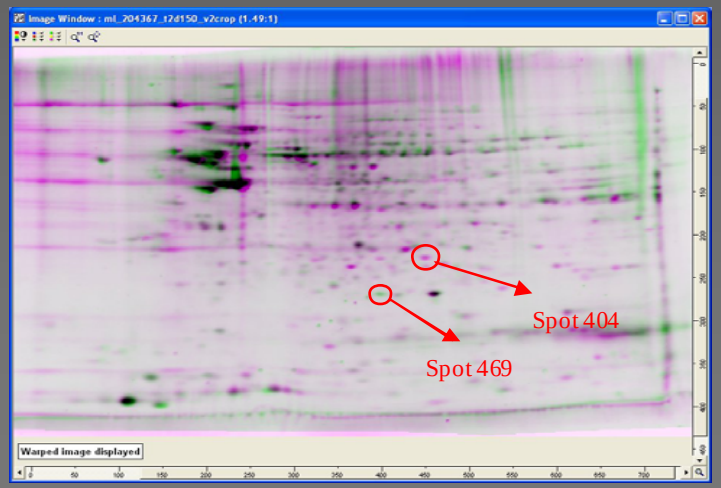
Apolipoprotein A-I and -IV

UP REGULATED

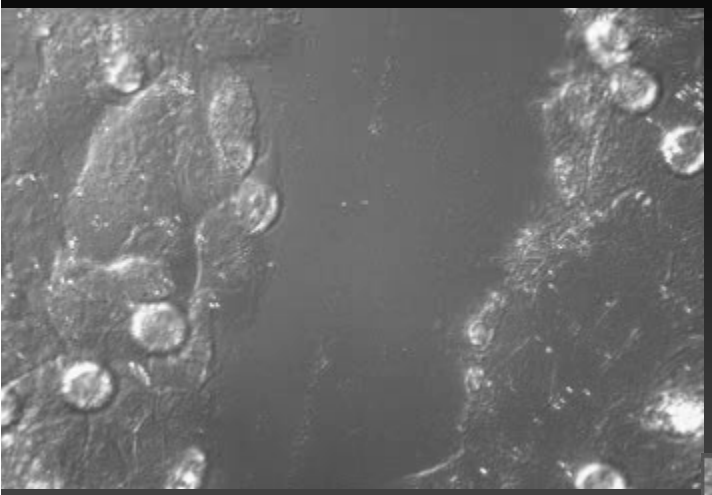
Peroxiredoxin-6

Enolase 1

2D Gel Protein Expression Analysis

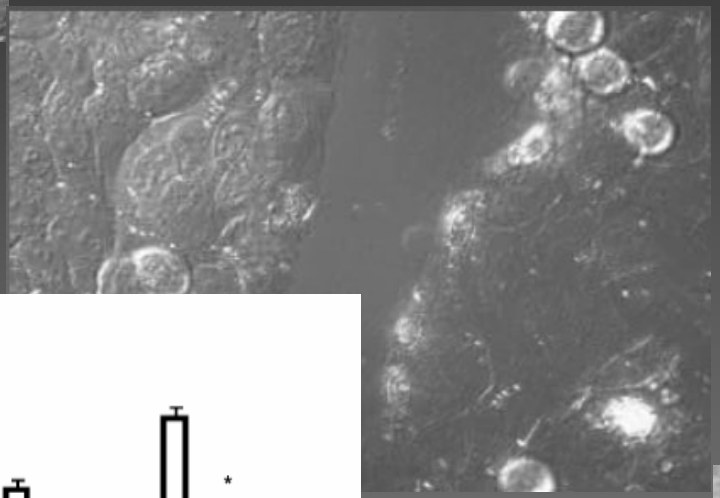


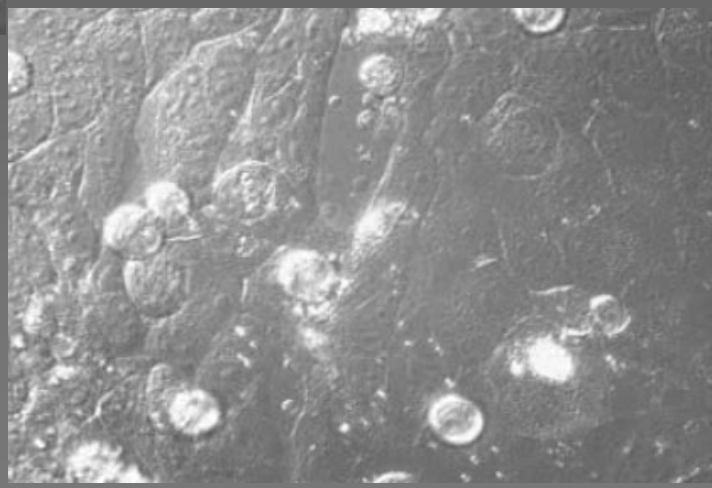
LC-MS/MS MUDPIT

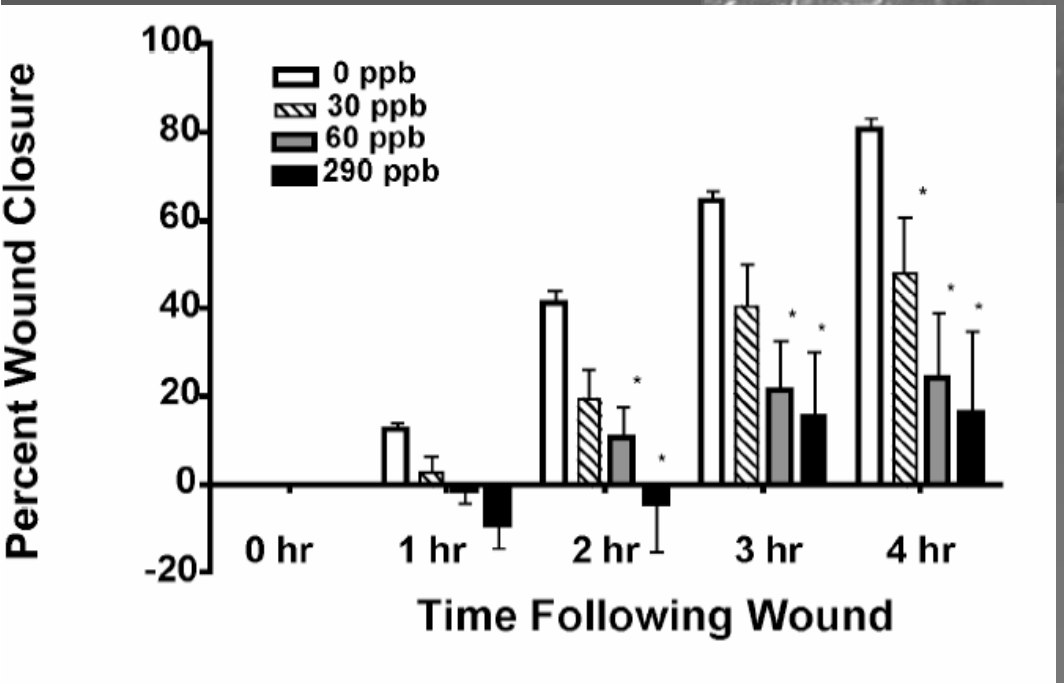


"Scratch wound healing" model

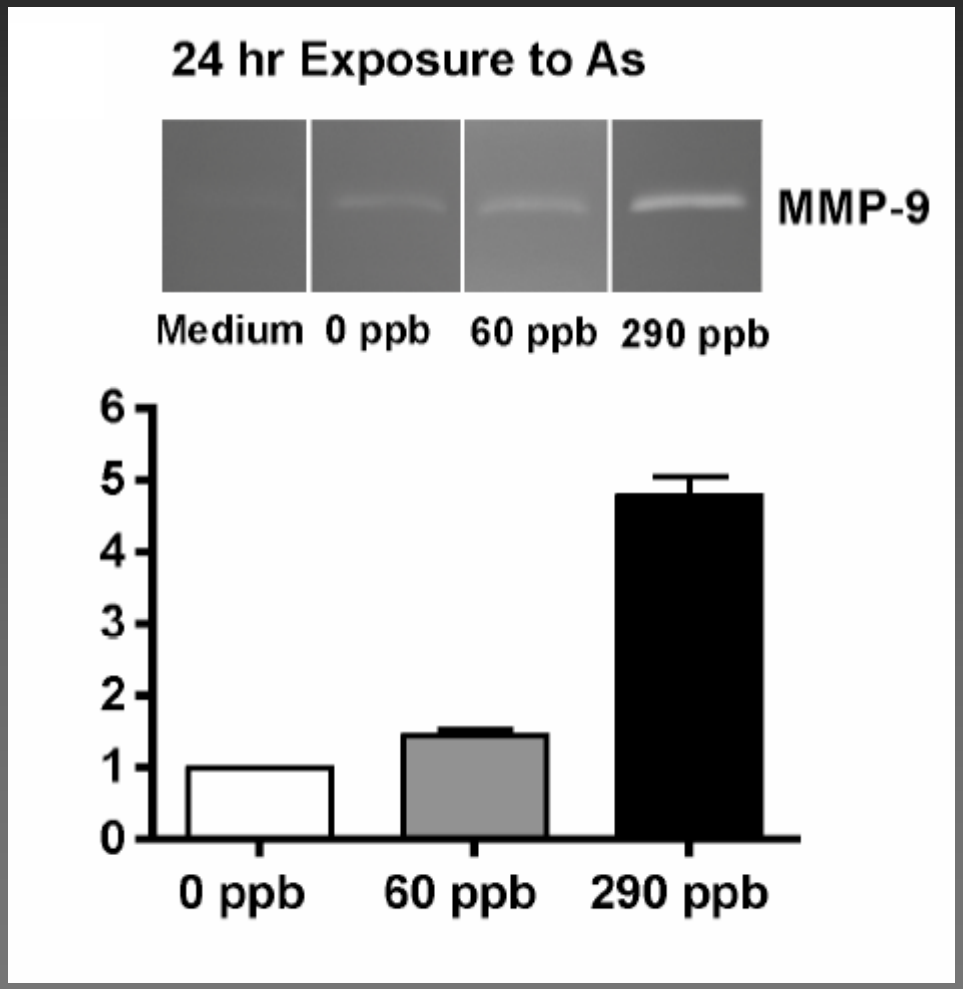
Cells were grown to confluence, exposed to arsenic (60 ppb) for 24 hrs and then plate was scraped to produce "wound"

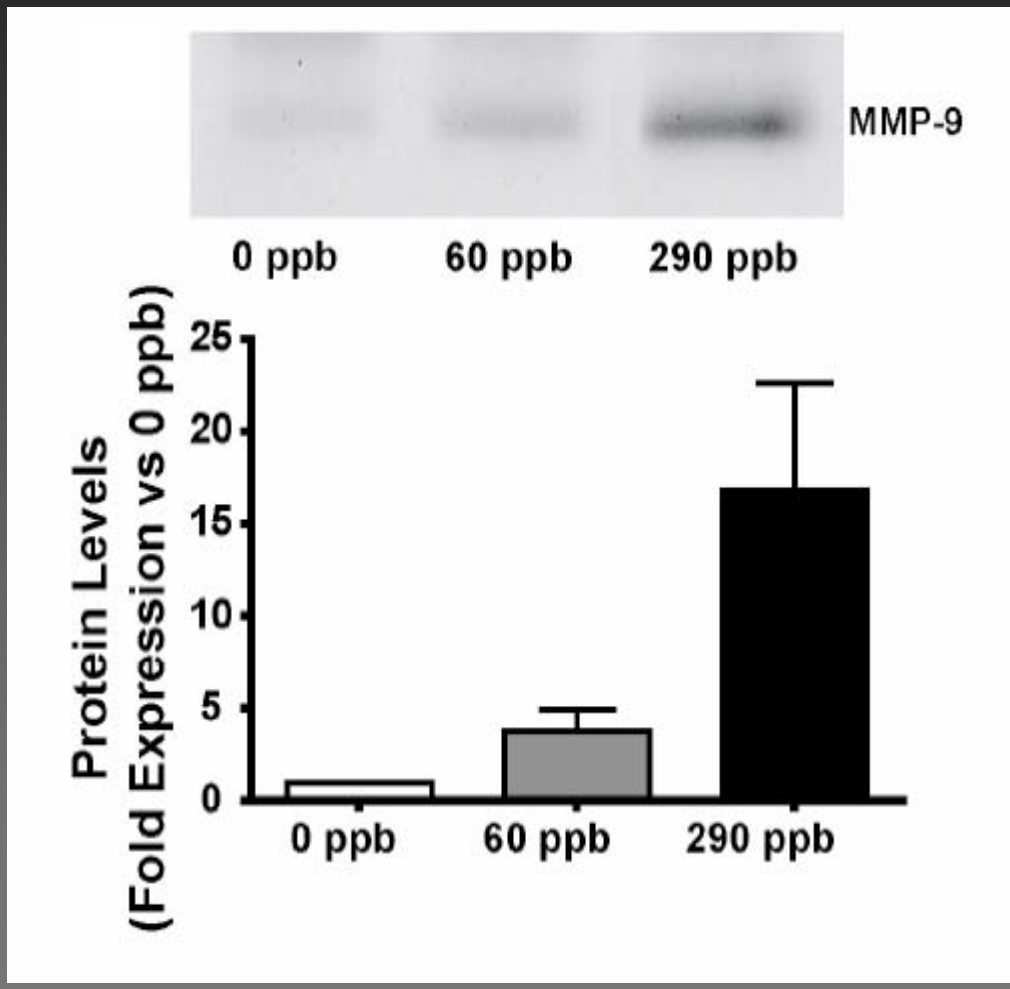






Effect on MMP-9 expression and activity





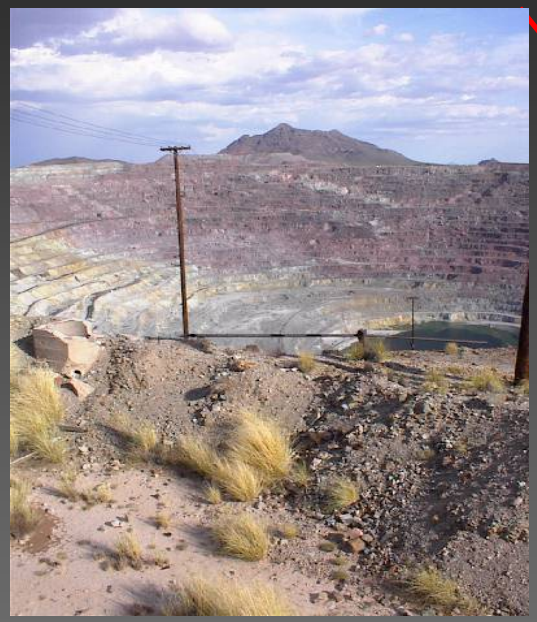
No effect of arsenic on MMP-2 or TIMP-1

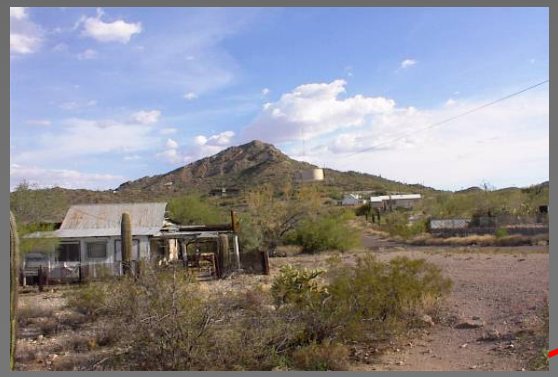
Conclusions

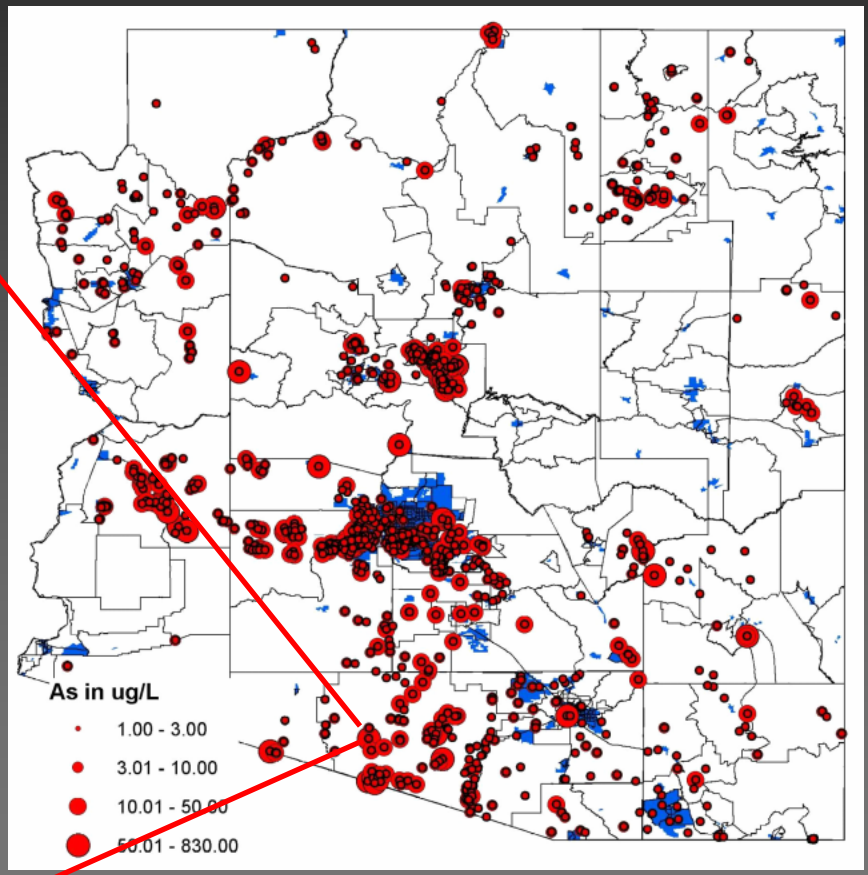
- Eight week exposure of adult male mice to arsenic in their drinking water results in
  - alteration of proteins associated with cell motility and wound healing
- *In vitro* exposure to arsenic leads to
  - decreased wound healing
  - increased expression and activity of MMP-9

Do these changes occur in a human population exposed to arsenic?

Correlation of results in human populations







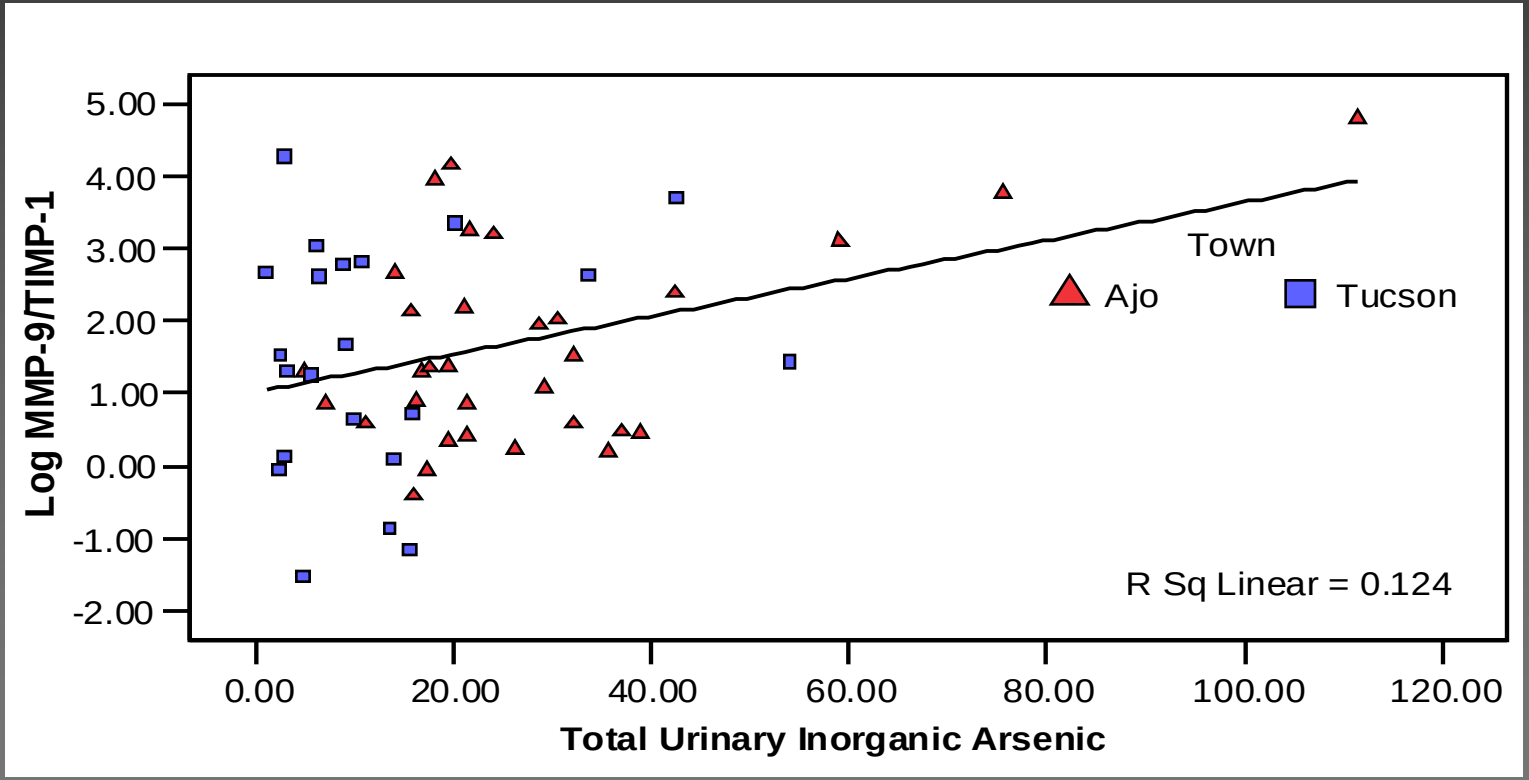
Population

- 73 individuals met inclusion criteria
  - 40 in Ajo, 33 in Tucson
  - Age range 30-92
  - Predominantly female, non-Hispanic white
- No difference between towns in age, gender, race, asthma or diabetes incidence or past smoking history
- Collected
  - First morning void urine to measure arsenic
  - Induced sputum (33 in Ajo, 23 in Tucson)
    - ELISA to measure protein levels
    - MMP-9, RAGE and α-1-antitrypsin

Effect of arsenic on sputum proteins

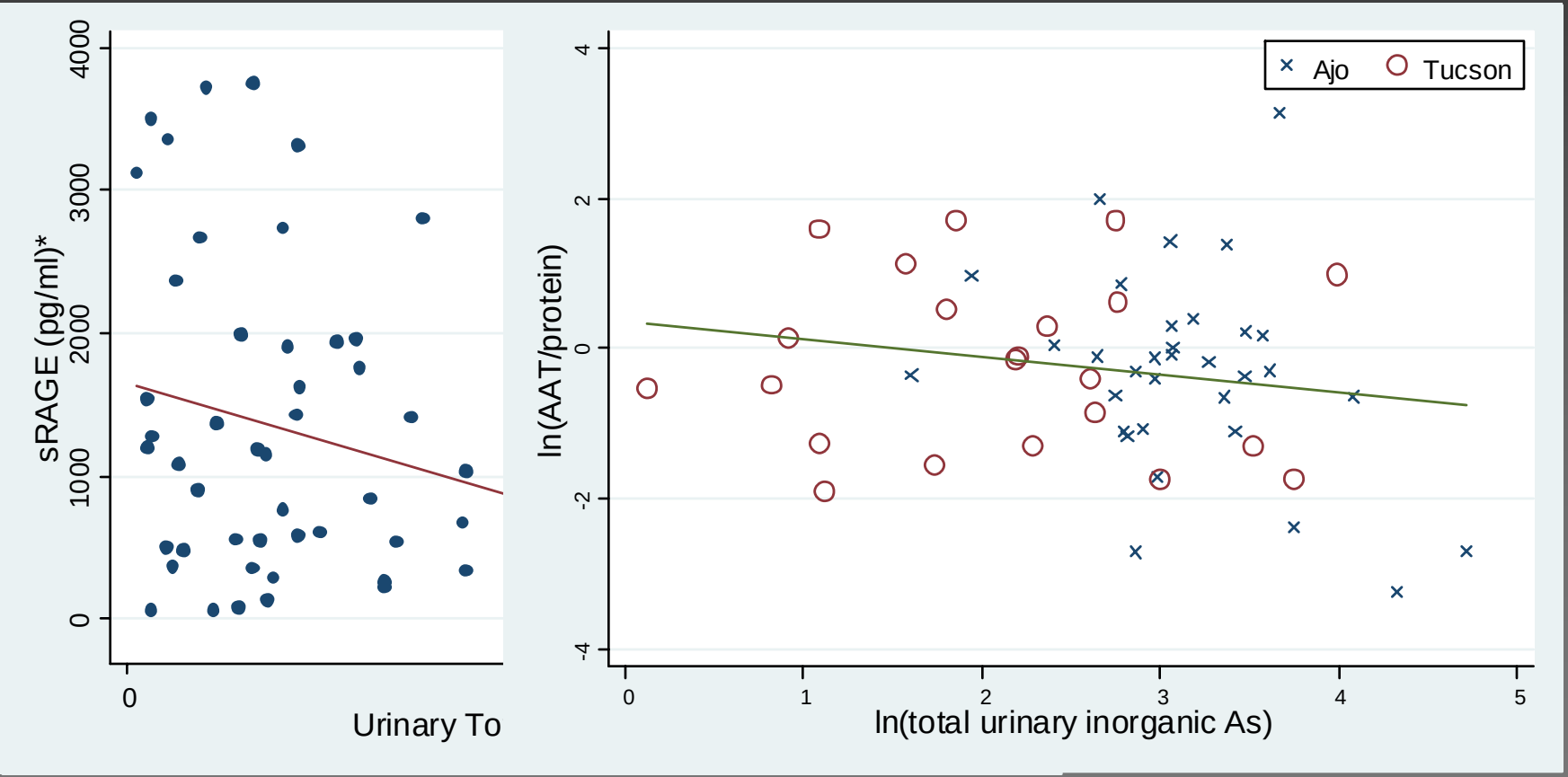
Are changes in induced sputum levels of MMP-9 associated with levels of urinary arsenic?

Levels were measured using ELISA.



Effect of arsenic on sputum proteins

Are changes in induced sputum levels of RAGE and AAT associated with levels of arsenic exposure?



Comparison of models versus human

Models

- Increase in MMP-9
- Decrease in RAGE
- Decrease in AAT

Humans

- Increase in MMP-9/TIMP1
- Decrease in RAGE
- Decrease in AAT

Human data are consistent with results from model systems

Conclusions

- Have identified unique protein biomarkers
  - Associated with wound healing
  - Cancer
- Use of *in vivo* and *in vitro* model systems to direct human studies has been a successful strategy

Future directions

	Condition	
Model	Homeostasis	Repair
In-vitro polarized epithelial cell culture	Confluent culture	Scratch
In-vivo mouse model	No insult	Naphthalene treatment
Human	General population (Sonora, Mexico)	COPD (Arizona, well water use)

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