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Dietary proteins modulates the gene expression in mice chronically exposed to arsenate.

Barbara Casati[1], Graziella Cimino-Reale[1], Roberta Brustio[1], Angelo Collotta[1], Raffaella Folgieri[2], Libero Clerici[1] and Erminio Marafante[1].
[1]Physical and Chemical Exposure Unit, Institute for Health and Consumer Protection, Joint Research Centre, 21020 Ispra, Italy
[2]Università degli Studi di Milano, Dipartimento di Scienze dell'Informazione, 20135 Milano

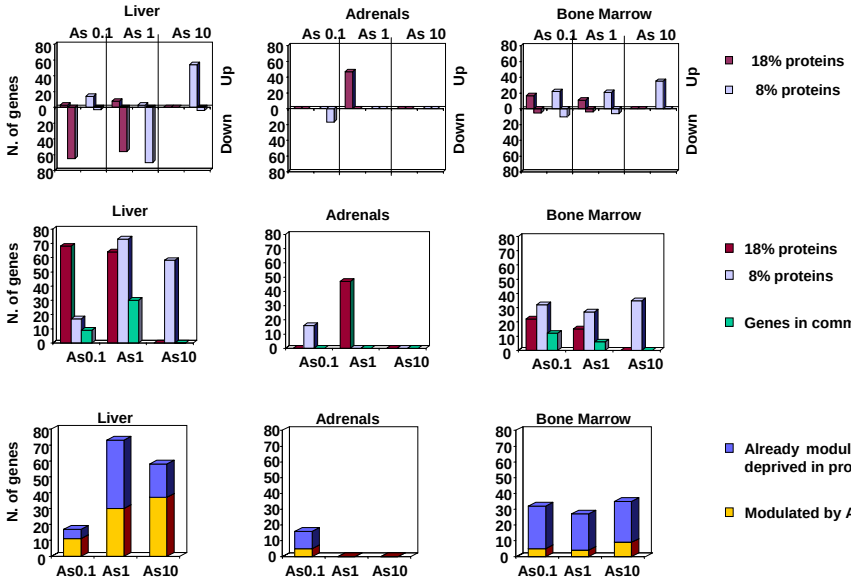
Introduction

In the frame of a project on the assessment of risk modifying factors modulating the health effects of environmental chemicals we are developing a toxicogenomic approach using an *"arsenic in mice"* experimental model, considering multistressors exposure, genetics, age, levels and length of exposure, etc.
In the present study, we used cDNA Macroarrays to investigate the effects of low protein intake on the expression of 1185 cancer-related genes in the liver of male and female mice transplacentary exposed to different levels of arsenate in drinking water during gestation and developmental age.

Materials and Methods

√ **Experimental animals***:* female CD-1 mice.
√**Treatment***:* *mothers:* female adult mice were fed either with standard rodent chow (18% protein rich) or with a protein deprived one (8%). Both groups of animals were also exposed to different concentrations of sodium arsenate in drinking water (0.1 mg As/L; 1 mg As/L; 10 mg As/L) for 10 days before mating and during gestation and the feeding period. *Offspring* were fed with the two different chows and exposed to different concentrations of arsenate in drinking water according to treatment of their mothers, up to two months of age.
√ **Total RNA Extraction** from tissues using RNeasy Qiagen kit.
√ **Retrotranscription and cDNA Labeling***:* Super Script III Reverse Transcriptase (Invitrogen), ^{33}P-dATP (Amersham), Mouse Cancer 1.2 CDS primer mix (Atlas™, Clontech, U.S.A.) and 1 μg total RNA.
√**cDNA Hybridization** on Mouse Cancer 1.2 Array (Atlas™, Clontec, U.S.A.) membranes (16 hours at 50°C).
√ **Image Acquisition***:* Cyclone instrument (Packard Camberra Instruments, U.S.A.) after exposure for 21 hours on a phosphor-image screen (Packard).
√ **Image Analysis***:* Atlas Image software (Atlas™). The pixel intensities of each spot were normalized as percentages of total pixels on the membrane.
√ **Data Analysis***:* Significance Analysis of Microarrays (SAM).

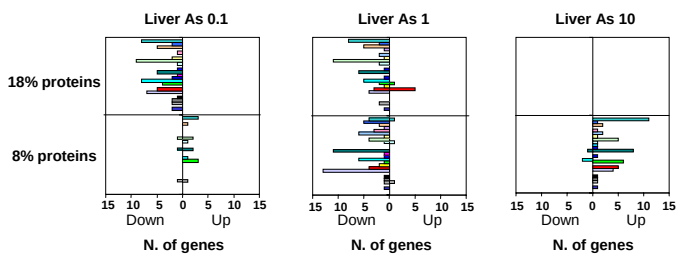
Results



Combined exposure:
Diet rich (18%■) or poor (8%□) in protein influenced the expression of different numbers of genes in tissues of mice.
Only less than 50% of the genes were modulated by arsenate (■), the major part being dependent on diet.

The reduction of protein content in diet affected the gene expression in untreated mice when compared to normal diet fed animals (■).
Moreover, only a fraction of genes modulated by arsenic (□) were not already affected by protein deprivation in control mice.

The modulation of gene families depended on proteins level in the diet. Here the liver as an example.



Functional gene families

- Adhesion
- Apoptosis
- Cell cycle
- Cell surface antigens
- Cytoskeleton/motility
- DNA synthesis/recombination/repair
- Extracell transporters/carriers
- G.F.,Cytok.,Chemokines (Cell signaling)
- HSP/chaperons
- IL&IFN (Cell signaling)
- Immune system
- Intracell modulator
- Matrix
- Membrane channels
- Metabolism
- Oncogenes&tumor suppressors
- Post-Translational modification
- Protein turnover
- Receptors
- RNA processing/turnover/transport
- Signaling/extracell communications
- Traffiking/targeting
- Transcription
- Translation
- Transporters (xenobiotic)
- Unclassified

Conclusion

The results of this study support the relevance of dietary factors in modulating the physiological responses in gene expression following chronic exposure to xenobiotics.
In mice chronically exposed to arsenate in drinking water, the modulation of gene expression in different tissues was not only depending on the levels of the xenobiotic under investigation, but mainly regulated by the content of proteins in diet.