Physiological and nutritional factors modulating the gene expression in the liver of mice transplacentary exposed to arsenate.

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Introduction

There are suggestions that inorganic arsenic, a major drinking water contaminant in several countries, could act as a transplacental carcinogen in mice (Walkees et al.2004).
There are also evidences that toxic responses following exposure to arsenic are strongly influenced by nutritional, physiological and genetic factors (Vahter, 2000).

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 a *"arsenic in mice"* experimental model, considering multistressors exposure, genetics, age, levels and length of exposure, etc.

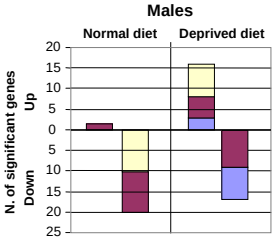
In the present study, we used cDNA Macroarrays to investigate the effects of low protein intake on the expression of 1185 cancer-related genes in the liver of male and female mice transplacentary exposed to different levels of arsenate in drinking water during gestation and developmental age.

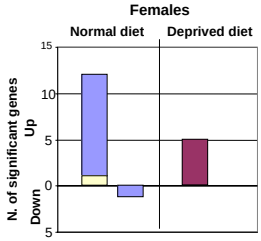
Materials and Methods

√ **Experimental animals***:* male and 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 livers 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 mg 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).
√ **Data Selection Criteria***: **1)*** changes must be ≥ 20% higher/lower than expression in proper control; ***2)*** radioactivity of each spot must exceed 0.1 % of total radioactivity on membrane; ***3)*** these criteria have to be met in at least 3 out of the 4 samples.

Results: differential gene expression at 2 days of age





Males

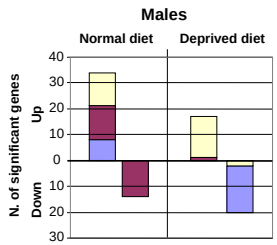
	Normal diet		Deprived diet	
	Up	Down	Up	Down
As 0,1	13		16	2
As 1	13	14	1	
As 10	8			18

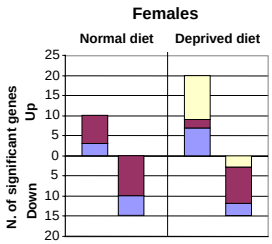
Females

	Normal diet		Deprived diet	
	Up	Down	Up	Down
As 0,1	11	1		
As 1			5	
As 10	1			

In the liver of newborn mice at two days of age, there were significant differences in the gene expression between male and female mice. For both sexes the deprived diet significantly altered the modulation of hepatic gene expression induced by exposure to arsenate.

Results: differential gene expression at 2 months of age





Males

	Normal diet		Deprived diet	
	Up	Down	Up	Down
As 0,1	8			18
As 1	13	14	1	
As 10	13		16	2

Females

	Normal diet		Deprived diet	
	Up	Down	Up	Down
As 0,1	3	5	7	3
As 1	7	10	2	9
As 10			11	3

Major effects on the gene expression were observed in the liver of offspring whose exposure to arsenate in drinking water was continued for other two months after birth. There were strong effects of the proteins deprivation in the diet on the modulation of gene expression induced by arsenic exposure.

Conclusions

The results of this study support the relevance of host factors in modulating the physiological responses following chronic exposure to xenobiotics. In the liver of mice chronically exposed to arsenate in drinking water, the modulation of gene expression was not only depending on the levels and length of exposure, while differently regulated also by sex, age and diet.
The main gene functional gene families modulated by the exposure to arsenate, from in-utero to adult age, covered a wide range of biochemical and physiological regulations, like cell cycle modulation, cell adhesion, apoptosis, xenobiotic metabolism, DNA repair, protein turnover and oncogenes, supporting the needs for coherent and specifically designed studies to assess the effects of long term exposure to low levels of environmental xenobiotics.

Bibliography

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