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Clonogenicity and gene expression modulation in the bone marrow of mice chronically exposed to arsenic and atrazine.

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Introduction

Studies on the relevance of host factors in modulating the physiological responses following chronic exposure to xenobiotics were carried out according to a toxicogenomic model on arsenic in mice. This model is focused on chronic exposure to arsenate given alone or in combination with other xenobiotics, to assess potential "cocktail effects" and related cumulative risks.
The clonogenicity of myeloid progenitors (CFU-GM) and the modulation of gene expression of 1185 cancer-related genes by DNA-macroarrays in bone marrow were used to investigate in male and female mice the combined effects of continuous exposure to arsenate and atrazine in drinking water.

Materials and Methods

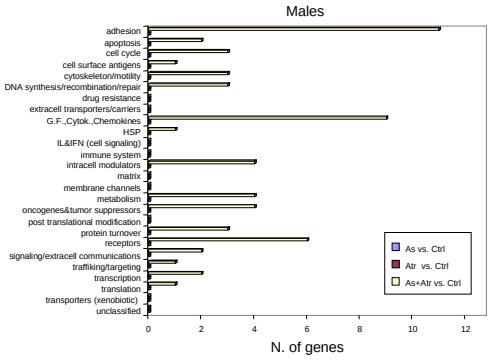
√ **Experimental animals:** male and female CD-1 mice.
√ **Treatment:** Female adult mice were treated with arsenate in drinking water (1 mg As/L) for 10 days before mating and during the gestation. Separate groups of arsenic exposed males and females offspring were exposed for 2 months to 1 mg As/L of additional arsenate (As). Control male and female mice without any treatment were also analysed (Ctrl).
√ **Total RNA was extracted** from tissues using RNeasy Qiagen kit and 1 µg was converted into [^{33}P]-labelled cDNA using Super Script III Reverse Transcriptase (Invitrogen) and ^{33}P-dATP (Amersham), Mouse Cancer 1.2 CDS primer mix (Atlas™, Clontech, U.S.A.).
√ **cDNA Hybridization** on Mouse Cancer 1.2 Array (Atlas™, Clontec, U.S.A.) membranes (16 hours at 50°C).
√ **Image Analysis:** After acquisition by Cyclone instrument (Packard Camberra Instruments, U.S.A.), the images were analyzed by Atlas Image software (Atlas™).
√ **Data Analysis:** SAM (Significance Analysis of Microarrays), PANTHER (Protein ANalysis THrough Evolutionary Relationships).

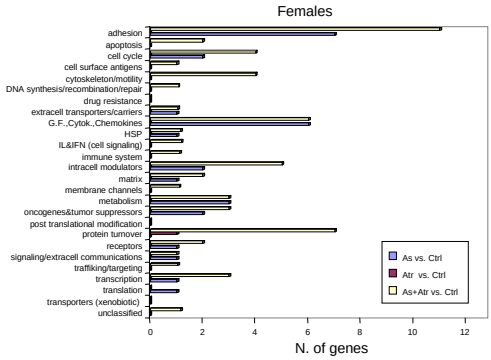
Results

In male mice the exposure to arsenate (As) or to atrazine (Atr) alone did not result in significant changes on the gene expression in bone marrow cells, whereas, co-exposure to arsenic and atrazine (As+Atr) resulted in a significant up-modulation of gene expression.

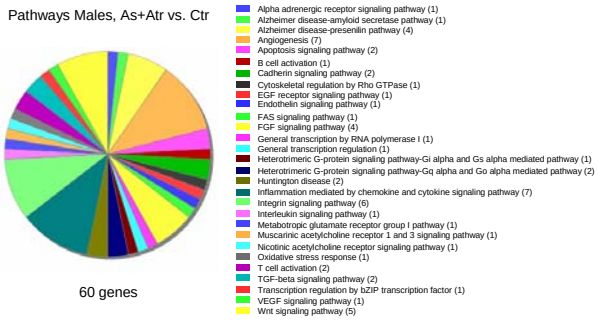
In female mice, the co-exposure to arsenic and atrazine (As+Atr) resulted in a significant up-modulation of gene expression. In As-treated mice we found 29 genes with higher expression and only one up-modulated gene in Atr-treated.

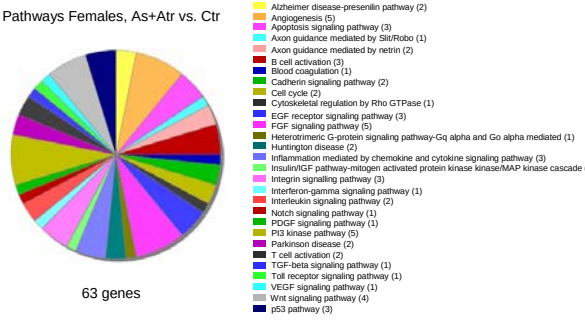
	Genes	As	Atr	As+Atr
Male	total	0	0	338
	significant	0	0	60
Female	total	587	1	491
	significant	29	1	63





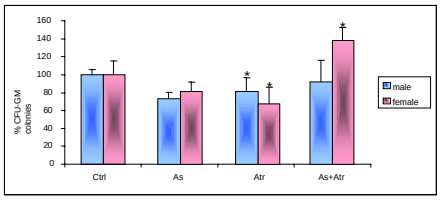
In both genders the main functional role exerted by the up-modulated genes were in cell adhesion, in the biosynthesis of chemokines and cytokines, protein turnover, and receptors.





PANTHER classifies protein products of genes into functional categories and allows to visualize them graphically. The genes modulated by co-exposure to arsenate and atrazine in males and females were classified according to pathways.

Clonogenicity of myeloid progenitors (CFU-GM)



The use of a methylcellulose colony-forming unit-granulocyte/macrophage (CFU-GM) assay was used in this study to evaluate the haematotoxicity of atrazine and arsenate in mice.
In male mice the percentage of CFU-GM weakly decreased after exposure to individual compounds, while the co-exposure did not change the clonogenicity of the progenitors.
In females the percentage of CFU-GM decreased significantly after atrazine exposure, did not change with arsenic treatment, but dramatically increased after the combined administration.

Conclusions

This study supports the need for novel toxicological approaches considering co-exposure and related cocktail effects.
The results indicate that the stimulation of clonogenicity and the modulation of gene expression in bone marrow were mainly observed in mice co-exposed to arsenic and atrazine and were not as evident in mice exposed in drinking water to arsenic or atrazine alone.
Furthermore strong sex differences were evident in both the clonogenicity and the modulation of gene expression, as confirmed by the differences in molecular pathways in the two sexes.

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