RIKILT WAGENINGEN UR

Assessment of the usefulness of the murine thymoma cell line EL-4 for immunotoxicity screening by transcriptomics

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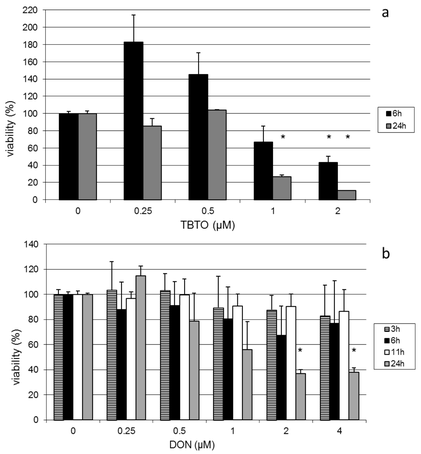
Introduction

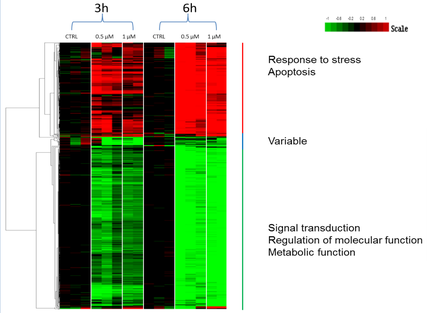
For immunotoxicity testing, there is a big demand to replace animal testing by in vitro experiments. Whole genome mRNA expression analysis on cells exposed to test chemicals in vitro promises to be a valuable option for this aim. The immunological response involves many different cell types. It is therefore likely that multiple immune cell types have to be included to enable adequate evaluation of immunotoxic hazard.
In this study, the value of the mouse thymoma EL-4 cell line as an in vitro screening model for immunotoxicity was assessed using two immunotoxic model compounds, the organotin compound tributyltin oxide (TBTO) and the mycotoxin deoxynivalenol (DON). TBTO induces endoplasmatic reticulum (ER) stress, DON induces ribotoxic stress.

Viability of EL-4 cells after exposure to (a) TBTO and (b) DON

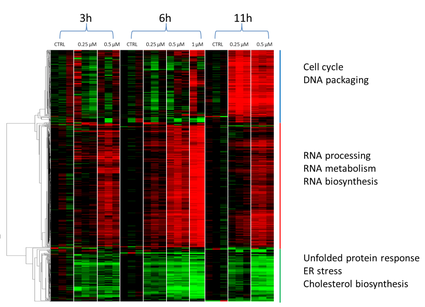


Dose selection for microarray analysis was based on a decrease in viability (WST-1 assay) of <20% after 24 h. A second dose was selected on >20% decrease of viability.
TBTO: 0.5 and 1.0 μM
DON: 0.25 and 0.5 μM

Unsupervised hierarchical clustering and Metacore pathway analysis

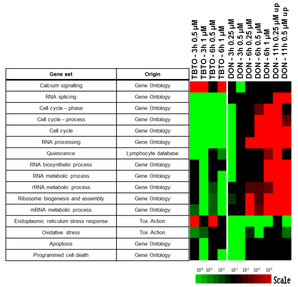


TBTO: 708 spots that were > 1.6 times up or downregulated by TBTO in at least 3 out of 16 arrays.



DON: 717 spots that were > 1.6 times up or downregulated in at least 3 out of 29 arrays.

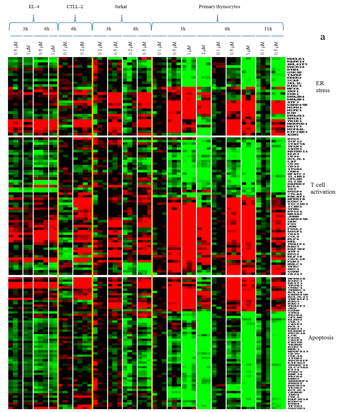
Gene Set Enrichment Analysis (GSEA)



DON, but not TBTO, induces RNA processing and ribosome biogenesis which is in line with the known mode of action of ribotoxic stress induction.

TBTO (0.5 μM) induces ER stress as described before.

Effects on individual genes involved in RNA biosynthesis, ER stress, T cell activation and apoptosis



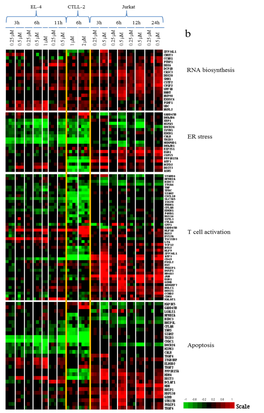


Table 1. Overview of processes affected by TBTO and DON in this study and previous transcriptomics studies

TBTO	EL-4 (this study)	CTLL-2	Jurkat	Primary thymocytes
ER stress	+	+	+	+
T cell activation	0	+	+	+
Apoptosis	0	+	+	+

DON	EL-4 (this study)	CTLL-2	Jurkat	Thymus *in vivo*
Ribotoxic stress	+	+	+	+
ER stress	-	-	+	+
T cell activation	0	-	+	+
Apoptosis	-	-	+	+

+ upregulated, - downregulated, 0 not regulated.

Conclusion

EL-4 cells lack factors that link ribotoxic stress to ER stress and fail to elicit a T cell activation response upon TBTO or DON exposure.
The EL-4 cell line has a limited value for immunotoxicogenomics-based screening.

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