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Protein phosphorylation profiling identifies potential mechanisms for direct immunotoxicity

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Background/objective

Signalling networks are essential elements that are involved in diverse cellular processes. One group of fundamental components in various signalling pathways concerns protein tyrosine kinases (PTKs). PTK are of widespread interest not only because of their intrinsic physiological functions and being drug targets in the treatment of malignant diseases, but also because of their role in the toxicity of various (immuno-)toxicants. In a previous study we assessed the effects of a wide range of compounds on the transcriptome of the human Jurkat T-cell line *in vitro* to identify common mechanisms underlying direct immunotoxicity (Shao et al. 2013 , Toxicol Sci. 135(2):328-346). The present work aimed to identify cellular signalling pathways that are commonly modulated by immunotoxicants at the protein phosphorylation level.

Methods

- Pathscan RTK Signalling antibody arrays (Cell Signalling Technology, Danvers, MA): to assess the effects of five immunotoxicants (lindane, ochratoxin A, TBTC, TBTO, and DON), two immunosuppressive drugs (rapamycin and mycophenolic acid), and two non-immunotoxic control chemicals (urethane and mannitol) on the phosphorylation of 28 receptor tyrosine kinases and 11 crucial signalling nodes in Jurkat T cells.
- Western blotting: to examine the effect of TBTO on components of the mTOR-p70S6K-RPS6 pathway.
- Pathway analysis: gene set enrichment analysis (GSEA) and molecular concept analysis of transcriptomics data.
- Chemotaxis analysis (Fig. 1).

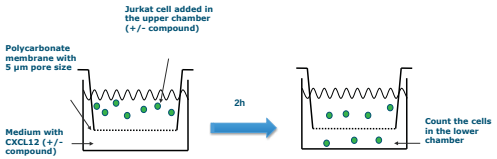

Figure 1. Schematic overview of the Transwell migration assay with the chemokine CXCL12.

Results

- The antibody array study showed that phosphorylation of RPS6, the kinases Akt, Src, and p44/42 is affected by at least three of the immuno-toxicants and immuno-suppressive drugs, with the largest effect observed for RPS6 which is part of the mTOR-p70S6K-RPS6 pathway (Fig. 2).

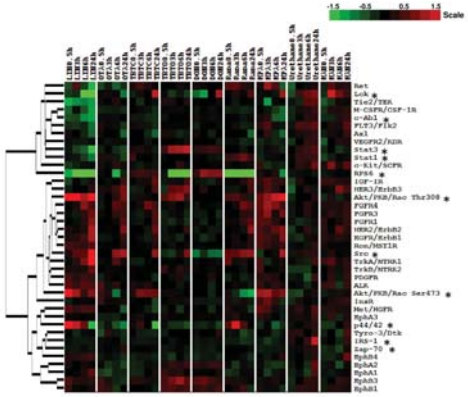

Figure 2. Heatmap visualizing the outcome of unsupervised hierarchical clustering of the phosphorylation of 28 receptor tyrosine kinases and 11 important signalling nodes (the latter are indicated with *).

- Western blotting revealed that both TBTO and the mTOR inhibitor rapamycin inactivate RPS6 but via different mechanisms (Fig. 3).

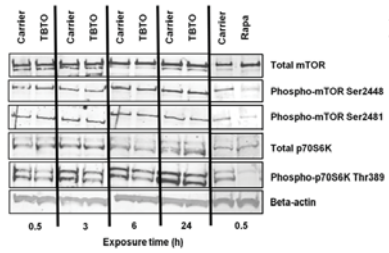

Figure 3. Effects of TBTO (100 nM) and rapamycin (20 nM) on protein expression levels of mTOR signalling intermediates.

Protein	Phosphorylation site	Relative ratio (phospho/ total, DMSO set at 100%)				
		TBTO 0.5h	TBTO 3h	TBTO 6h	TBTO 24h	Rapamycin 0.5h
mTOR	Ser2448	104.8	103.22	101.5	105.9	48.7
	Ser2481	102.6	96.7	101.6	102.0	46.7
P70S6K	Thr389	78.6	75.5	66.6	77.9	34.2

- Comparison of protein phosphorylation to transcriptome data resulted in a good correlation at the pathway level and indicated that TBTO affects ribosome biogenesis and leukocyte migration (Fig. 4)

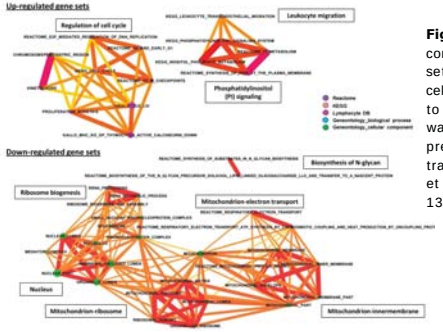

Figure 4. Molecular concept analysis of gene sets affected in Jurkat cells after 6 hr exposure to 100 nM TBTO. GSEA was performed on previously obtained transcriptome data (Shao et al. 2013, Toxicol Sci. 135(2):328-346).

- Upon exposure for 24 hr, TBTO decreased the percentage of migrated cells to 76.5% (Fig. 5)

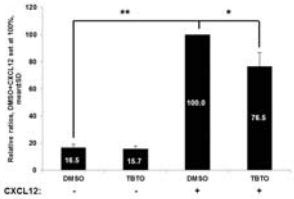

Figure 5. Effects of 100 nM TBTO on Jurkat cell chemotaxis toward CXCL12. *p< 0.05; **p< 0.01: two sample equal variance T-test, two-tailed.

Conclusions

- Various immunotoxicants affect the phosphorylation of RPS6, an important downstream effector of the mTOR pathway, in the Jurkat cell line.
- TBTO affects the kinase p70S6K (upstream regulator of RPS6) but via a different mechanism than the immunosuppressive drug and mTOR inhibitor rapamycin.
- TBTO decreases CXCL12-mediated chemotaxis of Jurkat cells.

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