Unraveling immunotoxic mechanisms of environmental contaminants and drugs by applying OMICs technology

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Introduction

Many chemicals that can be contaminants of food and drinking water, as well as certain drugs, are known to cause suppression of the immune system. Clear insights into the immunosuppressive mechanisms of these chemicals however is lacking. Therefore this project focuses on elucidating these mechanisms and identifying biomarkers of direct immunotoxicity using OMICs techniques.

The following experiments, in which immune cells are exposed to a diverse set of immunosuppressive chemicals, will be performed in order to gain such mechanistic insights:

• transcriptome, miRNA and proteome expression profiles will be generated, *in* vitro as well as *in vivo*. We will start-off with the transcriptome profiling.

• functional genomics, such as silencing of key regulatory genes, will be applied, based on novel mechanistic information.

• the relative abundance of the various cell populations within PBMCs will be analyzed by using FACS. These results will be related to serum markers and clinical outcome.

• mechanism-based *in vitro* models of direct immunotoxicity will be designed.

Materials & Methods

Human leukemia cell lines:

• Jurkat lymphoblastic T-cells, and MUTZ-3 CD34+ myeloid cells

Viability assays:

For determining sub-cytotoxic concentrations of the immunotoxic chemicals and drugs:

• WST-1 (Roche)

• ATPLite (Perkin Elmer)

totRNA isolations, including miRNA molecules:

• Trizol (Invitrogen), phenol / chlorofom (Ambion) extraction, and miRNAeasy (Qiagen)

• QC by Experion analysis (Biorad).

mRNA expression profiling:

• Pre-defined target genes of immunotoxicants will be quantified by Q-RT-PCR (MyIQ, Biorad)

• Transcriptomes will be analyzed by using the Affymetrix U133A platform.

ROS assay:

• C-DCFDA (C-369, Invitrogen) for the determination of reactive oxygen species (ROS)

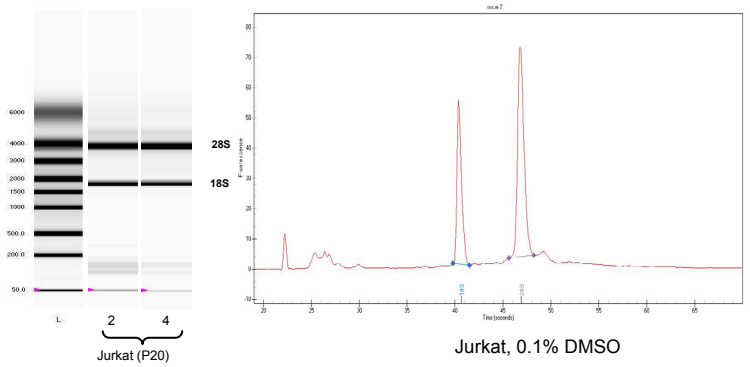

Fig. 1 tQC of totRNA molecules from Jurkat cells, Experion

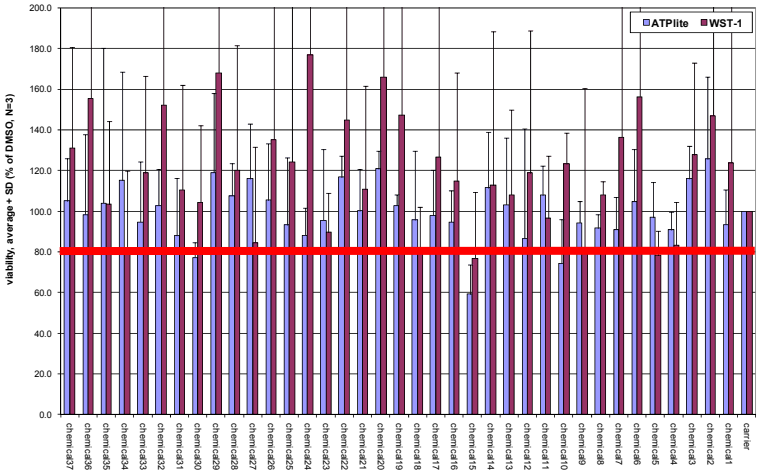

Fig. 2 Viability of Jurkat cells (comparison of WST-1 and ATPlite assay, 24h exposure)

Progress

I. A selection of 37 immunotoxic chemicals and immunosuppressive drugs has been made. This selection is based on (i) the severity of immunotoxicity, (ii) the environmental abundance, and (iii) the known molecular targets.

II. The combined isolation of miRNA/mRNA molecules, including QC, has been achieved (Fig. 1).

III. In the Jurkat cells the sub-cytotoxic concentrations have been found for all of these chemicals (Fig. 2). The threshold for these sub-cytotoxic concentrations was set at >80% viability after 24 hours of exposure to the respective chemical.

IV. Protocols have been established for *in vitro* exposures to the chemicals.

V. The immunotoxic compounds have been divided into molecular target-specific groups, as based on literature findings.

VI. Q-RT-PCR primers have been designed for sets of human genes.