INTEGRATION OF TRANSCRIPTOMIC, PROTEOMIC AND METABOLOMIC PROFILES IN MICROFLUIDIC BIOARTIFICIAL ORGANS APPLIED TO MECHANISTIC INTERPRETATION OF ACETAMINOPHEN INJURY

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ABSTRACT

Microfluidic bioartificial organs allow the reproduction of *in vivo*-like properties such as cell culture in a 3D dynamical micro environment [1]. In this work, we established a method and a protocol for performing the integration of transcriptomic, proteomic and metabolomic profiles in order to investigate xenobiotic toxicity. For that purpose, we present the example of the analysis of HepG2/C3A treated with acetaminophen (APAP) and cultivated in a liver microfluidic biochip.

KEYWORDS : liver biochip, transcriptomic, proteomic, metabolomic, microfluidic, acetaminophen

INTRODUCTION

A variety of approaches are available for describing the behavior and activity of cells as they react to stress, such as during exposure to a drug. Transcriptomic, proteomic and metabolomic techniques are part of those [1] (Boverhof et al., 2006). Genomic and transcriptomic methods can provide a near-complete analysis of the hereditary material of living organisms. Proteomics assay proteins contributing to the structure and function of a cellular compartment, a cell, a tissue or a whole living organism. Lastly, metabolomics, have also been proposed to analyze concurrently all the small intermediate or final metabolites produced by chemical reactions taking place in cells or whole organisms. Metabolomics can potentially identify all the changes in biochemical composition and metabolism occurring after exposure to a given substance. All these "omic" approaches can therefore help understanding how a substance acts, at various levels, on an organism.

To investigate the potential of integrating systems biology and microfluidic biochip technology, we present here the interaction between the transcriptomic, proteomic and metabolomic profiles of the hepatic HepG2/C3a cell line cultivated in a microfluidic PDMS biochip and exposed to acetaminophen (APAP). In the liver, APAP is metabolized by the cytochromes CYP2E1, CYP1A2 and CYP 3A4 to a toxic intermediate N-acetyl-p-benzoquinone imine. Secondary metabolism is mediated by glutathione (GSH), sulfo- and glucurono- conjugations. Thanks to the microfluidic culture conditions, we were able to identify the major biological pathways involved in APAP toxicity to hepatocytes. Finally, a comparison with published *in vivo* studies lead to a similar interpretation of APAP toxicity mechanism, as opposed to the results that we obtained from the conventional plate analysis.

EXPERIMENTAL

To fabricate the biochips, we used the Polydimethylsiloxane polymer (PDMS). The biochips were coated with fibronectin before carrying out the cultures. The cells were cultivated in the biochips under static conditions during 24 h for adhesion. Then, a flow rate of 10 µL/min of medium was applied for 72 h.. The cells were seeded at a density of $2.5x10^5$ cells/cm^2 in plates and in biochips (this corresponds to $5x10^5$ cells/biochip). For APAP treatment, 1 mM of acetaminophen was loaded in the plates and the biochip circuits just before the start of perfusion.

At the end of the culture, the cells were collected from three independent experiments (resulting to 3x3=9 samples per control and APAP treated biochips and plates) to perform the mRNAs extraction and transcriptomic profiling. Additional four experiments were used to extract intracellular proteins (from 4x3=12 samples) for the proteomic analysis. The culture medium was sampled from all experiments to perform the metabolomics analysis. Detailed protocols are presented in reference [2].

RESULTS

The transcritpomic comparison between the biochip and Petri (without APAP treatment) revealed that "drug and lipid metabolisms" and "molecular transport" appeared in the "Top networks" significantly affected by the microfluidic

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culture conditions. Then, in the "ToxList" we found the RXR/PXR activation pathway (involved in drug metabolism) and an inflammatory response via the NRF2 pathway (table 1). Consequently, genes and proteins involved in xenobiotic metabolism (phase 1 and phase 2 enzymes, phase 3 transporters) were particularly over expressed in the biochip (Figure 1). Since HepG2/C3a cells derived from a hepatoblastoma, the "cell compromise" and "cancer pathway" were also extracted.

	Molecular and cellular functions	Top canonical pathway	Top tox lists
Treatment effect in biochip	Cell morphology Cellular movement DNA replication, recombination and repair Cell cycle Lipid metabolism	D-arginine and D-ornithine Metabolism Sphingolipid metabolism VDR/RXR activation Glycosaminoglycan degradation Pyrimidine metabolism	VDR/RXR activation CYP450 panel Hormone receptor regulated cholesterol metabolism
Treatment effect in Petri	Cell cycle DNA replication, recombination and repair Cellular assembly and organisation Cellular growth and proliferation Cell compromise	Role of BRCA1 in DNA Damage Response Role of CHK Proteins in Cell Cycle Checkpoint Control p53 Signaling Mitotic Roles of Polo-Like Kinase Pyrimidine Metabolism	P53 signaling G2/M transition of the cell cycle G1/S transition of the cell cycle AHR signaling Pro-apoptosis

Table 1: Ingenuity analysis of the transcriptomic data related to the treatment effect in the biochip and in Petri.

Integration of the transcriptomics, proteomics and metabolomics analysis confirmed the impact of the microenvironment on the cellular behavior. Indeed, 26 protein markers detected in the proteomic analysis also appeared at the gene levels (Figure 1) and are involved in hepatic functions. Whereas specific extracellular biomarkers (such as the 3-hydroxybutyrate) confirmed the cytoprotective response at the metabolome level, due to the microfluidic biochip cultures.

Induction of the drug metabolism pathway in the biochip results in a higher metabolism of the acetaminophen (APAP) when compared to Petri cultures. The filtered fold change gene lists in the APAP treated conditions led to 1236 genes differentially expressed between the Petri dishes and the microfluidic biochip (730 up-regulated and 506 down-regulated). Thus, we observed 50% growth inhibition of cell proliferation at 1mM in the biochip, which appeared similar to human plasmatic toxic concentrations reported at 2mM (Figure 2).

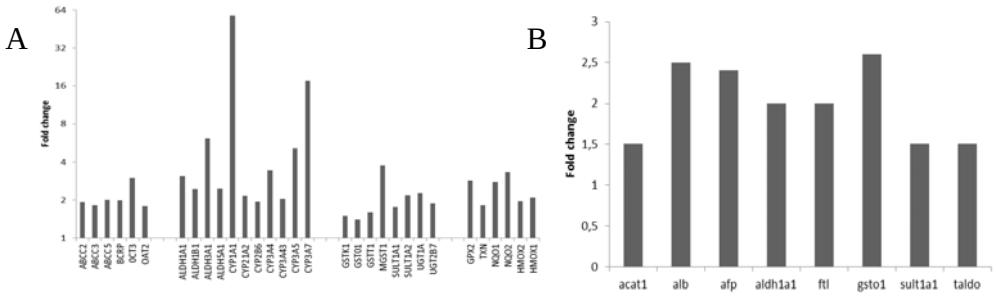

Figure 1: (A) Main genes and proteins (B) differentially expressed by the environment condition and involved in hepatic differentiated function

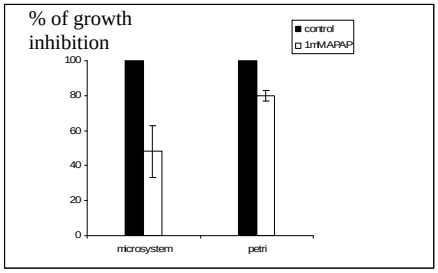

Figure 2: Comparison of the cell growth in biochip and Petri dishes in untreated and treated conditions with 1mM of APAP after 96h of cultures

The metabolic signature of APAP toxicity in the biochip showed similar metabolic pathways as those reported *in vivo*, such as the calcium homeostasis, lipid metabolism and reorganization of the cytoskeleton, at the transcriptome and proteome levels (which was not the case in Petri dishes), as shown in table 1. At the metabolome level, the mechanism of toxicity was confirmed by the important production of 2-hydroxybutyrate and 3-hydroxybutyrate, and the methionine, cystine, histidine consumptions in treated biochips. Interestingly, those metabolites correspond to specific biomarkers of hepatotoxicity and glutathione depletion, as reported in the literature.

The integration of the transcriptomic, proteomic and metabolomics profiles allowed the reconstruction of a network describing the mechanism of action of APAP (Figure 3). The results demonstrate a network closed to situations found *in vivo* [3].

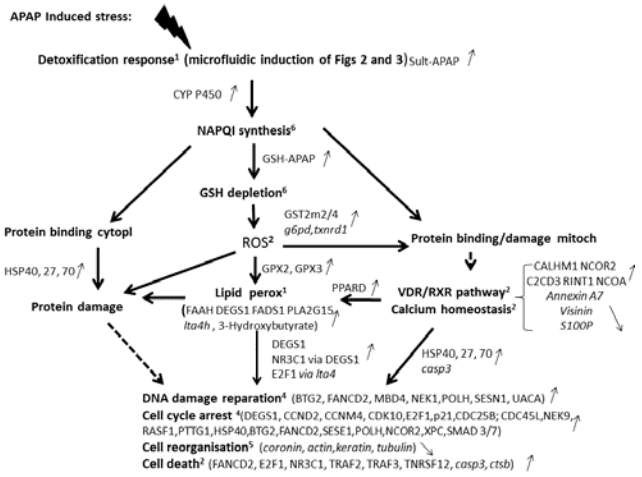

Figure 3: APAP toxicity pathway reconstruction from biochips experiments [2]

CONCLUSION

In summary, we have characterized the transcriptomic, proteomic and metabolomic profiles of HepG2/C3AC3a cells cultivated in a microfluidic environment. The toxicological response of HepG2/C3A cells in biochips cultures to APAP injury could be correlated to glutathione depletion and to the apparition of NAPQI. That led to a perturbation of calcium homeostasis via mitochondrial perturbations, to lipid peroxidation and to cell death. In addition, we also illustrated the applicability of an exploratory spectroscopic phenotyping assay to identify metabolic biomarkers of xenobiotics exposure and toxicological insults in mammalian cells thanks to microfluidic cultures. Therefore, the "systems biology on chip' approach we propose, has the potential to allow serendipitous discovery of cell-specific dose-response markers, while reducing the use of laboratory animals. Finally, our finding provide an important insight into the use of microfluidic biochips as new tools in biomarker research in therapeutic drug studies and predictive toxicity investigations.

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