

# Toxicoepigenomics

## Transcriptional and epigenetic profiles of primary liver cells and *in vitro* models

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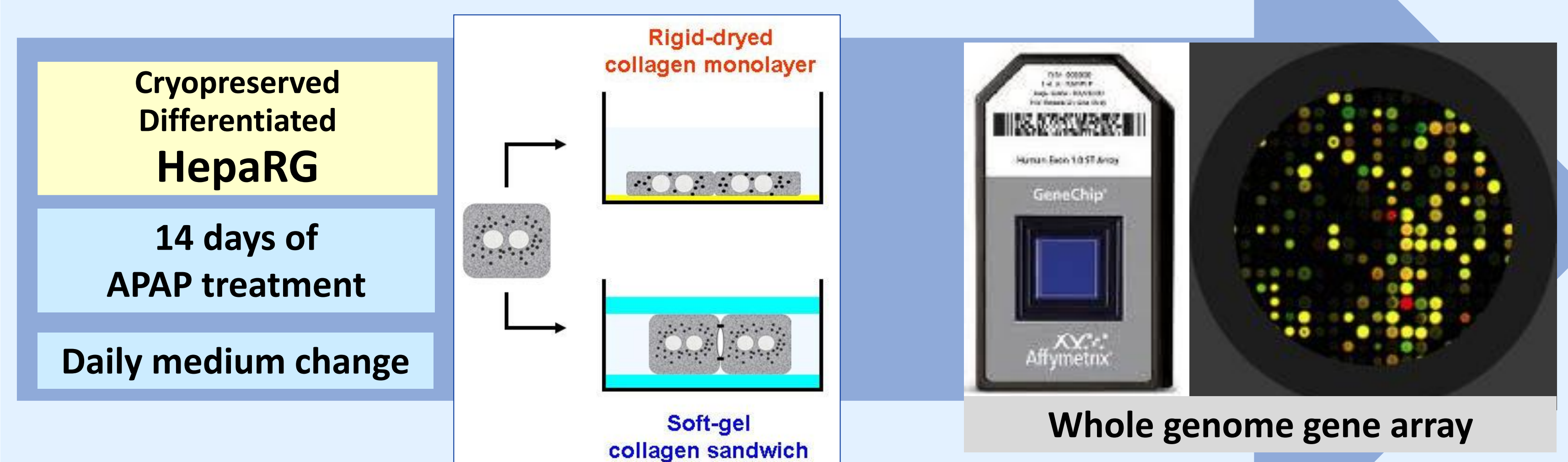
### Introduction

A major interest of cosmetic and pharmaceutical industry is the long term exposure to potentially toxic ingredients in low concentrations. Given the imperious need to replace animals for chronic toxicity testing, the NOTOX project is focused in establishing robust *in vitro* assays for predicting long term toxicity.

### Experimental Approach

As a model for chronic toxicity, we treated HepaRG cells in a collagen monolayer and in collagen sandwich culture with a daily dose of 200  $\mu$ M acetaminophen (APAP) over two weeks, and analyzed alterations in mRNA expression by Affimetrix whole genome gene arrays.

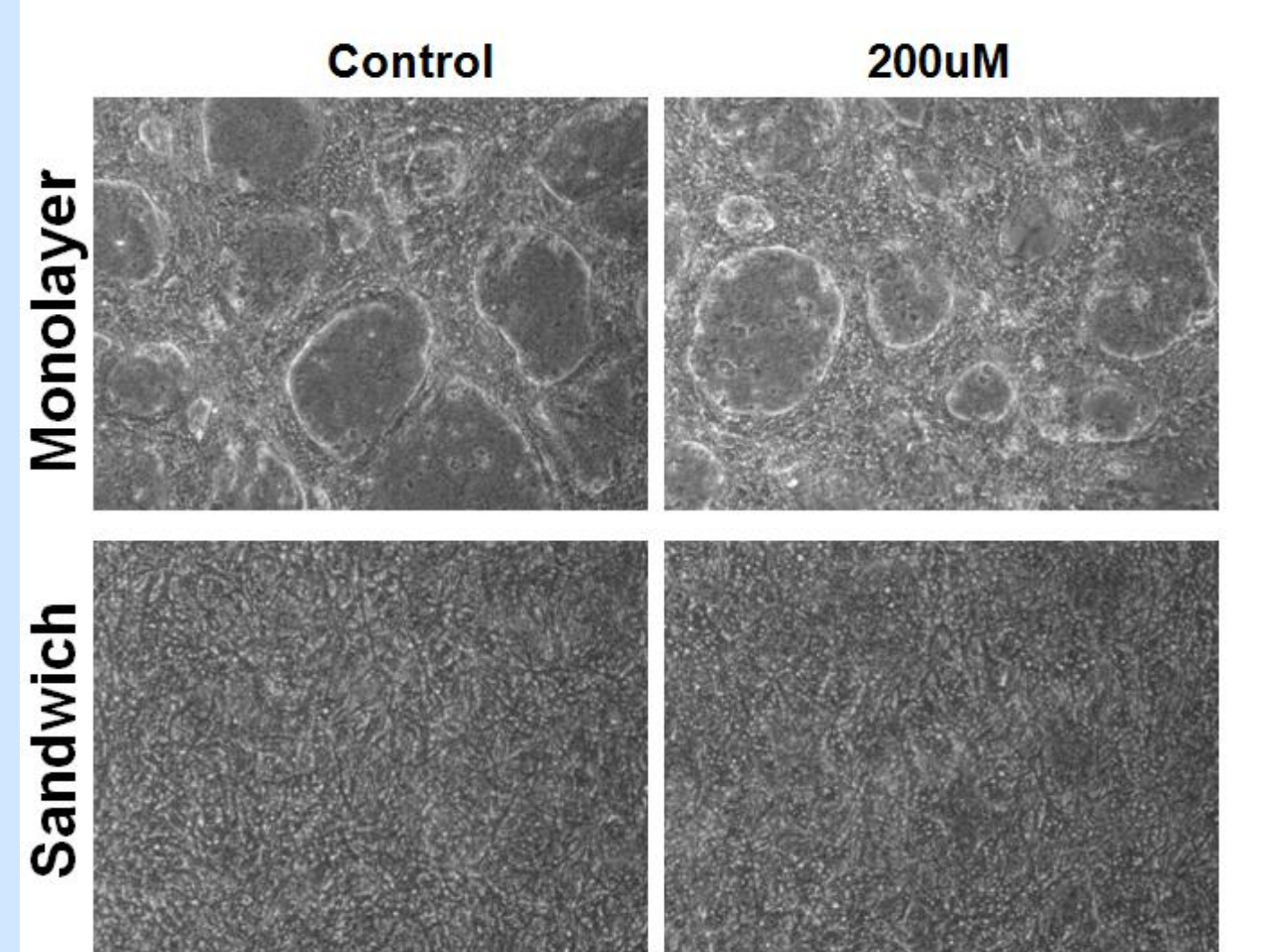
**Fig. 1: Experimental Approach**



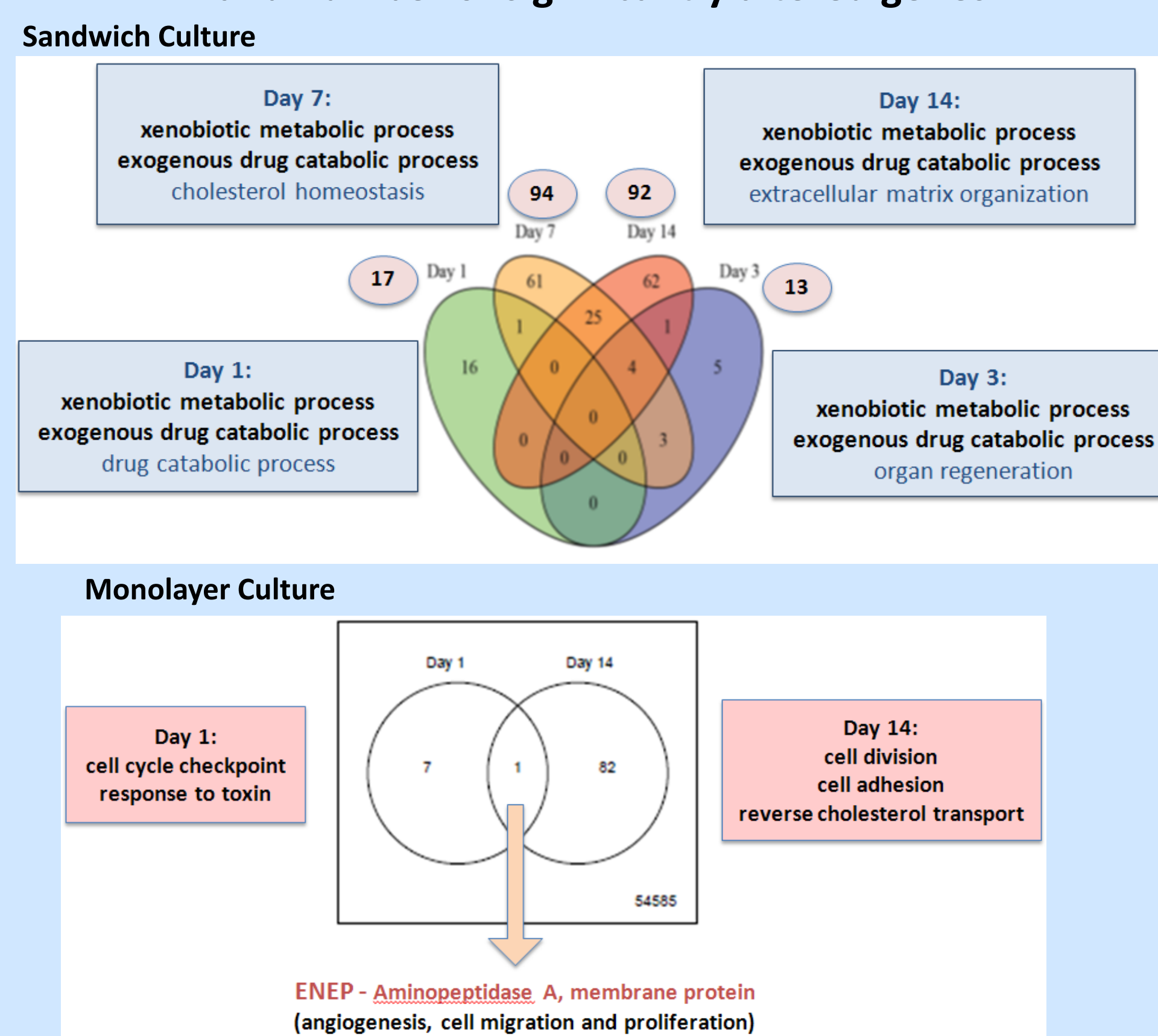
**Fig. 2: Results**

#### A. Morphology

HepaRG cells after 14 days exposure to APAP



#### B. GO ontology classification and number of significantly altered genes\*



(\*)  $\geq 1.5$ -fold change, p-values  $< 0.01$

### RESULTS

- The two cultivation systems result in a distinct morphology of HepaRG cell line. Established cultivation protocols allow performing reliable long-term studies with APAP exposure.
- In sandwich culture, significant increase in de-regulated genes was induced by chronic treatment in a time-dependent manner. Specifically, only 17 and 13 genes were de-regulated at day 1 and 3 of treatment, whereas at days 7 and 14 the number of total de-regulated genes increased to 94 and 92, respectively. Although different expressional changes have been triggered at different time-points, there seem to be a strong functional link to xenobiotic and drug response pathways (according to GO ontology classification indicated in frames).

We have as well observed a time-dependent increase in de-regulated genes in monolayer culture that could be mainly associated with cell division. However, there is only little overlap between day 1 and 14 of APAP exposures.

### CONCLUSIONS

Transcriptomics analysis revealed a time-dependent gene expression responses to APAP with a strong functional link to xenobiotic and drug response pathways for sandwich culture and cell division for monolayer culture. These results indicated that HepaRG cells in sandwich culture can be used as a sensitive model for APAP-induced gene expression.

### Perspectives

We are currently performing similar experiments with higher APAP concentrations in HepaRG cells and primary human hepatocytes. Furthermore, we have established protocols for the analysis of proteomics and epigenomics which will complement RNA expression data that will be used for mathematical modeling of toxicity, leading to a comprehensive assessment of molecular mechanisms of drug toxicity in a chronic exposure setup. Our main focus is identification of important toxic pathways regulating response to xenobiotics.