

GENE EXPRESSION PROFILES IN HEPARG CELLS TO DISCRIMINATE GENOTOXIC FROM NON GENOTOXIC COMPOUNDS

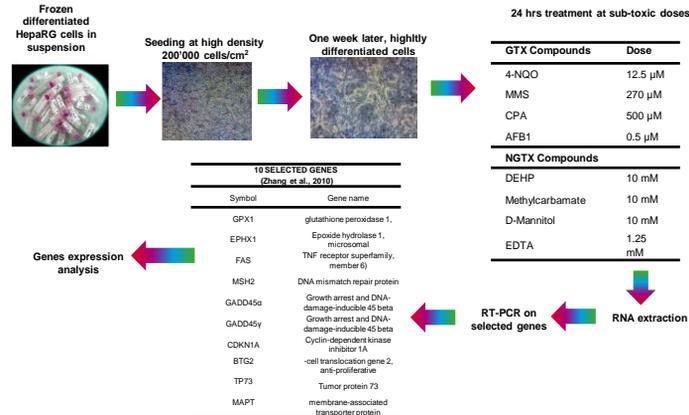
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Background

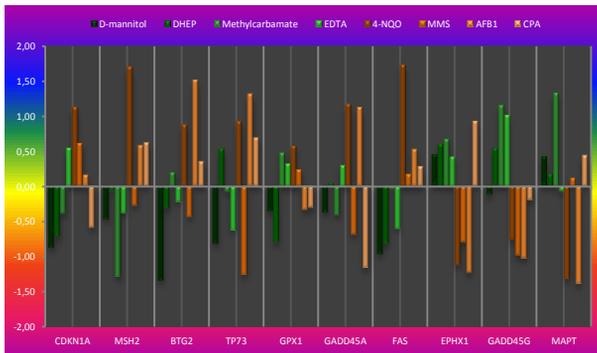
Because of metabolic bioactivation plays a major role in the genotoxic response of environmental human carcinogens, genotoxicity assays are performed with induced rat liver S9 fraction to mimic the liver metabolism. However, extrapolating of results from the metabolism rodent to humans is questionable. Among the human cell culture models mimicking human liver metabolism, the hepatoma HepaRG cells are certainly one of the most promising in vitro model for genetic toxicology. They express most of liver-specific functions (phase I and II enzymes, nuclear receptors, ...). In order to determine if the cryopreserved HepaRG model is suitable for detecting genotoxic carcinogens, we conducted a study to characterize gene expression signatures of exposure to 4 genotoxic compounds (GTX) (AflatoxinB1, MMS, 4-NQO and cyclophosphamide) and 4 non genotoxic compounds (NGTX) (D-mannitol, EDTA, DHEP, methylcarbamate). Principal component analysis (PCA) was applied to identify data patterns and highlight data differences between GTX and NGTX compounds. This study demonstrated the suitability of the HepaRG cell model for mutagenesis evaluation based on gene expression alterations.

RT-QPCR in HepaRG cells treated with GTX and NGTX



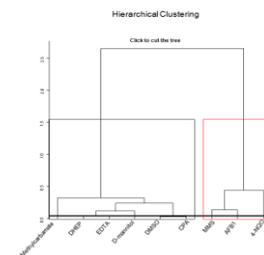
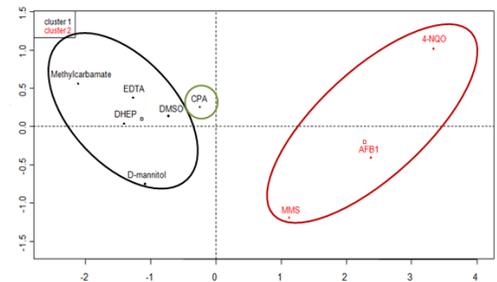
Results

Difference of selected gene expression between NGTX and GTX in HepaRG treated cells (n=3, 24hs of treatment)



Expression of 10 target genes was assessed in response to GTX and NGTX compounds in HepaRG cells. We found that 7 genes (CDKN1A, MSH2, BTG2, TP73, FAS, EPHX1 and GADD45y) showed similarity expression profiles that permit the discrimination between GTX and NGTX compounds. GTX compounds induced overexpression of CDKN1, MSH2, BTG2, TP73 and FAS genes and underexpression of GADD45y and EPHX1.

Principal component analysis to distinguish GTX from NGTX



Conclusion

These results showed that gene expression profiles of HepaRG cells could distinguish genotoxic from non-genotoxic compounds. Among the GTX compounds (MMS, 4-NQO, AFB1 and CPA) only CPA which was showed to induced DNA damage in HepaRG cells, failed to be distinguished from NGTX compounds (EDTA, Mannitol, DHEP and Methylcarbamate). Then, the combining the used of a human metabolic competent cell line like differentiated HepaRG cells and gene expression profiling may become a suitable method to identify human genotoxic carcinogens.

Principal component analysis (PCA) was applied to identify data patterns and to highlight data similarity and difference between the GTX and NGTX treated HepaRG cells. PCA of all 10 genes was unable to discriminate the GTX from the NGTX compounds. However, selecting 4 specific genes (FAS, MSH2, EPHX1, GADD45y), enables an optimal separation between the two types of compounds. We found that Methylcarbamate, DHEP, EDTA and DMSO exhibited a first principal component and MMS, AFB1 and 4-NQO a second principal component. However, CPA could not be distinguished from the NGTX compounds.