Detection of cytokeratin 18 in proliferating, primary-like upcyte® hepatocytes to predict drug-induced hepatotoxicity

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INTRODUCTION

Early detection of drug-induced liver injury (DILI) is essential during drug development to minimize the risk of adverse effects and subsequently drug withdrawal. To evaluate the safety profile of a drug candidate, there is a constant need for reliable in vitro test systems. While primary human hepatocytes remain the gold standard, limited throughput and a rapid loss of liver-specific functions limit their use. In the present study, we expanded primary human hepatocytes by lentiviral transduction with proliferation inducing genes. So-called upcyte® hepatocytes proliferated for up to 40 population doublings while maintaining several characteristics of primary cells, such as adult marker expression and phase I/II activities.

Recently, cytokeratin 18 (CK18) was suggested as a promising biomarker for DILI. Likewise, the M30 neoepitope generated upon caspase-dependent cleavage of CK18 is considered a robust biomarker for apoptosis. Here, we evaluated the use of upcyte® hepatocytes for M30-based in vitro hepatotoxicity assays by analyzing their expression of CK18 and the degree of caspase-dependent cleavage upon challenge with established hepatotoxic model compounds.

RESULTS

The upcyte® technology

expansion of primary hepatocytes using a defined cocktail of lentiviral vectors

We first generated a library of lentiviral vectors carrying proliferation-inducing genes, allowing primary human hepatocytes (pHep) to bypass senescence. Resulting upcyte® hepatocytes gained the ability to proliferate for up to 40 additional population doublings without losing functional and phenotypic characteristics of mature cells. All cells exhibited expected morphology patterns and were restricted by the presence of specific growth factors, contact inhibition and anchorage dependence.

suitability of upcyte® hepatocytes for overall toxicity and CK18-based apoptosis assays

CK18 cleavage status during cell death
CK18 expressed by epithelial cells constitutes an important stabilizing component of the cytoskeleton. Apoptosis leads to activation of caspases which in turn cleave CK18, resulting in formation of the neoepitope M30. This epitope is selectively recognized by the M30 antibody. During necrosis, full-length and cleaved CK18 are released, both detectable by the M5 antibody. VLVbio offers both M5 and M30-based ELISA kits to detect total CK18 levels or caspase-cleaved CK18, respectively.

mRNA expression analysis of differentiated upcyte® hepatocytes

upcyte® hepatocytes were cultured for 4 days at confluence and analyzed by immunofluorescence microscopy for expression of the lateral surface marker E-Cadherin (green) counter-stained against DAPI (blue) and α-actin or albumin (red). Differentiated cultures after 5 days revealed distinct polarized cell nodules amidst E-Cadherin negative cells. Both polarized and non-polarized cells show strong albumin staining, demonstrating their hepatocyte origin (bar: 50 μm).

upcyte® hepatocytes express full-length CK8/18

upcyte® hepatocytes were previously illustrated to express the characteristic adult marker proteins serum albumin and α-antitrypsin while lacking embryonic markers such as α-fetoprotein. Importantly, cells revealed strong expression of CK8 and CK18, indicating their potential use for CK18-based apoptosis assays.

drug-induced CK18 cleavage in upcyte® cells

We next investigated basal and drug-induced cleavage of CK18 in upcyte® cells using the M30 Apoptosense® ELISA. We observed different basal levels of cleaved CK18 (CK18*) in different donors, with donor 653-03 showing the highest signal-to-noise ratio. The majority of CK18 was found in the supernatant of cells challenged with hepatotoxic model compounds such as cyclosporin A and diclofenac. Importantly, toxicity was also observed for substances exerting toxicity after biotransformation, such as acetaminophen.

SUMMARY & CONCLUSION

In conclusion, we developed a comprehensive platform enabling the controlled expansion of primary hepatocytes for up to 40 population doublings. Importantly, upcyte® cells maintained many features of primary hepatocytes such as phase I and II activities. upcyte® hepatocytes further revealed marked expression of CK18. Exposure to established hepatotoxic model compounds markedly increased caspase-cleaved CK18 levels as determined by M30 Apoptosense® ELISA. We thus conclude that upcyte® hepatocytes and the M30 Apoptosense® ELISA represent a promising tool for quantification of drug-induced apoptosis, potentially facilitating throughput and reproducibility of cell-based hepatotoxicity assays.