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Development of automated image-based gH2AX and micronucleus assays for efficient genotoxicity and mutagenesis screening in the advanced 3D human hepatocytes HepoidR model

The liver is essential in the elimination of environmental and food contaminants. Given the significant inadequate data resulting from interspecies differences between rodents and humans in xenobiotic metabolism, the development of novel relevant in vitro human models is crucial to investigate the genotoxicity and mutagenesis of compounds that undergo metabolic activation. The in vitro gold standard model primary human hepatocytes suffer from a limited lifespan and lack of proliferation while the differentiation of the hepatic HepaRG cell line requires high concentration of dimethyl sulfoxide which restricts its usefulness for drug-metabolism studies.

We have developed a DMSO-free advanced 3D model of human hepatocytes named HepoidR, that allows concomitant proliferation and differentiation of human hepatocytes (primary and HepaRG cells) cultured in collagen matrix. Cells rapidly organize into characteristic polarized hollow spheroids of differentiated hepatocytes exhibiting high levels of liver-specific functions and xenobiotic metabolism enzymes expression and activities after a few days of culture and for at least 4 weeks. Traditional genotoxicity assays are labour intensive and time consuming. Here, we have applying machine learning models from the open access software Fiji and develop computational approaches to automate the quantification of gH2Ax and micronucleus from the 3D HepoidR model, thus reducing analysis time and minimizing human bias. We have studied the effects of well-known DNA reactive carcinogenic (MMS, MMC, colchicine, vinblastine, DMH, AFB1) and negative compounds (ethionamide, DEHP, methylcarbamate) to validate the methods and show that they can be used to efficiently discriminate the genotoxic effects of various class of molecules.

Taken together, our results show that the highly differentiated HepoidR model associated with automized analysis is an adapted tool for acute and long-term genotoxic in vitro assays and environmental chemicals risk assessment.