Next-generation human iPS cell-derived hepatocytes for metabolic disease modeling and drug discovery

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Human pluripotent stem (hPS) cell-derived hepatocytes have the potential to serve as predictive human in vitro model systems for drug discovery, drug metabolism research, and hepatotoxicity studies, provided they possess relevant hepatocyte functions. Importantly, some hepatocyte applications, like chronic toxicity testing, demand a 2-week usage window, an order of magnitude outside standard culturing practices. Here, we show data for a newly developed maintenance medium allowing culturing of the hPS cell-derived functional hepatocytes for 14 days, and thus enables their use for new applications with longer culture times. We have performed multiple analyses, including RT-qPCR, immunostainings, and functional assays, to investigate if our hepatocyte differentiation and maintenance system will 1) generate mature hepatocytes from multiple hPS cell lines, and then 2) to support their functionality during an extended culture time. Importantly, the hPSC-derived hepatocytes expressed important genes of the drug metabolizing machinery, such as CYPs, phase II enzymes and transporters during the entire culture time. Next, we exposed these novel hPS cell-derived hepatocytes to known hepatotoxins for up to 14 days and found they respond correctly to these toxic compounds with an increasing sensitivity upon longer exposure, demonstrating their utility for chronic toxicology studies. The hiPS cell-derived hepatocytes also respond to insulin, and they can take up and store lowdensity lipoproteins and fatty acids. Since we observed that our new maintenance medium substantially extended the lifespan of hPS cell-derived hepatocytes, we tested if it also could extend the lifespan of human primary hepatocytes. Interestingly, we found that cryopreserved human primary hepatocytes cultured in the new maintenance medium were viable and showed stable activities of several key CYP enzymes for several weeks in conventional 2D cultures, sharply contrasting existing commercially available hepatocyte maintenance media. Thus, the novel maintenance medium enables the use of human primary hepatocytes in conventional 2D cultures for applications requiring longer culture times. We hope that the increased assay window of functional hepatocytes in 2D cultures will advance the use of hPS cell-derived hepatocytes in metabolic disease modeling, and empower new areas of liver research and applications.