cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTERS

Epigenetic Reprogramming of Human Hepatoma Cells: A Low-Cost Option for Drug Metabolism Assessment

Primary cultures of hepatocytes are widely considered the gold standard for evaluating the hepatic metabolism of pharmacologic molecules.[1] However, limited access to human hepatocytes has led to the development of various alternative models.[2–5] The procedures used to generate these cells remain complex and time-consuming. In addition, the resulting hepatocytes frequently display heterogeneous hepatic gene expression, rapidly dedifferentiate, and lose their metabolic functions, which are essential for biological, pharmacologic, and toxicologic studies. Strategies that reverse epigenetic alterations in malignant cells offer a unique opportunity for cellular reprogramming,[6–11] which is valuable for the bioengineering of surrogate hepatic models. In this study, we aimed to develop a robust method that enables the recovery of a differentiated phenotype in hepatoma cells and significant drug-metabolizing features for drug metabolism studies. The procedure applied is based on a continued exposure to the demethylating agent 5-azacytidine (5-AZA).

The human HepG2 cell line was used for the first part of this study. Despite their low metabolic capacity, HepG2 cells are frequently used for in vitro studies as a replacement for human hepatocytes.[12] Characterization of HepG2 cells in response to the 5-AZA reprogramming regimen showed extensive storage of lipids and glycogen compared with the control cells (Supplementary Figure 1). Moreover, the treated cells showed a gradual increase in the expression of genes associated with lipid metabolism, carbohydrate metabolism, and amino acid metabolic processes. Interestingly, our data also indicated that the demethylating treatment led to global enrichment in the expression of the cytochrome P450 (*CYP*) genes (Figure 1*A*). We analyzed the activity of 4 of the most important phase I drug-metabolizing enzymes before and after epigenetic reprogramming. The data showed that CYP activity was induced dramatically in reprogrammed HepG2 cells (Figure 1*B*). Notably, metabolism of midazolam by CYP3A4 showed the strongest induction in reprogrammed cells. Of the various members of the CYP superfamily, CYP3A4 certainly plays the most important role in the metabolism of marketed drugs,[13] supporting the relevance of epigenetically reprogrammed hepatic cells for the study of xenobiotic detoxification.

Next, we established a turnkey protocol for the epigenetic reprogramming of human hepatoma cells and CYP activity assessment. In contrast to hepatocyte cultures, the 5-AZA–treated cells showed persistent proliferation rates (Figure 1*C*), thereby allowing their expansion by serial passaging (Figure 1*D*). The reprogramming method was based on a 2-step procedure as shown in Figure 1*E*. First, hepatoma cells were exposed to the demethylating agent 5-AZA (2.5 μmol/L) for 7 days. Next, reprogrammed cells were re-seeded at a high density, maintained in 5-AZA, and treated with CYP inducers for 72 hours. We evaluated dexamethasone (Dex)- and phenobarbital sodium (PB)-mediated CYP3A4 metabolic capacity using reprogrammed HepG2 and Huh-7 cells. Cultures of cryopreserved hepatocytes were used as a reference. CYP3A4 activity was not obviously induced in HepG2 and Huh-7 cells before reprogramming (Figure 1*F*). Conversely, Dex and PB stimulation induced CYP3A4 activity by 5.29 ± 1.00-fold and 1.93 ± 0.11-fold, respectively, in reprogrammed HepG2 cells and by 6.51 ± 0.45-fold and 2.84 ± 0.55-fold, respectively, in reprogrammed Huh-7 cells ($P < .001$, t test vs solvent-treated control cells). The CYP3A4 activities measured in the 5 lots of human hepatocytes were relatively heterogeneous and ranged from 2.04 ± 0.24-fold to 70.45 ± 2.66-fold for Dex and ranged from 1.93 ± 0.23-fold to 19.25 ± 0.54-fold for PB, respectively. Notably, the CYP induction values obtained in reprogrammed hepatoma cells were relatively close to the average activities of the 5 lots of human hepatocytes after Dex and PB induction (30.24-fold and 7.28-fold, respectively). Furthermore, we found that CYP3A4 functions were more stable in reprogrammed hepatoma cells because the cryopreserved hepatocytes tended to extensively dedifferentiate over time (Supplementary Figure 2). Indisputably, the use of demethylating compounds for differentiating liver progenitors and cancer cells has been reported extensively in the literature.[10,14–16] However, none of these studies considered the actual functionality and drug-metabolizing capability of the treated cells, which supports the significance and novelty of our finding.

To address whether 5-AZA acts by directly influencing the methylation state of the *CYP3A4* gene, the correlation between DNA methylation and *CYP3A4* expression levels was evaluated in reprogrammed and control hepatoma cells. Gene expression measurement showed that *CYP3A4* expression showed a marked augmentation in the reprogrammed cells (Figure 2*A*). In silico genomic analysis showed that *CYP3A4* contained a CpG-rich region in its promoter (Supplementary Figure 3). We performed combined bisulfite restriction analysis to examine the methylation status of the identified CpG sites and found prominent hypermethylation of the *CYP3A4* promoter in Huh-7, Hep3B, and HepG2 cells compared with human hepatocytes (Figure 2*B*). By contrast, reprogrammed hepatoma cells showed significant demethylation of the analyzed CpG sites (Figure 2*C*), which was consistent with the re-expression of the *CYP3A4* gene observed after 5-AZA treatment.

In conclusion, the epigenetic reprogramming method offers several advantages. First, reprogrammed hepatic cells can be expanded without