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Comprehensive Analysis of Gene Expression using *3D-Gene*® in Rifampicin-treated Cryopreserved Human Hepatocytes

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Purpose

Drug interaction guideline recommends that in vitro cytochrome P450 (CYP) induction potency of new drug applications should be evaluated based on mRNA expression. Although its level is generally evaluated by using real-time PCR, it is difficult to simultaneously analyze multiple genes by the PCR. On the other hand, high sensitive DNA chip, *3D-Gene*®, is known to be a useful tool for integrated analysis of gene expression; in this study, we comprehensively analyzed CYP-related, non-CYP metabolic enzyme-related, and transporter-related gene in the rifampicin-treated hepatocytes by using *3D-Gene*®.

Materials

Rifampicin and dimethylsulfoxide (DMSO) were purchased from Wako Pure Chemical Industry. Human cryopreserved hepatocytes and medium were purchased from Thermo Fisher Scientific (Life Technologies). DNA chip, *3D-Gene*® (mRNA and miRNA) was purchased from Toray Industries.

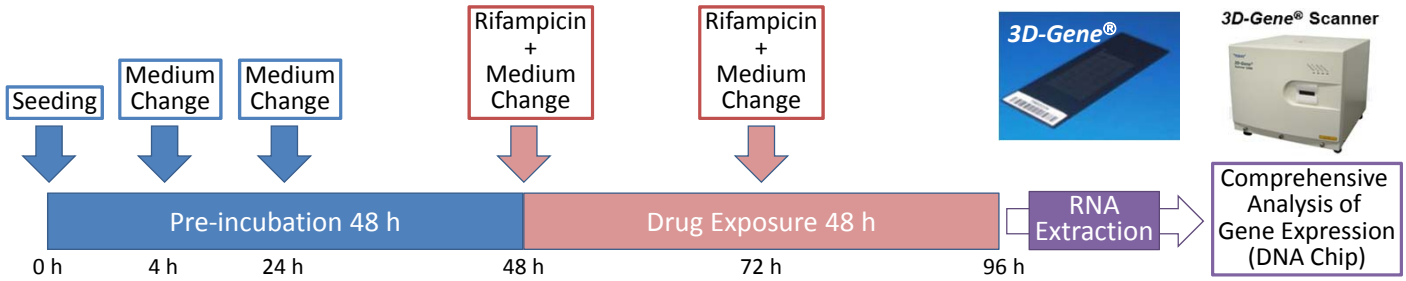
Donor Information

Donor ID	Gender	Race	Age
HU8148	Female	Caucasian	55

3D-Gene® Product Name		On-board number of genes
mRNA	Human Oligo chip 25k	24460
miRNA	Human miRNA Oligo chip	2565

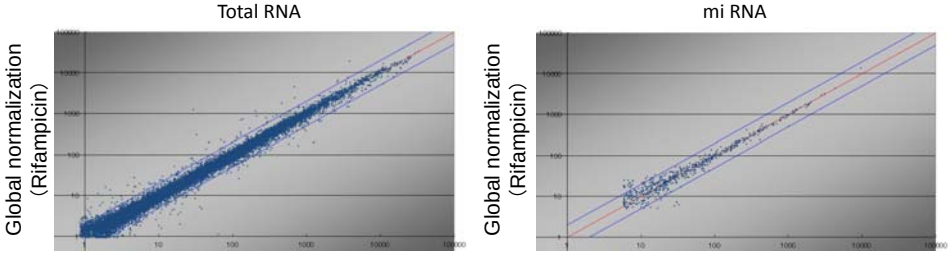
Methods

Human cryopreserved hepatocytes were seeded into 24-well plates at 3.5×10^5 cells/well and maintained in medium.
After 48 h, the hepatocytes were treated with 20 µmol/L rifampicin (CYP2C and CYP3A inducer) for 48 h.
Total RNAs were isolated from the rifampicin-treated hepatocytes, and applied to *3D-Gene*® for gene (human mRNA Oligo chip) and micro RNA (human miRNA Oligo chip) analysis. The 0.1% DMSO -treated hepatocytes were used as control.



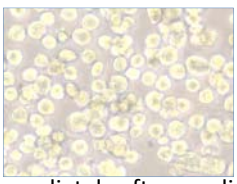
Results

Comprehensive Analysis of Gene Expression

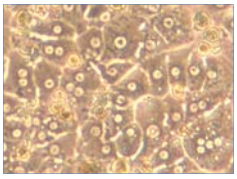


We found that 125 genes including CYP2C8, CYP2C9, CYP2C19, CYP3A4, UGT1A1 and ABCB1 were increased (>2-fold) and 155 genes including CYP2E1 and SLC22A7 were decreased (>0.5-fold) among 24460 genes, whereas 23 micro RNAs were increased (>2-fold) and 10 micro RNAs were decreased (>0.5-fold) among 2565 micro RNAs by the rifampicin treatment. Up-regulations of CYP3A and CYP2C gene by the rifampicin treatment indicated that the experimental system worked properly.

Cell morphology

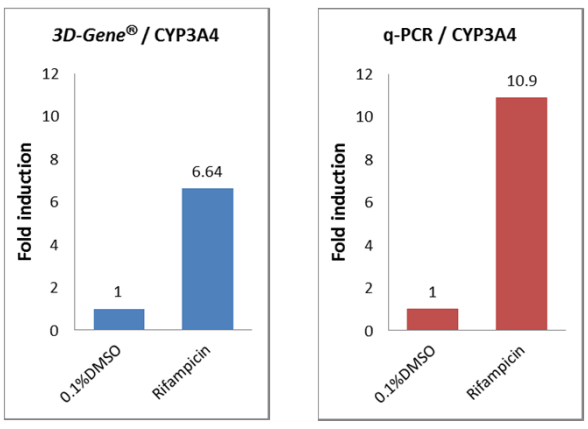


Immediately after seeding



52 hours after seeding

Comparison of 3D-Gene and quantitative PCR



Conclusions

3D-Gene® made it possible to comprehensively and sensitively analyze drug-induced gene expression and micro RNA change in human hepatocytes; therefore, this method could be used to analyze not only pharmacokinetic- related change but also toxicity- or pharmacology- related change in human hepatocytes caused by test drugs.