The HCA assay protocol and evaluation in HepaRG and HepG2 cells

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Introduction

High Content Analysis (HCA) is a high-throughput imaging-based cellular analysis technology which enables a highly sensitive detection of multiple parameters obtained simultaneously from identical cells, and therefore has a strong ability to detect detailed cellular toxic changes. In the field of the hepatotoxicity, many research reports using HCA have provided helpful information to detect hepatotoxicity, including its mechanisms. HepaRG cells, a human liver cell line, are considered to be one of the best tools to evaluate the human hepatotoxicity of chemicals due to maintained hepatic functions and higher reproducibility of experimental results when compared with HepG2 cells. In this study, we have established the HCA assay protocol and evaluation in HepaRG as well as HepG2 by comparing toxic responses to clinical DILI or Non-DILI compounds between HepaRG and HepG2. HCA included oxidative stress parameters such as reactive oxygen species (ROS) production, glutathione (GSH) consumption, and mitochondrial membrane potential (MMP) attenuation as well as cell viability (ATP). Our in-house case study with HCA is also presented.

DILI and Non-DILI Compounds Tested

The hepatotoxicity of clinical DILI and non-DILI compounds was evaluated in HepaRG and HepG2 cells to compare the toxicity responses between the two cell lines.

Results (HepaRG vs HepG2)

By considering exposure levels in clinic, each HCA positive parameter was quantified according to the right side. Total scores for each compound were determined by adding each quantified parameter's score of GSH, ROS, MMP, and ATP.

HepaRG cells showed stronger effects in a GSH depletion with Troglitazone and Disulfolane and in a ROS elevation with Benzbromarone when compared with HepG2.

Total Evaluation of DILI

Clinical DILI compounds showed a wide range distribution of total points above zero, whereas most Non-DILI compounds showed 0 points. The sensitivity/specificity for clinical DILI was 98.9% and 88.7% at a threshold of total scores for hepatotoxicity was set at 2 points.

The total scores of each HCA parameters improved both the sensitivity and specificity for the hepatotoxicity evaluation.

Conclusion

The HCA assay protocol and evaluation in HepaRG and HepG2 have been validated and established as a predictive in vitro hepatotoxicity test system by evaluating toxic responses to clinical DILI or Non-DILI compounds.

In our HCA assay, HepaRG and HepG2 respond differently to clinical DILI compounds, indicating a possible involvement of reactive metabolites through the drug metabolism enzymes highly maintained only in HepaRG.

Therefore, the comparison between HepaRG and HepG2 is considered valuable for a better understanding of DILI.

The total scores of each HCA parameters improved both the sensitivity and specificity for the hepatotoxicity evaluation.

These results encouraged us to implement the HCA assay in both HepaRG and HepG2 for evaluating the clinical DILI potential in the drug screening process.

Validity to Known Toxic Response

Reported changes in GSH, ROS, and MMP were mostly reproduced in our HCA assay. The specificities to clinical DILI for GSH, ROS, MMP, and ATP in both cells were 70% or greater.

Difference between HepaRG and HepG2

About a half of DILI compounds are known to cause liver injury through reactive metabolites, whereas HepaRG showed more GSH depletion in HepaRG than in HepG2.

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