Evaluation of Kinase Inhibitor Cardiotoxicity with Patient-Specific iPSC-CMs

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Abstract

Background: Kinase Inhibitors (KIs) are a novel class of oncology drugs with over 60 approved by the FDA and more under development; however, many KIs are associated with severe cardiotoxicity in a subset of patients, which cannot be predicted by conventional animal studies. Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) generated from individuals with diverse genetic backgrounds may represent the heterogeneity within the patient population and be a valuable model to identify patients with increased risk of KI cardiotoxicity. **Purpose:** To evaluate the value of a patient-specific iPSC-CM model for personalized KI cardiotoxicity prediction. **Methodology:** Twelve KIs with different targets and variable incidence of cardiotoxicity in the population were tested on 24 lines of iPSC-CMs generated from unrelated, genetically diverse individuals from the HyperGEN cohort. Cardiomyocyte cytotoxicity and beating activity were evaluated with an impedance assay at clinically relevant concentrations over a 48-hour time period after drug exposure. The expression of ~25,000 genes was examined with targeted RNA sequencing technology. **Results:** The twelve KIs exhibited different inter-individual variability on cytotoxicity and the cytotoxic effects were cell line- and drug-specific. For KIs displaying remarkably varied cytotoxic effects, the reproducibility of the assay was evaluated with three batches of iCell cardiomyocytes and an additional line of iPSC-CMs that were generated with the same protocol. Smaller intra-individual than inter-individual variability confirmed the value of the patient-specific iPSC-CM model. Interestingly, based on the expression of specific panels of cardiac marker genes, the cell lines can be clustered into different groups with notably distinct baseline cell index, beating amplitude (AMP), and beating rate. For example, a panel of genes involved in cardiac hypertrophy stratified the cell lines into two groups with remarkably different AMP (0.072 ± 0.025 vs 0.158 ± 0.048, p <0.0001). **Conclusion**: Toxicity evaluation with a panel of iPSC-CMs from multiple donors may better reflect the inter-individual variability of KI cytotoxicity in a study population. Additional analyses will be done to determine if the phenotypes observed on the impedance assay correlated with genotypes/gene expression profiles of patient-specific iPSC-CMs. Potential correlations of functional parameters and gene expression profiles/signaling pathways will be further analyzed to evaluate the value of the iPSC-CM model for personalized KI cardiotoxicity prediction.

Introduction

- KIs have revolutionized cancer treatment; however, some KIs induced severe cardiotoxicity in a subset of patients, which were not observed in conventional animal studies
- Human iPSC-CMs have spurred a great interest for integrated cardiac safety evaluation of new drug candidates and personalized cardiac safety prediction
- Results from studies using patient-specific iPSC-CMs in predicting susceptibility to clinical doxorubicin- or trastuzumab-induced cardiotoxicity suggest that genetic factors may predispose certain patients to oncology drug-induced cardiotoxicity
- A panel of patient-specific iPSC-CMs may be a valuable model to assess the inter-individual variability of KI cardiotoxicity in the population

Materials and Methods

iPSC-CMs

From 250 lines of iPSC-CMs that were generated from selected donors of the HyperGEN cohort by Fujifilm Cellular Dynamics International (FCDI) with the standard protocol used for iCell® cardiomyocytes (CMs), a panel of 24 lines of iPSC-CMs from donors with different ethnic backgrounds and clinical phenotypes were chosen to represent the heterogenicity of the study population. Three batches of iCell-CMs and an additional line of iPSC-CMs were used to evaluate the intra- and inter-individual variability of the assay.

Changes of Impedance Signals after KI Treatment

Twelve KIs with different targets and variable incidence of cardiotoxicity were tested on the 24 lines of iPSC-CMs at three concentrations (1-, 3-, and 10- fold of *Cmax*). The changes of impedance signals before and 1, 6, 24, and 48 hours post compound addition were recorded with the RTCA Cardio instrument and data analyzed with its accompanying software (Agilent). Four biological replicates were run per each condition.

Targeted Transcriptome Sequencing

At 48 hours after drug treatment, total RNA was isolated from each well of cells and a barcoded cDNA library was prepared with 10 ng of total RNA per well. The expression of >20,000 genes was examined using the AmpliSeq for Illumina Transcriptome Human Gene Expression Panel, following the manufacturer's instructions (Illumina, Inc). Gene expression analysis was done using the R/Bioconductor package DESeq2.

Results

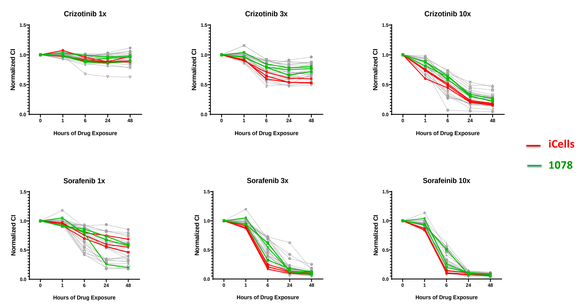

Figure 1. Intra- and inter-individual variability. Three batches of iCell-CMs (in red) and an additional line of iPSC-CMs (1078, in green) showed relatively smaller intra-individual variability on cytotoxicity to crizotinib and sorafenib as compared to the inter-individual differences across the panel of 24 lines of iPSC-CMs (in gray).

Results

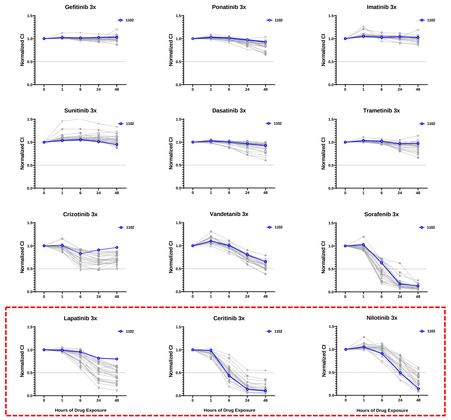

Figure 2. The twelve KIs exhibited different inter-individual variability on cytotoxicity. Individual cell lines were treated with 12 KIs at 3x *Cmax*. The variable cytotoxic responses of the panel of iPSC-CMs suggested that KI cytotoxicity was cell line- and drug-specific. For example, line 1102 (in blue) was more sensitive to ceritinib and nilotinib, but not lapatinib.

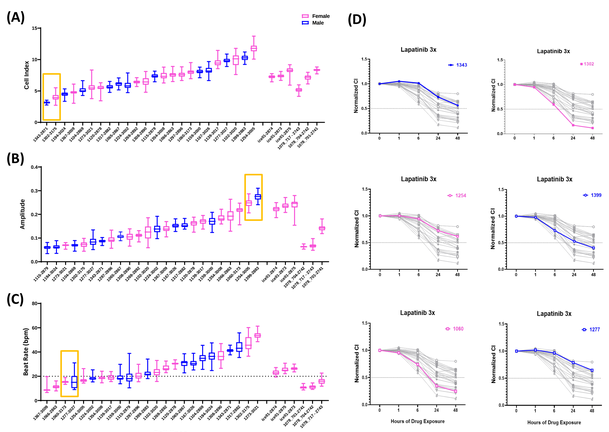

Figure 3. Baseline beating activity of human iPSC-CMs were not a predictor of KI cytotoxicity. (A-C) Box-and-Whisker plots showed variable baseline impedance signals including CI, impedance amplitudes (AMP), and beating rate respectively, among the panel of iPSC-CMs examined in this study. (D) The baseline parameters did not seem to be correlated with the sensitivity for KI cytotoxicity as shown by an example of the differential responses of select sell lines to lapatinib at 3x *Cmax*.

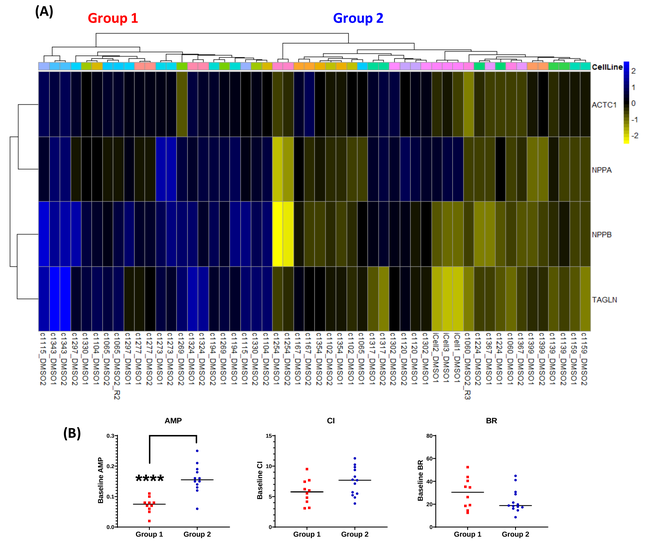

Figure 4. A panel of cardiac hypertrophy marker genes stratified the cell lines into two groups with remarkably different baseline beating amplitude. (A) The heatmap showed the scaled expression of a panel of hypertrophy marker genes in DMSO-treated iPSC-CMs and the clustering of the cell lines. (B) Baseline beating activity of the two groups of cell lines.

Conclusion

- ❑ Toxicity evaluation with a panel of iPSC-CMs from multiple donors may better reflect the inter-individual variability of KI cardiotoxic potential in a study population
- ❑ The baseline phenotypes observed in the impedance assay may be determined by genotypes/gene expression profiles of patient-specific iPSC-CMs
- ❑ Additional analysis is currently underway to evaluate potential correlations of functional parameters and gene expression profiles/signaling pathways that will be useful in assessing the value of the iPSC-CM model for personalized KI cardiotoxicity prediction

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