Cardiotoxicity remains the number one reason for drug withdrawal from the market, and Food and Drug Administration issued black box warnings, thus demonstrating the need for more predictive preclinical safety screening, especially early in the drug discovery process when much chemical substrate is available. Whereas human-ether-a-go-go related gene sequence has become routine to mitigate proarrhythmic risk, the development of in vitro assays predicting additional on- and off-target biochemical toxicities will benefit from cellular models exhibiting true cardiomyocyte characteristics such as native tissue-like mitochondrial activity. Human stem cell–derived tissue cells may provide such a model. This hypothesis was tested using a combination of flux analysis, gene and protein expression, and toxicity-profiling techniques to characterize mitochondrial function in induced pluripotent stem cell (iPSC) derived human cardiomyocytes in the presence of differing carbon sources over extended periods in cell culture. Functional analyses demonstrate that iPSC-derived cardiomyocytes are (1) capable of utilizing anaerobic or aerobic respiration depending upon the available carbon substrate and (2) bioenergetically closest to adult heart tissue cells when cultured in galactose or galactose supplemented with fatty acids. We utilized this model to test a variety of kinase inhibitors with known clinical cardiac liabilities for their potential toxicity toward these cells. We found that the kinase inhibitors showed a dose-dependent toxicity to iPSC cardiomyocytes grown in galactose and that oxygen consumption rates were significantly more affected than adenosine triphosphate production. Sorafenib was found to have the most effect, followed by sunitinib, dasatinib, imatinib, lapatinib, and nilotinib.

**Key Words:** iPSC cardiomyocytes; carbon source; long-term culture; mitochondrial function; kinase inhibitors; bioenergetics; cardiotoxicity screening.

Drug-induced adverse cardiovascular events are the number one cause of drug withdrawal, dose limitations, or black box warnings (Lawrence et al., 2008). The first drug withdrawn from the market for cardiac liability was steritoline in 1991 due to QT prolongation. Several additional drugs have been withdrawn because of adverse effects on cardiac electrophysiology, biochemistry, or physiology including fen-phen, terfenadine, cisapride, rofecoxib, pergolide, sibutramine, and propoxyphene. These drugs were used to treat a variety of disorders, acted through diverse mechanisms, and possessed different structural chemical backbones. Furthermore, members of many different drug classes such as the anthracyclines, nonsteroidal anti-inflammatory drugs, and nucleoside reverse transcriptase inhibitors, while not withdrawn, carry black box warnings limiting their use (Dykens and Will, 2007). Oftentimes, toxic effects were not observed in preclinical or regulatory animal studies (Lasser et al., 2002; Lexchin, 2005). Therefore, the Food and Drug Administration has expanded requirements for cardiotoxicity testing during the preclinical drug development.

An ideal place to start cardiovascular assessment is early in the drug discovery process, when chemical matter is available and Structure Activity Relationship (SAR) can be achieved. Several hurdles currently exist to this approach, including a paucity of relevant tissue cells and imperfect translation of surrogate in vitro and animal model data to predicting cardiotoxicity in humans (Mandenius et al., 2011). In recent years, the utility of stem cells in a variety of different biomedical applications has been the subject of increasing scientific research as they can be maintained and expanded indefinitely in culture and differentiated into a variety of terminal and progenitor cell types (Chapin and Stedman, 2009; Davila et al., 2004). Human cardiomyocytes derived from induced pluripotent stem cells (iPSCs) exhibit many of the characteristics of in vivo cardiac myocytes, including appropriate ion channels, receptors, transporters, and syncytial and contractile activities, and expected electrophysiological and biochemical responses upon exposure to environmental stimuli (Cohen et al., 2011; Guo et al., 2011; Ma et al., 2011). Thus, iPSC-derived cardiomyocytes hold potential as a relevant cellular model for in vitro assessment of human cardiotoxicity (Anson et al., 2011).

Native cardiac tissue is energetically active (Pedersen, 2009) and undergoes a developmental transition from using glycolysis to fatty acid based oxidative phosphorylation (OXPHOS).
as the major and preferred energy source (Allard et al., 1994; Kinnuma et al., 2002; Lopaschuk and Jaswal, 2010). In mature cardiac tissues, mitochondria are the primary sources of cellular bioenergetics, where they account for at least 20% of the myocyte volume and provide the constant energy required to meet the electrical and mechanical demands (Brand and Nicholls, 2011; Hill et al., 2009; Wallace, 2005). In contrast, in vitro cellular models are generally cultured in elevated non-physiological levels of glucose without fatty acids and thus glycolytic. Most cells possess metabolic flexibility to shift their relative reliance on glycolysis versus OXPHOS in response to nutrient availability and thus rely on glycolysis in the presence of glucose despite having fully functional mitochondria (Gohil et al., 2010; Marroquin et al., 2007; Rossignol et al., 2004).

Although human iPS-derived cardiomyocytes possess many attributes of native myocytes, little is known about their glycolytic/OXPHOS profile, their preferred substrate for energy utilization, or their bioenergetic adaptability. To address this unknown, mitochondrial function in iPS cardiomyocytes was characterized when maintained in culture media containing different levels and combinations of glucose, galactose, and fatty acids. Substrate effects were assayed by measuring adenosine triphosphate (ATP) production, the relative contributions of glycolysis, and OXPHOS to the bioenergetic profile, as well as mitochondrial and glycolytic gene expression profiles. Once the model was fully characterized, a variety of kinase inhibitors with known clinical cardiotoxicity liabilities were tested.

MATERIALS AND METHODS

Consumable Materials

Cell culture reagents were obtained from Sigma (St Louis, MO) or Invitrogen (Carlsbad, CA) unless noted otherwise. iPS-derived cardiomyocytes (iCell Cardiomyocytes), plating medium, and carbon source-free basal media (DMEM) with serum were obtained from Cellular Dynamics International (CDI; Madison, WI). The CellTiter-Glo kit was purchased from Promega (Madison, WI). Hoechst 33342 and propidium iodide dyes were purchased from Invitrogen. RNeasy micro kit was obtained from Qiagen (Valencia, CA). The Genomic DNA Elimination Kit, custom PCR (polymerase chain reaction) arrays, cDNA synthesis kit, and SYBR Green/ROX master mix were purchased from Invitrogen. Ribonuclease (RNase) reagent was obtained from Promega (Madison, WI). Hoechst 33342 and propidium iodide at 15 µg/ml final concentration were purchased from Thermo Fisher Scientific Cellomics (Madison, WI). Hoechst 33342 at 30 µg/ml final concentration and propidium iodide at 15 µg/ml final concentration for 30 min at 37°C in clear black collagen-coated 96-well plates prior to acquiring cell counts on a ThermoFisher Scientific Cellomics array scan. Cellular viability was assessed by adding 100 µl CellTiter-Glo (Promega) reagent to each well, agitating for 10 min in the dark at room temperature, prior to acquiring luminescence levels on an EnVision plate reader (PerkinElmer, Waltham, MA).

Measurement of Oxygen Consumption Rate and Extracellular Acidification Rate of iPS-Derived Cardiomyocytes

OXPHOS and glycolysis in iPS-derived cardiomyocytes were assayed by measuring oxygen consumption rates (OCRs) and extracellular acidification rates (ECARs) in real-time using an XF96 Extracellular Flux Analyzer (Seahorse Biosciences) per the manufacturer’s protocols. Low-buffered media for measuring ECAR was composed of DMEM without bicarbonates supplemented with 2mM glutamine, 30mM NaCl, 1mM sodium pyruvate, and carbon source to match the HG, LG, LG + FA, GAL, or GAL + FA (pH 7.4) carbon-source culture media used to maintain iPS-derived cardiomyocytes. Briefly, following maintenance in carbon-sourced media for 7, 14, or 21 days, iPS-derived cardiomyocytes were equilibrated in 150 µl HG, LG, LG + FA, GAL, or GAL + FA low-buffer media for 90 min at 37°C incubator without CO2 prior to obtaining readings. Measurements of oxygen and pH were periodically made over 5 min and OCR and ECAR were obtained from the slopes of concentration change in these parameters versus time. To test the effect of a single compound, four baseline rate measurements of the OCR and ECAR were made using a 2-min mix and 5-min measure cycle. The compounds were then injected pneumatically into each well, mixed, and six OCR and ECAR measurements were made using the 2-min mix and 5-min measure cycle. To test the effect of two compounds, four baseline rate measurements of OCR and ECAR were taken, then the first compound was injected followed by three OCR and ECAR measurements, then the second compound was injected, which was followed by another four OCR and ECAR measurements.

RNA Isolation, RT Profiler PCR Array, and Gene Expression Analysis

Total RNA was isolated using the RNeasy microkit according to the manufacturer’s instructions with an additional genomic DNA elimination step via a Genomic DNA Isolation Kit. In brief, 500 ng RNA was incubated with genomic
Western Blotting

Protein was extracted from iPSC-derived cardiomyocytes using the mammalian cell lysis kit with protease inhibitor (Invitrogen) and quantified using the Bio-Rad Bradford assay (Life Science, Hercules, CA) according to the following thermal cycle conditions: 95°C for 10 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. Each array contained wells that allowed for control of genomic DNA contamination, and first cDNA synthesis efficacy. Genes that had Ct values > 30 were not considered in the data analysis process. Gene expression values were normalized to ACTB, GAPDH, HPRT1, PPIA, and RPS18 housekeeping genes in the custom array (CAPH10095) and B2M, HPRT1, GAPDH, ACTB, and RPL13A housekeeping genes in the human mitochondrial energy metabolism array (PAH-008).

Data Analysis

Gene expression differences and up-down fold regulation (ΔΔCt) were calculated using the online SABiosciences data analysis software. The p values were calculated based on a Student’s t-test of the replicate (2−ΔΔCt) values for each gene in the control group and treatment groups, and p values < 0.05 were considered significant and used for the calculation of fold regulation. The fold-change (2−ΔΔCt) is the normalized gene expression (2−ΔΔCt) in the test sample divided by the normalized gene expression (2−ΔΔCt) in the control sample. Fold regulation values greater than 2 were considered as upregulation; fold regulation values less than −2 were considered as downregulation.

For all other experiments, statistical analysis was performed using two-way ANOVA. A p value < 0.01 was considered statistically significant.

RESULTS

Intra- and Interassay Variation of the OCR and ECAR of iPSC-Derived Cardiomyocytes

To ensure experimental consistency, we first determined the intra- and interassay variation of the OCR and ECAR rates of iPSC-derived cardiomyocytes. The intraassay (within plate) variation of the OCR was 13–17% and the average interassay (day-to-day) variation was 15.4% (Supplementary table 1A). The interassay variation of the ECAR was 11–14%, and the average interassay variation was 12.2% (Supplementary table 1B). The OCR and ECAR rates of iPSC-derived cardiomyocytes from the same batch were very consistent and reproducible measured on 5 separate days. Due to the consistency in the data, further experiments were conducted as two separate experiments.

ATP Levels of iPSC-Derived Cardiomyocytes

Mitochondria are the primary energy suppliers of the cell, providing energy in the form of ATP. Approximately 95% of ATP is produced during aerobic respiration by mitochondria, the rest being made during glycolysis in the cytosol. In order to establish appropriate culture conditions and time points for toxicity testing, iPSC-derived cardiomyocytes were thawed according to the manufacturer’s instructions and maintained in media with different carbon sources containing either HG, LG, LG + FA, GAL, or GAL + FA for up to 21 days. Cardiomyocytes grown in each of the five different media did not show obvious morphological differences (Fig. 1), and ATP content normalized to cardiomyocyte number was approximately equal across all media conditions and showed a consistent intergroup increase over days 7, 14, and 21 in culture (Fig. 2). For experimental details, please refer to Materials and Methods section.

Bioenergetics of iPSC-Derived Cardiomyocytes

Mitochondria are essential for making almost 95% of the cellular energy supply in the form of ATP by OXPHOS. The relative contribution of OXPHOS and glycolysis to ATP production in iPSC-derived cardiomyocytes was determined by simultaneous measurement of dissolved oxygen in the media (expressed as OCR) and (ECAR), respectively, as detailed in Materials and Methods section. The processes underlying OCR were further investigated through modulating mitochondrial function and measuring the relative increases and decreases in oxygen consumption. OCR under basal conditions is due to OXPHOS where electron transport through the protein complexes I–IV is coupled to ATP synthesis (phosphorylation) catalyzed by the ATP synthase (complex V). Inhibition of the ATP synthase by oligomycin decreases OCR leaving only proton leak across the electron transport chain, cell surface oxygen consumption, peroxisosmal oxygen consumption, and substrate oxidation (Abe et al., 2010). Mitochondrial and nonmitochondrial respiration can be further separated by addition of the complex I and II inhibitors rotenone and antimycin A, respectively, to stop proton leak. Conversely, maximal mitochondrial respiration can be investigated by the addition of carbonyl cyanide p-[trifluoromethoxy]-phenylhydrazo (FCCP). Addition of FCCP uncouples respiration and represents the mitochondrial capacity to respond to increased energy demands such as utilization of various substrates under conditions of cell stress. Sequential addition of oligomycin and FCCP illustrates the reserve and maximum respiratory capacities of the mitochondria. These bioenergetic parameters in iPSC-derived cardiomyocytes are shown in...
FIG. 1. Fluorescent microscope images of iPSC-derived cardiomyocytes grown in (A) HG, (B) LG, (C) LG + FA, (D) galactose, and (E) GAL + FA media on day 7 postplating.

FIG. 2. ATP levels were measured and normalized to their respective cell numbers in iPSC-derived cardiomyocytes grown in HG, LG, LG + FA, GAL, and GAL + FA on day 7, 14, and 21 postplating. Each data point represents the mean ± SEM of 10 wells, n = 2 separate experiments. There was no statistically significant difference between media conditions at all three time points.
Figure 3. Detailed data for the mitochondrial bioenergetics profiles at days 7, 14, and 21 can be found in Supplemental tables 2–4, whereas Figures 3–6 only show representative examples of detailed bioenergetics parameters measured.

Basal Respiration and Glycolytic Rates of iPSC-Derived Cardiomyocytes

OCR and ECAR rates were measured in iPSC-derived cardiomyocytes grown in HG, LG, LG + FA, GAL, or GAL + FA at days 7, 14, and 21 postthaw. The OCR/ECAR ratio was determined to assess the relative contribution of glycolysis and mitochondrial respiration to energy generation (Fig. 4). A higher OCR/ECAR ratio indicates that energy is mainly generated through mitochondrial OXPHOS, whereas a lower OCR/ECAR ratio indicates that energy is predominantly generated by glycolysis. The OCR/ECAR ratios of iPSC-derived cardiomyocytes grown in HG, LG, and LG + FA were significantly lower compared with iPSC-derived cardiomyocytes grown in GAL and GAL + FA (Fig. 4). Furthermore, the OCR/ECAR ratio increased over time in iPSC-derived cardiomyocytes grown in media containing GAL and GAL + FA, suggesting that under these conditions iPSC-derived cardiomyocytes increased their mitochondrial function relative to glycolysis.

Reserve Capacity and Maximum Respiratory Capacity

To investigate a causal effect between energy substrate and mitochondrial reserve or maximum respiratory capacity, these two parameters were measured from cardiomyocytes grown...
on each of the five test media. The reserve capacity (Fig. 5A) was significantly higher in cardiomyocytes grown in GAL and GAL + FA for 7 and 21 days than in cells grown in HG, LG, or LG + FA ($p < 0.01$). In addition, the reserve capacity of the LG media group was significantly higher on day 21 than on day 7. Similar observations were made for the maximum respiratory capacity (Fig. 5B). Cardiomyocytes grown in GAL and GAL + FA had significantly higher values than cells grown in HG, LG, or LG + FA for 7 and 21 days. In addition, just as for the reserve capacity, the maximum respiratory capacity in the low-glucose media group was significantly higher on day 21 than on day 7.

Effect of Mitochondrial OXPHOS Modulators on the ATP Production

The contribution of OXPHOS to ATP production under the various growth substrates was determined by assessing the effects of the ATP synthase inhibition (oligomycin; Fig. 6A) or inhibition of complexes I, III, and V (rotenone, antimycin, and oligomycin, respectively; Fig. 6B) on the ATP levels of iPSC-derived cardiomyocytes growing in different substrate media at days 7 and 21. As shown in Figure 6A, inhibition of ATP synthase significantly reduced ATP levels of iPSC-derived cardiomyocytes grown in GAL and GAL + FA, but did not reduce ATP levels in cells grown in HG, LG, or LG + FA. Similar results were observed for inhibition of complexes I, III, and V (Fig. 6B).

Metabolism-Related Gene Expression in iPSC-Derived Cardiomyocytes Growing in Different Substrate Media

Commercial and custom RT² profiler PCR arrays were used to define transcriptional correlates of iPSC-derived cardiomyocyte behavior: potential genomic components of bioenergetic adaptability, protective processes, and differences between the in vitro model and native tissues were investigated by comparing the expression levels of genes involved in mitochondrial OXPHOS, glycolysis, fatty acid/cholesterol metabolism, apoptosis, and oxidative stress in iPSC cardiomyocytes across HG, LG, LG + FA, GAL, or GAL + FA culture media as well as to the expression levels present in adult cardiac tissue. The fold change of these genes across the five media compared with adult tissue is represented by the heat map in Figure 7 and Supplementary table 5. The first observation was that the
examined genes did not show significantly different expression levels as a function of culture media (Fig. 7). Secondly, compared with the adult human heart tissue, ATP synthase, cytochrome C oxidase, and NADH dehydrogenase genes were under expressed, whereas genes involved in cholesterol metabolism (PRKAG1 and PRKAG2) and protection against apoptotic and oxidative stress processes (BCL2L1 and SOD1, respectively) were equal or over expressed.

Protein Expression of iPSC-Derived Cardiomyocytes Grown in Different Substrate Media

Protein expression analysis was used to further define the mechanisms underlying the bioenergetic behavior of iPSC-derived cardiomyocytes. Levels of the mitochondrial complexes I (NDUFB8 CI-20), II (FeS CII-30), III (CIII-Core 2), IV (CIV-II), and V (ATP synthase alpha subunit); COX 4; and COX 6A were determined for cardiomyocytes grown in each of the five media (Fig. 8). Growth in GAL or GAL + FA resulted in higher levels of mitochondrial complexes I–V than the growth of iPSC-derived cardiomyocytes in HG, LG, or LG + FA media. COX 4 protein was equally expressed across all five media conditions, whereas COX 6A levels were higher in LG + FA, GAL, or GAL + FA media versus LG or HG media. In comparison to adult cardiac tissue, the levels of mitochondrial complexes I–V and COX 6A were significantly less in iPSC-derived cardiomyocytes, whereas the level of COX 4 protein was similar. There was no observable expression of SOD1 and BCL2L1 protein in human heart, whereas there was slight expression of these proteins in iPSC-derived cardiomyocytes growing in all the media types.
Effect of Tyrosine Kinase Inhibitors (Antineoplastic Agents) on ATP Production and OCRs

The previous results suggested that maintaining cardiomyocytes in GAL or GAL + FA resulted in ATP production predominately through mitochondrial respiration, rather than glycolysis. This hypothesis was confirmed by observing that inhibition of complex I with rotenone reduced the OCR of iPSC-derived cardiomyocytes growing in all different substrate media, however cell viability was only depleted in cardiomyocytes growing in GAL or GAL + FA (Fig. 9). As ATP production through mitochondrial respiration most closely mimics the native condition, a selection of six anticancer drugs (sorafenib, sunitinib, dasatinib, imatinib, lapatinib, and nilotinib) were tested for their effects on oxygen consumption and ATP content in iPSC-derived cardiomyocytes grown in media containing GAL or GAL + FA at day 21 (Fig. 10). Sorafenib reduced OCR and ATP content of the cells in dose-dependent manner (Fig. 10A). Sunitinib had moderate effect on OCR while drastically reducing ATP content (Fig. 10B). Imatinib and dasatinib had moderate effects on both OCR and ATP content; predominantly observed at 100µM (Figs. 10C and D, respectively). Nilotinib and lapatinib did not have any major effect on either OCR or ATP content of the cells (Figs. 10E and F, respectively). Similar effects were observed when iPSC-derived cardiomyocytes were maintained in GAL + FA medium. The effect of kinase inhibitors on OCRs and cell viability on iPSC-derived cardiomyocytes growing in GAL + FA can be found in Supplementary figure 1.

DISCUSSION

The data presented here highlight several important and novel findings regarding the use and suitability of iPSC-derived cardiomyocytes as an experimental model. Several metabolic, transcriptional, and protein-based investigations defined the
FIG. 7. Heat map of expression analysis of genes involved in the response to mitochondrial oxidative phosphorylation, glycolysis, fatty acid and cholesterol metabolism, oxidative stress, and apoptosis. Fold regulation was calculated using the $-\Delta\Delta C_t$ method comparing over treatment to RNA prepared from human heart tissue. An increase (fold regulation) in gene expression is depicted in red color, whereas a decrease in gene expression is represented by blue color. No differences in expression are depicted in black.
overall bioenergetic profile, metabolic processes, and underlying mechanisms of iPSC-derived cardiomyocytes and examined their responses to therapeutic compound with mitochondrial liabilities. To our knowledge, this is the first study to examine these endpoints and together this information provides a timely dataset that will aid in developing and implementing more relevant in vitro models.

Off- and on-target cardiotoxicity is a primary cause of compound attrition during drug development, and postlaunch restrictions, and withdrawal (Force and Kolaja, 2011). Drug-induced adverse cardiac effects include ion channel block, arrhythmia, altered contractility, ischemia, and many more. Therefore, there is an increasing need for relevant cardiotoxicity assessment throughout the drug development process and

FIG. 8. Western blotting analysis of (A) mitochondrial complexes I–V and human heart tissue; (B) SOD1, BCL2L1, COX 6A, COX 4, and human heart tissue. GAPDH was used as a loading control. (C) Quantification of mitochondrial complexes I–V, SOD1, BCL2L1, COX 6A, COX 4, and human heart tissue. Results are presented as means ± SD, n = 3 separate experiments. Statistical significance changes (*p < 0.001) from adult human heart tissue are reported.
FIG. 9. The percentage change in OCR and cell viability of iPSC-derived cardiomyocytes growing (at 21 days) in (A) HG, (B) LG, (C) GAL, (D) LG + FA, and (E) GAL + FA containing media after addition of rotenone. Each data point represents the mean ± SD, n = 3 separate experiments. *p < 0.001 compares cell viability values to the OCR at various drug concentrations.
FIG. 10. The percentage change in OCR and cell viability of iPSC-derived cardiomyocytes growing on galactose containing media at day 21 after addition of (A) sorafenib, (B) sunitinib, (C) imatinib, (D) dasatinib, (E) nilotinib, and (F) lapatinib. Each data point represents the mean ± SD, n = 3 separate experiments. *p < 0.01 compares cell viability values to the OCR at various drug concentrations.
in particular during the early stages of drug discovery when chemical matter is available that can be subjected to SAR medicinal chemistry efforts. In recent years, it has become routine for most pharmaceutical companies to evaluate cellular toxicity in basic immortalized cell lines. However, it is nearly impossible to predict particular organ toxicity from such a reduced approach (Lin and Will, 2012). For example, assessment of potential cardiac electrophysiological effects is often performed using various immortalized cell lines over expressing a single ion channel, such as human-ether-a-go-go related gene potassium channels (Moller and Witchel, 2011).

Although such models provide a robust tool for examining single-ion channels, they miss potential protein-protein interactions. As a consequence of the disadvantages inherent in current models, recent approaches to predicting arrhythmic effects have utilized cardiomyocytes derived from human iPSCs (Guo et al., 2011; Moretti et al., 2010). Similarly, more holistic cellular approaches have been taken with H9c2, HL-1, neonatal rat and mouse ventricular myocytes, and adult rat/human cardiomyocytes for assessing nonelectrophysiological cardiotoxicity assessment, and the advantages and disadvantages have been discussed recently (Force and Kolaja, 2011).

Predicting specific biochemical and/or metabolic cardiotoxities has been hampered by non-native cell culture conditions where most immortalized cell lines are cultured in high concentrations of glucose in order to facilitate rapid growth and generate energy. Cells in this environment generate ATP primarily through glycolysis, are less dependent on mitochondrial oxidative phosphorylation, and thus have minimal utility in assessing metabolism-related cardiotoxicity as native cardiac myocytes primarily use fatty acids and are highly oxidative in their ATP production (Pedersen, 2009).

Here, we evaluated iPSC-derived cardiomyocytes grown in cell culture across differing carbon substrates for extended durations as a suitable in vitro model with an appropriate bioenergetic profile.

ATP levels in iPSC-derived cardiomyocytes were monitored for 21 days in days in culture and found to increase over time (Fig. 2), which may be due to postdifferentiation development and maturation of the metabolic machinery. Although iPSC-derived cardiomyocytes are fully differentiated cardiomyocytes, they have been shown to exhibit certain characteristics of maturation over time in culture using both electrophysiological and genomic markers (Babiarz et al., 2012; Sartiani et al., 2007).

Carbon source in the media was shown to influence the relative levels of active mitochondrial oxidative phosphorylation and glycolysis, with media containing galactose (GAL) or galactose and fatty acids (GAL + FA) showing the highest level of OXPHOS and thus most closely recapitulating the in vivo condition (Fig. 4). Relative levels of OXPHOS activity also showed an increase over time in culture, with GAL + FA reaching a maximum at day 14. Interestingly, when both glucose and fatty acids were available as a substrate (LG + FA), iPSCs remained fairly glycolytic. Despite the availability of FA, iPSCs appeared to have a preference for glucose under the conditions that we used. The dependence upon mitochondrial respiration as the primary source of energy in cardiomyocytes grown in GAL or GAL + FA was confirmed by a decrease in oxygen consumption upon exposure to the mitochondrial modulators rotenone, oligomycin, and antimycin (Fig. 6) as well as a rotenone mediated loss in cell viability (Fig. 9); in agreement with work by Marroquin et al. (2007) and Will et al. (2007), who also demonstrated that cells grown in media containing galactose were susceptible to mitochondrial modulators.

The bioenergetic parameters of mitochondrial reserve and maximum respiratory capacity also showed a similar dependence upon carbon source and duration in culture. Mitochondria play a very important role in mediating the cellular response to oxidants formed during acute and chronic cardiac dysfunction (Pedersen, 2009). When cells are subjected to a variety of different stress elements, mitochondria are capable of drawing upon a “reserve capacity” which is available to serve the increased energy demands for maintenance of organ function, cellular repair, or detoxification (Hill et al., 2009). iPSC-derived cardiomyocytes grown in GAL or GAL + FA media have a higher reserve and maximum respiratory capacity suggesting that these cardiomyocytes have an appropriate “reserve capacity” relative to cardiomyocytes grown in glucose-containing media (Fig. 5). Taken together, these data demonstrate that when cultured under appropriate media conditions and durations, iPSC-derived cardiomyocytes exhibit an aerobiocally poised metabolism that recapitulates the in vivo condition and provides a suitable model for interrogating several aspects of basal and stressed mitochondrial respiration either as a potential therapeutic or toxic outcome.

iPSC-derived cardiomyocytes showed differing levels of OXPHOS and glycolytic activities as a function of the carbon source in the media (Fig. 4). The expression levels of key genes involved in these processes were lower than those of adult cardiac tissue and did not show significant differences across the different media (Fig. 7). However, iPSC-derived cardiomyocytes growing in GAL and GAL + FA did show higher levels of mitochondrial OXPHOS proteins compared with cells growing in rest of the media conditions (Fig. 8). These results suggest that iPSC-derived cardiomyocytes bioenergetic adaptability occurs posttranscriptionally, and these cells provide a model whereby that adaptability can be modulated or monitored for therapeutic or toxic outcomes.

Compared with adult human heart, iPSC-derived cardiomyocytes showed increased gene and protein expression of the antiapoptotic protein BCL2L1 and SOD1 (Figs. 7 and 8). Overexpression of BCL2L1 has been reported in other stem cell-derived lines and may promote survival and growth in vitro (Amps et al., 2011; Ardehali et al., 2011), whereas SOD1 represents the first line of defense against oxidative stress and may promote survival (Landis and Tower, 2005; Parker et al., 2004). Together, these data suggest iPSC-derived cardiomyocytes in culture may have adaptive processes related to their in vitro environment.
The data demonstrate that iPSC-derived cardiomyocytes grown in GAL or GAL + FA media mature and increase their mitochondrial contribution to energy generation over time and are most reliant on OXPHOS between day 14 and day 21. We tested several kinase inhibitors on iPSC-derived cardiomyocytes growing on this bioenergetically favorable media at day 21. The cytotoxicity rank order of these kinase inhibitors was sorafenib > sunitinib > dasatinib > imatinib > lapatinib > nilotinib, which is in agreement with published literature that discusses these kinase inhibitors leading to some level of clinical cardiotoxicity (Force and Kolaja, 2011). Will et al. (2008) have also studied the effect of multtargeted tyrosine kinase inhibitors sorafenib, sunitinib, and imatinib on mitochondrial function in isolated rat heart mitochondria and in H9c2 cells and showed evidence that these kinases caused mitochondrial liabilities.

In summary, we have characterized iPSC-derived cardiomyocytes growing on different carbon substrates growth over an extended period of time up to 21 days. We conclude that iPSC-derived cardiomyocytes growing in HG, LG, and LG + FA rely primarily on glycolysis for their ATP production, whereas iPSC-derived cardiomyocytes growing on GAL or GAL + FA primarily rely on mitochondrial OXPHOS for their ATP production. The contribution of OXPHOS to ATP production, as well as the appearance of “reserve capacity” components increases over time, reaching a peak between 7 and 14 days in culture with GAL + FA. Gene expression analysis confirmed that these although overall expression levels of mitochondrial OXPHOS genes are lower than in native tissue, these cardiomyocytes are capable of adjusting posttranscriptionally in real-time to different carbon sources. Moreover, iPSC-derived cardiomyocytes growing in GAL and GAL + FA were more susceptible to mitochondrial toxicants suggesting that may be more proficient in identifying cardiotoxicants that will induce mitochondrial liabilities. These data taken together demonstrate that iPSC-derived cardiomyocytes can provide an aerobically poised cellular model that recapitulates native myocyte behavior suitable for cardiomyocyte-based screening.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

ACKNOWLEDGMENTS

We would like to thank Dr William Pennie and Dr Sashi Nadanaciva for their editorial input.

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