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Pluripotent Stem Cell Modeling of Anticancer Therapy–Induced Cardiotoxicity

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Abstract

Purpose of Review In this article, we review the different model systems based on human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) and how they have been applied to identify the cardiotoxic effects of anticancer therapies.

Recent Findings Developments on 2D and 3D culture systems enabled the use of hiPSC-CMs as screening platforms for cardiotoxic effects of anticancer therapies such as anthracyclines, monoclonal antibodies, and tyrosine kinase inhibitors. Combined with computational approaches and higher throughput screening technologies, they have also enabled mechanistic studies and the search for cardioprotective strategies.

Summary As the population ages and cancer treatments become more effective, the cardiotoxic effects of anticancer drugs become a bigger problem leading to an increased role of cardio-oncology. In the past decade, human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) have become an important platform for preclinical drug tests, elucidating mechanisms of action for drugs, and identifying cardioprotective pathways that could be further explored in the development of combined treatments. In this article, we highlight 2D and 3D model systems based on hiPSC-CMs that have been used to study the cardiotoxic effects of anticancer drugs, investigating their mechanisms of action and the potential for patient-specific prediction. We also present some of the important challenges and opportunities in the field, indicating possible future developments and how they could impact the landscape of cardio-oncology.

Keywords Anticancer therapy · Chemotherapy · Cardiotoxicity · Cardio-oncology · Human induced pluripotent stem cell · Cardiomyocyte

Introduction

With a combination of an aging population and increased shares of cancer survivors, potential side effects of anticancer treatments become more evident and prevalent [1]. The clinical picture for patients ranges from mild and temporary reduction in ejection fraction to overt heart failure depending on the chemotherapy agent and pre-existing conditions. That some cardiac malignancies are thought to be reversible is another important factor taken into consideration when choosing a chemotherapy treatment regimen. However, we still lack clear data on both fronts: why some patients develop cardiotoxic effects, and why they might be temporary on some while lead to complete heart damage in others? To address these questions, cardio-oncology has become an emerging field in biomedical research that aims to the address toxic effects of both cancers and anticancer therapeutics [2]. Standard preclinical strategies have, to date, proven unable to provide a clear picture of the likelihood of cardiotoxic effects of chemotherapeutic agents as they largely rely on animal models that provided limited translatability of results into clinical settings [3]. The advancements in human-induced pluripotent stem cells (hiPSCs) and their differentiation into cardiomyocytes (hiPSC-CMs) in the past 12 years possibly can bridge this gap in knowledge. hiPSC-CMs created a new

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powerful tool to replace some of the common practices in preclinical drug development and enabled personalized medicine, yielding a mechanistic understanding of why anticancer agents generate cardiotoxic responses. In this review, we will highlight different model systems based on hiPSC-CMs and how they are used to understand cardiotoxic responses.

Model Systems

Modeling comprises selecting specific components that will provide us with informative elements to further probe a question or to reevaluate the use of tools already in place. In the case of cardiotoxicity studies with hiPSCs and hiPSC-CMs, models range from 2D to 3D culture systems and from tissues formed primarily of cardiomyocytes to combinations of multiple cell types. In the following subsections, we will detail their applications.

2D Cultures

2D cultures are the standard tissue culture platforms used in the early stages of preclinical drug development. hiPSCs are grown in plates, dishes, or coverslips, with little to moderate control of tissue architecture. To obtain a simple monolayer of cardiomyocytes, hiPSCs are cultured in dishes or wells until confluency (60–80% recommended [4]) and differentiated following multiple protocols [5, 6]. These tissues will present varying degrees of contractility, cell-cell junctions, cardiac protein, and gene expression, and electrophysiological properties measurable using microscopy, blotting, PCR, RNA-seq, and patch clamping. When patient-specific samples are used, it is possible to recapitulate more reliably the clinical phenotype, in addition, to identify potential mechanisms for the cardiotoxic response.

Doxorubicin has proven to be the prototypical drug to be studied in hiPSC-CM cardiotoxicity models. We previously used this system to show that hiPSC-CMs recapitulate the clinical phenotype seen in doxorubicin-induced cardiotoxicity (DIC) and have expanded the analysis to identify markers associated with this clinical phenotype. DIC cells presented sarcomeric disarray, increased levels of arrhythmias and ROS, decreased levels of glutathione (GSH), and reduced viability. Additionally, we were able to identify differentially expressed genes between samples from patients without DIC and those with DIC, illustrating the potential of this model system as a mechanistic investigation tool [7]. Similarly, Cui et al. used a monolayer approach to investigate how DIC-related effects are associated with the level of maturity of hiPSC-CMs. Using 30-day-old and 60-day-old hiPSC-CMs, they showed that ROS levels are more exacerbated in older cells than in younger ones due to increased mitochondrial population, in addition to showing that different levels of topoisomerase 2A and 2B (TOP2A and TOP2B) explain the extent of DNA damage in immature cells compared with their more mature counterparts [8]. Knowles, Burrows, and colleagues used hiPSC-CMs from 45 healthy controls to study the differential dose-response to doxorubicin and detected response-expression and response-splicing quantitative trait loci (QTLs) which were also enriched in anthracycline-induced cardiotoxicity genome-wide association studies (GWAS) [9•]. Other studies also investigate the differential expression post-doxorubicin treatment to identify potential biomarkers for DIC and a panel of candidate genes that should be further investigated when designing new chemotherapeutic agents [10–12].

Monoclonal antibodies such as trastuzumab have also been studied using the hiPSC-CM cardiotoxicity model. For example, Necela et al. treated hiPSC-CMs with either trastuzumab or lapatinib and performed differential gene expression analyses to identify mechanisms associated with their cardiotoxic phenotypes. Genes associated with small-molecule metabolism were consistently downregulated in the trastuzumab-treated group; furthermore, these cells presented decreased glucose uptake compared with healthy controls [13]. Similar metabolic downregulation was identified by Kitani et al. in models that used both healthy controls treated with trastuzumab and patient-derived hiPSC-CMs generated from cohorts with moderate and severe trastuzumab-induced cardiotoxicity. In addition to altered metabolism, they identified impaired contractility and autophagy in the samples from the severely impacted patients [14]. De Lorenzo and colleagues evaluated the different responses of pertuzumab and trastuzumab-emtansine (TDM1), two novel anti-ERBB2 agents. TDM1 presented stronger negative effects on cell viability compared with pertuzumab and trastuzumab [15].

A third group of drugs, small-molecule tyrosine kinase inhibitors, have also been studied in 2D. Cohen et al. investigated the cardiotoxic effects of sunitinib, identifying that AMPK and RSK inhibition are not the primary components of sunitinib-mediated cardiotoxicity, but a combined inhibition of multiple kinases simultaneously [16]. Wang and colleagues utilized hiPSC-CMs to investigate the different cardiotoxic responses to an array of tyrosine kinase inhibitors (TKIs) identifying particularly strong effects on metabolism. Specifically, hiPSC-CMs treated with sorafenib presented decreased oxidative phosphorylation and upregulated glycolysis. Cells treated for a week in lapatinib and sunitinib also presented decreased levels of ATP, while those in erlotinib maintained relatively stable levels of mitochondrial membrane potential and ATP [17]. Similarly, a higher throughput screening of TKIs performed by Sharma et al. using samples derived from healthy individuals and cancer patients was performed using hiPSC-CM monolayers. TKIs were assessed for changes in cardiac viability, contractility, calcium handling, electrophysiology, and signaling. From the response profile for each TKI, a "cardiac safety index" was established to reflect more directly the cardiotoxic effects of each compound

and ease clinical translation of the results. Interestingly, cardioprotective effects were observed with co-treatment with exogenous insulin or IGF1, indicating the usefulness of this 2D model to identify mechanistic features of disease and potential protective routes [18, 19••].

Together, these results suggest that 2D hiPSC-CMs are suitable for modeling the full range of anthracycline, monoclonal antibody, and small molecule–based anticancer therapies. It would appear that toxicity of drugs that specifically affect cardiomyocytes, especially those that attenuate mitochondrial-related pathways, is particularly well recapitulated in 2D.

3D Cultures

To further recapitulate the macroscopic properties of cardiac tissue and their effects on cardiotoxic response, 3D tissue culture systems have been developed. They present varying degrees of control of tissue properties and to recapitulate physiology, and they provide informative insights into how cells and tissues respond to drug treatments.

The simplest 3D culture system is cardiac spheroids. In this case, the number of cells and possibly the extracellular matrix they are embedded in are controlled, but little to no architectural constraints are provided to force tissue alignment and cellular distribution. It is also possible to control the cellular population via differentiation techniques and purification of cells before the formation of the spheroids/organoids [20, 21]. This type of system has been used to study the effects of doxorubicin [22–24] and sunitinib [25], and could provide a physiologically relevant response to the compounds.

Differently from cardiac spheroids, engineered heart tissues present a greater degree of control in cellular organization. For example, hiPSCs might be seeded onto or mixed with extracellular matrix proteins that are cast to silicon molds leading to the formation of tissue bundles [26–28] or "tissue rings" [29], ring-like structures [30, 31], or mini-ventricular chambers [32]. An advantage of these types of constructs is to induce cardiomyocyte alignment either via mechanical forces or matrix deposition and organization. A 3D microtissue architecture was used to show that increased afterload on cardiomyocytes was associated with increased sunitinib-induced cardiotoxicity [26].

So far, efforts on modeling cardiotoxic effects of anticancer therapeutics in 3D have validated these model systems but have not yet proven the need for a more complex model. 3D models also introduce variabilities in genomic assessment and make some types of imaging, for example, calcium transients and mitochondrial membrane potential, more difficult, when not impossible. Additionally, the heterogeneity and hindrance of reproducibility that 3D systems might introduce complicate their deployment as the go-to platform for preclinical drug screening and to identify mechanisms behind cardiotoxic effects of drugs. There still might exist applications where they are needed and provide better insights than the present 2D models or more complex 2D systems such as those that incorporate tissue alignment and matrix engineering, especially on drugs that have heterogeneous effects on cells based on distribution and location.

Multicellular Systems

Cardiomyocytes interact with other cell types in the heart mechanically, electrically, and chemically [33]. Therefore, recapitulating parts of those interactions might be essential to understand potential cardiotoxic or cardioprotective responses when modeling chemotherapy-induced cardiotoxicity. Kurokawa et al. combined hiPSC-CMs and endothelial colony-forming cell-derived endothelial cells (ECFC-ECs) to study the protective effects of neuregulin-1 (NRG1) in DIC. They showed that, for hiPSC-CMs alone, the addition of trastuzumab partially negates the protective effects of NRG1. When combining hiPSC-CMs and ECFC-ECs, the addition of exogenous NRG1 does not improve DIC, while co-treatment with trastuzumab and doxorubicin decreases cell viability [34]. It is possible to expand this multicellular monolayer approach to create 3D constructs. Amano et al. used a layer-by-layer technique to fabricate a 3D vascularized cardiac tissue and to investigate its potential for pharmaceutical assays. They combined hiPSC-CMs, endothelial cells, and fibroblasts, and assessed doxorubicin effect on capillary area and cardiac contraction [35]. Another study from the same group utilized a similar technique to probe the effects of doxorubicin on cell viability [36].

Instead of directly seeding together different cell types, microfluidics could be employed to create connecting channels seeded with different cell types. Weng et al. applied this technique to create a cardiac-endothelial-tumor-on-a-chip model and assess the effects of doxorubicin and oxaliplatin on these cell types. They assessed hiPSC-CMs contractility as a proxy for cardiotoxic effects and were able to observe reductions on spontaneous beating rates of myocytes for doxorubicin concentrations near or at the IC_{50}. Moreover, they did not identify cardiotoxic effects for oxaliplatin below the IC_{50} concentration [37]. Another exploration of multiplexing systems using microfluidics included compartments for four systems, heart, brain, liver, and skeletal muscle, and assessed pharmacological responses to known compounds. In the case of doxorubicin, they identified decreased viability and cell beating, similar to other in vitro and in vivo models [38]. Similar designs were also tested with primary cells, where a microfluidic system was used to study the interactions between hepatocarcinoma cells and primary cardiomyocytes. In this case, the cells were just fluidically connected, while residing in chambers that were sufficiently isolated from each other. Interestingly, this study showed cardiotoxic effects for doxorubicinol but not for doxorubicin [39]. With the increased availability of stem cell–derived

tissues, these systems can be further expanded with multiplexing multiple organs-on-chips or organoids to create a "person-on-chip" to recapitulate systemic responses to drugs that are clinically known to induce cardiotoxic effects [40, 41]. Other complex fabrication techniques such as the use of 3D printing and bioprinting can be applied to create either new multiplexing systems with embedded sensors [42, 43] or multicellular systems such as vascularized cardiac patches [44].

Multicellular systems add a level of complexity that might be necessary to study drugs with known or hypothesized multisystem effects. For example, if an anticancer therapeutic was clinically identified to have both cardiotoxic and muscle cachexia effects, modeling the systems together might be useful to perform therapeutic screening and testing, besides identifying possible signaling mechanisms. Additionally, some more complex systems might be warranted when studying cardioprotective drugs that are known to have off-target effects on other organs, such as novel ACE inhibitors that affect kidneys. However, unless a clear need to study organ interactions or to identify simultaneously the effects on two or more organs arise, individualized 2D systems are still advantageous versus multiplexed or multicellular ones.

Challenges and Opportunities

Even though hiPSC-CMs have improved the relevancy of data acquired in preclinical stages of drug development and created new tools to investigate the mechanisms of action/off-target effects of anticancer drugs, important limitations remain. hiPSC-CMs still present a relatively immature phenotype that might not recapitulate to the full extent metabolic responses to pharmacological insults [45], and it is not feasible or cost-effective to keep cells in culture for very long periods [46]. To solve this problem, different maturation media compositions have been developed; however, they are not fully optimized and largely rely on non-fully chemically defined elements [47–50]. Chemically defined compositions are also important if the goal is to multiplex various organ systems into a "person-on-chip," as the same media would perfuse the different organs and carry paracrine signaling from one chamber to another [51]. Furthermore, chemically defined compositions would make results between different research groups more comparable, improving the reproducibility of research. Lastly, fully defined compositions that use commercially available reagents without animal-derived sera can lower costs of science, increasing laboratory throughput and reducing the entry barrier for researchers in different countries.

Another important area of improvement is partially bridging the gap between 2D and 3D systems, potentially increasing the complexity of monolayers by making them more physiologically relevant with the addition of tissue architecture cues and matrix elements. These components have the potential of improving the maturity of hiPSC-CMs [52–55], similarly to chemical cues present in the media described above. Also, this type of system can improve sarcomere alignment

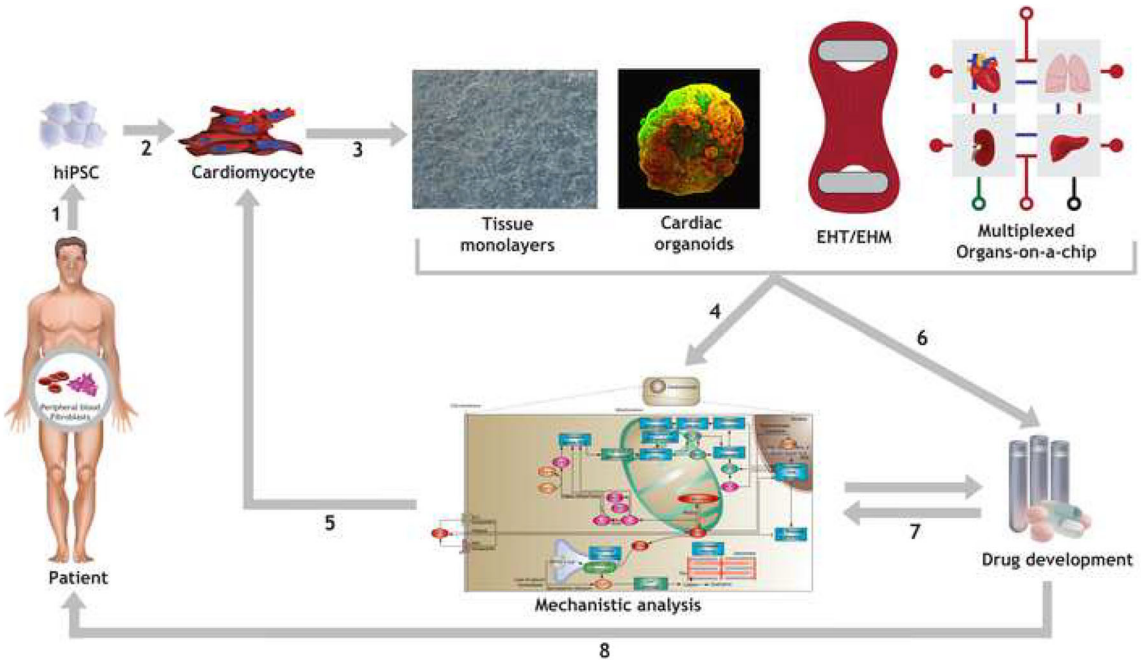

Fig. 1 Pluripotent stem cell modeling of anticancer drug-induced cardiotoxicity. (1) Patient samples, either peripheral blood mononuclear cells or fibroblasts, are isolated and reprogrammed into hiPSCs. (2–3) hiPSCs are differentiated into hiPSC-CMs and models of the heart or body are generated. (4–5) Mechanistic insights are derived from the model and tested in new sets of samples to refine findings. (6–7) Model results and mechanistic insights inform drug development and therapeutic selection. (8) A patient-tailored treatment is selected and applied aiming to reduce cardiotoxic side-effects

[56, 57], nuclear morphology [58], and mitochondrial organization in hiPSC-CMs [59] that potentially affect functional responses [60, 61]. To study myocardial function, tissue engineering techniques for recapitulating cellular architecture, matrix rigidity, and composition are extremely useful and could be further expanded [62, 63]. Those approaches also enable the embedding of sensors into culture substrates instead of indirectly computing metrics using microscopy [43]. Increasing the complexity, but remaining using 2D tissue culture techniques, helps to identify key components of cardiac tissue organization that are essential for the results seen in vivo, while keeping costs low and the systems replicable.

As mentioned before, hiPSC-CMs enable mechanistic studies on human-relevant platforms. Additionally, they enable the discovery or validation of protective pathways that are identified in animal models and are still elusive. For example, Eldridge et al. used hiPSC-CM monolayers to study the protective role of ERBB2 in cardiomyocytes in a model trastuzumab-induced cardiotoxicity. They identified that downregulation of ERBB2 expression attenuated NRG-induced AKT and ERK1/2 phosphorylation, worsening the DIC damage [64]. With increased availability of patient-specific samples, especially from cohorts with differential responses to the same drug, large-scale genomic analyses could be employed to identify both negative and protective pathways. However, more standardize testing, labeling of data, and data analysis approaches are necessary to guarantee correct interpretation of the biological findings and the connectivity between experimental settings.

Conclusion

The advances in hiPSC culture and differentiation in the past decade, combined with the need for a better understanding of the cardiotoxic effects of anticancer treatments, have significantly impacted research in cardio-oncology. Different model systems were developed that benefit from hiPSC-CMs as cell source and finally enabled the identification of the mechanisms behind clinical observations (see Fig. 1). Additionally, the ability to characterize patient-specific responses broadens the scope of science, as personalized approaches will become available for different conditions. As the field further advances, the validation of present techniques and further developments will dictate if and how hiPSC-CMs will keep revolutionizing cardio-oncology and what will be the impact on patient standard of care.

Compliance with Ethical Standards

Conflict of Interest Davi M. Lyra-Leite and Paul W. Burridge declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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