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Special issue on recent progress with hPSC-derived cardiovascular cells for organoids, engineered myocardium, drug discovery, disease models, and therapy

Kenneth R. Boheler¹ · Albano C. Meli² · Huang-Tian Yang^{3,4,5}

Over 35 years ago, Doetschman et al. were the first to describe the *in vitro* differentiation of cardiomyocytes (CMs) from mouse embryonic stem cells (mESCs) [7]. Then, just as today, the leading causes of adult morbidity and mortality in Western Societies were cardiovascular diseases [2]. Doetschman's original findings and those subsequently from other investigators led to the hope that *in vitro* derived CMs could facilitate the improved study of mammalian cardiac development, the creation of new and informative cardiac disease models, and advance testing of drug efficacy and toxicology [37]. Initially, the *in vitro* differentiated CMs from mESCs were described as being small and immature, retained some proliferative capacity, and displayed a developmental continuum in terms of ion channel expression and function. With time, these cells facilitated a reductionist biology approach; i.e., the mESC lines were amenable

to genetic manipulation, which facilitated the analysis of specific gene defects on developmental processes *in vitro* and *in vivo* [4, 36]. While these mouse model systems were instrumental in determining some genetic causes of human cardiac disease, profound physiological and developmental differences between mouse and human (e.g., beating rates, action potentials, ion channel expression, and current profiles) confounded data interpretation.

Ultimately, it was the generation of human ESC (hESC) lines and the emergence of human induced pluripotent stem cells (hiPSCs) that led to an explosion in research activity [31, 32]. Human pluripotent stem cell (hPSC)-derived cardiovascular cells held promise not only for developmental studies and model development of human cardiac disorders that are often complex, polygenic conditions strongly influenced by environmental and genetic factors, but also for cardiac repair and regenerative medicine [6, 8, 16, 23, 36]. From a basic science perspective, these hPSC derivatives had numerous advantages over other mammalian systems for the cellular study of development, physiology, disease,

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and pharmaceuticals. Similar to mESCs, human PSCs are amenable to gene targeting and gene editing using advanced technologies (e.g., CRISPR/Cas 9) [10, 34]. Advances with hPSCs and the generation of isogenic controls have already improved our understanding of genetic factors that contribute to development, rhythm disorders, cardiomyopathies, and valvular and vascular disorders and to metabolic risk factors contributing to cardiomyopathies and ischemic heart disease [24, 26]. During the course of these studies, however, a number of limitations have been identified that have tempered the great expectations for human PSC-derived cardiomyocytes (hPSC-CMs), particularly for regenerative medicine [17, 24]. As noted with mouse, human PSC-CMs are generally immature and retain traits most typical of embryonic or fetal cardiomyocytes [28]. These cells in two-dimensional (2D) cultures also undergo a developmental block in vitro that limits their ability to form more mature, adult-like CMs suitable for drug screening and for model development of adult syndromes [12, 21, 22, 33]. When transplanted into suitable mammalian model systems, only very early in vitro differentiated cells retain the ability of forming cardiomyocytes with “adult-like” morphologies with well-defined contractile properties [11, 12, 29]. Problematically, immature in vitro differentiated hPSC-CMs transplanted into adult hearts contribute to arrhythmias, which may limit their regenerative potential [19]. Genome-wide profiling has shown that 5–46% of hiPSC phenotype variability, including differentiation capacity and cellular morphology, results from differences among individuals [14]. We now recognize that an understanding of the mechanisms underlying cardiovascular cell biology requires defined cell types with knowledge of the molecular species present, how they interact, and how they respond to stimuli both at a cellular and a complex organismal level [3, 12]. Furthermore, studies of molecular and cellular cardiology are challenged by the diversity, complexity, heterogeneity, and dynamic range of cells and molecules present within the cardiovascular system that result in experimental bias according to the constraints of the approaches used. Recent advances in technologies that improve our ability to identify, quantify, localize, characterize, and manipulate the cells, their genomes, their metabolism, and their carbohydrates are paramount in determining how they behave in health and disease.

New cell applications and the emergence of innovative technologies used in the study of hPSC-CMs for basic biology, bioengineering, and regenerative medicine are rapidly emerging. In this special issue entitled “Recent Progress with hPSC-derived Cardiovascular Cells for Organoids, Engineered Myocardium, Drug Discovery, Disease Models and Therapy,” a series of reviews, research perspectives, and primary research articles summarize the current state of hPSC-CM research and describe emerging technologies that are likely to overcome existing limitations to the use of

hPSC-CMs for research and clinical applications. In choosing these topics, it was our aim to introduce the readers to both the current state of research as well as to cutting edge approaches that may represent the driving force for new discoveries and physiological applications of human stem cells to the cardiovascular system.

Issues of cell heterogeneity are addressed in a review by Boheler and Poon [5]. These authors provide an exhaustive overview of some of the current limitations associated with hPSC-CM heterogeneity arising from in vitro differentiation protocols. This includes issues of contaminating non-cardiomyocyte cells and issues of maturation, genetic variability due to diversity among hiPSC lines, cell reprogramming, and culture conditions. The authors describe the heterogeneity in cardiac maturation and current efforts to promote in vitro maturation. They describe current approaches to sort hPSC-CM from non-cardiomyocytes using non-genetic strategies, including the use of the mitochondrial potentiometric dye, lactate metabolism in glucose- and pyruvate-free conditions, temperature control for hPSC-CM enrichment, as well as molecular beacons. The authors then describe how immunophenotyping coupled with cell sorting is a validated approach for overcoming many of these limitations of heterogeneity and provide an exhaustive review of mostly positive selection techniques. Highlights include discussions of cell surface proteins like activated leukocyte cell adhesion molecule (ALCAM), signal regulatory protein alpha (SIRPA, CD172a), and vascular cell adhesion protein 1 (VCAM1) for cardiomyocyte purification and CD36 for isolation of cardiomyocytes with a defined maturation state.

In the research article by Luo et al. [20], the authors used the fluorescent protein Venus to determine whether myosin light chain 2 protein (MLC2v) could serve as a useful marker to distinguish between atrial and ventricular CMs derived from hESCs. This is relevant as MYL2 in mouse is present developmentally in the atrioventricular canal and outflow tract, even though MYL2 is dominantly localized to ventricular myocardium [25]. The authors report a developmental continuum of MYL2 expression of hESC-CMs. At early differentiation stages, both MLC2v-Venus⁻ and MLC2v-Venus⁺ CMs displayed ventricular-like traits, but the ventricular-like cells from MLC2v-Venus⁺ hESC-CMs displayed more developed action potential (AP) properties than those from MLC2v-Venus⁻ hESC-CMs. About half of the MLC2v-Venus⁻ CMs also displayed traits typical of atrial-like cells. At later stages, almost all MLC2v-Venus⁺ cells have ventricular-like characteristics, and relative to the MLC2v-Venus⁻ cells, the Venus⁺ cells had improved sarcomeric structures, calcium handling properties, and mitochondrial function. The MLC2v-Venus⁺ cells were also more sensitive to hypoxic stress. Their findings suggest that early expression of MYL2 may be informative as an early marker of chamber-specific cardiomyocytes. In a separate

research article by Giannetti et al. [9], the authors perform a comprehensive study of the hyperpolarization-activated “funny” current (I_f) in hiPSC-derived CMs with pacemaker activity. This is a timely publication, as the I_f channel is expressed in hPSC-CMs well after the initiation of spontaneous contractions. Their extensive electrophysiological analyses coupled with pharmacological interventions firmly establish the pacemaker CMs as a suitable model for studying human I_f -related sinus arrhythmias.

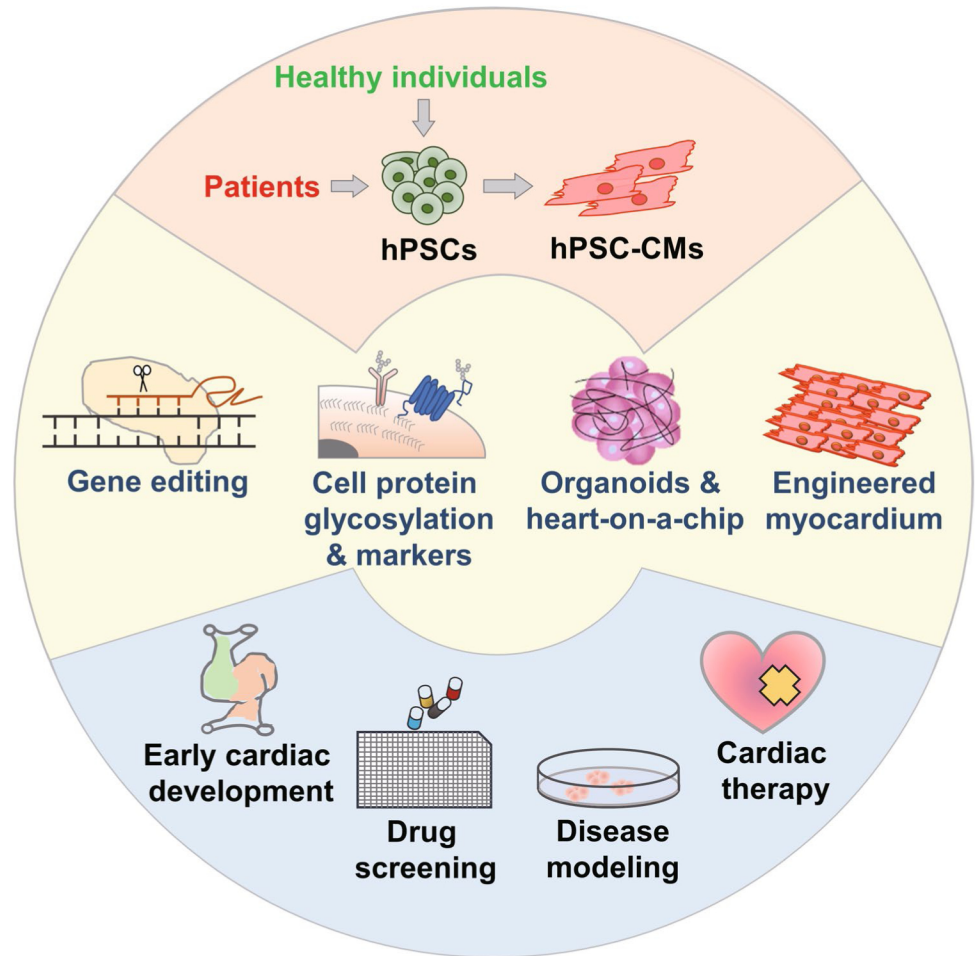
A new and evolving field of research that is poorly defined is glycomics, which is the comprehensive study of the entire complement of sugars present on complex molecules. Because the process of glycosylation depends on cell type and development, the study of glycomics may have profound biological significance on pathways critical for differentiation and maturation of hPSC-CMs. To address this issue, Kelly et al. [13] contributed a perspective article consisting of a review of protein glycosylation (N- and O-linked) coupled with new experimental results showing the dynamics of glycosylation in hPSC-CMs with time of *in vitro* differentiation. The authors provide a comprehensive overview of protein glycosylation, and they discuss how protein glycosylation is affected during CM development and in disease. They discuss strategies required to assess protein glycosylation and review published reports on protein glycosylation during hPSC-CM differentiation. They make a compelling case describing how protein glycosylation is dynamic in hPSC-CMs and how changes in protein glycosylation may critically impact hPSC-CM function and maturation. By combining their findings with RNA-seq analyses, they further demonstrate that proteins and enzymes critical for glycosylation (e.g., beta-galactoside alpha-2,3-sialyltransferase 4) are dynamically present in hPSC-CMs with time *in vitro* differentiation. These findings offer mechanistic insights into how glycan structures vary with time of cultivation and contribute to functional heterogeneity.

Applications of hiPSC-derived CMs for drug screening are provided by Andrysiak et al. [1] and by Liu et al. [18] who emphasized the fact that potential therapeutic compounds must be validated during preclinical and clinical trials. To overcome the expense and differences between animal models and humans, cellular models should facilitate high-throughput screening of chemical compounds. Primary human cardiac cells, however, are difficult to obtain, except when differentiated from hPSC lines. Andrysiak et al. [1] discuss the current state of *in vitro* differentiation of hPSCs to ventricular-, atrial-, and pacemaker-like CMs and described methods to improve CM maturity and functionality. They advocate for the use of hPSC-CMs in conjunction with 3D cardiac structures, including organoids, and 2D heart on a chip models that can promote cell maturation and thus are advantageous for drug screening. The assessment of drugs using hPSC-CMs requires extensive analyses,

including electrophysiological techniques, calcium imaging microscopy, measurement of the cellular contractile properties, and gene expression profile. The authors argue that a comprehensive approach is necessary to truly model cardiac disorders and to delineate possible drug effects using high throughput testing of drug libraries on cardiac 2D and 3D models apt for the study of diverse cardiac diseases. From a different perspective, Liu et al. [18] discuss the lack of suitable humanized preclinical models for drug discovery and development. They review underlying mechanisms implicated in the development of ischemic, inherited, and drug-induced heart disease and then discuss the value of hPSC-CMs to model cardiovascular disorders and for preclinical drug screening. They discuss the relevance of specific hPSCs-based cardiac disease models to understand the complexities of inherited cardiac diseases, including monogenic channelopathies and cardiomyopathies as well mitochondrial cardiac disease. Moreover, they argue that the metabolic state of hPSCs-CMs for cardiac disease modeling is of relevance not only to pathological conditions like metabolic cardiomyopathy induced by type 2 diabetes mellitus, but also for cardiotoxicities induced by chemotherapeutic drugs such as doxorubicin and trastuzumab. They conclude by discussing the challenges associated with hPSC-CMs, and they provide emerging solutions, such as the use of 3D organoid structures and heart-on-a-chip, that may overcome some of these problems.

Svobodova et al. [30] and King et al. [15] review the use of hPSC-CMs in disease models and discuss how improved physiological models that incorporate biomechanical environments with hPSC-models may advance the field. Svobodova et al. [30] utilized Duchenne muscle dystrophy (DMD) as an exemplar for iPSC cardiac disease modeling. DMD is an X-linked disease with either dysfunctional or an absence of dystrophin to cause compromised skeletal and cardiac muscle function. Importantly, the cardiac syndrome associated with DMD is poorly mimicked in mouse models, thus illustrating how and why hiPSC-derived models are so valuable to the understanding of disease processes. *In vitro*, DMD cardiomyocytes from human display modified mechanical and electro-chemical properties, as well as intracellular calcium handling disturbances; however, the hiPSC-derived DMD CMs suffer from incomplete maturation. The authors go on to discuss how these cells may be apt for future therapeutic applications and for patient-targeted drug treatments. King et al. [15] review the physiological relevance of vascularized stem cell derived myocardium and discuss how the generation of biomechanically compatible environments are necessary for improved human models. This comprehensive review discusses cell mechanics, microvasculature, and the convergence of tissue engineering with stem cell biology. They discuss how biomechanics drive human iPSC-derived cardiomyocyte maturation and how

Fig. 1 Human pluripotent stem cells-derived cardiomyocytes (hPSC-CMs) for drug discovery, organoids, disease models, and therapy. Human PSCs can be generated from healthy individuals or patients and edited to create mutant cell lines or to correct existing mutations. The identification of protein glycosylation and cell markers in hPSC-CMs, the construction of organoids as well as heart-on-a-chip, and the engineered myocardium further expand potential applications of hPSC-CMs in early cardiac development, drug screening, disease modeling, and cardiac therapy



interactions between myocardial and microvascular components are necessary to recapitulate physiological biomechanics in vitro. Additional topics covered in this review include discussions of cell polarization, cell–cell communication, and crosstalk. They also discuss how microfluidic systems and bioprinting can promote better endothelial-cardiomyocyte interactions. By using complex bioengineering approaches to create physically relevant biomechanical conditions, they posit that researchers are moving closer to the creation of vascularized human myocardial models with biomechanics that better approximate those of human hearts.

Finally, topics focused on precision medicine are described by Wang et al. [36] and Pan et al. [27]. Precision medicine is best described as “an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle” (<https://ghr.nlm.nih.gov/primer/precisionmedicine/definition>). Wang et al. [36] describe the use of patient-specific iPSCs for the study of cardiomyopathies (involving structural and non-structural proteins), cardiac channelopathies (including those that involve potassium and sodium channels), and other cardiovascular diseases (e.g., DMD,

supravalvular aortic stenosis, and pulmonary arterial hypertension). They discuss the benefits of employing engineered heart tissues for disease modeling and how these systems can be invaluable for drug screening using patient-specific iPSC lines. Pan et al. [27] expand upon this subject by discussing the value of using hiPSC-CM models for the study of rare cardiovascular diseases (RCDs), which affect millions of people worldwide. Although not always uncommon, many of these RCDs are misdiagnosed or overlooked, which creates obstacles to effective treatment. In the past, studies of these rare diseases have often been limited to the use of patient samples or animal models, but the use of hiPSC-CMs has been critical to recent advances for several channelopathies (such as long QT syndrome, short QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia), idiopathic cardiomyopathies, and lysosomal storage diseases. The authors go on to indicate how these diseases can be better understood using precision medicine-based approaches that rely on hiPSC model systems.

In conclusion, we hope that the readers of this special issue will benefit from our selection of topics describing

the current and evolving state of hPSC-derived cardiovascular cells for organoids, engineered myocardium, drug discovery, and disease models (Fig. 1). Of significance, the original research articles included in this issue are meant to stimulate insights into the characteristics of protein glycosylation and cell markers, as well as in the unique value of hPSC-CMs generated from healthy individuals or patients in drug discovery, disease modeling, and therapy. A better understanding of the heterogeneity of the hPSC-CM pool should also promote the development of novel and relevant approaches for generating and characterizing cardiomyocyte subtypes, as in the analysis of the I_f channel. Progress in the construction of hPSC-CM with heterotypic endothelial cell base organoids, heart-on-a-chip, and engineered myocardium will ultimately reveal new insights regarding cardiac development, physiology, drug screening, cardiac disease modeling, and therapy, well beyond the purview of 2D cultures or single hPSC-CMs analyses. By sharing the information on emerging technologies (e.g., glycomics) and on developing technologies necessary to promote research excellence in a more time- and cost-effective manner, this collection should assist readers in the development of innovative research strategies to overcome many of the limitations surrounding the current use of hPSC-CMs. Finally, we suggest that these and other advances will contribute to faster translation of research findings from hPSC-CMs into clinical applications.

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Declarations

Conflict of interest The authors declare no competing interests.

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