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Previews

Uncovering the origins and lineage markers of human heart fields

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In this issue *of Cell Stem Cell*, Yang et al. devise a protocol for induction and differentiation of human heart-field precursors, drawing on inspiration from *in vivo* development. Intricate computational analyses uncovered conserved factors governing heart-field segregation, which facilitate enhanced study of human heart development and disease in the dish.

Since the advent of *in vitro* differentiation of pluripotent stem cells (PSCs), landmark findings—inspired by *in vivo* development—have continued to advance and refine the field. The days of the Hanging Drop, or spontaneously differentiating aggregates of PSCs, were replaced by targeted differentiation approaches, facilitating precise differentiation of multiple cell types and cell subtypes for more efficient study of the cellular diversity of the human body. Yang and The Keller Lab—a pioneer in the field of cardiac differentiation and development—have continued to work toward the goal of generating the cell types and subtypes of the heart through clever cross-species (human versus mouse) and cross-systems (human *in vivo* versus human *in vitro*) computational analyses and rigorous *in vitro* testing.

Heart chambers, valves, and vessels are derived from multiple sources. The two primary sources—the first and second heart fields—develop into the majority of the heart, with some contributions from the neural crest cell lineage (Buckingham et al., 2005). The first heart field (FHF), which is located in the cardiac crescent and populates the left ventricle, and the second heart field (categorized further into the anterior and posterior second heart fields [aSHF; pSHF]), which is positioned dorsal to the FHF and populates the right ventricle and atria, are distinct groups of cells that are specified during early development. Recent studies in mice have suggested that the precursors of these cells are specified around the time of gastrulation (Lescroart et al., 2018). However, less is known about this process in humans due to the inability to routinely access fetal tissues from this developmental window. Diversity in cell source seems to be highly relevant throughout heart development and maturation, as many diseases affect certain portions of the heart—arrhythmogenic right ventricular cardiomyopathy, hypoplastic left heart syndrome, and a variety of valve diseases, to name a few. This cell-type- and cell-source-specific nature of many heart diseases highlights the need to develop systems to study each cell subtype, specifically and precisely, to begin to develop effective therapies and cures.

In this elegant work, Yang et al. (2022) build upon their group's previous efforts to control differentiation of ventricular or atrial cells through modulation of Bmp and Activin signaling (Lee et al., 2017). Here, the authors began by performing single-cell RNA-sequencing (scRNA-seq) of human precardiac mesoderm cells that were induced with varying levels of Bmp4 and Activin A. Interestingly, high levels of Bmp and Activin led to the induction of FHF precursors, while lower levels led to the formation of a heterogeneous population of cells that contained aSHF and pSHF precursors. This distinction at such an early developmental time point (d3 of differentiation) represents an important landmark for the field by capturing this pre-heart field segregation in *ex vivo* cardiac mesoderm and capturing distinct aSHF and pSHF precursors *in vitro* (Figure 1). Furthermore, continued differentiation and culture of these heart-field progenitor cell populations resulted in a variety of cardiomyocyte (CM)-subtypes—right ventricle (RV)-CMs, left ventricle (LV)-CMs, atrial (A)-CMs, atrioventricular canal (AVC)-CMs, sinus venosus (SV)-CMs, and outflow tract (OFT)-CMs—which were verified to undergo appropriate heart-field-specific differentiation by scRNA-seq.

An interesting thread throughout this story is the temporal tracking of cell subtype identity based on scRNA-seq trajectories. This analysis showed that these human heart-field precursors could be distinguished at early development—around the mesoderm induction stage—and that these distinctions persist throughout differentiation and maturation. For example, pre-aSHF cells were distinct from both pre-FHF and pre-pSHF cells, and their progeny (RV-CMs) were transcriptomically and functionally distinct from A-CMs and LV-CMs. The authors also used their trajectory analysis to infer the status of their *in vitro* heart-field progenitors along developmental time. By comparing scRNA-seq data of their hPSC differentiation with previous scRNA-seq from a human gastrulating embryo, they found that the FHF progenitors appeared to be more developmentally advanced than the aSHF and pSHF, whose transcriptomic signature resembled that of nascent mesoderm and primitive streak, respectively. These findings are in agreement with our lab's findings in murine-PSCs (Andersen et al., 2018), which showed sequential induction of FHF and SHF, and those in an *in vivo*

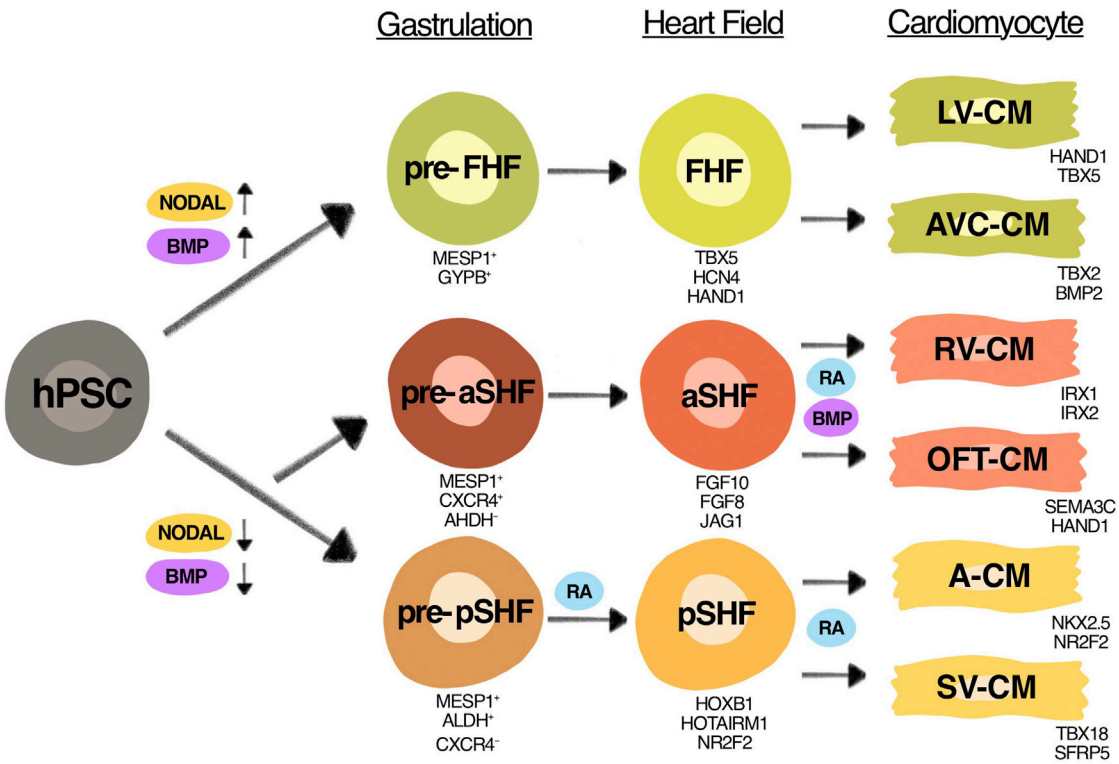

Figure 1. Pre-heart-field cell induction and differentiation in human pluripotent stem cell culture
hPSC, human pluripotent stem cells; FHF, first heart field; aSHF, anterior second heart field; pSHF, posterior second heart field; CM, cardiomyocyte; LV, left ventricular; AVC, atrioventricular canal; RV, right ventricular; OFT, outflow tract; A, atrial; SV, sinus venosus; RA, retinoid acid.

murine study (Ivanovitch et al., 2021), which showed similar sequential FHF, aSHF, and pSHF induction, suggesting a conserved deployment of heart field development across mammals and systems.

After verifying through multiple methods the tight control of their heart-field-specific differentiations, the authors then engineered a method to purify these cell subtypes at the precardiac mesodermal stage. Through identification of FACS-compatible candidates in their scRNA-seq dataset, Yang and colleagues found a combination of fluorescent antibodies to isolate progenitors of each heart field. $CXCR4^{+}/ALDH^{-}$ cells represented the aSHF, $CXCR4^{-}/ALDH^{+}$ cells represented the pSHF, and $GYPB^{+}$ cells represented the FHF. This advance not only allowed for separation of heart-field progenitor populations, but it also maintained the differentiation potential of the isolated heart-field progenitor cells, as these progenitors were able to continue differentiating into their respective, developmentally appropriate lineages. This approach will be especially useful for transcriptomic and functional analysis of chamber-specific CMs *in vitro* in the future.

The authors' application of their machine learning algorithm for identifying cell subtypes to existing differentiation systems was particularly thought provoking. When they applied their algorithm to data from a differentiation protocol that utilized Chir (a widely utilized Wnt agonist) as the main induction agent, the primary identified CM-subtype was RV-CM, with a notable absence of LV-CMs. This finding raises many questions about the CM-subtypes present in a "typical" differentiation. Do differentiations that solely modulate Wnt signaling (GiWi) during induction result in CMs from the SHF lineage alone? Are there batch effects that confound cross-study comparison of scRNA-seq data? Has variability in the heart-field source of CMs affected disease modeling *in vitro*? These uncertainties emphasize the importance of careful phenotyping of CMs—to both cell type and subtype—depending on differentiation conditions when conducting experiments.

These findings from Yang and colleagues represent a significant advance in the quest to more completely elucidate human heart development and disease in the dish by reproducibly obtaining progenitors and CMs from the FHF, aSHF, and pSHF lineages. Application of this approach in a relevant cardiac organoid or engineered heart tissue model (Miyamoto et al., 2021) will be crucial for studying heart-field-heart-field interactions and 3D dynamics of cardiac tissue. A recent preprint by Schmidt et al. begins to address this issue; the authors engineer spheroids composed of adjacent pSHF-like, FHF-like, and aSHF-like cells and pattern them together into one unit to mimic a heart tube with atria, LV, and RV compartments (Schmidt et al., 2022). Whether the process of integrating spheroids from separate differentiations, rather than co-development utilized in the gastruloid system (Rossi et al., 2021), is relevant to *in vivo* biology remains to be seen. Harmonization of studies of this nature and others will likely be an important strategy going forward to capitalize on the important findings reported here.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Fission impossible: Mitochondrial dynamics direct muscle stem cell fates

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Muscle stem cells (MuSCs) exhibit different metabolic profiles depending on their activity, however the mechanisms by which mitochondria affect MuSC fate has been understudied. In this issue of *Cell Stem Cell*, Hong et al. (2022) and Baker et al. (2022) demonstrate that defects in mitochondrial dynamics hinder proper MuSC activation and impair muscle regeneration.

Muscle stem cells (MuSCs) are resident adult stem cells in skeletal muscles that are indispensable for regeneration. These cells sustain quiescence in homeostatic conditions, and once activated by injury, they demonstrate remarkable proliferative and regenerative capacity, leading to repair of damaged muscle. Inappropriate MuSC activation leads to reduced proliferation, delayed differentiation, and impaired regeneration. Although the transition of MuSCs from quiescence to activation is tightly regulated, the molecular components involved in transitioning between MuSC states have not been fully delineated. Recently, autophagy, secreted factors, epigenetic modifications, changes of the stem cell niche (reviewed in Relaix et al., 2021), and remodeling of MuSC cytoskeletal protrusions (Ma et al., 2022; Kann et al., 2022), have been identified as key components of MuSC fate transitions. RNA sequencing (RNA-seq) analysis has shown that quiescent MuSCs utilize mitochondrial fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) rather than glycolysis. Contrarily, proliferating MuSCs express lower OXPHOS and FAO levels, indicating that the metabolic preference in proliferating cells has been shifted toward glycolysis (Pala et al., 2018). However, the exact mechanisms that regulate the mitochondrial elements involved in governing adult MuSC fates during adult regeneration remain elusive.

Two new studies in this issue of *Cell Stem Cell* from Hong et al. (2022) and Baker et al. (2022) attempt to overcome this knowledge gap. Using different genetic and pharmacological methods, they discovered that mitochondrial