Guide to the heart: Differentiation of human pluripotent stem cells towards multiple cardiac subtypes

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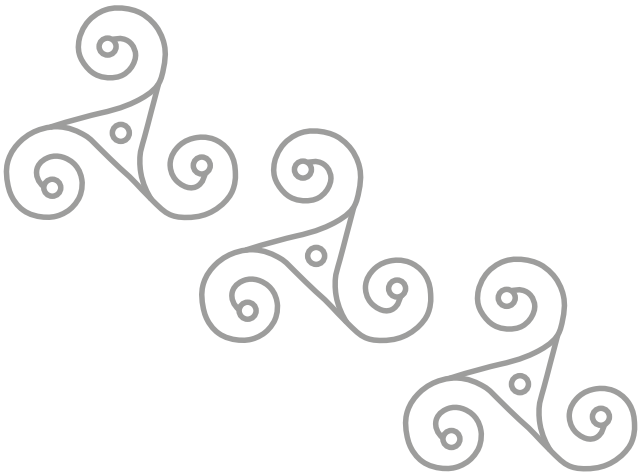

Chapter 1:

General Introduction

Cardiac Development

The first functional organ during human embryonic development is the heart (Figure 1.1). Already early after onset of gastrulation, about 18 days after fertilization, pro-angiogenic and primitive myocardial progenitor cells within the splanchnic mesoderm of the bilateral plates form the first cardiogenic region, the cardiac crescent or first heart field (FHF) (Moorman et al., 2003; Schoenwolf et al., 2015; Sylva et al., 2013). In response to bone morphogenetic protein (BMP) and wingless type (WNT) signaling cues, released by adjacent endodermal and ectodermal layers, clusters of myoblasts and blood islands on each lateral side of the embryo then coalesce to endocardial cords. These quickly develop into two endocardial tubes enveloped by a two-cell thin myocardial layer of primitive cardiomyocytes (CMs) (Schoenwolf et al., 2015; Sedmera and McQuinn, 2008; Sylva et al., 2013). Upon progressive folding of the embryo, both endocardial tubes converge towards the midline and fuse to form the primitive tubular heart before onset of first peristaltic contractions (Schoenwolf et al., 2015; Sylva et al., 2013). Expansion of the linear heart tube, segmented into sinus venosus, primitive atrium and ventricle, bulbus cordis and truncus arteriosus, primarily relies on de novo differentiation of uncommitted CMs from surrounding mesoderm, the second heart field (SHF). The SHF contributes to the arterial outflow tract, part of both atria and the muscular portion of the right, but not the left ventricle (Buckingham et al., 2005; Schoenwolf et al., 2015; Sylva et al., 2013). Upon activation of the left/right asymmetry cascade at around day 23, looping of the heart is initiated. The venous part of the heart tube folds dorsally, which bends the bulbus cordis – future right ventricle and outflow tract - and primitive ventricle ventrally before rightward looping of the bulbus cordis. Only upon rightward looping of the heart, CMs of atrial and ventricular chambers enter their distinct transcriptional programs, manifested by the upregulation of early chamber-specific markers followed by atrial- and ventricular-enriched transcription factors, structural proteins and ion channels (Sylva et al., 2013). Following looping of the heart tube, atrial and ventricular chambers grow by re-initiation of proliferation in CMs at the venous pole for atrial and at the outer curvature for ventricular chamber expansion (Sylva et al., 2013). Simultaneously, the thin myocardial layer of the early heart tube thickens into compact multi-layered myocardium with trabecular protrusions by clonal expansion of single CMs (Schoenwolf et al., 2015; Sedmera and McQuinn, 2008; Sylva et al., 2013). Faster expansion of the right ventricle and outflow tract by contribution from the SHF, as well as

growth of endocardial cushion tissues at the atrioventricular junction and outflow tract, contribute to properly align the future right and left atrium with their respective ventricles, as well as the connection between the future left ventricle and the outflow tract (Schoenwolf et al., 2015; Sylva et al., 2013). To complete septation into a four-chambered heart, endocardial cushion tissues at the atrioventricular junction fuse to build the atrioventricular septum and membranous parts of the interatrial and interventricular septa, while muscular regions develop by proliferation and migration of CMs in the myocardial walls (Anderson et al., 2003; Schoenwolf et al., 2015; Sylva et al., 2013).

In the primitive embryonic heart tube unidirectional, slowly propagating contraction waves are generated in the sinus venosus myocardium to pump fetal blood from the venous to the arterial pole. In contrast, in the mature heart, the electrical trigger that initiates cardiac contraction originates from the intrinsic and fast depolarization of CMs within the sinoatrial node, the primary pacemaker of the heart, located in the posterior wall of the right atrium. From the sinoatrial node impulses are propagated via the right and left atrium to the atrioventricular node and the conduction system of the ventricles (Sedmera and McQuinn, 2008). In spite of commonly accepted differences in cardiac physiology of rodents and humans, such as heart rate and distribution of contractile proteins or ion channels (Kaese and Verheule, 2012; De Sousa Lopes et al., 2006), most knowledge about the development of the cardiac pacemaker has been gained from animal studies. In murine and avian hearts, specialized CMs of the sinoatrial node either arise from a third cardiogenic mesoderm or are formed by a minor proportion of CMs which retain their automaticity in the posterior wall of the right atrium, while surrounding CMs acquire atrial identity (Bakker et al., 2010; Bressan et al., 2013; Christoffels et al., 2006, 2010; Mommersteeg et al., 2010; Spater et al., 2013).

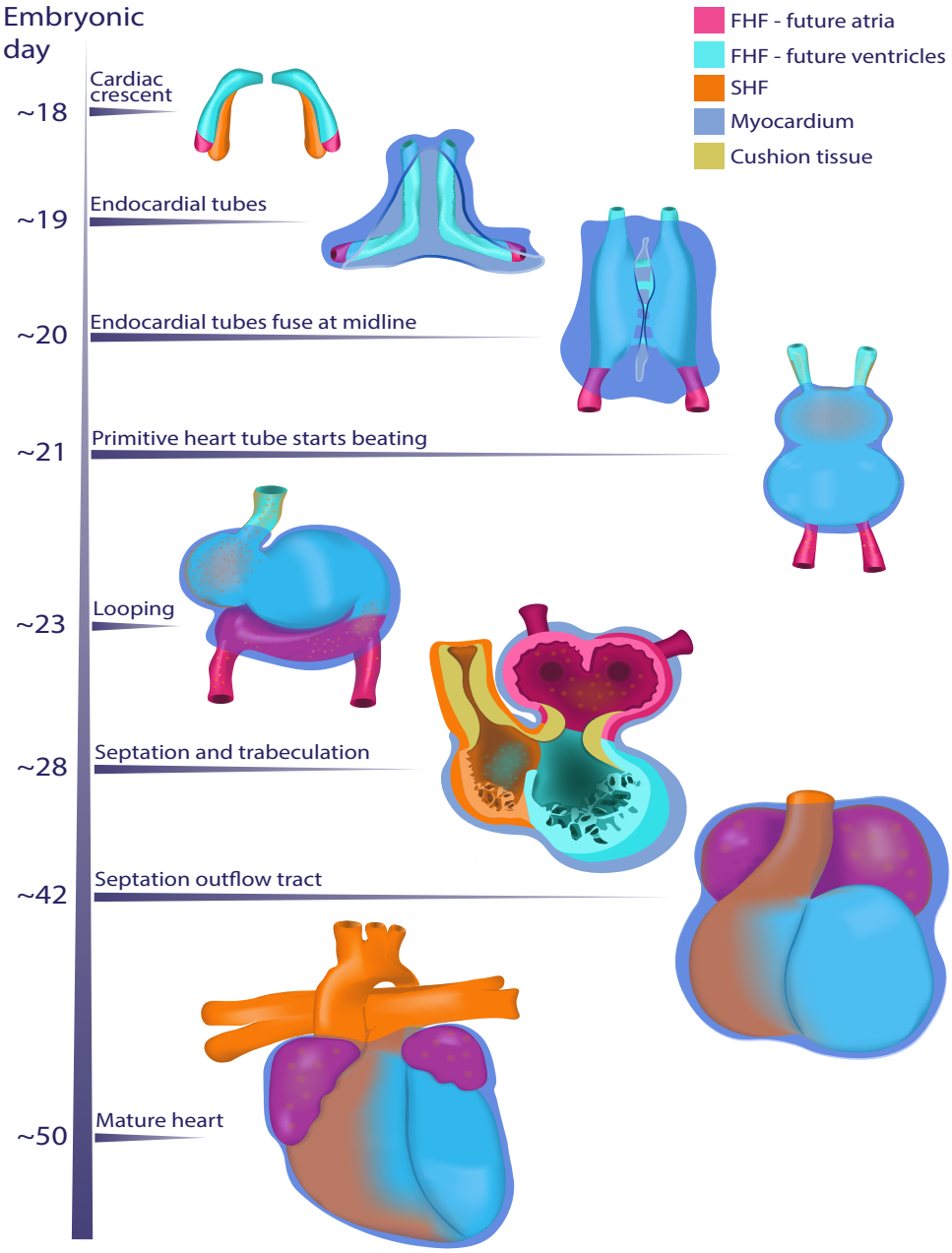

Figure 1.1: Cardiac development

Era of human pluripotent stem cells

Human pluripotent stem cells (hPSCs), both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), possess the remarkable abilities to self-replicate indefinitely and differentiate into every cell type of the three germ layers: ectoderm, mesoderm and endoderm, by recapitulating molecular events that are crucial for *in vivo* embryonic development (Figure 1.2). Importantly, since the first isolation of hESCs from the inner cell mass of human blastocysts in 1998 (Thomson et al., 1998), these properties have been valuable for studying developmental biology, as well as disease modeling *in vitro*, drug screening and discovery, as well as regenerative medicine. About a decade later – in 2007, the next milestone was achieved with the generation of hiPSCs by reprogramming somatic cells into pluripotent stem cells through transient overexpression of only four transcription factors: OCT4, SOX2, c-MYC and KLF4 (Takahashi et al., 2007). Induction of pluripotency in cells with a patient-specific genetic background permits the development of *in vitro* disease models recapitulating clinical features of inherited diseases and specific response to treatment, which is beneficial for understanding disease and the development of new therapies. In the cardiac field, the derivation of cardiac progenitor cells (CPCs) and CMs from human pluripotent stem cells (hPSCs) has substantially advanced our knowledge about molecular mechanisms underlying cardiac specification and disease development in human (Beqqali et al., 2009; Davis et al., 2011). Importantly, because of the inaccuracy of current drug prediction models, hPSC-derived CMs (hPSC-CMs) have rapidly been considered as a promising alternative in safety-pharmacology and pre-clinical drug discovery (Sala et al., 2016). More advanced models to study complex interactions between different cell types or cell-matrix-communication within the heart necessitate development of three dimensional (3D) *in vitro* engineered heart models. Apart from these applications, another more long-term goal of hPSCs and their derivatives are cell-based repair and tissue engineering for organ replacement of tissues with limited regenerative capacity, as for example the heart, with an annual CM turnover of less than 1% (Bergmann et al., 2009). HPSC-CMs pose the possibility for cardiac repair either by transplantation into injured areas after myocardial damage to restore contractile function, or by *in vitro* tissue engineering of artificial heart transplants for replacement. However, transplantation of hPSC-CMs in rodent models of heart disease only improved short-term cardiac function, but had no beneficial long-term effects (van Laake et al., 2007; Laflamme et al., 2007). Although hPSC-CMs

survived and engrafted, grafts failed to couple to host myocardium which is a pre-requisite for synchronized contraction (van Laake et al., 2007). Myocardial injection of hPSC-CMs and allogeneic iPSC-CMs into non-human primate hearts post myocardial infarction (MI) was capable to remuscularize injured myocardium and to electrically couple to the host, but also led to transient non-fatal, but life-threatening ventricular arrhythmias (Chong et al., 2014; Liu et al., 2018; Shiba et al., 2016). Arrhythmic activity in the graft persisted only within an initial adaptation period and vanished upon further *in vivo* maturation of the transplanted hPSC-CMs (Chong et al., 2014; Liu et al., 2018). However, despite major progress, safety concerns and technical hurdles on the transplantation of hPSCs and their differentiated progeny have made their routine clinical application more challenging than initially anticipated. Until now, only in a single clinical case, purified, clinically-grade hPSC-derived CPCs have been transplanted after MI in 7 patients with severe heart failure (Menasché et al., 2015, 2018). Transplantation of CPCs embedded in a fibrin patch to the epicardial layer of the failing heart has proven to partially improve systolic heart function without adverse effects, including arrhythmias or tumorigenicity, within 18-month follow-up (Menasché et al., 2015). Importantly, transplanted CPCs have been generated by short-term BMP4 treatment which has not been proven to faithfully commit to the cardiac lineage. *In vitro* engineered 3D artificial hearts could provide an alternative for the shortage in available donor hearts for transplantation after heart failure. However, in contrast to gut, brain or kidney organoids, hPSC-CMs are unable to self-organize into complex tissue structures (Clevers, 2016). Also decellularized mouse hearts with architectural and extracellular matrix clues of native heart failed to deliver necessary force to pump blood after repopulation with hPSC-CMs (Lu et al., 2013), indicating the need for an optimal microenvironment and defined cardiac subtypes, including atrial- ventricular, as well as CMs of the conduction system, in combination with non-cardiac cells for complex 3D tissue engineering of the heart. Overcoming technical challenges, such as (1) generation of high numbers of defined cardiac subtype-specific cells with high purity and (2) creating the biological niche that mimics native heart to boost *in vitro* maturation towards an adult phenotype, may pave the way for hPSCs-based regenerative medicine and tissue engineering in the future. In this regard it is very promising that hPSCs have been implemented in clinical phase I or II trials for a variety of disease conditions, including age-related macular degeneration, diabetes, Parkinson disease and spinal cord injury (Trounson and DeWitt, 2016).

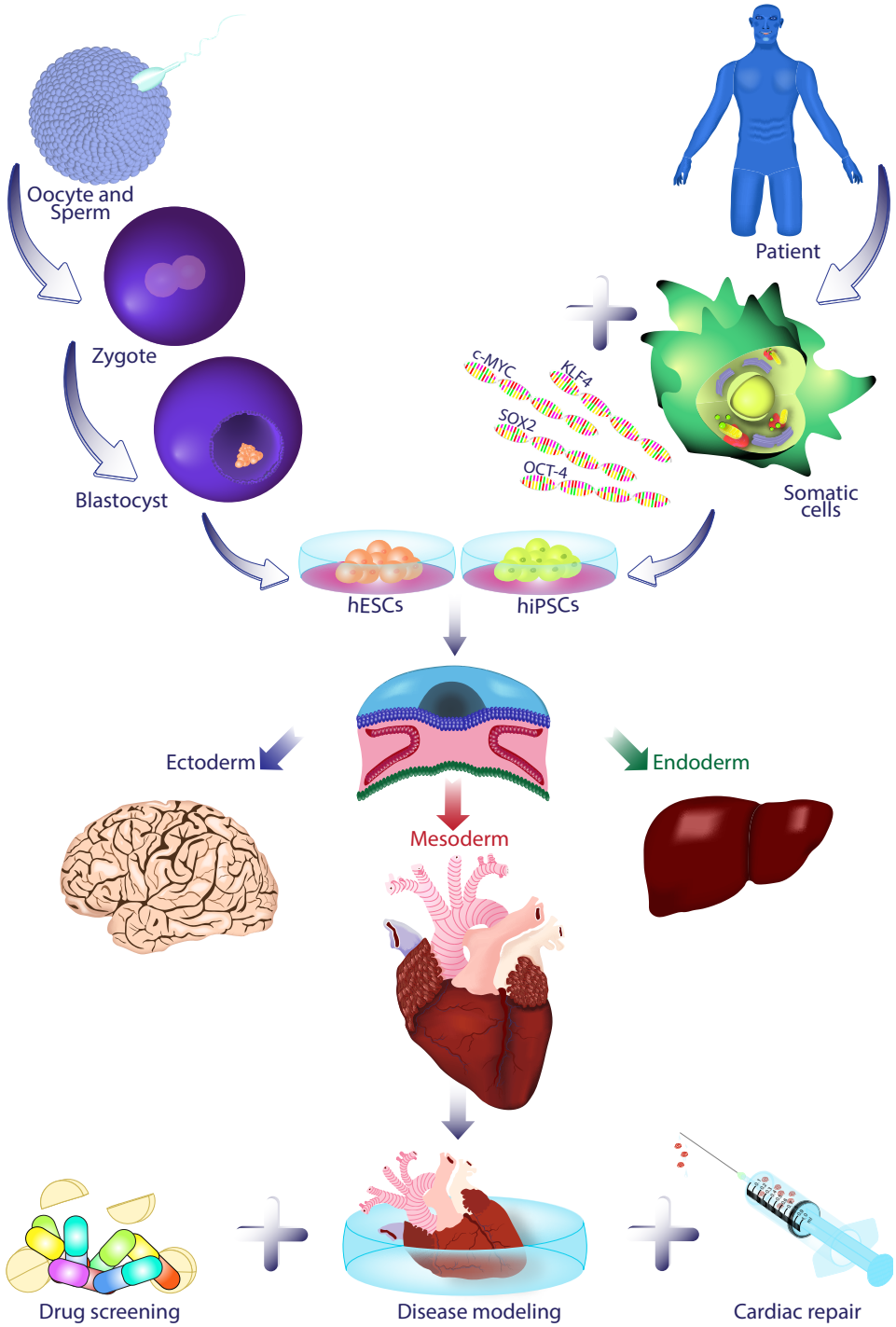

Figure 1.2: Derivation of hESCs and hiPSCs and their biomedical applications

Scope of this thesis

General aim of this thesis was to explore the generation of multiple human pluripotent stem cell (hPSC)-derived cardiac subtypes and their application for selective pharmacology, understanding human cardiac development and cardiac repair.

In **chapter 2** of this thesis, we describe approaches for the differentiation of hPSCs to cardiomyocytes (CMs) followed by magnetic bead-based purification from heterogeneous cultures.

In **chapter 3**, we focused on the derivation and characterization of hPSC-derived CMs with atrial identity and their application as pre-clinical pharmacological tool.

In **chapter 4**, we aimed to identify atrial- and ventricular specific surface markers for the separation of atrial and ventricular CMs.

In **chapter 5**, we developed a human atrial reporter line by CRISPR/Cas9-mediated knockin of red fluorescent mCherry into the genomic locus of atrial-enriched COUP-TFII to select atrial CMs. In addition, we evaluated the importance of COUP-TFII for atrial differentiation of hPSC *in vitro*.

In **chapter 6**, we described the potential of hPSC-derived cardiac progenitor cells (CPCs) to improve cardiac function, ventricular remodeling and fibrosis after transplantation to the heart after acute myocardial infarction in mice.

In **chapter 7**, we reviewed the native cardiac environment and current knowledge regarding extracellular matrix preferences for engineering cardiac tissues from hPSC-CMs.

In **chapter 8**, we discussed each chapter of this thesis and future perspectives.

References

Anderson, R.H., Webb, S., Brown, N. a, Lamers, W., and Moorman, A. (2003). Development of the heart: (2) Septation of the atriums and ventricles. Heart 89, 949–958.

Bakker, M.L., Christoffels, V.M., and Moorman, A.F.M. (2010). The cardiac pacemaker and conduction system develops from embryonic myocardium that retains its primitive phenotype. J. Cardiovasc. Pharmacol. 56, 6–15.

Beqqali, A., Van Eldik, W., Mummery, C., and Passier, R. (2009). Human stem cells as a model for cardiac differentiation and disease. Cell. Mol. Life Sci. 66, 800–813.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabé- Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., et al. (2009). Evidence for Cardiomyocyte Renewal in Humans. Science (80-.). 324, 98–102.

Bressan, M., Liu, G., and Mikawa, T. (2013). Early Mesodermal Cues Assign Avian Cardiac Pacemaker Fate Potential in a Tertiary Heart Field. Science (80-.). 340, 744–748.

Buckingham, M., Meilhac, S., and Zaffran, S. (2005). Building the mammalian heart from two sources of myocardial cells. Nat. Rev. Genet. 6, 826–835.

Chong, J.J.H., Yang, X., Don, C.W., Minami, E., Liu, Y.-W., Weyers, J.J., Mahoney, W.M., Van Biber, B., Cook, S.M., Palpant, N.J., et al. (2014). Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature 510, 273–277.

Christoffels, V.M., Mommersteeg, M.T.M., Trowe, M.O., Prall, O.W.J., De Gier-De Vries, C., Soufan, A.T., Bussen, M., Schuster-Gossler, K., Harvey, R.P., Moorman, A.F.M., et al. (2006). Formation of the venous pole of the heart from an Nkx2-5-negative precursor population requires Tbx18. Circ. Res. 98, 1555–1563.

Christoffels, V.M., Smits, G.J., Kispert, A., and Moorman, a. F.M. (2010). Development of the pacemaker tissues of the heart. Circ. Res. 106, 240–254.

Clevers, H. (2016). Modeling Development and Disease with Organoids. Cell 165, 1586–1597.

Davis, R.P., van den Berg, C.W., Casini, S., Braam, S.R., and Mummery, C.L. (2011). Pluripotent stem cell models of cardiac disease and their implication for drug discovery and development. Trends Mol. Med. 17, 475–484.

Kaese, S., and Verheule, S. (2012). Cardiac electrophysiology in mice: A matter of size. Front. Physiol. 3 SEP, 1–19.

van Laake, L.W., Passier, R., Monshouwer-Kloots, J., Verkleij, A.J., Lips, D.J., Freund, C., den Ouden, K., Ward-van Oostwaard, D., Korving, J., Tertoolen, L.G., et al. (2007). Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction. Stem Cell Res. 1, 9–24.

Laflamme, M. a, Chen, K.Y., Naumova, A. V, Muskheli, V., Fugate, J. a, Dupras, S.K., Reinecke, H., Xu, C., Hassanipour, M., Police, S., et al. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat. Biotechnol. 25, 1015–1024.

Liu, Y.W., Chen, B., Yang, X., Fugate, J.A., Kalucki, F.A., Futakuchi-Tsuchida, A., Couture, L., Vogel, K.W., Astley, C.A., Baldessari, A., et al. (2018). Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. Nat. Biotechnol. 36, 597–605.

Lu, T.-Y., Lin, B., Kim, J., Sullivan, M., Tobita, K., Salama, G., and Yang, L. (2013). Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nat. Commun. 4, 2307.

Menasché, P., Vanneaux, V., Hagège, A., Bel, A., Cholley, B., Cacciapuoti, I., Parouchev, A., Benhamouda, N., Tachdjian, G., Tosca, L., et al. (2015). Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: First clinical case report. Eur. Heart J. 36, 2011–2017.

Menasché, P., Vanneaux, V., Hagège, A., Bel, A., Cholley, B., Parouchev, A., Cacciapuoti, I., Al-Daccak, R., Benhamouda, N., Blons, H., et al. (2018). Transplantation of Human Embryonic Stem Cell–Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular Dysfunction. J. Am. Coll. Cardiol. 71, 429–438.

Mommersteeg, M.T.M., Domínguez, J.N., Wiese, C., Norden, J., De Gier-De Vries, C., Burch, J.B.E., Kispert, A., Brown, N.A., Moorman, A.F.M., and Christoffels, V.M. (2010). The sinus venosus progenitors separate and diversify from the first and second heart fields early in development. Cardiovasc. Res. 87, 92–101.

Moorman et al. (2003). Development of the heart (1) formation of the cardiac chambers and arterial trunks. Heart 89, 806–814.

Sala, L., Bellin, M., and Mummery, C.L. (2016). Integrating cardiomyocytes from human pluripp-

otent stem cells in safety pharmacology: has the time come? Br. J. Pharmacol. 1–17.

Schoenwolf, G., Bleyl, S., Brauer, P., and Francris-West, P. (2015). Larsen's Human Embryology.

Sedmera, D., and McQuinn, T. (2008). Embryogenesis of the Heart Muscle. Heart Fail. Clin. 4, 235–245.

Shiba, Y., Gomibuchi, T., Seto, T., Wada, Y., Ichimura, H., Tanaka, Y., Ogasawara, T., Okada, K., Shiba, N., Sakamoto, K., et al. (2016). Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. Nature 538, 388–391.

De Sousa Lopes, S.M.C., Hassink, R.J., Feijen, A., Van Rooijen, M.A., Doevendans, P.A., Tertoolen, L., De La Rivière, A.B., and Mummery, C.L. (2006). Patterning the heart, a template for human cardiomyocyte development. Dev. Dyn. 235, 1994–2002.

Spater, D., Abramczuk, M.K., Buac, K., Zangi, L., Stachel, M.W., Clarke, J., Sahara, M., Ludwig, a, and Chien, K.R. (2013). A HCN4+ cardiomyogenic progenitor derived from the first heart field and human pluripotent stem cells. Nat Cell Biol 15, 1098–1106.

Sylva, M., Van den Hoff, M.J.B., and Moorman, A.F.M. (2013). Development of the human heart. Am. J. Med. Genet. Part A 164, 1347–1371.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131, 861–872.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. Science (80-.). 282, 1145–1147.

Trounson, A., and DeWitt, N.D. (2016). Pluripotent stem cells progressing to the clinic. Nat. Rev. Mol. Cell Biol. 17, 194–200.


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