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Differentiation, Characterization and Applications of Human Embryonic Stem Cell – Derived Cardiomyocytes

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1. Introduction

The term "embryonic stem (ES) cell" was introduced in 1981 to distinguish embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonal carcinoma (EC) cells (Martin 1981). First ES cells were derived from mouse intracellular mass (ICM) in the same year (Evans and Kaufman 1981) and in 1994 Bongso and co-workers reported the successful isolation of human ICM cells and their continued culture for at least two passages in vitro (Bongso, Fong et al. 1994). The first permanent human embryonic stem cell (hESC) lines were derived more than a decade ago by Thomson and co-workers (Thomson, Itskovitz-Eldor et al. 1998) and these lines are still widely used.

hESCs are capable of proliferating extensively at undifferentiated state in vitro and have the ablity to differentiate towards all three germ layers and furthermore can, in principle, give rise to all cell types of the body. Adult human cardiomyocytes have limited capability to regenerate and the heart tissue cannot undergo extensive repair needed for example after myocardial infarction. Therefore, the rapid development of stem cell technology has raised hopes for new treatments for tissue damage of cardiac and other tissues with limited regenerative capacity. Human embryonic stem cells (hESC) have the ability to differentiate into functional cardiomyocytes by multiple differentiation methods. Traditionally hESC-derived cardiomyocytes (hESC-CM) are differentiated spontaneously in embryoid bodies (EB) or in co-culture with mouse endodermal cell like (END-2) cells (Kehat, Kenyagin-Karsenti et al. 2001; Mummery, Ward-van Oostwaard et al. 2003). In addition, more defined differentiation methods using growth factors have been developed (Laflamme, Chen et al. 2007; Yang, Soonpaa et al. 2008). However, the cardiac differentiation is still quite uncontrolled and inefficient and even though new more defined differentiation methods have been published, spontaneous differentiation in EBs and differentiation in co-culture with END-2 cells are still widely used as they are rather inexpensive and functioning for most of the hESC lines.

At the end of the year 2009, the total number of hESC lines worldwide has been estimated to be 1071 (Loser, Schirm et al. 2010). Even though the number of hESC lines has increased steadily, two lines, H1 and H9 (WiCell Research Institute), are the most used ones in stem cell research (Guhr, Kurtz et al. 2006; Scott, McCormick et al. 2009; Loser, Schirm et al. 2010).

After reporting the concept of induced pluripotent stem cells (iPS cells) in 2007 (Takahashi, Tanabe et al. 2007), these mature cell derived pluripotent stem cells have rapidly emerged in stem cell studies with hESCs or replacing them.

According to comparison studies, hESCs have been shown to be similar, but not identical according to the expression of pluripotency markers (Adewumi, Aflatoonian et al. 2007). Greater variation between cell lines has, however, been observed after cells start to differentiate - the expression of differentiation markers and differentiation propensity varies between different hESC lines (Adewumi, Aflatoonian et al. 2007; Kim, Kim et al. 2007; Osafune, Caron et al. 2008; Pekkanen-Mattila, Kerkela et al. 2009; Pekkanen-Mattila, Pelto-Huikko et al. 2010). Due to this observation, it has been suggested, that the most suitable hESC line should be chosen according to its propensity to differentiate towards lineage of interest (Osafune, Caron et al. 2008).

Pluripotent stem cell derived cardiomyocytes can be obtained in cell culture, but their therapeutic use is in its infancy. Functional cardiomyocytes can, however, be obtained with various differentiation methods and already at the moment they can be used to study cardiac differentiation, the effects of different exogenous factors on they behaviour and potentially in the near future also cardiac side effects in pharmacological and toxicological industry.

2. Spontaneous cardiac differentiation in EBs

hESCs can be differentiated spontaneously as embryoid bodies (EB). In principle, during EB formation the culture condition for stem cells is changed from two-dimension into three-dimensional. First pluripotent stem cells are either enzymatically or mechanically dissociated to small cell clumps or single cells. Secondly cells are let to form aggregates in suspension and factors needed to keep them pluripotent are removed. After few days, EBs are formed and they are plated down on matrix coated cell culture plates (Kurosawa 2007). When hESCs are removed from the environment which supports the undifferentiatied state (feeder cells and the growth factor FGF), they start to differentiate towards three germ layers in the cell aggregates (Itskovitz-Eldor, Schuldiner et al. 2000). During the early stages of suspension culture, the cell aggregate transforms into cystic body and trilayer shell composed of extra cellular proteins forms around EB (Sachlos and Auguste 2008). The paracrine and endocrine signaling determine the stem cell fate. Similarly as in embryo this signaling may lead to the formation of concentration gradient in the EBs and further influence the cell differentiation (Sachlos and Auguste 2008).

EB formation has similar characteristics as embryonal development (Keller 1995) and therefore the interplay of different germ layers and their influences into cell differentiation can be studied in EB cultures. The EB differentiation, such as cardiac differentiation, is particularly well documented with mouse ES cells (Hescheler, Fleischmann et al. 1997; Boheler, Czyz et al. 2002). However, the EB formation of hESC and the spontaneous differentiation has proven to be more difficult and more inefficient if compared to mouse counterpart (Wobus, Wallukat et al. 1991; Kehat, Kenyagin-Karsenti et al. 2001). When mouse ES cells are differentiated in EBs, beating areas appear 1 day after plating, and, within 2–10 days, 80–90% of EBs show spontaneous beating (Wobus, Wallukat et al. 1991). In the hESC differentiation beating areas are observed later and the differentiation efficiency is traditionally much lower, usually under 10% (Kehat, Kenyagin-Karsenti et al. 2001).

Cardiomyocyte differentiation from hESC and iPS cells in EBs has been described in many reports (Itskovitz-Eldor, Schuldiner et al. 2000; Kehat, Kenyagin-Karsenti et al. 2001; Zhang,

Wilson et al. 2009). EB differentiation is widely used also in production of other cell types such as neuronal cells, hematopoietic cells, adipocytes and chondrocytes (Pera and Trounson 2004). For the whole existence of hESC, EB differentiation has been widely used differentiation method for its relatively simple and inexpensive nature.

There are multiple methods for EB formation (Kurosawa 2007). Suspension culture in bacterial-grade cell culture dishes was first developed for mouse ES cells (Doetschman, Eistetter et al. 1985) and has later been used in cardiomyocyte differentiation from hESCs (Itskovitz-Eldor, Schuldiner et al. 2000; Kehat, Kenyagin-Karsenti et al. 2001). In this method enzymatically dissociated cells aggregate when cultured unattached in the culture medium. hESCs are more vulnerable to dissociation to the single cell stage (Thomson, Itskovitz-Eldor et al. 1998; Amit, Carpenter et al. 2000; Kehat, Kenyagin-Karsenti et al. 2001; Xu, Police et al. 2002) and therefore hESCs have been tradionally dissociated into small clumps of cells to retain the cell to cell contact (Amit, Carpenter et al. 2000; Pyle, Lock et al. 2006), but current technology enables also single cell stage with hESCs (Amit, Chebath et al. 2010). To scale up EB formation in suspension cultures, bioreactors and spinner flasks have also been used (Messina, De Angelis et al. 2004; Kurosawa 2007; Yirme, Amit et al. 2008).

Cardiomyocytes have also been differentiated by hanging drop-method, where single cell suspension is pipetted to small drops onto petri dish cover and cover is then inverted on top of a dish (Takahashi, Lord et al. 2003; Burridge, Anderson et al. 2007). The drop hangs because of surface tension and provides a good environment for the cells to aggregate and form the EB. Hanging drop-method is not suitable for long term EB differentiation because the medium change is impossible (Kurosawa 2007). Overall hanging drop-method is very laborious and therefore it is not suitable for large scale experiments.

Recent studies indicate that the EB size has an effect on cardiomyocyte differentiation as well as differentiation in general (Burridge, Anderson et al. 2007; Bauwens, Peerani et al. 2008; Mohr, Zhang et al. 2010). Therefore the amount of cells should be measurable in order to optimize differentiation. Hanging drop method enables to standardize the initial amount of hESC. However, Ng and coworkers developed more robust method compared to hanging drops, a forced aggregation (FA) or spinEB system for hematopoietic differentiation and it has been used also in cardiomyocyte differentiation (Ng, Davis et al. 2005; Burridge, Anderson et al. 2007). FA mimics the hanging drop method, the cells are forced to aggregate by centrifugation in a round bottomed, low-adherence 96-well plate wells. The medium change is possible to the wells and therefore longer culture times can be used and also differentiation inducing agents can be added to the culture medium (Burridge, Anderson et al. 2007). Two-dimensional cell pieces can also be produced by microprinting technique, where standard-size colonies are formed and then scraped into suspension culture (Bauwens, Peerani et al. 2008; Niebruegge, Bauwens et al. 2009). EB differentiation techiques are summarized in Table 1.

3. Cardiac differentiation in END-2 co-cultures

More directed way to differentiate cardiomyocytes from hESCs is in co-culture with mouse endodermal-like cells (END-2), particularly in a absence of serum and with ascorbic acid (Mummery, Ward-van Oostwaard et al. 2003; Passier, Oostwaard et al. 2005). The differentiation inducing factors are secreted from END-2 cells and therefore the END-2 conditioned medium can also be used in cardiomyocyte differentiation (Graichen, Xu et al. 2008). END-2 cells support the differentiation towards endodermal and mesodermal

EB differentiation techniques					
Method description	**Hanging drop**	**Forced aggregation (FA)**	**Suspension culture**	**Microprinting technique**	**Manual**
hESC colony dissociation	Enzymatic dissociation			Detachment of microprinted colonies	Manual cutting
EB formation	Single cells/small aggregates form EB in a hanging drop	Cell suspension is aggregated to EB by centrifuging in a 96-well plate	Spontaneous aggregation in suspension	One cell colony or cell colony piece forms an EB in suspension	
EB culture	Formed EBs transferred for suspension culture		Suspension culture continues		
	After suspension culture EBs are plated on a coated cell culture plate				
Advantages	Gentle EB formation in a drop because of gravity	Scalable, straightforward, cell number per EB easy to standardize	Straightforward	Cell number per EB easy to standardize	Gentle, non-enzymatic hESC colony dissociation
Disadvantages	Laborious, non-scalable	hESC colonies have to be dissociated to single cell stage	Forming EBs randomly sized	Need for microprinting technique for colony formation	Laborious, non-scalable
Reference	(Takahashi, Lord et al. 2003)	(Ng, Davis et al. 2005)	(Doetschman, Eistetter et al. 1985)	(Bauwens, Peerani et al. 2008; Niebruegge, Bauwens et al. 2009)	Pekkanen-Mattila, Peltohuikko et al., 2010)

Table 1. Summary of the EB differentiation techniques.

derivatives (Mummery, Ward-van Oostwaard et al. 2003; Passier, Oostwaard et al. 2005; Beqqali, Kloots et al. 2006) which is in accordance with embryonal development studies, which show that anterior visceral endoderm is essential in normal heart development (Lough and Sugi 2000).

The mechanism or the specific factors inducing cardiac differentiation by END-2 cells are, however, not clearly known. Systematic testing of END-2 conditioned medium revealed that END-2 cells were able to clear insulin from the medium (Xu, Graichen et al. 2008). Insulin has been shown to inhibit cardiac differentiation by suppressing endoderm and mesoderm formation and favouring ectoderm differentation (Freund, Ward-van Oostwaard et al. 2008). Insulin acts via the insulin-like growth factor-1 receptor (IGF-1R) and phosphatidylinositol 3-kinase (PI3K/Akt) pathway and has been suggested to inhibit epithelial-to mesenchymal transition by elevated levels of E-cadherin (Freund, Ward-van Oostwaard et al. 2008). However, IGF/PI3K/Akt has also been shown to have a role in proliferation of immature

cardiomyocytes (McDevitt, Laflamme et al. 2005) which suggests that this pathway has a dual role in cardiomyogenesis. Additionally, END-2 cells are not the only type of cells which clear insulin from the culture media. Similar phenomenon has been observed with MES1-cells (Mummery, Feijen et al. 1986) and mouse embryonic fibroblasts (MEFs) which do not have the cardiac differentiation inducing effect (Xu, Graichen et al. 2008). Therefore insulin depletion is not likely the cardiac inducing factor of END-2 cells. A more promising cardiac differentiation inducer of END-2 cells has been suggestested to be prostaglandin I2 (PGI2). END-2 cells have been reported to secrete more PGI2 than other type of mouse cells which lack the cardiac inductive effect. In fact, addition of PGI2 to differentiation medium has been reported to result in similar levels of cardiac differentiation as END-2 conditioned medium (Xu, Graichen et al. 2008).
In addition to the PGI2, inhibition of p38 mitogen activated protein kinase (MAPK) increases cardiac differentiation rate (Graichen, Xu et al. 2008). Selective MAPK inhibitors (molecules SB203580 and SB202190) (Cuenda, Rouse et al. 1995) were found to increase the differentiation rate when added to END-2 conditioned medium. However, the inductive effect of these molecules was concentration dependent, at high concentrations (>15 μM) cardiomyocyte formation was decreased and finally inhibited (Xu, Graichen et al. 2008). The use of p38 inhibitor PD169316 also causes mouse ES cells to differentiate towards neural linage while the cardiac mesoderm formation is inhibited (Aouadi, Bost et al. 2006). Therefore the inhibition of MAPK has a partially opposite effect on mouse and human cells and its exact role in cardiac differentiation remains to be revealed.

4. Differentiation with defined growth factors

Cardiac differentiation is controlled by a complex signalling network and currently there is no single factor that would direct stem cells to differentiate effectively towards cardiac lineage. Laflamme and co-workers have used the combination of activin A and BMP-4 in cardiomyocyte differentiation (Laflamme, Chen et al. 2007). This cascade of factors enhances mesoendoderm formation, an early precursor cell lineage which gives rise to mesoderm and endoderm. Mesoderm is the origin of cardiac cells and it has been shown that cardiac differentiation inducing signals are in large extent arising from endoderm (Lough and Sugi 2000). Therefore mesoendoderm induction would yield more efficient human embryonic stem cell-derived cardiomyocyte (hESC-CM) differentiation.
Stepwise differentiation protocol was also developed by Yang and co-workers (Yang, Soonpaa et al. 2008). This protocol involves induction of primitive streak-like population, in addition to formation of cardiac mesoderm and expansion of cardiac lineages. Protocol is based on EB differentiation and is comprised of three stages. Growth factors BMP-4, FGF, activin A, vascular endothelial growth factor (VEGF) and dickkoptf homolog 1 (DKK1) were used in varying combinations.
Mesoendoderm formation has also been induced by Wnt3A, an activator of the canonical Wnt/β-catenin signalling pathway (Tran, Wang et al. 2009).
Taken together, even though the use of growth factors and other chemicals may enhance the cardiac differentiation, pure populations of cardiomyocytes can not be currently produced and enrichment methods are still needed. Due to multi-phased differentiation protocols, the high costs of growth factors, and the complexity of defining right concentrations and exposure times to different factors, the simple and functional EB differentiation method needs widely used method in cardiomyocyte production. Also the current differentiation

methods produce beating aggregates, but for many purposes cardiac differentiation in monolayer would be more optimal, but this method is still to be developed further.

5. Characterization of differentiated cardiomyocytes

5.1 Structural analysis

Differentiated hESC-CM have the capacity to beat spontaneously and are, thus, easily detected in the culture as beating areas (Kehat, Kenyagin-Karsenti et al. 2001; Mummery, Ward-van Oostwaard et al. 2003). Beating cells are at early stage relatively small and round and situated in circular accumulations in the EBs. At later stages, EBs gradually develop larger and the cells turn into more elongated in shape and tend to accumulate in strands. Electron microscopy studies reveal that cardiomyocytes contain myofibrils which are first randomly and in a varying manner distributed throughout the cytoplasm. However, organised sarcomeric structures occur at later stages of differentiation with A, I, and Z bands. In the vicinity of the sarcomeres, mitochondia are also present. In addition, cells have intercalacted disks with gap junctions and desmosomes (Kehat, Kenyagin-Karsenti et al. 2001; Snir, Kehat et al. 2003; Pekkanen-Mattila, Kerkela et al. 2009).

5.2 Estimation of differentiation efficiency and expression of cardiac markers

The number of hESC-CM containing beating areas has been used in quantifying differentiation efficiency of different hESC lines (Passier, Oostwaard et al. 2005; Pekkanen-Mattila, Kerkela et al. 2009; Pekkanen-Mattila, Pelto-Huikko et al. 2010). However, Passier and co-workers reported a large variation in the number of cells in the beating areas, ranging from 1-2500 cells (Passier, Oostwaard et al. 2005). Therefore, to determine the amount of hESC-CM more accurately, cytospin analysis has been used in the estimation of percentage of cardiac marker positive cells per total amount of cells (Graichen, Xu et al. 2008; Xu, Graichen et al. 2008).

Cardiac troponin T (cTnT) is encoded by the TNNT2 gene (Thierfelder, Watkins et al. 1994) and is the tropomyosin-binding subunit of the troponin complex and therefore can be used in characterizing hESC-CM (Figure 2E). Troponin complex is located on the thin filament of striated muscles and regulates muscle contraction in response to alterations in intracellular calcium ion concentrations as revieved (Farah and Reinach 1995; Tobacman 1996). In addition to cTnT, other cardiac spesific structural proteins are used for confirming cardiac phenotype of the beating hESC-CM such as cardiac troponin I, myosins (Figure 2F) or cardiac α-actinin (Kehat, Kenyagin-Karsenti et al. 2001; Mummery, Ward-van Oostwaard et al. 2003). Even though many cardiac markers exist, there is a lack of cardiac specific surface proteins and therefore lack of antibodies for making sorting by fluorescence-activated cell sorting (FACS) possible (Mummery 2010).

Another approach to estimate the cardiac differentiation efficiency and follow the differentiation is to quantify the mRNA levels of cardiac markers and cardiac differentiation markers. hESC-CM differentiation can be predicted by the transient expression of the early mesodermal marker Brachyury T. Brachyury T expression peak is detected at day 3 in END-2 co-cultures (Beqqali, Kloots et al. 2006; Pekkanen-Mattila, Kerkela et al. 2009) and a day later in EBs (Bettiol, Sartiani et al. 2007; Pekkanen-Mattila, Pelto-Huikko et al. 2010). Brachyury T belongs to the family of transcription factors which are encoded by the T-box genes (Showell, Binder et al. 2004). Brachyury T can be nominated as a classical transcription factor, it is localized in the nucleus and is an endogenous activator of mesodermal genes (Conlon, Lyons et al. 1994; Kispert, Koschorz et al. 1995; Showell, Binder et al. 2004). In the embryo,

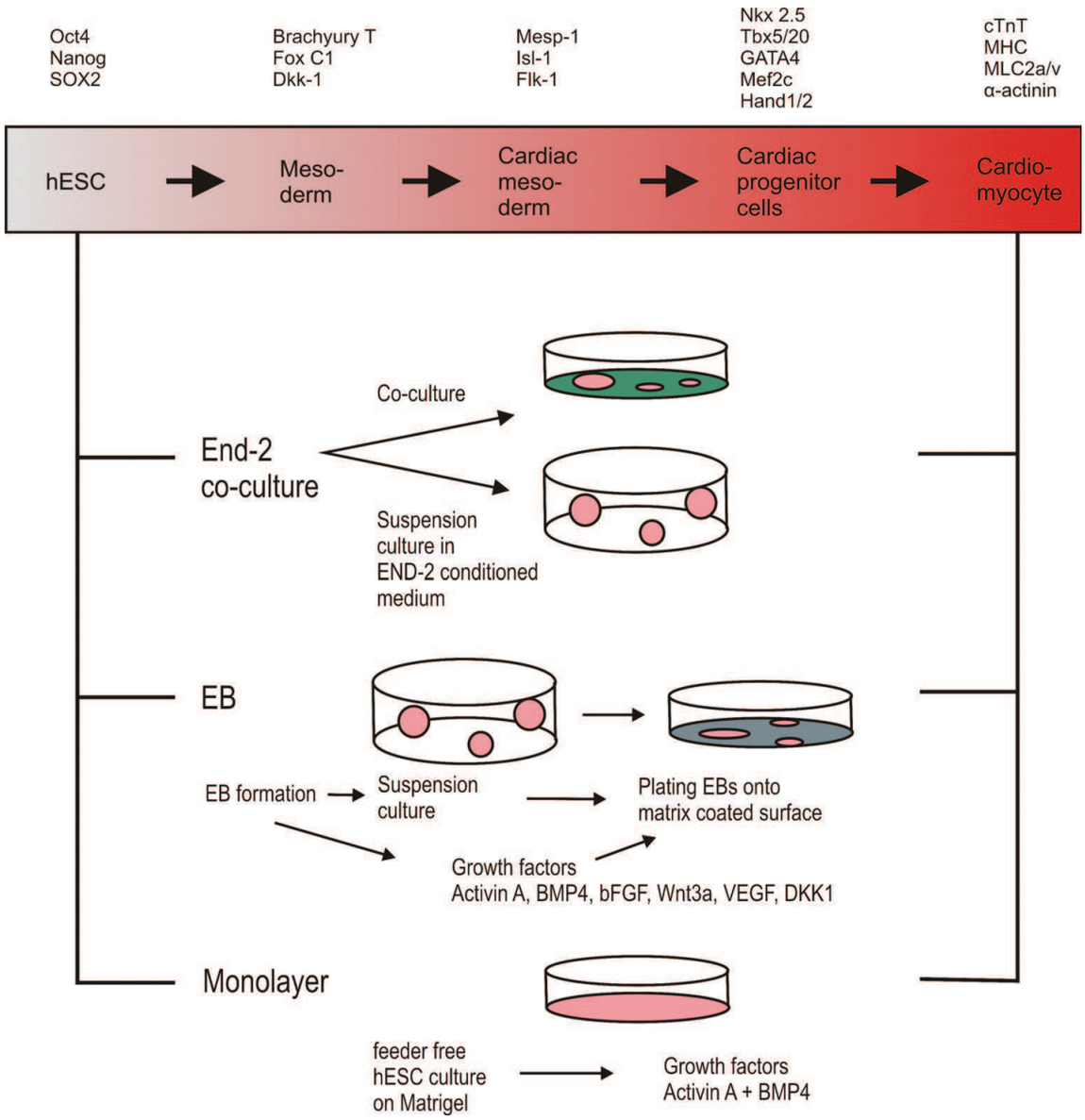


Fig. 1. ***Cardiac differentiation steps and differentiation methods.*** From the top; Markers for different stages of cardiac differentiation, steps in cardiac differentiation and schematic view of differentiation methods. **END-2** differentiation has two variables, hESC are either plated on top of END-2 cell layer or hESC are cultured as EBs in suspension in END-2 conditioned medium. **EB method**, differentiation can be performed spontaneously or with differentiation inducing growth factors. **Monolayer differentiation** is initiated with feeder free hESC cultures. Culturing of hESC and differentiation with activin A and BMP-4 is performed on top of Matrigel.

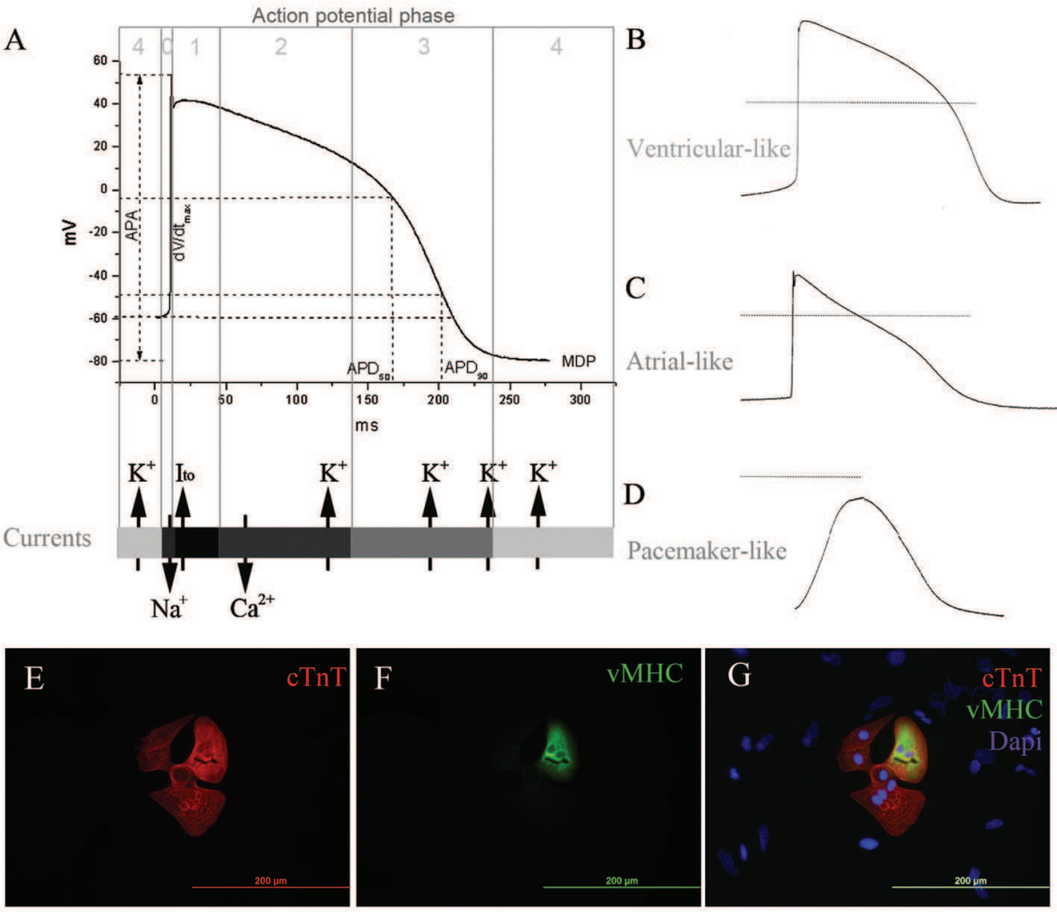


Fig. 2. ***Cardiac action potential parameters and phases, and characteristics of different cardiomyocyte subtypes***. **A.** Action potential (AP) parameters: Action potential amplitude (APA), maximum rate of rise of the action potential (dV/dtmax), action potential delay (ADP) and membrane diastolic potential (MDP). AP phase 0 is a rapid depolarization phase when the sodium channels are activated and membrane permeability is increased to Na+. Rapid depolarisation is followed by rapid repolarization phase 1 and plateau phase 2, where Ca2+ ions are entered to the cell through L-type calcium channels. At phase 3, calcium channels are inactivated and repolarization is caused by outward potassium currents. Repolarization is due to the currents carried mainly by the slow Iks and rapid Ikr components of the delayed rectifier potassium channels. The Ikr current is produced by hERG channel (encoded by the human ether-à-go-go-related gene). By contrast, inward potassium current contributes to the maintenance of the resting membrane potential, phase 4. **B-D** Classification of ventricular (B), atrial (C) an pacemaker-like (D) action potentials. Ventricular action potential has a prominent plateau phase whereas atrial action potential is more triangularly shaped. Pacemaker-like cells are characterized by slower upstroke velocity and amplitude if compared to ventricular and atrial type of cells. **E-G** Immunocytochemical staining of hESC-CM by (E) cardiac Troponin-T, (F) ventricular specific myosin heavy chain, (G) Merged image with nuclei stain Dapi. Scalebar 200 µm.

Brachyury T expression is suggested to be induced by TGFβ and FGF signalling (Hemmati-Brivanlou and Melton 1992; Amaya, Stein et al. 1993). Overall, very few direct targets for T-box genes have been identified. However, embryonic FGF (eFGF) (Casey, O'Reilly et al. 1998), Brachyury-induced homeobox Bix4 (Tada, Casey et al. 1998) and XWnt11 (Tada and Smith 2000) have been suggested to be downstream targets for Brachyury T.

Differentiation cascade can be followed further by the expression of the cardiac regulatory transcription factors such as Islet-1 (Isl-1), Mesp 1, GATA-4, Nkx2.5 and Tbx6 (Graichen, Xu et al. 2008; Yang, Soonpaa et al. 2008). Markers for cardiac differentiation steps are illustrated in Figure 1.

The gene expression profiles of the hESC during cardiac differentiation (Beqqali, Kloots et al. 2006; Synnergren, Adak et al. 2008) and the differentiated hESC-CM have been studied by DNA microarray (Cao, Wagner et al. 2008; Synnergren, Akesson et al. 2008; Kita-Matsuo, Barcova et al. 2009; Xu, Soo et al. 2009). These studies reveal that the molecular signature of hESC-CM resembles the cardiomyocytes from the human heart (Vidarsson, Hyllner et al. 2010).

5.3 Electrophysiology

hESC-CM exhibit heterogenic action potential (AP) morphologies which can be divided into nodal, atrial and ventricular subtypes (Figure 2) (He, Ma et al. 2003; Zhang, Wilson et al. 2009). According to the immunocytochemisty by ventricular specific marker and action potential studies, most of the differentiated cardiomyocytes were ventricular type of cells (Pekkanen-Mattila, Chapman et al. 2010). Slight variation in the amount of ventricular type of cells has been observed in different cell lines (Moore, Fu et al. 2008) and the amount has also been observed to vary between cardiac differentiation methods (80% and 100% of END-2 and EB-derived cardiomyocytes, respectively) (Pekkanen-Mattila, Chapman et al. 2010). If compared to the human neonatal or adult atrial or ventricular cardiomyocytes, hESC-CM have relatively positive maxium diastolic potential (MDP) and slow maxium rate of rise of the AP (dV/dtmax) and therefore resemble embryonal atrial- and ventricular like cells (He, Ma et al. 2003). However, even one third of our hESC-CMs exhibited a more mature phenotype with MDPs of < -70 mV and upstroke velocities >140 V/s. The demonstration of the presence of cardiomyocytes with dV/dtmax of over 150 and MDP of close to -80 is very important and suggests that mature human cardiac cells can be produced from hESCs, but the optimal conditions are still to be defined.

Traditionally patch clamp method has been used in analyzing the AP and electrophysiological properties of cardiomyocytes. Micro-electrode array (MEA) technology provides another useful platform to study cell electrophysiology, especially for ES-derived cardiomyocytes (Hescheler, Halbach et al. 2004; Reppel, Pillekamp et al. 2004; Pekkanen-Mattila, Kerkela et al. 2009). In MEA, cells are plated on top of electrodes in a cell culture well-type platform and they can be cultured and measured repeatedly for a long period of time. In addition, MEA can be utilized in testing the effects of pharmaceutical agents on hESC-CM (Braam, Tertoolen et al. 2010).

6. Applications for hESC-CM

6.1 Human cardiac cell/tissue model

Since the establishment of the first permanent hESC line (Thomson, Itskovitz-Eldor et al. 1998) there has been a great hope for replacing damaged heart tissue by hESC derived

cardiomyocytes. However, many severe problems need to be solved before hESC-CM are usable in clinics. Before clinical use becomes reality, it is likely that the hESC-CM would be applicable for drug discovery and safety pharmacological applications (Braam, Passier et al. 2009). Nevertheless, cardiac differentiation and the beating cells are already a useful tool for developmental biology and to study the pathophysiology of human cardiac diseases. In addition, iPS technology enables the production of patient specific cell lines which broadens the potential use even more.

6.2 Pathophysiology of cardiac diseases

Many cardiac diseases are caused by gene mutations or gene-enviroment interactions. Until today, these severe diseases have been studied in animal models, especially using transgenic mice. Even though mouse models can yield valuable information, differences between human and mouse physiology limit the applicability of the results, for example remarkably faster beating rate of the mouse may override the effects of arrhythmias which would be severe for human (Freund and Mummery 2009).

Cardiomyocytes derived from genetically modified hESC could be used as a disease model. To construct mutated hESC line and the disease model, the hESC line needs to be genetically manipulated. However, genetic manipulation of hESCs has proven to be more challenging if compared to mouse ES cells and only a limited amount of reports of successful gene targeting and manipulation exist (Braam, Denning et al. 2008; Giudice and Trounson 2008).

To obtain disease specific lines, the genetic manipulation step can be circumvented by deriving pluripotent stem cells from patients with genetic diseases using iPS cell technology (Park, Arora et al. 2008; Ebert, Yu et al. 2009; Freund, Davis et al. 2010). The differentiation of these model iPS-cells to desired cell type enables studying the development and the pathophysiology of the disease. In addition, the factors that affect the development and the progress of the disease can be studied (Freund, Davis et al. 2010). However, iPS-cell technology is still in its infancy and it remains to be seen if differentiated cells really manifest the disease phenotype of the mutation they carry and serve as a real disease model (Freund and Mummery 2009).

6.3 Safety pharmacology and drug discovery

The heart has been proven to be very sensitive to the side effects of pharmaceutical compounds. Severe reactions, such as syncope, arrhythmia and sudden death, related a special ventricular tachycardia, torsade de pointes (TdP), have led to the refusal of approval or the withdrawal from the market of many pharmaceutical agents (Roden 2004). In the absence of a complete understanding and direct analysis of TdP, the regulatory authorities have adopted the QT prolongation as a marker for the possible development of drug-induced TdP even though it is not a perfect marker for arrhythmogenesis (Finlayson, Witchel et al. 2004). Prolongation of the QT interval resulting from a delay in ventricular repolarization, whether drug-induced or for instance congenital arising from mutation of genes (to date LQT1-12), can be associated with TdP (Roden 2004; Zareba and Cygankiewicz 2008), though the relationship is complex (Shah and Hondeghem 2005). However, the QT interval is the cornerstone of the guidelines for the assessment of new chemical compounds in regard to proarrhythmic potential (ICH 2005; ICH 2005). Delayed rectifier potassium current (IKr) is one of the ion channels responsible for the repolarization of the action potential and the channel protein is encoded by the human ether-to-go-go-related gene

(hERG) (Vandenberg, Walker et al. 2001; Pollard, Valentin et al. 2008). Inhibition of this hERG channel (KV11.1) and the following inhibition of the IKr, is the predominant basis of drug-induced QT prolongation and TdP (Redfern, Carlsson et al. 2003; Hancox, McPate et al. 2008). Currently a number of preclinical models and assays have been employed by pharmaceutical companies (Carlsson 2006; Pollard, Valentin et al. 2008). These assays include in vivo QT assays, such as ECG telemetry of conscious dogs (Miyazaki, Watanabe et al. 2005), and in vitro assays, such as of repolarization assay which detects changes in the action potential delay (APD) of cardiac tissues (isolated animal purkinje fibers, papillary muscles or cardiac myocytes) or the hERG channel assay where hERG current expressed in heterologous cell system (such as CHO- or HEK293-cells) or native IKr is characterized (Finlayson, Witchel et al. 2004; Martin, McDermott et al. 2004).
Current methods are not fully adequate (Redfern, Carlsson et al. 2003; Lu, Vlaminckx et al. 2008). In addition, they are costly and the in vivo assays are ethically doubtful because of the large number of animals is used. Therefore there is a need for an in vitro method based on human cardiac cells that would bring additional value and reliability for testing novel pharmaceutical agents.
Cardiomyocytes derived both from hESC and iPS-cells have many potential applications in pharmaceutical industry including target validation, screening and safety pharmacology. These cells would serve as an inexhaustible and reproducible human model system and preliminary report of validation of hESC-CM system already exist (Braam, Tertoolen et al. 2010). However, a lot of optimization and development remains to be done, especially because of the immature phenotype of these cells and problems due to the differentiation efficiency, heterogeneous hESC-CM populations and enrichment methods (Braam, Passier et al. 2009).

6.4 Regenerative medicine

In principle, it would be possible to restore the function of the damaged heart by transplanting differentiated hESC. However, this may be one of the most challenging tasks to put into practise. The amount of transplantable cells needed is high and they should be immunocompatible. In addition, the transplanted graft should integrate into host myocardium, receive blood flow to remain vital, electrically couple with host myocardium and contract in synchrony in response to the conduction system (Braam, Passier et al. 2009).
hESC-CM have been transplanted into healthy myocardium of rodents. The cells were reported to survive, form myocardial tissue and proliferate but they were usually separated from the rodent myocardium by a layer of fibrotic tissue (Laflamme, Gold et al. 2005; van Laake, Passier et al. 2007). When transplanted to infracted rat or mouse hearts, some beneficial effects for the function of the heart have occurred (Laflamme, Chen et al. 2007; van Laake, Passier et al. 2007). However, after longer follow-up period, positive effects were not present anymore (van Laake, Passier et al. 2007; van Laake, Passier et al. 2008; van Laake, Passier et al. 2009). It is questionable wheather these temporal benefits are due to the formed myocardium or paracrine effects, like has been proposed for adult stem cells.
Even though some information concerning transplantation can be obtained by using rodent models, studies with larger animals (pigs, goats and sheep) are warranted to give more accurate results from safety issues, electrical coupling and cardiac function. Usage of the iPS-cells or ESC from the same species would eliminate the xeno barriers (Braam, Passier et al. 2009).

In addition to the above-mentioned issues, the timing of cell therapy and the delivery methods still need to be determined. It is likely that cells need supportive material during transplantation and therefore biomaterial research is also needed before clinical studies can be properly defined (Passier, van Laake et al. 2008).

7. Future perspectives

The ultimate goal for stem cell research is to cure patients with diseases caused by the loss of functional tissue such as myocardial infarction. However, it has become clear that pluripotent stem cell derived cardiomyocytes are not ready for the clinical use in the near future and a lot of basic research on cardiac differentiation and directed differentiation of cardiomyocyte subtypes (atrial, ventricular and conduction cells) is still needed. The cardiac differentiation is still inefficient and uncontrolled. Therefore effective methods for differentiation that supply homogenous populations of cardiomyocytes of sufficient quality, reproducibility and in large quantities are prerequisite for applications in pharmaceutical industry as well as for clinical use.

The differentiated cardiomyocytes are a mixed population consisting of non-cardiac cells and cardiomyocytes with several subtypes and maturation stages. For studies of development of disease or testing new potential drug molecules the cardiomyocyte population should be of one subtype (e.g. ventricular) and they should have mature, adult-like phenotype.

It is not only a disadvantage to have non-cardiac cells present in the differentiated cell populations. If aiming at cardiac tissue model, other cell types such as fibroblasts and endothelial cells are needed for proper cardiac differentiation (Kim, Majdi et al. 2010). However, the population should, as mentioned, be standardized and composed of desired cells with right ratios. It is likely, that cells can not form three-dimensional tissue model structure by themselves and some, perhaps extra cellular matrix mimicing biomaterial is needed to give cells support, attachment surfaces and nutrition.

Even though the pluripotent stem cell derived CM are not yet ready for clinical use and many obstacles have to be overcome before their use in drug discovery, they possess already now tremendous opportunities for basic research and pharmaceutical industry.

8. References

Adewumi, O., B. Aflatoonian, et al. (2007). "Characterization of human embryonic stem cell lines by the International Stem Cell Initiative." Nat Biotechnol 25(7): 803-16.

Amaya, E., P. A. Stein, et al. (1993). "FGF signalling in the early specification of mesoderm in Xenopus." Development 118(2): 477-87.

Amit, M., M. K. Carpenter, et al. (2000). "Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture." Dev Biol 227(2): 271-8.

Amit, M., J. Chebath, et al. (2010). "Suspension culture of undifferentiated human embryonic and induced pluripotent stem cells." Stem Cell Rev 6(2): 248-59.

Aouadi, M., F. Bost, et al. (2006). "p38 mitogen-activated protein kinase activity commits embryonic stem cells to either neurogenesis or cardiomyogenesis." Stem Cells 24(5): 1399-406.

Bauwens, C. L., R. Peerani, et al. (2008). "Control of human embryonic stem cell colony and aggregate size heterogeneity influences differentiation trajectories." Stem Cells 26(9): 2300-10.

Beqqali, A., J. Kloots, et al. (2006). "Genome-wide transcriptional profiling of human embryonic stem cells differentiating to cardiomyocytes." Stem Cells: 2006-0054.

Bettiol, E., L. Sartiani, et al. (2007). "Fetal bovine serum enables cardiac differentiation of human embryonic stem cells." Differentiation 75(8): 669-81.

Boheler, K. R., J. Czyz, et al. (2002). "Differentiation of Pluripotent Embryonic Stem Cells Into Cardiomyocytes." Circ Res 91(3): 189-201.

Bongso, A., C. Y. Fong, et al. (1994). "Isolation and culture of inner cell mass cells from human blastocysts." Hum Reprod 9(11): 2110-7.

Braam, S. R., C. Denning, et al. (2008). "Feeder-free culture of human embryonic stem cells in conditioned medium for efficient genetic modification." Nat Protoc 3(9): 1435-43.

Braam, S. R., R. Passier, et al. (2009). "Cardiomyocytes from human pluripotent stem cells in regenerative medicine and drug discovery." Trends Pharmacol Sci 30(10): 536-45.

Braam, S. R., L. Tertoolen, et al. (2010). "Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes." Stem Cell Res 4(2): 107-16.

Burridge, P. W., D. Anderson, et al. (2007). "Improved human embryonic stem cell embryoid body homogeneity and cardiomyocyte differentiation from a novel V-96 plate aggregation system highlights interline variability." Stem Cells 25(4): 929-38.

Cao, F., R. A. Wagner, et al. (2008). "Transcriptional and functional profiling of human embryonic stem cell-derived cardiomyocytes." PLoS ONE 3(10): e3474.

Carlsson, L. (2006). "In vitro and in vivo models for testing arrhythmogenesis in drugs." J Intern Med 259(1): 70-80.

Casey, E. S., M. A. O'Reilly, et al. (1998). "The T-box transcription factor Brachyury regulates expression of eFGF through binding to a non-palindromic response element." Development 125(19): 3887-94.

Conlon, F. L., K. M. Lyons, et al. (1994). "A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse." Development 120(7): 1919-28.

Cuenda, A., J. Rouse, et al. (1995). "SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1." FEBS Lett 364(2): 229-33.

Doetschman, T. C., H. Eistetter, et al. (1985). "The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium." J Embryol Exp Morphol 87: 27-45.

Ebert, A. D., J. Yu, et al. (2009). "Induced pluripotent stem cells from a spinal muscular atrophy patient." Nature 457(7227): 277-80.

Evans, M. J. and M. H. Kaufman (1981). "Establishment in culture of pluripotential cells from mouse embryos." Nature 292(5819): 154-6.

Farah, C. S. and F. C. Reinach (1995). "The troponin complex and regulation of muscle contraction." Faseb J 9(9): 755-67.

Finlayson, K., H. J. Witchel, et al. (2004). "Acquired QT interval prolongation and HERG: implications for drug discovery and development." Eur J Pharmacol 500(1-3): 129-42.

Freund, C., R. P. Davis, et al. (2010). "The first reported generation of human induced pluripotent stem cells (iPS cells) and iPS cell-derived cardiomyocytes in the Netherlands." Neth Heart J 18(1): 51-4.

Freund, C. and C. L. Mummery (2009). "Prospects for pluripotent stem cell-derived cardiomyocytes in cardiac cell therapy and as disease models." J Cell Biochem 107(4): 592-9.

Freund, C., D. Ward-van Oostwaard, et al. (2008). "Insulin redirects differentiation from cardiogenic mesoderm and endoderm to neuroectoderm in differentiating human embryonic stem cells." Stem Cells 26(3): 724-33.

Giudice, A. and A. Trounson (2008). "Genetic modification of human embryonic stem cells for derivation of target cells." Cell Stem Cell 2(5): 422-33.

Goumans, M. J., T. P. de Boer, et al. (2007). "TGF-beta1 induces efficient differentiation of human cardiomyocyte progenitor cells into functional cardiomyocytes in vitro." Stem Cell Res 1(2): 138-49.

Graichen, R., X. Xu, et al. (2008). "Enhanced cardiomyogenesis of human embryonic stem cells by a small molecular inhibitor of p38 MAPK." Differentiation 76(4): 357-70.

Guhr, A., A. Kurtz, et al. (2006). "Current State of Human Embryonic Stem Cell Research: An Overview of Cell Lines and their Usage in Experimental Work." Stem Cells.

Hancox, J. C., M. J. McPate, et al. (2008). "The hERG potassium channel and hERG screening for drug-induced torsades de pointes." Pharmacol Ther 119(2): 118-32.

He, J.-Q., Y. Ma, et al. (2003). "Human Embryonic Stem Cells Develop Into Multiple Types of Cardiac Myocytes: Action Potential Characterization." Circ Res 93(1): 32-39.

Hemmati-Brivanlou, A. and D. A. Melton (1992). "A truncated activin receptor inhibits mesoderm induction and formation of axial structures in Xenopus embryos." Nature 359(6396): 609-14.

Hescheler, J., B. K. Fleischmann, et al. (1997). "Embryonic stem cells: a model to study structural and functional properties in cardiomyogenesis." Cardiovasc Res 36(2): 149-62.

Hescheler, J., M. Halbach, et al. (2004). "Determination of electrical properties of ES cell-derived cardiomyocytes using MEAs." Journal of Electrocardiology 37(Supplement 1): 110-116.

ICH (2005). "E14: the clinical evalution of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs."

ICH (2005). "S7B: The nonclinical evalution of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals."

Itskovitz-Eldor, J., M. Schuldiner, et al. (2000). "Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers." Mol Med 6(2): 88-95.

Kehat, I., D. Kenyagin-Karsenti, et al. (2001). "Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes." J Clin Invest 108(3): 407-14.

Keller, G. M. (1995). "In vitro differentiation of embryonic stem cells." Curr Opin Cell Biol 7(6): 862-9.

Kim, C., M. Majdi, et al. (2010). "Non-cardiomyocytes influence the electrophysiological maturation of human embryonic stem cell-derived cardiomyocytes during differentiation." Stem Cells Dev 19(6): 783-95.

Kim, S. E., B. K. Kim, et al. (2007). "Comparative analysis of the developmental competence of three human embryonic stem cell lines in vitro." Mol Cells 23(1): 49-56.

Kispert, A., B. Koschorz, et al. (1995). "The T protein encoded by Brachyury is a tissue-specific transcription factor." Embo J 14(19): 4763-72.

Kita-Matsuo, H., M. Barcova, et al. (2009). "Lentiviral vectors and protocols for creation of stable hESC lines for fluorescent tracking and drug resistance selection of cardiomyocytes." PLoS One 4(4): e5046.

Kurosawa, H. (2007). "Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells." J Biosci Bioeng 103(5): 389-98.

Laflamme, M. A., K. Y. Chen, et al. (2007). "Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts." Nat Biotechnol 25(9): 1015-24.

Laflamme, M. A., J. Gold, et al. (2005). "Formation of human myocardium in the rat heart from human embryonic stem cells." Am J Pathol 167(3): 663-71.

Loser, P., J. Schirm, et al. (2010). "Human embryonic stem cell lines and their use in international research." Stem Cells 28(2): 240-6.

Lough, J. and Y. Sugi (2000). "Endoderm and heart development." Dev Dyn 217(4): 327-42.

Lu, H. R., E. Vlaminckx, et al. (2008). "Predicting drug-induced changes in QT interval and arrhythmias: QT-shortening drugs point to gaps in the ICHS7B Guidelines." Br J Pharmacol 154(7): 1427-38.

Martin, G. R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proc Natl Acad Sci U S A 78(12): 7634-8.

Martin, R. L., J. S. McDermott, et al. (2004). "The utility of hERG and repolarization assays in evaluating delayed cardiac repolarization: influence of multi-channel block." J Cardiovasc Pharmacol 43(3): 369-79.

McDevitt, T. C., M. A. Laflamme, et al. (2005). "Proliferation of cardiomyocytes derived from human embryonic stem cells is mediated via the IGF/PI 3-kinase/Akt signaling pathway." Journal of Molecular and Cellular Cardiology 39(6): 865-873.

Messina, E., L. De Angelis, et al. (2004). "Isolation and Expansion of Adult Cardiac Stem Cells From Human and Murine Heart." Circ Res 95(9): 911-921.

Miyazaki, H., H. Watanabe, et al. (2005). "QT PRODACT: sensitivity and specificity of the canine telemetry assay for detecting drug-induced QT interval prolongation." J Pharmacol Sci 99(5): 523-9.

Mohr, J. C., J. Zhang, et al. (2010). "The microwell control of embryoid body size in order to regulate cardiac differentiation of human embryonic stem cells." Biomaterials 31(7): 1885-93.

Moore, J. C., J. Fu, et al. (2008). "Distinct cardiogenic preferences of two human embryonic stem cell (hESC) lines are imprinted in their proteomes in the pluripotent state." Biochem Biophys Res Commun 372(4): 553-8.

Mummery, C. (2010). "Sorting cardiomyocytes: a simple solution after all?" Nat Methods 7(1): 40-2.

Mummery, C., D. Ward-van Oostwaard, et al. (2003). "Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells." Circulation 107(21): 2733-40.

Mummery, C. L., A. Feijen, et al. (1986). "Establishment of a differentiated mesodermal line from P19 EC cells expressing functional PDGF and EGF receptors." Exp Cell Res 165(1): 229-42.

Ng, E. S., R. P. Davis, et al. (2005). "Forced aggregation of defined numbers of human embryonic stem cells into embryoid bodies fosters robust, reproducible hematopoietic differentiation." Blood 106(5): 1601-3.

Niebruegge, S., C. L. Bauwens, et al. (2009). "Generation of human embryonic stem cell-derived mesoderm and cardiac cells using size-specified aggregates in an oxygen-controlled bioreactor." Biotechnol Bioeng 102(2): 493-507.

Oh, H., S. B. Bradfute, et al. (2003). "Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction." Proc Natl Acad Sci U S A 100(21): 12313-8.

Osafune, K., L. Caron, et al. (2008). "Marked differences in differentiation propensity among human embryonic stem cell lines." Nat Biotechnol 26(3): 313-5.

Park, I. H., N. Arora, et al. (2008). "Disease-specific induced pluripotent stem cells." Cell 134(5): 877-86.

Passier, R., D. W. Oostwaard, et al. (2005). "Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures." Stem Cells 23(6): 772-80.

Passier, R., L. W. van Laake, et al. (2008). "Stem-cell-based therapy and lessons from the heart." Nature 453(7193): 322-9.

Pekkanen-Mattila, M., H. Chapman, et al. (2010). "Human embryonic stem cell-derived cardiomyocytes: demonstration of a portion of cardiac cells with fairly mature electrical phenotype." Exp Biol Med (Maywood) 235(4): 522-30.

Pekkanen-Mattila, M., E. Kerkela, et al. (2009). "Substantial variation in the cardiac differentiation of human embryonic stem cell lines derived and propagated under the same conditions--a comparison of multiple cell lines." Ann Med 41(5): 360-70.

Pekkanen-Mattila, M., M. Pelto-Huikko, et al. (2010). "Spatial and temporal expression pattern of germ layer markers during human embryonic stem cell differentiation in embryoid bodies." Histochem Cell Biol 133(5): 595-606.

Pera, M. F. and A. O. Trounson (2004). "Human embryonic stem cells: prospects for development." Development 131(22): 5515-25.

Pollard, C. E., J. P. Valentin, et al. (2008). "Strategies to reduce the risk of drug-induced QT interval prolongation: a pharmaceutical company perspective." Br J Pharmacol 154(7): 1538-43.

Pyle, A. D., L. F. Lock, et al. (2006). "Neurotrophins mediate human embryonic stem cell survival." Nat Biotechnol 24(3): 344-350.

Redfern, W. S., L. Carlsson, et al. (2003). "Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development." Cardiovasc Res 58(1): 32-45.

Reppel, M., F. Pillekamp, et al. (2004). "Microelectrode arrays: a new tool to measure embryonic heart activity." J Electrocardiol 37 Suppl: 104-9.

Roden, D. M. (2004). "Drug-induced prolongation of the QT interval." N Engl J Med 350(10): 1013-22.

Sachlos, E. and D. T. Auguste (2008). "Embryoid body morphology influences diffusive transport of inductive biochemicals: a strategy for stem cell differentiation." Biomaterials 29(34): 4471-80.

Scott, C. T., J. B. McCormick, et al. (2009). "And then there were two: use of hESC lines." Nat Biotechnol 27(8): 696-7.

Shah, R. R. and L. M. Hondeghem (2005). "Refining detection of drug-induced proarrhythmia: QT interval and TRIaD." Heart Rhythm 2(7): 758-72.

Showell, C., O. Binder, et al. (2004). "T-box genes in early embryogenesis." Dev Dyn 229(1): 201-18.

Snir, M., I. Kehat, et al. (2003). "Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes." Am J Physiol Heart Circ Physiol 285(6): H2355-63.

Synnergren, J., S. Adak, et al. (2008). "Cardiomyogenic gene expression profiling of differentiating human embryonic stem cells." J Biotechnol 134(1-2): 162-70.

Synnergren, J., K. Akesson, et al. (2008). "Molecular signature of cardiomyocyte clusters derived from human embryonic stem cells." Stem Cells 26(7): 1831-40.

Tada, M., E. S. Casey, et al. (1998). "Bix1, a direct target of Xenopus T-box genes, causes formation of ventral mesoderm and endoderm." Development 125(20): 3997-4006.

Tada, M. and J. C. Smith (2000). "Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway." Development 127(10): 2227-38.

Takahashi, K., K. Tanabe, et al. (2007). "Induction of pluripotent stem cells from adult human fibroblasts by defined factors." Cell 131(5): 861-72.

Takahashi, T., B. Lord, et al. (2003). "Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes." Circulation 107(14): 1912-6.

Thierfelder, L., H. Watkins, et al. (1994). "Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere." Cell 77(5): 701-12.

Thomson, J. A., J. Itskovitz-Eldor, et al. (1998). "Embryonic stem cell lines derived from human blastocysts." Science 282(5391): 1145-7.

Tobacman, L. S. (1996). "Thin filament-mediated regulation of cardiac contraction." Annu Rev Physiol 58: 447-81.

Tran, T. H., X. Wang, et al. (2009). "Wnt3a-induced mesoderm formation and cardiomyogenesis in human embryonic stem cells." Stem Cells 27(8): 1869-78.

van Laake, L. W., R. Passier, et al. (2009). "Improvement of mouse cardiac function by hESC-derived cardiomyocytes correlates with vascularity but not graft size." Stem Cell Res 3(2-3): 106-12.

van Laake, L. W., R. Passier, et al. (2008). "Human embryonic stem cell-derived cardiomyocytes and cardiac repair in rodents." Circ Res 102(9): 1008-10.

van Laake, L. W., R. Passier, 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(1): 9-24.

Vandenberg, J. I., B. D. Walker, et al. (2001). "HERG K+ channels: friend and foe." Trends Pharmacol Sci 22(5): 240-6.

Vidarsson, H., J. Hyllner, et al. (2010). "Differentiation of human embryonic stem cells to cardiomyocytes for in vitro and in vivo applications." Stem Cell Rev 6(1): 108-20.

Wobus, A. M., G. Wallukat, et al. (1991). "Pluripotent mouse embryonic stem cells are able to differentiate into cardiomyocytes expressing chronotropic responses to adrenergic and cholinergic agents and Ca2+ channel blockers." Differentiation 48(3): 173-82.

Xu, C., S. Police, et al. (2002). "Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells." Circ Res 91(6): 501-8.

Xu, X. Q., R. Graichen, et al. (2008). "Chemically defined medium supporting cardiomyocyte differentiation of human embryonic stem cells." Differentiation 76(9): 958-70.

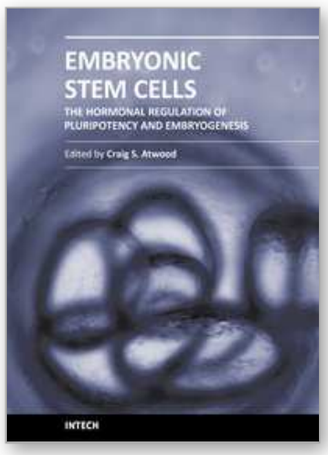
Xu, X. Q., S. Y. Soo, et al. (2009). "Global expression profile of highly enriched cardiomyocytes derived from human embryonic stem cells." Stem Cells 27(9): 2163-74.

Yang, L., M. H. Soonpaa, et al. (2008). "Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population." Nature 453(7194): 524-8.

Yirme, G., M. Amit, et al. (2008). "Establishing a dynamic process for the formation, propagation, and differentiation of human embryoid bodies." Stem Cells Dev 17(6): 1227-41.

Zareba, W. and I. Cygankiewicz (2008). "Long QT syndrome and short QT syndrome." Prog Cardiovasc Dis 51(3): 264-78.

Zhang, J., G. F. Wilson, et al. (2009). "Functional cardiomyocytes derived from human induced pluripotent stem cells." Circ Res 104(4): e30-41.



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Pluripotency is a prerequisite for the subsequent coordinated differentiation of embryonic stem cells into all tissues of the body. This book describes recent advances in our understanding of pluripotency and the hormonal regulation of embryonic stem cell differentiation into tissue types derived from the ectoderm, mesoderm and endoderm.

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