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Transcriptional Networks of Embryonic Stem Cell-Derived Cardiomyogenesis

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1. Introduction

Embryonic stem cells are pluripotent cells that, if cultured under specific conditions, give rise to clusters of beating cardiomyocytes. Beating cardiomyocytes, also dubbed beating areas, display gene expression profiles and functional properties similar to adult cardiomyocytes. In line with this, a wide heterogeneity has been observed in embryonic stem cell-derived cardiomyocytes, resembling thus the distinct characteristics of atrial, ventricular and conductive cardiomyocytes.

It has been recently demonstrated that two distinct cardiogenic precursor cells contribute to the developing heart. The first heart field contributes to the cardiac linear straight tube while a second population of cells adds cells to both arterial and venous pole of the cardiac tube, delimiting thus the second heart field. As cardiogenesis advances the first heart field mainly gives rise to the left ventricle, whereas the second heart field contributes to the right ventricle, outflow tract and the atrial chambers. A third population of cells, with distinct gene expression fingerprint has been demonstrated to form the inflow tract, suggesting the possible existence of a third heart field.

Over the last years we have gained insights about the transcriptional mechanisms that govern the distinct heart fields, however, our understanding about if such endogenous program is recapitulated in embryonic stem cell-derived cardiomyogenesis remains elusive. Within this review we elaborate about the current state-of-the-art of the transcriptional networks that operate during embryonic stem cell-derived cardiomyogenesis, with special emphasis on the development of heart field transcriptional networks.

2. Cardiogenic potential of embryonic stem cells

Embryonic stem cells are pluripotent stem cells derived from the inner cell mass of the developing blastocyst. *In vitro* culture of embryonic stem cells, if nurtured under specific conditions, can give rise to distinct cell types of endodermal, mesodermal and ectodermal lineages, including thus beating cardiomyocytes (Miller-Hance et al. 1993). Such capabilities have raised the possibility of using embryonic stem cells as a source to heal the damaged heart (Chinchilla & Franco, 2006; Franco et al., 2007). However, several constrains has obstructed this purpose. Leaving apart ethical concerns, mainly applicable to human

embryonic stem cells, technical and scientific obstacles also have contributed to slow down this quest. We shall update in this book chapter the state-of-the-art progress made to conquer the challenging aim of converting embryonic stem cells into beating cardiomyocytes as suitable therapeutical tools.

It is incredible to observe that a subset of cells hunted from the inner mass cells of the developing blastocyst, set in appropriate cell culture conditions, are able to progressively proceed *in vitro*, differentiating into neurons, fibroblasts, cardiomyocytes as well as in several other cells types (Lanza et al., 2004). The real question is then; does this occur mimicking the early step of embryogenesis? Several studies have demonstrated that upon the initial phases of *in vitro* embryonic stem cells culture, the aggregating cells that form the embryonic bodies in the hanging drops acquire a rather well organized three-dimensional structure, by which externally located cells express ectodermal markers, while internally located cells express mesodermal and endodermal makers (Doetschman et al., 1985, Wobus & Boheler, 1999; Boheler et al., 2002). Thus, these findings support the notion that cell specification and determination of embryonic stem cells into a discrete cell type would mainly follow the endogenous signal pathways. Therefore, in order to understand how stem cells can lead to cardiomyocytes, efforts should be made to learn the natural routing of a mesodermal cell that will contribute to the heart. In essence we need to learn how cardiac development is achieved.

3. Transcriptional control of cardiac muscle development

Over the last two decades, our understanding of the cellular and molecular mechanisms that govern cardiac development has greatly advanced. Initial steps of mesoderm commitment from the lateral plate mesoderm to the forming heart are mainly directed by interplay between Bmp, Fgf and Wnt signaling (Barron et al., 2000; Lopez-Sanchez et al., 2002; Marques et al., 2008). As soon as the cardiogenic mesoderm is committed, several transcription factors, such as Nkx2.5, Gata4, Srf, Hand2 and Mef2c are activated, which play crucial roles during cardiogenesis as revealed by loss-of-function experiments in mice (Lyons et al., 1995; Srivastava et al., 1995; Kuo et al., 1997; Lin et al., 1997). Cardiogenesis proceeds by the formation of two concentrical tissue layers, an external myocardial and internal endocardial layer. To date it remains elusive how each cardiogenic lineage is distinctly established and whether they share a common progenitor (Linask & Lash, 1993; Eisenberg & Bader, 1995) or, on the contrary, they are distinctly derived from separate precursors (Cohen-Gould & Mikawa, 1996; Wei & Mikawa, 2000). As the myocardium is configured, it has been recently demonstrated that two distinct cell populations contribute to the developing heart; the first heart field (FHF) will contribute to the linear heart tube and subsequently will give rise mainly to the left ventricle (Kelly & Buckingham, 2002), whereas a second population of cells is subsequently recruited, namely the second heart field (SHF), contributing to the rest of the developing heart (Kelly et al., 2001; Cai et al., 2001; Waldo et al., 2001; Kelly & Buckingham, 2002). FHF derivatives express Nkx2.5 but are negative from islet-1, while SHF cells express both Nkx2.5 and islet-1. In addition, SHF derivates can be subdivided in two distinct regions, according to their entry site to the developing heart; a) anterior SHF leads to the formation of the right ventricle and outflow tract, and its contribution is governed by signaling emanating from Fgf8- and Fgf10-expressing cells (Watanabe et al. 2010), as well as contribution from Tbx1 signaling at the arterial/pharyngeal pole of the heart (Huynh et al., 2007; Liao et al., 2008), b) posterior SHF

will contribute to the atrioventricular canal as well as right and left atria, and its contribution is directed by Fgf10 signaling at the venous pole of the heart (Kelly et al., 2001). In addition, complex regulatory networks are operative in the embryonic heart providing differentiation cues to the developing myocardium, as illustrated by the complex expression pattern of T-box genes, including therein Tbx2, Tbx3, Tbx5, Tbx18 and Tbx20 (Singh & Kispert, 2010; Greulich et al., 2011), Hand1 and Hand2 providing systemic *vs* pulmonary cues (Srivastava et al., 1995; Thomas et al., 1995) as well as left/right positional cues as illustrated by Pitx2 (Franco & Campione, 2003; Chinchilla et al., 2011). Thus, shared and distinct transcriptional pathways are governing first and second heart field deployment, leading in both cases to activation of a core cardiac transcriptional regulatory network as illustrated in **Figure 1**.

In addition, a third population of cells, with distinct gene expression hallmark as compared to first and second heart field cardiac precursors, has been demonstrated to form the inflow tract of the heart, suggesting the possible existence of a third heart field (Mommersteeg et al., 2010). Nkx2.5 /islet-1 negative cells, but positive for Tbx18, contribute to the formation of the caval veins (Mommersteeg et al., 2010). However, recent Cre-based lineage tracing experiments have challenged this notion, since all myocardial component of the venous pole, including the atrial appendages and the caval and pulmonary veins, have been reported to be derived from islet-1 and Nkx2-5 positive cells (Ma et al., 2008).

Concomitant with the deployment of the first and second heart field precursor cells, differentiation into distinct cardiomyocyte cell types is occurring, providing thus distinct working chamber and conduction system myocardium. Tbx2 and Tbx3 have been reported to play a fundamental role controlling gene expression pattern within the atrioventricular node (Bakker et al., 2008; Aanhaanen et al., 2009, 2011). Shox2, Tbx3 and Tbx18 have been demonstrated to play a crucial role on the sinoatrial node formation (Hoogaars et al., 2007; Wiese et al., 2009; Espinoza-Lewis et al. 2009). Furthermore, cardiomyocyte subtypes progressively emerge during cardiogenesis, such as distinct atrial and ventricular chamber myocardium, although the transcriptional regulation of such cell identities remains rather elusive. In chicken, Irx4 plays a crucial role in this step (Bao et al., 1999; Bruneau et al. 2000), however such function is not conserved in mice (Bruneau et al., 2001), which might be partially taken by Coup-tfII (Pereira et al., 1999; Wu et al., 2011). Similar events also are applicable for the cardiac conduction system, in which nodal and bundle branch fascicles are developed, each of them with distinct functional capabilities, yet their transcriptional regulation remains rather unexplored (Franco & Icardo, 2001; Miquerol et al. 2011).

In the adult heart, some of the developmental differences are progressively smoothened, in such a way that we can consider the adult heart being composed of two types of working myocardium (atrial and ventricular) and three distinct types of conductive cells (SAN and AVN node, His and bundle branches, and Purkinje fibers). Curiously, novel transmural differences emerge in the adult ventricular myocardium (Yan & Antzelevitch et al., 1996, 1999; de Castro et al., 2005), which are crucial for correct function of the heart (Constantini et al. 2005).

In its important to highlight that during cardiogenesis, the heart is progressively acquiring novel functional capabilities, which are reflected on the progressive onset of expression of contractile, conductive and cytoskeleton proteins. At the contractile level, sarcomeric genes such as myosins, actins and troponins, and cytoskeleton proteins as tropomyosin and actinins are differently expressed already at early stages of development (Lyons et al., 1990; Franco et al., 1998) providing functional heterogeneity to the developing myocardium, as

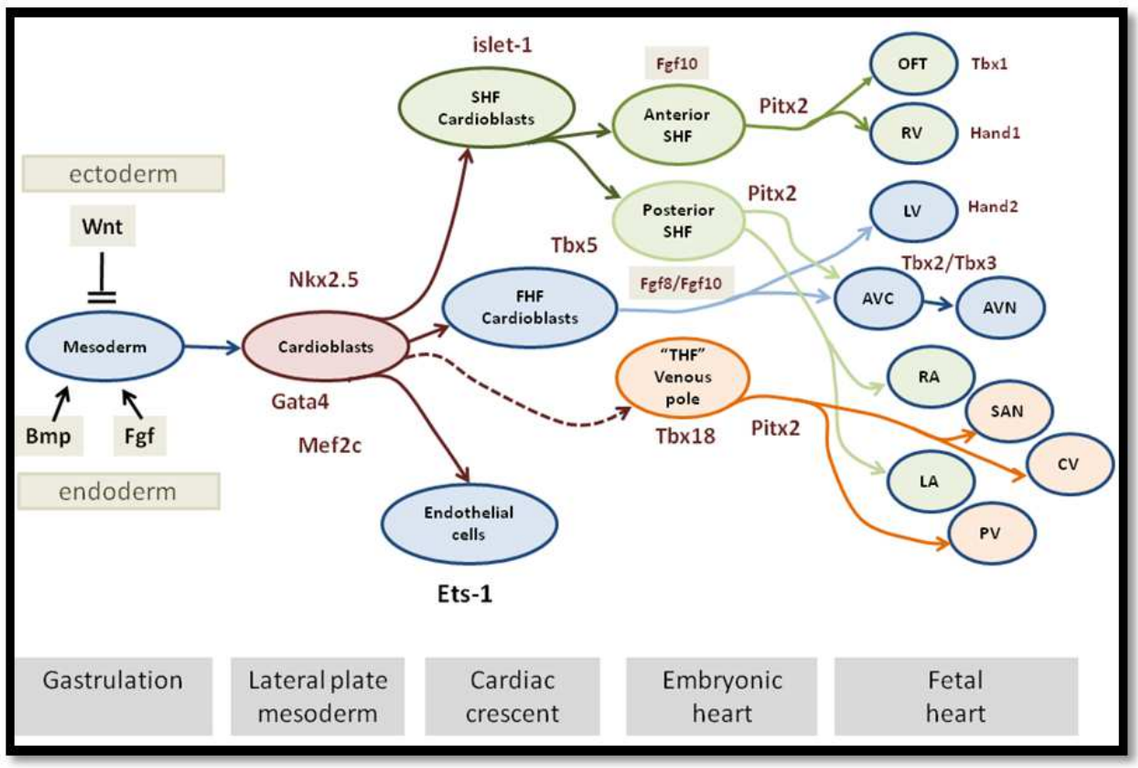
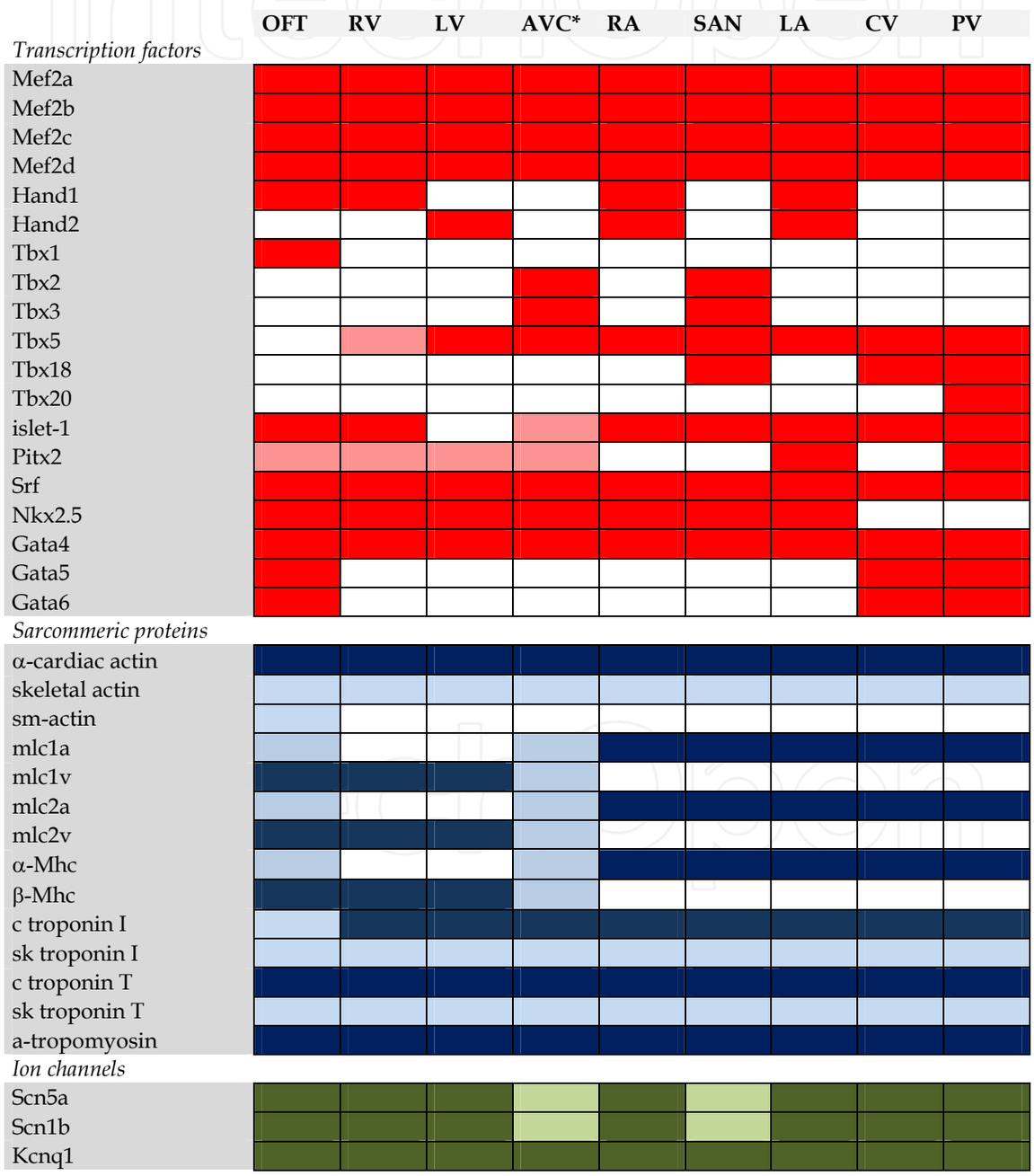


Fig. 1. **Schematic representation of embryonic cardiac development.** At gastrulation, nascent precardiac mesoderm listens to signal emanating from the surrounding ectodermal and endodermal tissues, committing the cells into the cardiogenic lineage, i.e. cardioblasts. Cardioblasts are characterized by the expression of core cardiac transcription factors, including Nkx2.5, Gata4 and Mef2c. Soon thereafter, cardiomyocyte and endothelial cell lineages emerged and within the cardiomyocyte lineage, two distinct populations of cells can be recognized, first heart field (FHF) and second heart field (SHF) cells. A third population of cells is originating soon thereafter, which lacks Nkx2.5 or islet-1 expression, yet, lineage tracing evidences using Cre/LoxP system suggested they have had a common ancestry (Cai et al., 2003; Zhou et al., 2008). FHF contributes mainly to the left ventricle and the atrioventricular canal (AVC). AVC will eventually be remodeled as septation proceeds, contributing therein to the formation of the atrioventricular node, which is characterized by Tbx2 and Tbx3 expression. Interestingly, SHF have two distinct components, an anterior SHF contributing to the outflow tract and right ventricle, listing to signal emanating from the pharyngeal arches domain, such as Fgf8 and Fgf10, and a posterior SHF leading to the right and left atrial chambers, listing to signals such as Fgf10. The "third" heart field contributes to the sinous venosus, forming therefore the caval veins and the sinoatrial node, and to the pulmonary myocardium which constitutes the atrial septa and the pulmonary veins (Franco et al., 2000). Importantly, almost all previously mentioned cardiac lineages listen to left/right signaling clues provided by the homeobox transcription factor Pitx2 (Campione et al., 1999; Franco & Campione, 2003).

detailed in **Table 1**. Similarly, ion channels and gap junctional proteins are progressively expressed in distinct regions of the developing heart (Gros & Jongsma, 1996; Franco et al. 2001; de Castro et al., 2005)(**Table 1**), providing the bases for the onset of a persitaltoid contraction in first instance and subsequently of synchronous contraction at late embryonic stage, displaying thus an apex-to-base pattern of activation. In essence, if we take thus as a reference our knowledge about cardiac development, it sounds reasonable that such information could be applicable to embryonic stem cells in order to obtain beating cardiomyocytes.



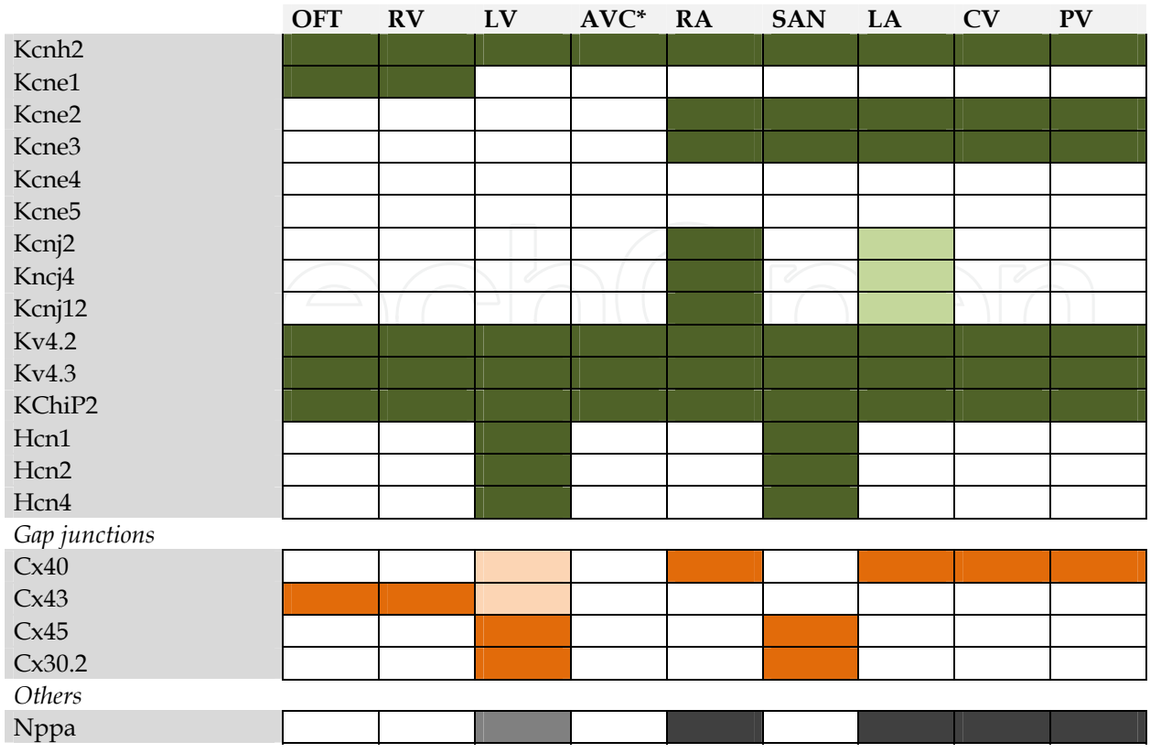


Table 1. Graphical respresentation of the expression profiles of cardiac-enriched transcription factors, sarcomeric proteins, ion channels and gap junctional proteins during embryonic heart development, in distinct regions of the embryonic/fetal heart. OFT, outflow tract; RV, right ventricle, LV, left ventricle, AVC, atrioventricular canal, RA, right atrium, LA, left atrium, SAN, sinoatrial node, CV, caval veins, PV, pulmonary veins. *AVC will lead in the adult heart to the atrioventricular node.

4. Understanding transcriptional control of *in vitro* cardiogenesis

The formation of beating cardiomyocytes from undifferentiated embryonic stem cell has been an important focus of scientific research, as it can be witnessed by the number of publication in this front. Several of the signaling pathways involved in *in vivo* cardiogenesis are also recapitulated *in vitro*, as depicted in **Figure 2**. Importantly, initial reports provided evidences that embryonic stem cell-derived cardiomyocytes display morphological, molecular and functional characteristics similar to adult cardiomyocytes, displaying therefore expression of sarcomeric and gap junctional proteins (Sachinidis et al., 2003; van Kempen et al., 2003; Fijnvandraaf et al., 2003ab). Additional experiments, demonstrated that most ion channels that are natively configure the cardiac action potential are also expressed during embryonic stem cell-derived cardiomyocytes (van Kempen et al., 2003). Furthermore, elegant spatio-temporal studies also nicely illustrate the progressive onset of the core transcriptional cardiac and ion channel expression during embryonic stem cell-derived cardiomyogenesis (Fijnvandraaf et al., 2003ab, van Kempen et al., 2003). However, several caveats were soon arising. Firstly, the fact that embryonic stem cell cultures, although capable of providing a source of cardiomyocytes, yield on average to a low percentage (<20%). Secondly, beating areas display distinct contraction rates, suggesting that large

heterogeneity was observed from beating area to beating area, which might have gone unappreciated since most studies were done using RT-PCR methods. Thirdly, it was unclear if all cells within a beating area were equally differentiated. Sorting out these key questions is compulsory before any therapeutical strategy could be envisioned since a large number of cardiomyocytes is required, which will need to be morphologically homogeneous in order to avoid the chance of generating arrhythmias, and sufficiently and adequately differentiated in order to limit the oncogenic propagation of undifferentiated or poorly differentiated embryonic stem cells.

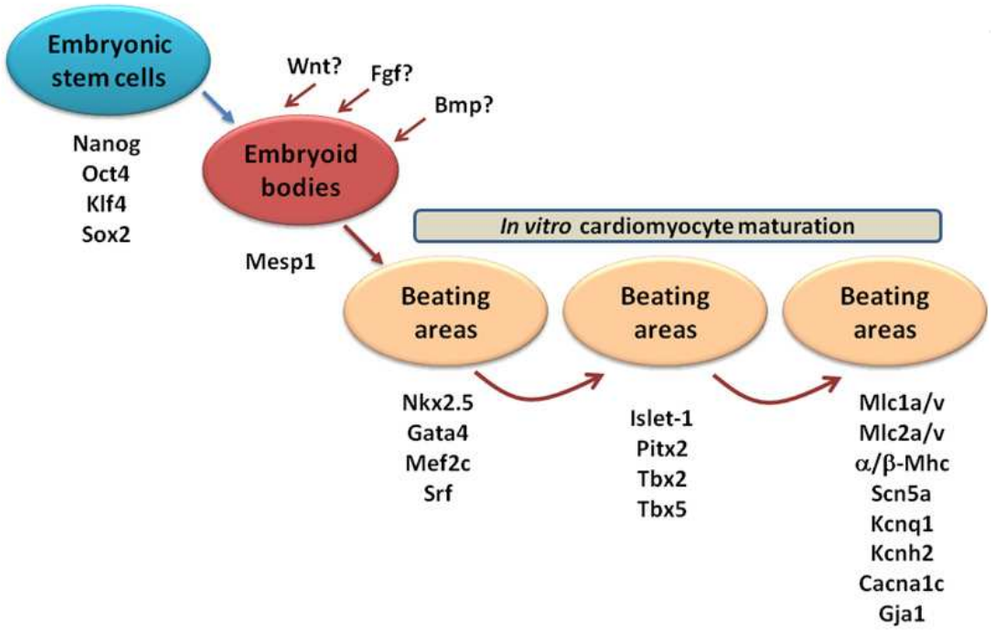
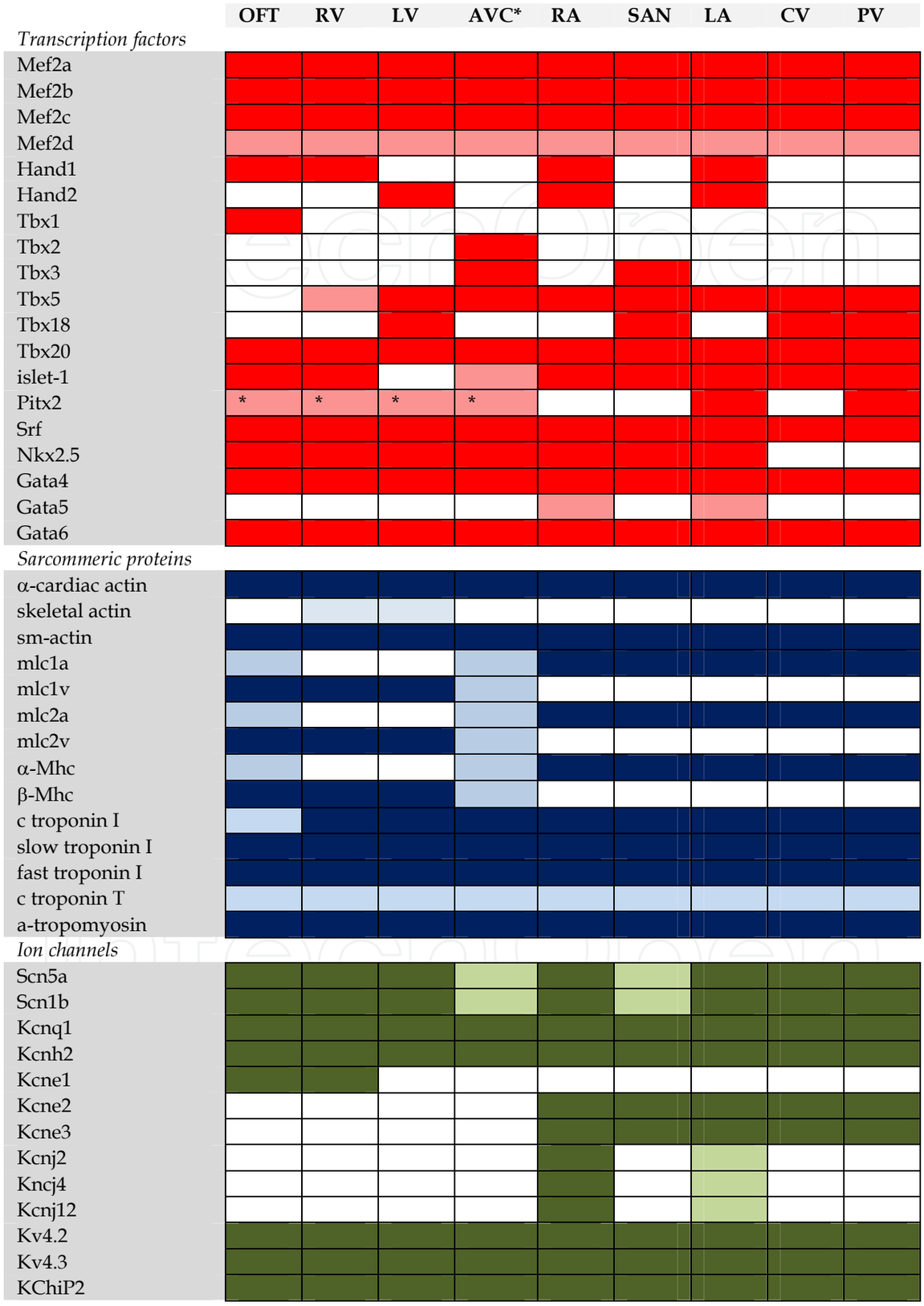


Fig. 2. **Schematic representation of the signaling pathways involved in embryonic stem cell–derived cardiogenesis.** Pluripotency makers such as Nanog, Oct4, Klf4 and Sox2 are expressed in undifferentiated embryonic stem cells. As soon as differentiation is initiated, these makers are down-regulated and mesoderm specification genes such as Mesp1 are expressed. Soon thereafter, as the beating areas are conformed, core cardiac transcriptional regulatory genes are up-regulated, and progressively the nascent cardiomyocytes acquire sacormeric and ion channel proteins, as depicted in **Table 2**.

First approaches to increase the efficiency of converting embryonic stem cells into cardiomycytes has been obtained by mimicking the inductive signals that naturally convert precardiogenic mesodermal cells into nascent cardioblasts (Hidai et al., 2003; Holtzinger et al., 2010). Bin et al. (2006) demonstrates that Bmp2 treatment of mouse embryonic stem cells increased the efficiency of obtaining beating areas, providing evidences that core transcriptional factor Nkx2.5 and structural proteins such as troponin-T and alpha-MHC were increased. Further evidences on the pivotal role of Bmp signaling in embryonic stem cell-derived cardiomyogenesis were reported by Rajasingh et al. (2007). More recently, Kim et al. (2008) provides similar evidences using human embryonic stem cells. However, other authors have reported that Bmp2 overexpression can also lead to induction of other mesodermal lineages such as smooth muscle cells (Blin et al., 2010) or condroblasts,


OFT
RV
LV
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Transcription factors
Mef2a
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Hand1
Hand2
Tbx1
Tbx2
Tbx3
Tbx5
Tbx18
Tbx20
islet-1
Pitx2
Srf
Nkx2.5
Gata4
Gata5
Gata6
Sarcommeric proteins
α-cardiac actin
skeletal actin
sm-actin
mlc1a
mlc1v
mlc2a
mlc2v
α-Mhc
β-Mhc
c troponin I
slow troponin I
fast troponin I
c troponin T
a-tropomyosin
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Scn5a
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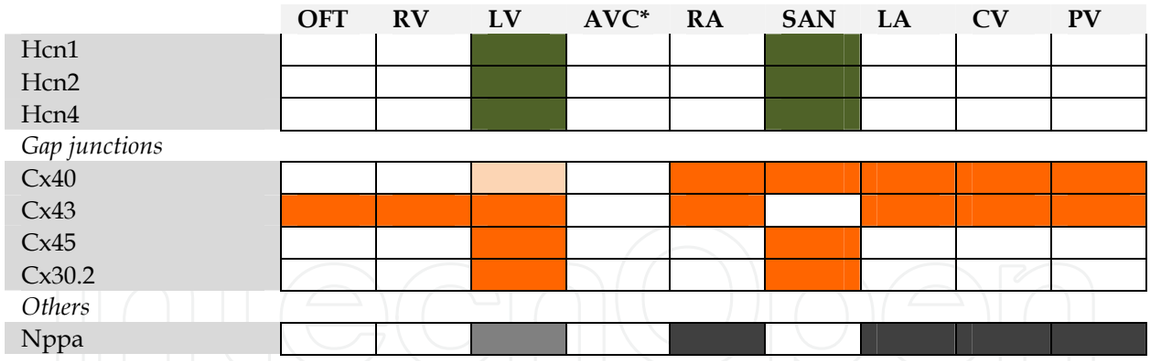

Table 2. Graphical respresentation of the expression profiles of cardiac-enriched transcription factors, sarcomeric proteins, ion channels and gap junctional proteins during embryonic stem cell differentiation. ESC, embryonic stm cells, EB, embryoid bodies, BA2, beating areas at 2 days of culture, BA7, beating areas at 7 days of culture, EB14, beating areas at 14 days of cultures.

osteoblasts and adipoblasts, if cultures with supplementary co-factors (zur Nieden et al., 2005). Thus, these data suggest that combinatorial treatments might even further enhance embryonic stem cell-derived cardiomyogenesis. In this context, Evseenko et al. (2010) has elegantly evaluated the initial stages of mesoderm commitment during human embryonic stem cell differentiation, demonstrating the presence of endogenous cardiogenic morphogens such as activin A, Bmp4, Vegf and Fgf2. Laflamme et al. (2007) demonstrate that Bmp4 treatment increased *in vitro* cardiomyogenesis using human embryonic stem cells and Paige et al. (2010) has elegantly shown a balanced interplay between activin A/Bmp4 and Wnt/β-catenin is needed to efficiently induce mesodermal lineage formation and subsequent cardiomyocyte development in human embryonic stem cells. Similar findings are also observed using mouse embryonic stem cells (Taha et al., 2006, 2007; Taha & Valojerdi, 2008; Takei et al., 2009; Verma & Lenka, 2010). In addition to the role of Bmp signaling, several studies have reported the pivotal role of Fgf signaling controlling mouse embryonic stem cell-derived cardiomyogenesis (Dell´Era et al., 2003; Ronca et al. 2009). Importantly, Fgf signaling, in conjunction with Bmp signaling enhances cardiomyocyte formation of other stem cell sources, such as bone marrow stem cells (Degeorge et al., 2008) or P19 carcinoma cell line (Hidai et al., 2003), reinforcing the notion that Fgf signaling is necessary (embryonic stem cells) and sufficient (other sources) to induce cardiomyocyte development. Curiously, novel signaling pathways, such as Sdf-1/Cxcr4 (Chiriac et al., 2010) and Vegf (Chen et al., 2006) also play a determinant role in cardiogenesis, although their links to Bmp, Fgf and Wnt signaling remains unexplored.

Understanding of the embryonic stem cell-derived cardiomyogenesis has also been largely unraveled by manipulation of several cardiac enriched transcription factors. Gata4 and Gata6 deficient mice suggested a pivotal role of this transcription factor in early cardiogenesis (Kuo et al., 1997; Xin et al., 2006). Gata4 deficient embryonic stem cells have been reported to disrupt visceral endoderm formation and thus hematopoiesis (Soudais et al., 1995; Bielinska et al., 1996, 1997; Morrisey et al., 2000; Pierre et al., 2009). Similar findings are also observed in Gata6 deficient embryonic stem cells (Pierre et al., 2009). Importantly, cardiomyogenesis is not affected in Gata4 deficient embryonic stem cells (Narita et al., 1997) but over-expression of Gata4 in embryonic stem cells enhances cardiogenesis (Grepin et al.,

1997) and visceral endoderm (Holtzinger et al. 2009) suggesting that Gata4 is not necessary but is sufficient to induce cardiomyocyte differentiation. Nkx2.5 deficient mice display cardiac embryonic lethality (Lyons et al., 1995), supporting a pivotal role for Nkx2.5 in cardiomyogenesis. In this context, over-expression of Nkx2.5 in embryonic stem cells increases the expression of cardiogenic markers at the expenses of hematopoietic markers such as Gata-1 (Caprioli et al., 2011). Similarly, enhanced expression of Mef2c in embryonic stem cells increases cardiomyogenic differentiation (Puceat et al., 2003), in line with its determinant role during heart development as demonstrated by genetic deletion in mice (Lin et al., 1997). Comparable over-expression approaches in embryonic stem cells have been described for the homeobox transcription factor Pitx2, a left-right signaling pathway determinant (Campione et al., 1999, 2002). Enhanced expression of Pitx2 leads to increased expression of cardiac markers (Lozano-Velasco et al., 2011) supporting a role of this transcription factor in cardiogenesis. In addition, overexpression of Tbx5, a pivotal transcription factor associated with Holt-Oram syndrome (Li et al., 1997), in P19 embryonic carcinoma cells (Fijnvandraat et al., 2003abc) display similar findings. Overall, these studies illustrate the pivotal role of distinct transcription factors with reported enhanced expression during cardiogenesis, as well as it also provides the entry to previously unknown transcription factors as key elements for cardiomyogenic lineage differentiation, as it is the case for the transcription factors hhLIM (Zheng et al., 2006) and Rb (Papadimou et al., 2005) or the GTPase Rac1 (Puceat et al., 2003).

Importantly, although these reports provided evidence of enhancing cardiomyocte formation by the usage of discrete growth factors and/or transcription factors, and subsequently identifying cardiac specific molecular markers such as Nkx2.5, Gata4 and sarcomeric proteins (i.e troponin T and alpha-actinin), it remains elusive if cardiomyocyte heterogeneity in terms of lineage origin (FHF, SHF), gene expression (atrial/ventricular/ nodal) or function (working/conductive) is observed. We have recently reported that mouse embryonic stem cell-derived cardiomyocytes display a dynamic temporal expression of FHF and SHF makers which are reminiscent of the *in vivo* cell lineage deployment (Lozano-Velasco et al. 2011). In addition, large heterogeneity in gene expression, displaying distinct atrial-, ventricular- and nodal-like patterns (Fijnvandraat et al. 2002, 2003abc) and functional heterogeneity (van Kempen et al., 2003) displaying distinct cardiac action potential configurations, have been extensively reported. In this context, is it important to highlight that overexpression of Pitx2 mainly directs the expression of SHF cardiomyocytes, since both islet-1 and Mef2c were up-regulated, whereas FHF marker Nkx2.5 was unaltered (Lozano-Velasco et al., 2011). Furthermore overexpression of Pitx2 enhances Tbx5 expression and thus Nppa (Anf) and Gja5 (Cx40)(Lozano-Velasco et al., 2011), in line with previous Tbx5 over-expression findings (Fijnvandraaf et al., 2003). Thus, it is plausible that enhance cardiomyocyte commitment by distinct inductive signals might generate cellular heterogeneity in a similar fashion as in the developing heart. If so, these observations might hindrance their therapeutical usage since, for example, engrafting nodal-like cells in the ventricular chambers might lead to ectopic electrical foci and thus to arrhythmias. In this context, searching for transcriptional factor cocktail which might homogenize the cardiomyocyte outcome is a plausible strategy, as recently reported to convert induced pluripotent fibroblasts into cardiomyocytes (Ieda et al. 2010) or hepatocytes (Huang et al., 2011).

Differentiation of embryonic stem cells into beating cardiomyocytes can therefore be naively observed by simply developing embryoid bodies or enhanced by supplementing these

embryonic stem cells and/or embryoid bodies with a subset of growth factors and/or transcription factors. **Table 2** summarizes the current state of the art knowledge about the dynamic expression of distinct transcription factors, sarcommeric proteins, ion channels and gap junctions during embryonic stem cell-derived cardiomyogenesis. However, the question arising is: are all beating areas differentiated into cardiomyocytes or are there remaining non-differentiated embryonic stem cells or contaminating cells that might differentiate into other embryonic lineages? This is a crucial question if we aim to use them *in vivo*. We have recently reported that fine-dissection of beating areas have minor contamination of endodermal- or ectodermal cells (Lozano-Velasco et al., 2011) at distinct developmental stages. Thus, it seems that a rather homogeneously differentiated cluster of cells is normally achieved. It remains to be elucidated if undifferentiated embryonic stem cells remain in those areas. *In vivo* approaches suggest that indeed this might be the case, yet titering the number of engrafted cells results in absence of teratomas at the long run (Behfar et al., 2005, 2007; Yamada et al. 2009).

5. Conclusions and perspectives

Over the last decade we have started to understand the molecular mechanisms that govern cardiac formation and we are translating these findings to the manipulation of embryonic stem cells opening promising avenues to enhance cardiomyocyte differentiation. We have learnt how to increase the number of cardiomyocyte produced, and we have learnt that embryonic development is faithfully recapitulated *in vitro*, including the onset of first and second heart field transcriptional programs. Manipulation of these transcriptional machinaries will be therefore the upcoming challenges for the next years to come in order to facilitate the generation of fully differentiated, structurally similar and functionally homogeneous cardiomyocytes. Searching for transcription factor cocktails or opening new strategies such as those emerging from the microRNA world (Ivey et al. 2008; Chinchilla et al., 2011) constitutes the next goals, as recently illustrated for miR-499 (Wilson et al., 2010). Thus, in summary, an important part of the route has been walked, and the way ahead seems promising with the reward of achieving therapeutically usable embryonic stem cell-derived cardiomyocytes.

6. Acknowledgments

We thank Jorge N. Dominguez for critical reading the manuscript. This work is supported by VI EU grant Heart Failure and Cardiac Repair (LSHM-2005-CT-018630) to DF, grants from the Ministry of Science and Innovation of the Spanish Government to DF (MICINN 2009-11566) and AA (MICINNN 2008-01217) and grants from the Junta de Andalucia Regional Council to DF (CTS-1614, CVI-06556) and AA (CTS-03878, CTS-446).

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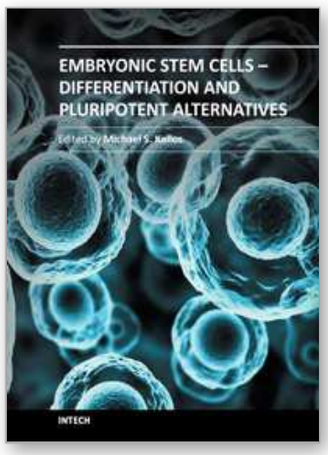

Embryonic Stem Cells - Differentiation and Pluripotent Alternatives
Edited by Prof. Michael S. Kallos

ISBN 978-953-307-632-4
Hard cover, 506 pages
Publisher InTech
Published online 12, October, 2011
Published in print edition October, 2011

The ultimate clinical implementation of embryonic stem cells will require methods and protocols to turn these unspecialized cells into the fully functioning cell types found in a wide variety of tissues and organs. In order to achieve this, it is necessary to clearly understand the signals and cues that direct embryonic stem cell differentiation. This book provides a snapshot of current research on the differentiation of embryonic stem cells to a wide variety of cell types, including neural, cardiac, endothelial, osteogenic, and hepatic cells. In addition, induced pluripotent stem cells and other pluripotent stem cell sources are described. The book will serve as a valuable resource for engineers, scientists, and clinicians as well as students in a wide range of disciplines.

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