Differential gene expressions in atrial and ventricular myocytes: insights into the road of applying embryonic stem cell-derived cardiomyocytes for future therapies

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Submitted 19 September 2009; accepted in final form 7 September 2010

Ng SY, Wong CK, Tsang SY. Differential gene expressions in atrial and ventricular myocytes: insights into the road of applying embryonic stem cell-derived cardiomyocytes for future therapies. Am J Physiol Cell Physiol 299: C1234–C1249, 2010. First published September 15, 2010; doi:10.1152/ajpcell.00402.2009.—Myocardial infarction has been the leading cause of morbidity and mortality in developed countries over the past few decades. The transplantation of cardiomyocytes offers a potential method of treatment. However, cardiomyocytes are in high demand and their supply is extremely limited. Embryonic stem cells (ESCs), which have been isolated from the inner cell mass of blastocysts, can self-renew and are pluripotent, meaning they have the ability to develop into any type of cell, including cardiomyocytes. This suggests that ESCs could be a good source of genuine cardiomyocytes for future therapeutic purposes. However, problems with the yield and purity of ESC-derived cardiomyocytes, among other hurdles for the therapeutic application of ESC-derived cardiomyocytes (e.g., potential immunorejection and tumor formation problems), need to be overcome before these cells can be used effectively for cell replacement therapy. ESC-derived cardiomyocytes consist of nodal, atrial, and ventricular cardiomyocytes. Specifically, for treatment of myocardial infarction, transplantation of a sufficient quantity of ventricular cardiomyocytes, rather than nodal or atrial cardiomyocytes, is preferred. Hence, it is important to find ways of increasing the yield and purity of specific types of cardiomyocytes. Atrial and ventricular cardiomyocytes have differential expression of genes (transcription factors, structural proteins, ion channels, etc.) and are functionally distinct. This paper presents a thorough review of differential gene expression in atrial and ventricular myocytes, their expression throughout development, and their regulation. An understanding of the molecular and functional differences between atrial and ventricular myocytes allows discussion of potential strategies for preferentially directing ESCs to differentiate into chamber-specific cells, or for fine tuning the ESC-derived cardiomyocytes into specific electrical and contractile phenotypes resembling chamber-specific cells.

atrial cardiomyocytes; ventricular cardiomyocytes; gene regulations; therapeutic application

MYOCARDIAL INFARCTION AND THE POTENTIAL OF ESC-DERIVED CARDIOMYOCYTES FOR TREATMENT OF MYOCARDIAL INFARCTION

Myocardial infarction has been the leading cause of morbidity and mortality in developed countries over the past few decades. Given the increasing tendency to eat a high-fat diet, together with a decline in exercise, myocardial infarction is considered to be a significant health concern for the 21st century. Patients who suffer from cardiac failure as a result of myocardial infarction have deteriorated heart function due to the significant loss of cardiomyocytes, especially ventricular cardiomyocytes. Hence, a large number of mature ventricular myocytes are needed for treatment. Although whole heart transplantation is the current method of treatment for cardiac failure as a result of myocardial infarction, cell replacement therapy, currently undergoing clinical trials, offers a potential new treatment. However, whole human hearts or cardiomyocytes are in extremely limited supply, hence there is a high demand for an alternative cardiomyocyte source (158).

There are a number of potential sources of cardiomyocytes for cell replacement therapies. Some endogenous or exogenous
stem cells, such as bone marrow-derived stem cells, hematopoietic stem cells, mesenchymal stem cells, resident cardiac stem cells, endothelial progenitor cells, embryonic stem cells (ESCs), and induced pluripotent stem cells with the capacity to differentiate into committed cardiomyocytes and repopulate lost myocardium, have been identified (158). However, there are significant controversies regarding both the efficiency and the reality of cardiac differentiation by many of these stem cell types (158). Among these cell sources, pluripotent stem cells unquestionably undergo the most efficient cardiogenesis (158).

ESCs have been isolated from the inner cell mass of blas-tocyst stage embryos (160, 192). They have two distinct characteristics, namely self-renewal and pluripotency. The ability to self-renew allows ESCs to propagate indefinitely in the primitive undifferentiated state. Pluripotency means that ESCs are able to develop into any of the three germ layers’ cell types. There are many reports describing the successful derivation of cardiomyocytes from ESCs (70, 78, 82, 88–90, 130, 136, 182, 213, 215). Characterization of these ESC-derived cardiomyocytes (ESC-CMs) is usually at the molecular, electrophysiological, and functional levels. At the molecular level, RT-PCR and immunostaining studies show that cardiac-specific markers, including transcription factors such as GATA-binding protein 4 (GATA4) and NK2 transcription factor-related locus 5 (NKX2.5), and structural proteins such as α-myosin heavy chain (α-MHC), cardiac troponin I (cTnI), cardiac troponin T (cTnT), atrial myosin light chain-2 (MLC-2a), and ventricular myosin light chain-2 (MLC-2v) (82, 88, 89, 130, 136, 213) are expressed at mRNA and protein levels. At the electrophysiological level, whole cell patch clamp (70, 136), extracellular electrogram recording (88–90, 215), and calcium measurement (89, 90, 136, 215, 224) have shown the presence of a cardiac-specific action potential (70, 88–90, 136, 215), intracellular calcium transient (89, 90, 136, 215, 224), and excitation-contraction (E-C) coupling (224) in ESC-CMs. At the functional level, ESC-CMs have been transplanted onto freshly isolated cardiomyocytes in a monolayer culture or injected into the infarcted hearts of small animals (90, 215). These electrically active ESC-CMs were able to integrate with and pace the electrical activity of ventricular cardiomyocytes both in vitro and in vivo (90, 215). In addition, some studies have shown that transplantation of these ESC-CMs into infarcted hearts can improve cardiac parameters such as ejection fraction and end-diastolic volume compared with animals receiving noncardiomyocyte derivatives (25, 151). These cells survived, integrated, and matured after intramyocardial injection in immunodeficient mice for up to 12 wk (199). All of these findings indicate that ESC-CMs are genuine cardiac cells that represent a novel source of cardiomyocytes for future cell replacement therapies.

CURRENT OBSTACLES TO THE CLINICAL USE OF ESC-CMS

Some technical difficulties need to be overcome before ESC differentiation derivatives can be used effectively for cell replacement therapy. Previous studies have reported the transplantation of undifferentiated human (h) ESCs (110, 211) and nonpurified hESC differentiation culture (or “committed” hESCs) (104, 110, 193) into myocardial infarcted hearts. Although there are functional improvements (104, 110) and formation of myocardial graft (104) in the infarcted hearts, findings from a number of mouse (m) ESC and hESC studies indicate a risk of tumor formation after transplantation of undifferentiated cells (15, 110, 145, 186). This is presumably due to the fact that transplanted undifferentiated ESCs (which are present in the nonpurified ESC culture) are not guided toward a cardiac fate in vivo (145); because of the ESCs’ self-renewal ability, they proliferate indefinitely and thereby generate tumors. By understanding the complex biology of ESCs, such as how they maintain their self-renewal characteristic, it should be possible to control their proliferation and thereby eliminate the risk of tumor formation.

Because ESCs do not originate in patients’ bodies, the problem of immune response also needs to be addressed. It has been reported that undifferentiated hESCs express low levels of class I major histocompatibility complex (MHC-I) molecules (a group of antigen-presenting proteins on the cell surface) (42, 43, 45, 209). This MHC-I expression increased ~10-fold with the induction of differentiation (45). These findings imply that following transplantation of undifferentiated ESCs and/or their differentiation derivatives, graft rejection may be elicited because the recipients’ allogeneic T cells may recognize the foreign MHC-I molecules present on the surface of the cells. A number of methods have been proposed to solve this immunorejection problem (44). Among them, derivation of ESCs that have their major histocompatibility complex [also called human leukocyte antigen (HLA) in humans] matched with the patient’s could be a potential method for eliminating immunorejection problems in ESC transplantation (91, 161). Future advancements in the development of induced pluripotent stem cells may also help to solve the immunorejection problem (147, 149, 150, 187, 188, 218).

Moreover, under conventional protocols, cells differentiated from ESCs are a mixed cell population with cardiomyocytes only contributing a very small proportion of the whole cell population (89, 104). Cardiomyocytes should be enriched for therapeutic application. By understanding the molecular and functional differences between atrial and ventricular myocytes, strategies may be developed for enriching chamber-specific myocytes from ESC differentiation culture and for preferentially differentiating ESCs into chamber-specific cells (e.g., ventricular myocytes for treatment of myocardial infarction). That will be discussed later in this review.

FUNCTIONAL DIFFERENCES BETWEEN ATRIAL AND VENTRICULAR MYOCYTES

The heart is responsible for pumping blood throughout the body. This pumping action requires the highly coordinated efforts of different kinds of cardiac cells, including nodal cells, atrial cells, and ventricular myocytes. Action potentials are originally generated from the sinoatrial (SA) nodal cells, or so-called pacemaker cells, which are located in the right atrium. These spontaneously firing action potentials from the SA node spread through the atria and ventricles; atrial and ventricular myocytes contract following the generation of the propagated action potential. Cells in nodal areas, the atria, and ventricles are morphologically, molecularly, and functionally distinct. They differ significantly in both their electrophysiological and contractile properties. Only a few genes that underlie the differences between the atrial and ventricular myocytes have been identified. The following section will begin...
with a review of the electrophysiology, contractile properties, and E-C coupling of atrial and ventricular cardiomyocytes. The genes that are uniquely expressed in the atria and ventricles will then be discussed. Finally, we will consider some potential strategies for enriching ESC-CMs and for preferentially differentiating ESCs into chamber-specific cells.

Electrophysiological Properties

Cardiac action potential has five distinct phases; it is initiated by a rapid upstroke (phase 0), followed by a transient repolarization (phase 1), and a slowly decaying plateau (phase 2). Afterward, rapid repolarization (phase 3) drives membrane potential back to the resting state (phase 4).

During phase 0, membrane is rapidly depolarized from a resting hyperpolarized potential (approximately −80 mV or −85 mV in atrial or ventricular myocytes, respectively) to a positive potential (approximately +30 mV) (171). When the atrial or ventricular myocytes are electrically stimulated, the initial membrane depolarization opens the fast-activating voltage-gated sodium (Na_v) channels, which are mainly composed of Na_v1.5 (121). Sodium influx through these Na_v channels in turn causes further depolarization, resulting in a fast upstroke of depolarization at a rate of 150–300 V/s (171).

After the initial fast depolarization (phase 0), a transient partial repolarization occurs during phase 1 when inward sodium current (i_{Na}) is rapidly inactivated and transient outward current (i_{to}) is activated. Duration and shape of early phase 1 repolarization are thought to be modulated by i_{to}, which consists of the slow (mediated by K_v1.4, K_v1.7, and K_v3.4) and fast (mediated by K_v4.2 and K_v4.3) transient outward currents (86, 152). Reduction of i_{to} leads to longer action potential duration, which results in stronger contraction force due to the increased calcium influx via L-type calcium channels. Atrial myocytes have lower and shorter action potentials than ventricular myocytes, partly due to the greater atrial i_{to} amplitude (62).

A few hundred milliseconds long depolarized plateau in phase 2 is a striking property of cardiac action potential. Its duration is also the most pronounced difference between atrial and ventricular action potential (62). Plateau of action potential in phase 2 is a delicate balance between the inward L-type calcium current (i_{Ca,L}) and outward delayed rectifier potassium current (i_K) (33, 84, 162). i_{Ca,L} has slower activation than i_{Na} but produces a longer lasting inward current (17). i_{Ca,L} is relatively strong in the early phase 2 and then lessens due to calcium- and voltage-dependent inactivation. This inward i_{Ca,L} is balanced by the outward i_K. A strong ultrarapid delayed rectifier (i_{Kur}) (mediated by K_v1.5) is expressed in atrial myocytes; this i_{Kur} has a very fast activation and a very slow inactivation and dominates the outward current in phase 2 of the atrium (50). i_{Kur} has been described in the atria of a variety of species (21, 205, 219); however, i_{Kur} was not detected in the ventricles, at least in ventricular myocytes from humans and dogs (111, 220). In contrast, currents with activation kinetics slower than i_{Kur} have been identified in both atrial and ventricular myocytes, including slow (i_{Ks}) and rapid (i_{Kr}) delayed rectifier (62, 219). These potassium currents were found to be stronger in atrial myocytes than in ventricular myocytes (62). Since the duration of phase 2 largely depends on the balance between the inward calcium currents and the outward potassium currents, atrial myocytes that express potassium channels with larger conductance and faster activation kinetics display shorter phase 2 than ventricular myocytes.

As the inward i_{Ca,L} diminishes at the end of phase 2, i_K, including i_{Kr}, i_{Kf}, and the inward rectifier current i_{K1}, becomes dominant and results in phase 3 repolarization. When the end of phase 3 is approached, channels for i_{Kr} and i_{Kf} eventually close due to the hyperpolarized membrane potential. i_{K1} persists to keep the steady resting membrane potential.

Phase 4 represents the resting membrane potential of atrial and ventricular myocytes. As ventricular myocytes show higher expression of inward rectifier than atrial myocytes, ventricular myocytes have a slightly more negative resting membrane potential than atrial myocytes (62, 171). Since the expression of funny current (i_h), carried by hyperpolarization activated, cyclic nucleotide-gated (HCN) channels, is relatively small/absent when compared with that present in the SA node, no spontaneous depolarization can be recorded in either atria or ventricles (13).

Contractile Properties

Myosin is the molecular motor of the heart. Cardiac myosin is the central participant in the cross-bridge cycling; it mediates both ATP hydrolysis and mechanical production of contractile force. Cardiac myosin includes two heavy chains and four light chains. In mammals, myosin heavy chain (MHC) exists in two isoforms: α and β. It is within these MHC subunits that ATPase activity resides. On the other hand, there are two types of myosin light chains (MLCs), namely, the essential MLCs and the regulatory MLCs. Essential MLCs have two main isoforms, the atrial myosin essential light chain (ALC-1) and ventricular myosin essential light chain (VLC-1) while regulatory MLCs also have two main isoforms, the MLC-2a and MLC-2v.

Atrial myocardium is known to have a higher maximum shortening velocity (V_{max}) when compared with ventricular myocardium (79, 154, 183). Both the rate of active tension generation and relaxation are faster in atrial than ventricular myofibrils, and this is believed to be related to the cross-bridge kinetics (154). In large mammals, the predominant MHC isoforms in adult atrium and ventricle are the α form and the β form, respectively (64, 77, 124, 128, 159, 170, 194). The ATPase activity of the α-MHC is higher than that of the β-MHC (156), and V_{max} is roughly proportional to ATPase activity. This is consistent with the observation that the actin filament sliding velocity generated by α-MHC in vitro motility assays is higher than that of β-MHC (102). This can also provide a reasonable explanation for the different kinetics of contraction and relaxation between atrial and ventricular working myocardium in large animals (79, 154, 183). However, young small rodents express the α-MHC both in the atria and the ventricles (32, 49, 112, 114, 142), and they also exhibit faster rates in the atrial myocardium (20). This suggests a role for the light chain isoforms in determining different kinetics. Myosin containing ALC-1 has faster cross-bridge kinetics and V_{max} than myosin containing VLC-1 (115, 132, 169). Since normal atrial and ventricular cardiomyocytes, respectively, express predominantly ALC-1 and VLC-1, respectively, this may help explain partially why atrial cardiomyocytes have a higher V_{max}. 

AJP-Cell Physiol • VOL 299 • DECEMBER 2010 • www.ajpcell.org
In addition to higher shortening rate, atrial working myocardium generates less active tension than the ventricular myocardium (154, 195). Since MLCs can control aspects of crossbridge cycling and alter force development (167), the difference in maximum tension development in atrium and ventricle may be attributed to the difference in expression of MLC isoforms (195). A previous study demonstrated that, with the same form of MHC, ventricular-type MLCs generated ~60% higher average force when compared with atrial-type MLCs (217). However, recent studies showed that the difference in tension developed between working atria and ventricles is likely due to lower density of myofibrils in the atrial myocytes (73, 139, 154).

On the other hand, atrial cardiomyocytes have been known to have lower resting tension compared with ventricular cardiomyocytes (154, 195). The lower resting tension in atrial myocytes suggests that they exhibit a lower resting stiffness than ventricular myocytes (4). It is known that part of the diastolic force of cardiac muscle is contributed by the extension of the I-band segment of titin. Previous studies showed that myocardio coexpressed two distinct titin isoforms: a smaller and less compliant isoform with the N2B element only (N2B titin) and a larger and more compliant isoform with both the N2B and N2A elements (N2BA titin). For a given sarcomer length, the longer extensible region of the larger N2BA titin would lead to a lower fractional extension length and hence a lower force. Since atria express a higher proportion of the larger N2BA isoform while ventricles express more of the smaller N2B isoform (1, 5), the difference in resting tension between atrial and ventricular myocytes is related to the differences in titin isoforms.

E-C Coupling

Calcium is the critical mediator for linking electrical depolarization upon arrival of an action potential with the cardiomyocyte contraction. The sarcoplasmic reticulum (SR) of cardiomyocytes is an intracellular calcium store. It contains the ryanodine receptors (RyRs), which are calcium release channels. When a cardiomyocyte is electrically stimulated, an action potential is triggered which in turn opens the voltage-gated calcium channels (VGCCs) on the plasma membrane. Calcium entering through VGCCs will further activate RyRs, which release more calcium from the SR through a process called calcium-induced calcium release (CICR). Depending on the cell’s condition, this CICR can represent an increase of around tenfold over the calcium influx through the cell surface VGCCs, and therefore represents an amplification of the original calcium signal (19). The calcium ions then bind to the troponin C of the myofibrils and trigger a contraction.

There are significant differences in the E-C coupling between atrial and ventricular myocytes; in small rodents, the differences are mostly due to cellular ultrastructure differences caused by the absence/low abundance of transverse tubules (T-tubules; the T-tubular invaginations of the cell surface plasma membrane) in atrial myocytes and their presence in ventricular myocytes (19, 190). In atrial myocytes of small rodents where the T-tubules are poorly developed or absent, meaning that the cell surface plasma membrane does not regularly protrude into the center of the atrial myocytes, the calcium influx through the cell surface membrane will trigger CICR through the RyRs located in the subsarcolemmal region. Therefore, depolarization leads to an increase in calcium signals that originates mostly at the cell periphery (19), leading to a “shell” of increased calcium level immediately beneath the cell surface membrane, with no immediate response deeper inside the cells (19). This CICR will then spread toward the center of the atrial myocytes by a propagated mechanism (190). However, this propagated CICR does not happen all the time because the subsarcolemmal calcium signal does not spread in a fully regenerative manner. The inward movement of calcium diminishes due to various events that “buffer” the change in calcium (e.g., activity of the SERCA). This propagated movement of calcium is ultimately controlled by several factors, such as the calcium content of the SR, the presence/absence of physiological positive inotropic agents, and inositol 1,4,5-trisphosphate infusion (19, 119).

In contrast, in ventricular myocytes of small rodents, the earliest rise in calcium level occurs in the Z-line upon arrival of the action potential (190), although this nonuniform calcium rise lasts for a very short period of time (around 4 ms). As the ventricular myocytes contain T-tubules, which serve to bring VGCCs on the cell surface membrane and RyRs on the SR into close proximity, the rise in calcium level becomes uniform throughout the cytoplasm in a rapid and global manner (within 10 ms after the onset of the action potential) (190). The calcium influx through the cell surface membrane induces the CICR through the RyRs at the junctional SR, which is located very close to the T-tubules. In other words, the calcium sparks are recruited largely in a synchronized and global fashion.

This difference in the presence/absence of T-tubules thus accounts for the spatial and temporal difference in the calcium pattern upon depolarization and thus the E-C coupling in the atrial and ventricular myocytes of small rodents (19, 190). To our knowledge, T-tubules have not yet been investigated in human atrial myocytes. However, T-tubules are very likely to be present in human atrial myocytes since atrial myocytes of large animals such as dogs and sheeps possess T-tubules (40, 41, 109). Further investigation is needed to dissect in details the mechanisms behind the differences in E-C coupling between atrial and ventricular myocytes in large animals.

Altogether, the atrial and ventricular myocytes differ significantly in both their electrophysiological and contractile properties, as well as the E-C coupling. In the following section, genes that are expressed in a chamber-specific manner will be discussed in detail; the available evidence on their functions, expression throughout development, and their regulation, will also be reviewed.

MOLECULAR DIFFERENCES BETWEEN ATRIAL AND VENTRICULAR MYOCYTES

Differential Expressions of Transcription Factors

Hairy-related transcription factor 1. Hairy-related transcription factors (HRTs), including HRT1 also named as (HEY1, HESR1, GRL1, HERP1) and HRT2, are basic helix-loop-helix transcriptional repressors (97). HRT1 is expressed in atrial cardiomyocytes but not in ventricular cardiomyocytes (55, 100, 138). Forced expression of HRT1 in the entire cardiac lineage of mice resulted in a reduction or loss of the atrioventricular (AV) canal (100). Furthermore, in these HRT1-misexpressing hearts, expression levels of bone morphogenetic protein 2...
(BMP2) (100, 165) and T-box transcription factor (TBX) 2 (TBX2), two AV canal-specific genes, were significantly reduced (100). These findings suggest that HRT1 is important for the precise formation of the heart’s AV boundary.

It is known that Notch signaling is required for multiple aspects of cardiovascular development (75, 144). In these studies, Notch1 activation in the mesodermal lineage led to myocardium abnormality. Also, in Notch1-activated hearts, HRT1 was strongly induced in both the ventricle and the AV canal. Interestingly, this myocardium abnormality could be rescued by knockout of the HRT1 gene from these Notch1-activated hearts (206). This study indicated that HRT1 was the downstream target of Notch1 signaling and that no/low expression of HRT1 in the ventricle was important for proper differentiation and functioning of the heart. Similarly, misactivation of Notch2, which specifically induced HRT1, and inhibition of Notch2, which specifically inhibited HRT1, suggests that HRT1 was the direct mediator of Notch2 (165). However, there were conflicting results that showed that expression of HRT1 and HRT2 were independent of Notch2 signaling (100). Some studies have suggested that mouse HRT1 and HRT2 genes are redundantly required to mediate Notch signaling in the developing cardiovascular system (98, 100). In vitro studies have revealed that the Notch signaling pathway directly regulates transcription of both HRT1 and HRT2 (210).

Concerning the downstream targets of HRT1, loss of HRT1 was found to increase the expressions of GATA4 and GATA6, whereas overexpression of HRT1 led to a strong repression of the activities of GATA4 and GATA6 promoters (56). Similarly, the promoter of activity in the GATA4/6 target gene atrial natriuretic factor (ANF) decreased in the presence of HRT1 (56). Protein interaction and mutation analyses found that HRT1 directly bound to GATA4 and GATA6; this interaction in turn decreased the binding of GATA4 and GATA6 to the ANF promoter and thereby decreased ANF transcription (56). In addition, analysis of both HRT1 knockout and HRT1 overexpressed animals demonstrated that HRT1 repressed the expression of BMP2 (100, 165) and TBX2 (100). Taken together, GATA4, GATA6, ANF, BMP2, and TBX2 were found to be the downstream targets of HRT1.

**Hairy-related transcription factor 2.** Hairy-related transcription factor 2 (HRT2) (also named as HEY2, HESR2, CHF1, GRL2, and HERP2) is a basic helix-loop-helix transcriptional repressor. It is expressed in ventricular cardiomyocytes but not in atrial cardiomyocytes, in contrast to HRT1 described above (55, 96, 100, 138, 212). Mice homozygous for a null mutation of HRT2 died perinatally (210) with a spectrum of cardiac malformations, including a dilated left ventricular chamber (210), markedly diminished fractional shortening of the left ventricle (99, 166, 210), abnormal ventricular septation (166), and abnormal AV valve formation (99). In addition, conditional knockout of HRT2 specifically in cardiomyocytes resulted in the ectopic activation of atrial genes in the ventricular myocardium, with an associated impairment of cardiac contractility. In contrast, the forced expression of HRT2 in atrial cardiomyocytes repressed atrial-specific gene expression (87, 212). Similar to HRT1, forced expression of HRT2 in a mouse heart led to reduction or loss of the AV canal, suggesting a critical role for HRT2 in AV canal formation (100). Interestingly, transgenic mice that overexpressed HRT2 in the myocardium showed an attenuation of hypertrophic response in response to α-adrenergic stimulation, suggesting that HRT2 functions as an antihypertrophic gene (210). Altogether, these studies suggest that HRT2 is important for mammalian cardiac development, especially left ventricular development, by suppressing atrial identity (96, 212). It is also important for maintaining normal myocardial contractility (99, 166, 212).

As mentioned previously, the Notch signaling pathway has been found to directly regulate transcriptions of both HRT1 and HRT2 (210), which worked redundantly to mediate Notch signaling (98, 100). In HRT2 knockout mice, ANF, TBX5, connexin 40 (CX40), atrial contractile genes ALC-1 and MLC-2a, all of which are found to be preferentially expressed in the atrium of normal mice, were expressed ectopically in the left ventricular compact myocardium (96). This suggests a general dysregulation of atrial gene expression as a result of knocking out HRT2. TBX5 was found to be a downstream target of HRT2, and HRT2 was found to normally suppress TBX5 expression in the left ventricular compact myocardium, which in turn suppressed the expression of ANF and CX40 (96). Moreover, HRT2 was found to inhibit expression of the ANF promoter in vitro (56, 87), during development (56), and during cardiac hypertrophy (210), through an interaction with GATA4. Similarly, loss of HRT1 and HRT2 in ESCs led to elevated GATA4/6 and ANF mRNA levels in embryoid bodies during differentiation, while forced expression of HRT2 strongly repressed activities of the GATA4 and GATA6 promoters in various cell lines (56). In addition, misexpression and knockout of HRT2 were also found to decrease and increase BMP2 expression, respectively (100, 165). The HRT2-misexpressing heart also had a reduced expression of TBX2 (100). In addition, protein interaction and mutation analyses suggested that HRT2 decreased ANF transcription by directly binding to GATA4 and GATA6, and thereby decreased their binding to the ANF promoter, similar to the case of HRT1 (56). Altogether, GATA4, GATA6, ANF, BMP2, TBX2, CX40, TBX5, ALC-1, and MLC-2a were found to be the downstream targets of HRT2.

**Iroquois homeobox gene 4.** Iroquois homeobox gene 4 (IRX4) is a member of the Iroquois family of homeobox transcription factors. Previous studies have shown that IRX4 plays a critical role in establishing chamber-specific gene expression in the developing heart (11, 22). Interestingly, the IRX4 protein has been shown to regulate the chamber-specific gene expression by activating the expression of the ventricle-specific gene and suppressing the expression of the atrial-specific gene in chicks. Overexpression of IRX4 led to a downregulation of atrial-specific gene atrial myosin heavy chain-1 (AMHC-1) and an upregulation of ventricular-specific gene ventricle myosin heavy chain-1 (VMHC-1) in the atria, whereas knockdown of IRX4 led to an upregulation of AMHC-1 and a downregulation of VMHC-1 in the ventricles (11). IRX4 has a ventricle-restricted expression pattern at all stages of chick, rat, and mouse heart development, beginning at the stage when the ventricular progenitor starts to appear (11, 22, 71).

IRX4 was reported to be downregulated in NKK2.5 or dHAND single mutants (22). In NKK2.5−/− dHAND−/− double mutants, ventricular dysgenesis occurred and there was a complete abolishment of IRX4 (216). This suggests that both NKK2.5 and dHAND cooperatively regulate the expression of IRX4. However, it is not yet known whether NKK2.5 and
Differential Expressions of Structural Genes

Atrial myosin light chain 2. Atrial myosin light chain 2 (MLC-2a), one of the essential MLC-2 isoforms, is a structural protein that is responsible for cardiac sarcomere formation and is therefore important for the contractile function of cardiac cells, similar to MLC-2a. In contrast to MLC-2a, which is expressed in atrial cardiomyocytes, MLC-2v is uniquely expressed in ventricular cardiomyocytes. It has been shown to be crucial for heart development (173). MLC-2v is ventricular specific from the onset of expression (122, 143, 146). Interestingly, MLC-2v knockout mice survived until E12.5. Although MLC-2a protein was found to express in the mutant ventricles, ultrastructural analysis revealed defects in sarcromeric assembly. Furthermore, an embryonic form of dilated cardiomyopathy characterized by a significantly reduced left ventricular contractility was detected (29), suggesting that MLC-2v has a unique function in the maintenance of ventricular contractility. In fact, MLC-2v isoform was found to contribute to the greater power-generating capability of the ventricle when compared with the atrium (24, 153).

During development, MLC-2v mRNA was found in the ventricular region at E8 by in situ hybridization. Its expression was high in the ventricular portion of the heart tube but was not detected in the atrial or sinus venous regions. A minimal level of MLC-2v mRNA was also found in the proximal outflow tract of the heart tube at this time. By E9–10, MLC-2v mRNA displayed a temporally and spatially distinct expression pattern in the proximal outflow tract region adjacent to the ventricular segment. By E11, before the completion of septation, its expression became restricted to the ventricular region and below the level of the AV cushion (146). Similarly, an in vitro study using differentiating ESCs also suggested that MLC-2v expression started from day 9 of the suspension culture, which is later than the expression of MLC-2a (103).

Studies in transgenic mice and on cultured ventricular myocytes have identified two regulatory elements, namely HF-1a and HF-1b/MEF-2, which are required to maintain the ventricular chamber-specific expression of the MLC-2v gene (106, 141, 164, 223). A further study showed that enhancer factor I subunit A [EFIA; known as Y box binding protein-1 (YB-1) in humans] bound to the HF-1a site in a sequence-specific manner (225) and produced a greater than threefold increase in the 250 bp MLC-2v promoter activity, specifically in the cardiac cell context (225). It was suggested that EFIA/YB-1, together with its partner p30, binds to the HF-1a site and, in conjunction with HF-1b/MEF-2, mediates ventricular-specific expression of the MLC-2v gene (225).

MEF-2, GATA4, and serum response factor (SRF) binding sites have been identified in the 5′ regulatory sequence of MLC-2a genes by sequence analysis (179), although experimental data are still lacking. In addition, MLC-2a was expressed ectopically in the left ventricular compact myocardium of HRT2 knockout mice, implying that HRT2 may play a role in the regulation of atrial gene expression (96).

Ventricular myosin-light chain 2. Ventricular myosin-light chain 2 (MLC-2v), one of the essential MLC-2 isoforms, is a structural protein that is responsible for cardiac sarcomere formation and is therefore important for the contractile function of cardiac cells, similar to MLC-2a. In contrast to MLC-2a, which is expressed in atrial cardiomyocytes, MLC-2v is uniquely expressed in ventricular cardiomyocytes. It has been shown to be crucial for heart development (173). MLC-2v is ventricular specific from the onset of expression (122, 143, 146). Interestingly, MLC-2v knockout mice survived until E12.5. Although MLC-2a protein was found to express in the mutant ventricles, ultrastructural analysis revealed defects in sarcromeric assembly. Furthermore, an embryonic form of dilated cardiomyopathy characterized by a significantly reduced left ventricular contractility was detected (29), suggesting that MLC-2v has a unique function in the maintenance of ventricular contractility. In fact, MLC-2v isoform was found to contribute to the greater power-generating capability of the ventricle when compared with the atrium (24, 153).

During development, MLC-2v mRNA was found in the ventricular region at E8 by in situ hybridization. Its expression was high in the ventricular portion of the heart tube but was not detected in the atrial or sinus venous regions. A minimal level of MLC-2v mRNA was also found in the proximal outflow tract of the heart tube at this time. By E9–10, MLC-2v mRNA displayed a temporally and spatially distinct expression pattern in the proximal outflow tract region adjacent to the ventricular segment. By E11, before the completion of septation, its expression became restricted to the ventricular region and below the level of the AV cushion (146). Similarly, an in vitro study using differentiating ESCs also suggested that MLC-2v expression started from day 9 of the suspension culture, which is later than the expression of MLC-2a (103).

Studies in transgenic mice and on cultured ventricular myocytes have identified two regulatory elements, namely HF-1a and HF-1b/MEF-2, which are required to maintain the ventricular chamber-specific expression of the MLC-2v gene (106, 141, 164, 223). A further study showed that enhancer factor I subunit A [EFIA; known as Y box binding protein-1 (YB-1) in humans] bound to the HF-1a site in a sequence-specific manner (225) and produced a greater than threefold increase in the 250 bp MLC-2v promoter activity, specifically in the cardiac cell context (225). It was suggested that EFIA/YB-1, together with its partner p30, binds to the HF-1a site and, in conjunction with HF-1b/MEF-2, mediates ventricular-specific expression of the MLC-2v gene (225).

MEF-2, GATA4, and serum response factor (SRF) binding sites have been identified in the 5′ regulatory sequence of MLC-2a genes by sequence analysis (179), although experimental data are still lacking. In addition, MLC-2a was expressed ectopically in the left ventricular compact myocardium of HRT2 knockout mice, implying that HRT2 may play a role in the regulation of atrial gene expression (96).
Review

SERCA2a is important for intracellular calcium distribution and is a determinant of the cardiac action potential, sarcolipin plays an important role in heart physiology.

In the species that have been studied, expression of sarcolipin mRNA was most abundant in the atria and was undetectable in the ventricles (127, 200). Interestingly, unlike most other atrial-restricted genes, sarcolipin was atrial specific from the onset of its expression at around E11.5 to E12.5 and increased abruptly at around E16.5 (127, 179). Expression of sarcolipin increased over time in the atria but was undetected in the ventricles at any of the developmental stages (127, 179).

In the adult stage, sarcolipin was still expressed exclusively in the atrium (6, 127, 200).

As far as we know, no transcription factor has been identified that binds to the promoter region of the sarcolipin gene. Interestingly, both mechanical stress (176) and hypertrophy (127) were found to downregulate the transcription of sarcolipin, although the messenger(s) involved in the process are currently unknown.

Differential Expressions of Ion Channels

**KvLQT1 (KCNQ1) regulator β-subunit MinK (KCNE1).** As previously discussed, a cardiac action potential is composed of several different phases in which the repolarization phase is dependent on the transmembrane delayed-rectifier cardiac potassium current. This potassium current consists of the slow \(I_{Ks}\) and rapid \(I_{Kr}\) components, respectively, generated by the pore-forming \(\alpha\)-subunits KCNQ1 and KCNH2, in association with their regulatory \(\beta\)-subunits. MinK, encoded by KCNE1, is a single transmembrane domain ancillary subunit for recapitulation of the \(I_{Kr}\) current. MinK was reported to associate with the KCNQ1 in the ventricles of mammals, including guinea pigs, mice, and humans (1, 122). MinK-KCNQ1 channels generated the \(I_{Kr}\) current, which contributed to human ventricular repolarization in which MinK functioned to regulate the kinetics of KCNQ1 (e.g., MinK slowed KCNQ1 activation, removed inactivation, increased unitary conductance, and was responsible for the pharmacology of the complex) (12, 53, 168, 175). Interestingly, mutations in MinK were found to be associated with human long-QT syndrome (204).

A previous study showed that during mouse heart development, KCNE1 expression was first observed at E9.5 throughout the entire myocardium. Progressively, by E16.5, KCNE1 expression was mainly confined to the compact ventricular myocardium (57). This was in contrast to the expression pattern of potassium channel \(\alpha\)-subunits, such as KCNQ1 and KCNH2, which were initially expressed at E9.5 and showed comparable levels of expression within the atrial and ventricular myocardium during the embryonic and fetal stages (57). In 10-wk-old mice, KCNE1 was expressed preferentially at a low level in the ventricle of an adult heart but remained undetectable in the atrium (122). It is worth noting that the expression patterns in a normal human heart seem to be significantly different (148). Some studies have found that mRNA of KCNE1 was present in the atrium of healthy human myocardium (116, 148).

Sequence analysis has shown the presence of several transcription factor binding sites in the promoter region of KCNE1, including binding sites for myogenic differentiation (MYOD), GATA6, SRF, and steroidogenic factor 1 (SF1) (117). In addition, site-directed mutagenesis of the GATA elements in the core promoter of KCNE1 reduced its activity by >50% and...
attenuated cardiomyocyte-preferential expression (137). In the same study, GATA4 knockdown with short-interfering RNA also inhibited around 40% of core promoter activity in neonatal rat cardiomyocytes (137), suggesting that GATA4 can bind the KCNE1 promoter and affect its transcriptional activity. A promoter study showed that stimulating protein 1 (Sp1) bound the core promoter of KCNE1 by interacting with the Sp1 cis-acting elements (118).

### Differential Expressions of Secretory Proteins/Peptides

**Atrial natriuretic factor.** ANF is a group of peptides that was originally isolated from the cardiac atria and has a number of important effects on blood pressure (by vasorelaxant activity), on renal function (by diuretic activity), and on salt balance (by natriuretic activity) (36, 37). ANF has been regarded as a naturally occurring “antihypertensive” regulator of blood pressure, volume status, and cardiovascular homeostasis.

Previous reports suggest that at the early stage of development, ANF is expressed in both the atrial and ventricular cardiomyocytes. By in situ hybridization, ANF mRNA was first detected in a subpopulation of myocardial cells at E8. From E9 onwards, ANF mRNA was detected in the atrium and the ventricle, but its expression became atrial specific immediately before birth (60, 80, 178, 221).

A 638 bp promoter region of the ANF gene was sufficient to drive correct chamber-specific expression in transgenic mice (51, 95, 174). However, distinct regions of this ANF 5’-flanking region were required for inducible expression of the ANF gene in the case of an adult mice ventricle subjected to pressure overload hypertrophy (95), indicating that there is a regulatory region outside this 5’-flanking region in the ventricle.

Regulation of ANF expression is controlled primarily at the transcriptional level. A number of transcription factors, including NKX2.5 (46, 59, 68, 76, 107, 180), GATA4 (46, 107, 133, 134, 180), SRF (134), myocardin (10, 202, 222), TBX5 (23), phenylephrine response element protein complex 1 (PEX1) (the rat homolog of human ZFP260) (38), and paired-like homeodomain transcription factor 2 (PITX2) (59) have been shown to activate ANF transcription by binding to their cis elements in the ANF enhancer region. On the other hand, a number of ANF repressors, such as TBX2 (68, 216), HRT factors (56, 210), Junmonji (92, 93, 108), HOP (28, 177), Friend of GATA-2 (FOG2) (185, 191), and cardiac ankyrin repeat protein (CARP)/Ankyrin repeat domain 1(ANKRD1) (31) were also identified.

GATA4 and NKX2.5 were found to interact physically and act synergistically to activate the ANF promoter (46, 107). Structure/function studies suggest that binding of GATA4 to the COOH-terminus autorepressive domain of NKX2.5 may induce a conformational change that unmasks NKX2.5 activation domains and activates the ANF promoter (46). An in vitro promoter study showed that both GATA4 and GATA6 bound to the GATA element of the ANF promoter, although with different binding affinities. GATA4 and GATA6 were found to activate ANF expression in a cooperative manner, and the activation was mediated through a single GATA-binding site (27). However, GATA6 could not be used to substitute GATA4 for interaction with NKX2.5 (46). Chamber-specific expression of ANF was mediated through binding of NKX2.5 and GATA4/GATA6 on the NKE and GATA sites, respectively (179). Mutation of either the NKE or GATA sites resulted in a failure to restrict ANF transcription in the atria. In agreement with this notion, mutation of the NKE site resulted in the upregulation of ANF promoter activity in cultured ventricular cardiomyocytes (47). As NKX2.5 and GATA4 were expressed in both the atrium and ventricles, it was suggested that NKE and GATA sites may serve as recognition sequences for a currently unidentified repressor complex, which may compete with NKX2.5 or GATA4 for NKE or GATA sites, respectively, thereby inhibiting ANF expression in the ventricular myocardium (179). Interestingly, consistent with this suggestion, NKX2.5 overexpression led to a persistent expression of ANF in the ventricle beyond the normal time of downregulation of ANF in the ventricle (189).

GATA factors also interact with other factors to control ANF expression. A previous study showed that GATA factors and SRF synergistically activate the ANF promoter that contained both GATA and SRF binding sites (134). In turn, an in vitro promoter study found that SRF interacted with myocardin to activate CArG box [CC(A/T)6GG]-containing ANF promoter (202). Consistent with this finding, myocardin has been shown to increase transcription of ANF (10, 222). Apart from GATA factors, NKX2.5 has also been found to work with other transcriptional factors to affect ANF transcription: TBX5 was found to bind and work synergistically with NKX2.5 to activate the ANF promoter through the T-box binding elements (TBEs) (76, 155).

Functional studies have found that PEX1/ZFP260, a nuclear zinc finger protein, binds to the phenylephrine response element (PERE) region of the ANF promoter (38). As the knock-down of PEX1/ZFP260 in cardiac cells has been shown to decrease endogenous ANF expression, it is likely that PEX1/ZFP260 is a transcriptional activator for ANF. The ANF promoter is also a target of PITX2 (PITX2A, PITX2B, and PITX2C isoforms), as they were found to bind the ANF promoter, although the isoforms differentially activated the ANF promoter (59). It is worth noting that only PITX2C can synergistically activate the ANF promoter in the presence of NKX2.5.

Concerning the repressors of the ANF promoter TBX2, another TBE site binding protein has been shown to inhibit ANF expression in the AV canal (68, 216). TBX2 bound to the TBE sites of the ANF promoter to act as a transcriptional repressor (68). In addition, as mentioned previously, a promoter study has shown that HRT factors inhibit ANF promoter activity (56). HRT factors interact with GATA4 and directly interfere with its DNA binding activity (56, 210). Loss of HRT factors also led to elevated ANF mRNA levels in both in vitro and in vivo studies, suggesting that HRT factors control ANF expression through their repressor activity.

Some less well-studied transcription repressor proteins have also been found to affect ANF transcription. Junmonji is a transcriptional repressor that is critical for normal heart development (93, 108). It was found to repress ANF gene expression by inhibiting the transcriptional activities of NKX2.5 and/or GATA4 (92). It is worth noting that although two Junmonji DNA-binding sites in the ANF enhancer were identified by gel mobility shift assays, these Junmonji-binding sites did not mediate ANF repression by Junmonji (92). Therefore, the effect of Junmonji on ANF expression probably does not occur through...
Differential Gene Expressions in Atrial and Ventricular Cardiomyocytes: Insights into the Potential Strategies of Increasing the Purity and the Yield of Ventricular Cardiomyocytes from ESC-CMs

Common disorders of the atria and ventricles include pacemaker disorder, arrhythmias, fibrillation (atrial and ventricular fibrillation), and myocardial infarction (see http://www.americanheart.org/). Implantation of an artificial electronic pacemaker is a commonly used treatment for pacemaker disorder. Current approaches to treat fibrillation include medications to slow down rapid heart rate, electrical cardioversion to restore normal heart rhythm with an electric shock (when medication does not improve symptoms), radiofrequency ablation to destroy tissue that triggers abnormal electrical signals or to block abnormal electrical pathways, and surgery to disrupt electrical pathways that generate fibrillation (see http://www.americanheart.org/). Patients who suffer from cardiac failure by myocardial infarction have deteriorated heart function owing to a significant loss of ventricular cardiomyocytes. As a result, a large number of mature ventricular myocytes are needed for the treatment of myocardial infarction. Therefore, while there are available and effective therapies for commonly occurring disorders of atrial cardiomyocytes, such as atrial fibrillation, loss of ventricular cardiomyocytes resulting from myocardial infarction cannot be effectively treated. Hence, there is a high demand for a source of ventricular cardiomyocytes.

Because there is no well-established cell surface marker for cardiomyocytes, to enrich ESC-CMs, several groups have employed the use of selection markers (e.g., fluorescence proteins or antibiotic resistant gene) driven under the control of cardiac-specific promoters (3, 14, 16, 52, 58, 61, 74, 82, 94, 101, 125, 135, 172, 208, 214). Klug et al. (94) were the first to demonstrate that a relatively pure population of cardiomyocytes (99%) could be selected from a heterogeneous population of mESC differentiation culture using this genetic approach. Since then, numerous studies have used different promoters with a similar strategy and showed the enrichment of ESC-CMs to different degrees, either in mESC studies (14, 16, 52, 61, 74, 94, 101, 125, 135, 172, 208) or, more recently, in hESC studies (3, 58, 82, 214). By using the information on the chamber-specific genes, a similar strategy to that described above can be used to enrich specifically atrial or ventricular cardiomyocytes. For instance, a lentiviral vector harboring the DNA

<table>
<thead>
<tr>
<th>Genes</th>
<th>Chamber in Which The Gene is Expressed</th>
<th>Functions</th>
<th>Upregulated By</th>
<th>Downregulated By</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT1</td>
<td>Atria</td>
<td>Basic helix-loop-helix transcriptional repressor; Repress GATA4/6, ANF, BMP2, TBX2; Precise formation of the AV boundary</td>
<td>Notch signaling</td>
<td></td>
</tr>
<tr>
<td>HRT2</td>
<td>Ventrices</td>
<td>Basic helix-loop-helix transcriptional repressor; Repress GATA4/6, ANF, BMP2, TBX2, CX40, TBX5, ALC-1, MLC-2a; Formation of the AV canal; Anti-hypertrophic; Maintain normal contractility of the ventricles</td>
<td>Notch signaling</td>
<td></td>
</tr>
<tr>
<td>IRX4</td>
<td>Ventrices</td>
<td>Homeobox transcription factor; Downregulate AMHC-1 (and slow MyHC3, the quail homolog of AMHC-1); Up-regulate VMHC-1; Cooperate with IRX5 to repress KCND2</td>
<td>NKX2.5, dHAND MEF2c(?)</td>
<td></td>
</tr>
<tr>
<td>MLC-2a</td>
<td>Atria</td>
<td>Form cardiac sarcomere; Maintenance of atrial contractility</td>
<td>HRT</td>
<td></td>
</tr>
<tr>
<td>MLC-2v</td>
<td>Ventrices</td>
<td>Form cardiac sarcomere; Maintenance of ventricular contractility</td>
<td>HF-1a, MEF-2, EFIA/YB-1 (together with p30), USF, SRF (together with myocardin)</td>
<td>TBX2, TBX5</td>
</tr>
<tr>
<td>CX40</td>
<td>Atria</td>
<td>Form gap junctions</td>
<td>GATA4, NKX2.5</td>
<td>Mechanical stress, hypertrophy</td>
</tr>
<tr>
<td>Sarcolipin</td>
<td>Atria</td>
<td>Regulate calcium cycling (decrease SR calcium transport by inhibiting SERCA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNE1</td>
<td>Ventricles</td>
<td>Accessory (β) subunit of the pore-forming (α) KCNQ1 which encodes the I_k</td>
<td>GATA4/6, SP1</td>
<td></td>
</tr>
<tr>
<td>ANF</td>
<td>Atria</td>
<td>Control blood pressure, renal function, salt balance</td>
<td>NKX2.5, GATA4/6, SRF, myocardin, TBX5, PEX1/ZFP260, PITX2</td>
<td>TBX2, HRT, Jumonji, HOP, FOG2, CARP/ANKRD1</td>
</tr>
</tbody>
</table>

(?) Conflicting data exist; *These information are from promoter sequence analysis. See text for details and definitions.
cassette “chamber-specific promoter-GFP” can be used for transduction of hESCs (130, 215), which can then be induced to differentiate. It is supposed that when cardiac differentiation occurs and chamber-specific myocytes appear, chamber-specific myocytes will preferentially express the GFP protein. Fluorescence-activated cell sorting can therefore be used for isolating the chamber-specific myocytes from the heterogeneous differentiation population. The atrial and ventricular myocytes enriched from this process can be used for later downstream studies, such as microarray analyses or proteomic studies, to identify the differences in gene expression profile between noncardiac cells and cardiac cells, or between myocytes from different chambers. This may eventually help identify cell surface marker(s) that are uniquely present in cardiomyocytes or chamber-specific myocytes. With the cell surface markers identified, antibody-based isolation of ESC-CMs can be carried out, as recently described (196). Further studies and future transplantation can therefore be facilitated.

Current strategies for identifying cardiomyocytes present in the ESC differentiation population are based either on their spontaneous beating phenotypes (70, 88–90, 130, 136, 182, 215) or by the selection markers (e.g., fluorescence proteins or antibiotic resistant gene) driven under the control of cardiac-specific promoters, as previously mentioned (3, 58, 82, 214). The cells obtained are mostly electrically active; that is, they spontaneously generate action potentials even without stimulation and are regarded as having the action potential profiles of “embryonic” nodal, atrial, and ventricular myocytes (18, 70, 131, 136). These cells, therefore, have a phenotype similar to that of native SA nodal cells in a mature heart. In addition, ultrastructural structural analysis and immunostaining of structural proteins reveals that some of the ESC-CMs have disorganized myofibrillar stacks, suggesting that ESC-CMs may have relatively immature contractile apparatus (89, 136, 182). Although these cells may be suitable for certain clinical applications, their electrically active phenotype and immature contractile apparatus are certainly not ideal for treating patients suffering from myocardial infarction, because these cells may overdrive the electrical activity of the whole heart and so may not couple well with the heart’s contractile activity. This asynchronized contraction could lead to arrhythmias that could be lethal. It is of critical importance for the myocardially infarcted heart, after cell replacement therapy with ESC-CMs, to be able to contract in a highly coordinated fashion. Although this asynchronized contraction problem may potentially be solved by a better electrical coupling between transplanted cells and the existing myocardium (163), it is highly desirable to find strategies to direct ESC differentiation to ventricular cardiomyocytes, which are electrically quiescent until electrically activated by propagated action potentials.

With an understanding of the natural signaling pathways that direct the multistep cardiomyogenic differentiation program, it is possible to control the sequential steps involved in cardiac differentiation (e.g., by adding defined small molecules) and therefore to “guide” the differentiation into cardiac lineage (207) or to chamber-specific cardiomyocytes. As mentioned previously, atrial and ventricular cardiomyocytes have differential expression of genes (transcription factors, structural proteins, ion channels, etc.) and are functionally distinct. A better understanding of the differential gene expressions in atrial and ventricular myocytes, their expression throughout development, and how these chamber-specific genes are regulated can provide insights into the potential strategies for directing ESCs to differentiate into chamber-specific cells or for fine-tuning the ESC-CMs into specific electrical and contractile phenotypes that resemble chamber-specific cells such as ventricular myocytes (Fig. 1). Previous studies in mouse P19 embryonic carcinoma cells (a cell type that has been shown to be capable of cardiac differentiation) showed that overexpression of cardiac-specific transcription factor(s) can direct differentiation preferentially to cardiac lineage and increase the yield of cardiomyocytes accordingly (66, 129). It will be tempting to investigate whether overexpression of a key ventricular-specific transcription factor (or a cocktail of key ventricular-specific transcription factors) and/or the knockdown of atrial-specific transcriptional factor/gene by an appropriate amount and/or at a suitable timing during cardiac differentiation, can genetically drive ventricular cardiomyocyte differentiation and thereby increase the yield of ventricular cardiomyocytes. Alternatively, knockdown or overexpression of a (or a combination of) key ion channel(s) and/or structural proteins may help to establish the ventricular electrophysiological and/or contractile profiles in other nonventricular cardiac cells (e.g., atrial myocytes) (Fig. 1).

Even if ESCs can be directed to differentiate preferentially into pure ventricular cardiomyocytes for treatment of myocardial infarction, there are still problems to be overcome for future therapies using these cells. For instance, the electrical conduction of the heart is not coupled well with the ESC-CMs, and therefore to “guide” the differentiation into cardiac lineage (207) or to chamber-specific cardiomyocytes. As mentioned previously, atrial and ventricular cardiomyocytes have differential expression of genes (transcription factors, structural proteins, ion channels, etc.) and are functionally distinct. A better understanding of the differential gene expressions in atrial and ventricular myocytes, their expression throughout development, and how these chamber-specific genes are regulated can provide insights into the potential strategies for directing ESCs to differentiate into chamber-specific cells or for fine-tuning the ESC-CMs into specific electrical and contractile phenotypes that resemble chamber-specific cells such as ventricular myocytes (Fig. 1). Previous studies in mouse P19 embryonic carcinoma cells (a cell type that has been shown to be capable of cardiac differentiation) showed that overexpression of cardiac-specific transcription factor(s) can direct differentiation preferentially to cardiac lineage and increase the yield of cardiomyocytes accordingly (66, 129). It will be tempting to investigate whether overexpression of a key ventricular-specific transcription factor (or a cocktail of key ventricular-specific transcription factors) and/or the knockdown of atrial-specific transcriptional factor/gene by an appropriate amount and/or at a suitable timing during cardiac differentiation, can genetically drive ventricular cardiomyocyte differentiation and thereby increase the yield of ventricular cardiomyocytes. Alternatively, knockdown or overexpression of a (or a combination of) key ion channel(s) and/or structural proteins may help to establish the ventricular electrophysiological and/or contractile profiles in other nonventricular cardiac cells (e.g., atrial myocytes) (Fig. 1).

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Fig. 1. Potential strategies for increasing the yield of ventricular myocytes derived from embryonic stem cells (ESCs). Spontaneous differentiation of ESCs would give rise to various cell types including cardiomyocytes. Higher yield of ventricular myocytes may be achieved by downregulation of atrial-specific transcription factor such as hairy-related transcription factor 1 (HRT1) (A), and/or overexpression of ventricular-specific transcription factors such as hairy-related transcription factor 2 (HRT2) or Iroquois homeobox gene 4 (IRX4) (B). With the help of atrial-specific promoter and ventricular-specific gene(s) or short hairpin RNA against atrial-specific genes, the electrophysiological and/or the contractile properties of atrial myocytes may become ventricular-like (C). An example is illustrated: atrial myocytes may be fine tuned to possess ventricular-like action potential by suitably altering the ion channels’ expressions.
communication between the transplanted cells and the host may need to be improved. Previous phase 2 clinical trials using skeletal myoblasts for cell replacement therapy of myocardial infarcted hearts showed that although contractile function was improved after transplantation, a significant number of patients suffered from lethal ventricular tachycardia (83, 181), probably due to the lack of connecting gap junctions between the transplanted skeletal myoblasts and the host myocardium. Overexpression of Cx43 has been employed to solve this problem (2, 123, 184). Furthermore, Cx43-encoded gap junctions were found to express in hESC-CMs (89, 130, 136) and at the interface between the hESC-CMs and host heart cells (215). However, when compared with intact human hearts, significantly slower conduction velocity was recorded within the differentiating human embryoid bodies (88). A recent study reported that the temporal expression level of Cx43 is an important determinant of human cardiac differentiation. Therefore, while the possibility of improving electrical conduction by overexpressing Cx43 remains to be tested, conditional Cx43 overexpression at specific differentiation time points may be needed for future genetically engineered hESC-derived cardiac grafts for improved electrical communication (130). A further study showed that although ESC-CMs survived, integrated, and matured after intramyocardial injection in immunodeficient mice for up to 12 wk (199), they were usually separated from the rodent myocardium by a layer of fibrotic tissue secreted by the transplanted cells themselves. The presence of fibrotic patches, together with the transplantation of immature cells and other types of cardiomyocytes, may also be risk factors for arrhythmia. Therefore, it is suggested that a tissue engineering approach, such as employing the use of a biodegradable scaffold together with a well-formulated mixture of cardiovascular cells (e.g., vascular endothelial cells together with ventricular myocytes), should be tested in the future.

ACKNOWLEDGMENTS

We thank Dr. Tian Xue for critical reading and comments on this manuscript.

GRANTS

S. Y. Ng and C. K. Wong were supported by postgraduate studentships from the Chinese University of Hong Kong (CUHK). The ESC research conducted in the authors’ lab was supported by the Competitive Earmarked Research Grant (474907) from the University Grants Committee of the Hong Kong Special Administrative Region, the Direct Grant for Research (2030371, 2030388, 2030407) from the CUHK, Lee Hysan Foundation Research Grant (474907) from the University Grants Committee of the Hong Kong Special Administrative Region, the Direct Grant for Research (2030371, 2030388, 2030407) from the CUHK, Lee Hysan Foundation Research Grant (CA11107, CA11122, CA11162), and a grant from the State Key Laboratory of Agrobiotechnology of the CUHK (1901073).

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