Journal of Cellular Biochemistry 104:119–128 (2008)

PROSPECT

Extrinsic Regulation of Cardiomyocyte Differentiation of Embryonic Stem Cells

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Abstract Cardiovascular disease is one of leading causes of death throughout the U.S. and the world. The damage of cardiomyocytes resulting from ischemic injury is irreversible and leads to the development of progressive heart failure, which is characterized by the loss of functional cardiomyocytes. Because cardiomyocytes are unable to regenerate in the adult heart, cell-based therapy of transplantation provides a potential alternative approach to replace damaged myocardial tissue and restore cardiac function. A major roadblock toward this goal is the lack of donor cells; therefore, it is urgent to identify the cardiovascular cells that are necessary for achieving cardiac muscle regeneration. Pluripotent embryonic stem (ES) cells have enormous potential as a source of therapeutic tissues, including cardiovascular cells; however, the regulatory elements mediating ES cell differentiation to cardiomyocytes are largely unknown. In this review, we will focus on extrinsic factors that play a role in regulating different stages of cardiomyocyte differentiation of ES cells. J. Cell. Biochem. 104: 119–128, 2008.

Key words: embryonic stem cells; cardiomyocyte; extrinsic factor; differentiation

Adult hearts have limited regenerative potential. Therefore, the loss of cardiomyocytes in ischemic heart disease is irreversible and results in progressive heart failure. The alternatives for treatment are limited: (i) heart transplantation is significantly hampered by inadequate numbers of donors and (ii) although several clinical trials attempted to regenerate heart muscle after a heart attack through the use of bone marrow stem cells, recent studies indicate little or no evidence of muscle regeneration from bone marrow stem cells. An alternative approach may be using embryonic stem (ES) cells as sources to generate cardiomyocyte progenitors. Transplantation of exogenous cardiomyocytes could provide functional cardiomyocytes, and therefore may be a viable therapeutic strategy to replace damaged myocardial tissue to restore cardiac function [Solloway and Harvey, 2003].

ES cells derived from the inner cell mass of the preimplantation embryo are pluripotent and capable of self-renewal. In vitro, ES cells can be cultured indefinitely, and have potential to differentiate to derivatives of all three primary germ layers. Therefore, ES cells have enormous potential as a source of therapeutic tissues, including cardiomyocytes that may be used to treat cardiovascular diseases and restore cardiac function. Because of the pluripotency, spontaneous ES cell differentiation in vitro generates multilineage cells, and only a small portion of differentiated ES cells contains contracting cardiomyocytes [Kehat et al., 2001]. A major challenge for clinical application of ES cells is to develop a differentiation protocol to generate sufficient cardiomyocytes in vitro [Sachinidis et al., 2003a]. Directing ES cells differentiation can be achieved by using extrinsic factors such as growth factors and chemicals [Schuldiner et al., 2000].

Here, we review the current status of extrinsic factors that participate in ES cells differentiation to cardiomyocytes. Among these extrinsic factors, some of them have been approved to be safe for clinical use or daily diet

Grant sponsor: National Institute of Health Grants; Grant numbers: K01DK064696, P20RR018789.

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Received 13 September 2007; Accepted 14 September 2007
DOI 10.1002/jcb.21604

supplementation. Studying the cardiomyogenesis promoted by extrinsic factors in ES cells will not only greatly benefit potential cardiomyocyte transplantation therapy, but also provide insights to enhance adult heart function by daily diet. In addition, ES cell differentiation may provide a unique system to study intrinsic signaling pathways that promote heart disease.

The heart is one of the first mesoderm-derived organs during embryonic development. Cardiac development involves several major stages, including (i) early mesodermal differentiation, (ii) generation of cardiovascular common progenitors (CVP) that have the potential to differentiate into all three of the major cell types of the heart: cardiomyocytes, smooth muscle cells, and endothelial cells [Kattman et al., 2006; Moretti et al., 2006; Wu et al., 2006], (iii) cardiac lineage commitment of cardiac progenitor (CP) cells, maturation of functional cardiomyocytes (MC), and (iv) morphogenesis of the chambered heart (Fig. 1). During cardiac development, specific genes are expressed at different stages. Many of them are transcription factors [Bruneau, 2002]. Brachyury, a T-box transcription factor, is a molecular marker of primitive mesoderm [Kubo et al., 2004; Kattman et al., 2006]. Genetic fate-mapping studies indicated that genes of isl1 (a LIM homeodomain transcription factor), Nkx2.5 (a homeobox containing transcription factor), and flk1 (VEGF receptor 2) are expressed in CVP [Moretti et al., 2006]; whereas c-kit+/Nkx2.5+ cells have bipotential to cardiomyocytes and smooth muscle cells [Wu et al., 2006]. The lineage commitment of cardiomyocyte progenitor cells is marked by the expression of GATA4 and Nkx2.5 [Lints et al., 1993; Redkar et al., 2001; Brewer et al., 2005]. The zinc finger transcription factor, GATA4, is expressed in anterior endoderm and mesoderm [Alsan and Schultheiss, 2002], and plays a role in activation of many myocardial differentiation genes [Molkentin, 2000]. Nkx2.5 is conserved and expressed in organisms that form hearts, and is often used to delineate CP cells [Redkar et al., 2001; Brewer et al., 2005]. Further differentiation of cardiomyocyte progenitor cells to mature cardiomyocytes is characterized by expression of functional sarcomeric-specific and chamber-specific cardiac genes, including α and β myosin-heavy chain (α- and β-MHC) [Metzger et al., 1995], myosin light chain-2 ventricular (MLC-2v) [Wobus et al., 1997], and ANF [Paquin et al., 2002; Small and Krieg, 2003].

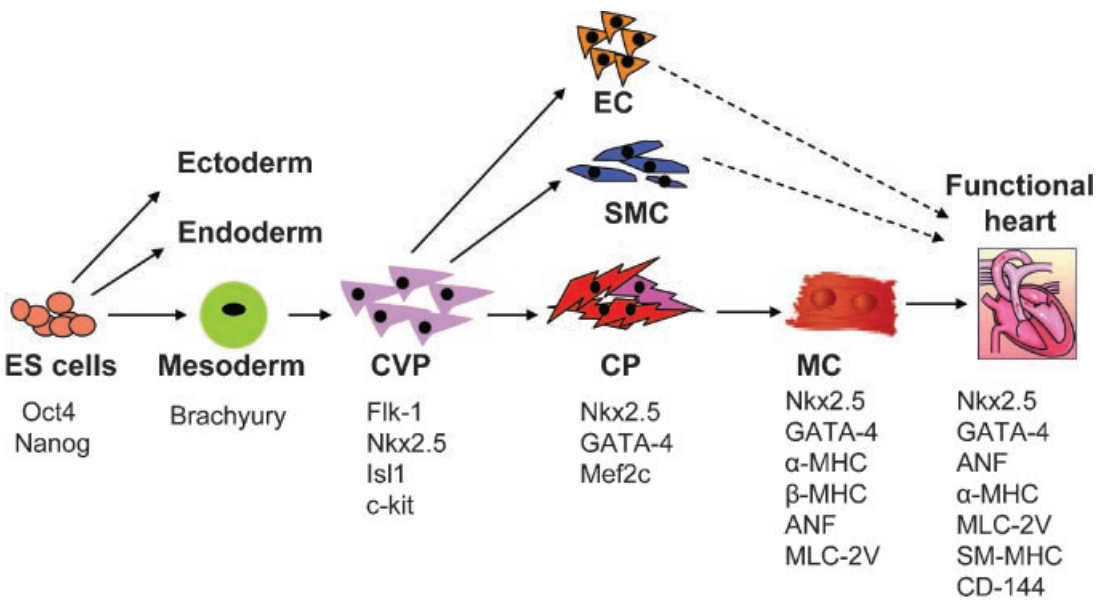

Fig. 1. Major steps during cardiomyocyte differentiation. Pluripotent ES cells differentiate to three germ layers: ectoderm, endoderm, and mesoderm. Common cardiovascular progenitors (CVP) have multipotential to give rise to endothelial cells (EC), smooth muscle cells (SMC), and cardiomyocyte progenitors (CP). The lineage committed cardiomyocyte progenitors further develop to functional mature cardiomyocytes (MC) that form contracting region in differentiated ES cells. Genes expressed in specific differentiation stages are listed.

EXTRINSIC FACTORS THAT MEDIATE ES CELL DIFFERENTIATION TO CARDIOMYOCYTES

Cell functions are often triggered by extrinsic signals in environment, resulting in intrinsic changes that affect cell proliferation, differentiation, apoptosis, and migration. We will focus on two groups of extrinsic factors: growth factors and chemical compounds.

Growth Factors

Bone morphogenetic proteins (BMPs). BMPs are members of the transforming growth factor-beta (TGF-β) super family that play a pivotal role in most morphogenetic processes during development [Ducy and Karsenty, 2000]. BMP signalings are required in mesodermal induction and cardiac differentiation [Winnier et al., 1995; Zhang and Bradley, 1996]. Application of BMP-2 or BMP-4 to explants of cardiac region or non-cardiac regions of chick embryos induces expression of early cardiac markers, such as GATA-4 and Nkx2.5, and promotes the cardiomyocyte beating phenotype. In addition, inhibition of BMP signaling blocks expression of Nkx2.5 and cardiac differentiation [Schultheiss et al., 1997; Andree et al., 1998; Ladd et al., 1998; Yamada et al., 2000]. BMP antagonists, including noggin and chordin, truncated versions of type I (tALK3) and type II (tBMPRII) BMP receptors, and Smad6 inhibitor, inhibit cardiac differentiation [Galvin et al., 2000; Nakajima et al., 2002; Tzahor et al., 2003]. However, transient inhibition of BMP signaling prior to mesoderm development by noggin induces cardiomyocyte differentiation in mouse ES cells [Yuasa et al., 2005]. TGF-β1 is a positive factor during cardiogenesis. Behfar et al. [2002] reported that priming of mouse ES cells with TGF-β1 and BMP-2 enhanced cardiomyocyte differentiation, resulting in increased contractile regions within embryoid bodies together with increased myofibrillogenesis. Combination of activin and BMP-4 also increases cardiomyocyte differentiation from human ES cells [Laflamme et al., 2007].

The effect of BMPs on cardiomyocyte differentiation from ES cells depends on culture medium. In serum-free or low serum medium, the addition of BMP-2 and BMP-4 enhances cardiomyocyte differentiation of Cynomolgus Monkey ES cells and human ES cells, whereas BMP-4 decreases cardiomyocyte differentiation of cynomolgus monkey ES cells in FBS-containing medium [Hosseinkhani et al., 2007; Laflamme et al., 2007; Pal and Khanna, 2007]. In addition to cardiac differentiation, BMP signaling may also be essential for migration and/or fusion of the heart primordia [Walters et al., 2001]. Taken together, BMPs are essential for at least two steps in the cardiomyocyte induction process: mesodermal induction and cardiomyocyte differentiation.

Wnts. Wnts are secreted cysteine-rich glycoproteins that regulate many key developmental processes in Drosophila (Wingless, homologues to Wnts) and vertebrates, including mediation of cell–cell communication in various developmental, morphogenesis, cell fate determination, cell growth, and survival processes [Dale, 1998].

The role of Wnt signaling during cardiogenesis is dependent on the developmental stages and model system. The canonical Wnt pathway (Wnt 1, 3, 3a), which uses β-catenin as a downstream molecule, inhibits cardiomyocyte differentiation in cardiac mesoderm [Marvin et al., 2001; Schneider and Mercola, 2001; Tzahor and Lassar, 2001]. Activation of Wnt/β-catenin signaling before gastrulation promotes cardiac differentiation, but inhibits heart formation during gastrulation [Ueno et al., 2007]. Wnt/β-catenin signaling is activated at the inception of mammalian cardiac myogenesis, and is indispensable for cardiac differentiation in P19 embryonic cells [Nakamura et al., 2003]. These studies suggest that Wnt/β-catenin signaling play a biphasic role in cardiomyocyte differentiation: activation is required to commit mesenchymal cells to the cardiac lineage; downregulation of β-catenin is needed for cardiomyocyte differentiation at later stages. Activation of Wnt/β-catenin during early EB formation enhances mouse ES cell differentiation into cardiomyocytes and suppresses the differentiation into hematopoietic and vascular cell lineages [Naito et al., 2006]. It will be important to test whether the addition of wnt antagonists and agonists at different time points can direct cardiomyocyte differentiation during ES cell differentiation.

Fibroblast growth factors (FGFs). The FGFs and FGF receptors (FGFRs) have been implicated in a variety of physiological and pathological conditions, including mesodermal development, tissue growth and remodeling, inflammation, tumor growth, and vascularization [Xu et al., 1999; Powers et al., 2000]. During development, commitment to a mesodermal

cardiac fate results from inductive interactions with adjacent endoderm during gastrulation [Sugi and Lough, 1994; Nascone and Mercola, 1995; Schultheiss et al., 1995]. An example is, FGF-2 secreted by endoderm promote cardiac myogenesis [Sugi and Lough, 1995]. In addition, BMP and FGF-specific pathways interact to specify the cardiac lineage [Lough et al., 1996; Ladd et al., 1998]. Inactivation of FGFR-1 in mice dramatically affects the expression of several cardiac transcription factors with a consequent impairment on the expression of structural myocardial genes and contractile foci formation [Dell'Era et al., 2003]. The addition of FGF-2 during ES cell differentiation upregulates Nkx2.5 expression in Mef2c+ cardiogenic mesodermal cells, suggesting that Nkx2.5 may represent one of the earliest direct FGF/FGFR targets during heart development [Dell'Era et al., 2003]. Combination of FGF-2 and BMP-2 substantially enhances cardiogenic activities during mouse ES cell differentiation [Kawai et al., 2004]. It is unclear whether FGF-2 promotes human ES cell differentiation to cardiomyocytes.

Hepatocyte growth factor (HGF). HGF is a potent mesodermal derived mitogen. During embryogenesis, HGF plays important roles in cell differentiation, proliferation, migration, and survival. In addition, after gastrulation, HGF is involved in several morphogenetic processes, including epithelial–mesenchymal interactions for liver differentiation [Weidner et al., 1993], dermis, and kidney [Sonnenberg et al., 1993; Santos et al., 1994]. HGF and its receptor, the proto-oncogene c-met, are expressed in immature and mature cardiomyocytes during cardiogenesis, suggesting that HGF is implicated in cardiac development [Rappolee et al., 1996]. Moreover, mesenchymal stem cells overexpressing HGF improve the function of infarcted myocardium by restoring local vascularization and regeneration of cardiomyocytes [Duan et al., 2003].

In mouse ES cells, HGF significantly and specifically enhances cardiomyocyte differentiation by increasing the number of beating EBs, and upregulating expression of the cardiac markers, including Nkx2.5, GATA 4, α-MHC, β-MHC, ANF, MLC2v, and Troponin T [Roggia et al., 2007]. The HGF-induced cardiomyocyte differentiation may involve the PI3 kinase/Akt pathway [Roggia et al., 2007].

Erythropoietin (EPO). EPO is a growth factor that promotes proliferation and differentiation of erythrocytes and megakaryocytes [Wu et al., 1999]. Studies of mice lacking EPO and EPO receptor (EPOR) demonstrate that EPO −/− and EPOR−/− animals suffer from ventricular hyperplasia and defects in the interventricular septum, suggesting that EPO signaling is also important in heart development [Wu et al., 1999]. Whether EPO promotes ES cell differentiation to cardiomyocytes in vitro is unknown.

Oxytocin (OT). OT, a nonapeptide largely expressed in the hypothalamus, has long been recognized as a female reproductive hormone that is necessary for uterine contraction during parturition, timing and amplification of labor, milk ejection during lactation, and ovulation [Gimpl and Fahrenholz, 2001]. In addition to its role of OT in reproduction, OT mediates heart development [Jankowski et al., 2000; Paquin et al., 2002; Jankowski et al., 2004]. OT and OT receptor are expressed in the developing heart [Paquin et al., 2002; Jankowski et al., 2004]. Administration of OT to the fetus impairs cardiac growth in humans and rats, and suppression of OT receptor by specific OT antagonists in the early stage of chicken egg development leads to cardiac malformation in the embryos [Chard et al., 1970; Schriefer et al., 1982]. In vitro, the addition of OT to P19 embryonic cells stimulates the production of beating cardiomyocyte colonies, and addition of OT antagonists completely inhibits the formation of cardiomyocytes [Paquin et al., 2002]. OT induced-cardiogenesis depends on nitric oxide (NO) [Danalache et al., 2007]. The promoting effect of OT in P19 cells is abolished by NO inhibitors, including N,G-nitro-L-argininemethyl- ester, 1,400 W, and ODQ [Danalache et al., 2007]. The stimulating effect of OT on cardiomyocyte differentiation was also demonstrated in mouse ES cells [Hatami et al., 2007].

Other growth factors. Besides above mentioned growth factors, other growth factors, such as insulin-like growth factors (IGFs), and platelet-derived growth factor-BB (PDGF-BB), also play positive roles in cardiogenesis. Antin et al. [1996] demonstrated that insulin and IGF-II promote cardiac development in vivo by both autocrine and paracrine mechanisms. Sachinidis et al. [2003b] reported that PDGF-BB is a potent factor that promotes

cardiogenesis in ES cells under serum-free conditions.

Chemical Compounds and Others

In addition to growth factors, a number of chemical compounds promote cardiomyocyte differentiation in vitro. Compared to growth factors, chemicals tend to be stable with a longer half-life, which is helpful for extending the time of in vitro cell culture over a longer period. Moreover, unlike proteins that have to be synthesized in living organisms and subjected to complex posttranslational modifications (i.e., glycosylation, conformational folding) for their activities, chemical compounds usually cost less, and are therefore affordable for long-term culture in vitro.

Dimethyl sulfoxide (DMSO). DMSO is a commonly used cryoprotectant that also induces cardiomyogenic differentiation in ES cells [Ventura and Maioli, 2000], as well as embryonal carcinoma cells [McBurney et al., 1982; Skerjanc et al., 1998]. DMSO induces the expression of cardiac specific transcription factors GATA-4 and Nkx2.5, and increases intracellular Ca^{2+} levels [McBurney, 1993; Morley and Whitfield, 1993; Skerjanc et al., 1998; Wilton and Skerjanc, 1999; Ventura and Maioli, 2000].

The molecular mechanism of DMSO-promoting cardiogenic activities is not well understood. DMSO treatment activates both the canonical Wnt pathway and the PI3K pathway independently [Naito et al., 2005]. OT and OT receptor are involved in DMSO-induced cardiogenesis [Paquin et al., 2002]. However, a study of human ES cells indicated that cardiomyocyte differentiation in human ES cells was not affected by DMSO [Xu et al., 2002].

Opioid. There are four broad classes of opioids: (i) endogenous opioid peptides, produced in the body; (ii) opium alkaloids, such as morphine (the prototypical opioid) and codeine; (iii) semisynthetic opioids such as heroin and oxycodone; and (iv) fully synthetic opioids, such as pethidine and methadone that have structures unrelated to the opium alkaloids. Adult cardiac myocytes express the prodynorphin gene that synthesizes secreted dynorphin B, a biologically active end product of k-opioid. P19 cells and murine ES cells also synthesize and secrete dynorphin B. Cardiac differentiation of mouse ES cells is associated with the opioid receptor ligand and complex subcellular redistribution of selected protein kinase C (PKC) isozymes, including PKC-α,-β1, -β2, -δ, -ε, and -ζ [Ventura et al., 2003]. PKC inhibitors prevent the expression of cardiogenic genes and dynorphin B in ES cells and abolish their development into beating cardiomyocytes [Ventura et al., 2003]. It is unclear whether other classes of opioids mediate cardiomyocyte differentiation.

Retinoic acid (RA). RA, the active derivative of vitamin A, by acting through retinoid receptors, is involved in signal transduction pathways regulating embryonic development, tissue homeostasis, and cellular differentiation and proliferation. The essential role of RA during early cardiovascular morphogenesis has been demonstrated in targeted gene deletion of RA receptors and in the vitamin A-deficient avian embryo [Pan and Baker, 2007].

RA has two subtypes: all-trans RA (ATRA) and 9-cis RA (9c-RA). Both of them increase the number of cardiomyocytes during mouse ES cell differentiation in the presence of serum [Wobus et al., 1991; Wobus et al., 1997]. The addition of RA to ES cell culture results in an increased levels of α-cardiac MHC and MLC-2v mRNA in early, but not in terminal developmental stages, which indicated that the RA-induced accelerated expression of cardiac-specific genes results in an enhanced development of ventricular cardiomyocytes [Wobus et al., 1997]. RXR agonist also enhances ES cell differentiation into cardiomyocytes in serum-free conditions [Honda et al., 2005].

RA receptors comprise two subfamilies composed of three RA receptors (RAR-a, b, and c) and three retinoid X receptors (RXR-a, b, and c) [Kastner et al., 1997]. Null mutation of the mouse RXR-a gene is lethal due to myocardial malformation [Kastner et al., 1994; Gruber et al., 1996].

Paquin et al. [2002] found that RA upregulated OT expression in the fetal heart, which is related to the OT—OT receptor system in P19 cell line. The OT gene promotor contains RA regulatory elements that respond to RA treatments in vivo and ex vivo in cultured cells [Richard and Zingg, 1991; Larcher et al., 1995]. These results suggest that OT is downstream of RA signaling during cardiac differentiation. However, cardiomyocyte differentiation in human ES cells is not affected by RA [Xu et al., 2002].

Clinical drug-related chemicals: verapamil, cyclosporine, and 5-Azacytidine. Clinical drugs have distinct advantages: they are tested in the preclinical experiments, and are considered suitable for patients. Sachinidis et al. [2006] reported that the L-type of Ca^{2+} channel blocker, verapamil, and an inhibitor of the protein phosphatase 2B, cyclosporin, have the most striking pro-cardiomyogenic effects in mouse ES cells. Both cyclosporin and verapamil specifically enhance the expression of early cardiac markers, Nkx2.5 and GATA4, as well as the mature cardiac marker, α-MHC; whereas the differentiation of smooth muscle, endothelial and neuronal lineages is not affected [Sachinidis et al., 2006]. In contrast, an adenylate cyclase stimulator, forskolin, inhibits cardiomyocyte differentiation in mouse ES cells [Sachinidis et al., 2006].

5-Azacytidine is a synthetic nucleoside drug that is commonly used as an inhibitor of DNA methylation to treat cancer patients. It is a potent inducer of cardiomyogenic differentiation in both embryonic [Xu et al., 2002] and adult stem cells, in particular bone marrow-derived mesenchymal stem cells [Heng et al., 2004]. For example, the cardiomyocyte differentiation of human ES cells is enhanced by treatment of 5-aza-2′-deoxycytidine (5-aza-dC) [Xu et al., 2002].

These studies indicate that when above drugs apply to patients, their side effect on heart function should be considered. Thus far, verapamil has been approved to treat arrhythmia and hypertension in clinic.

Vitamin C (ascorbic acid). Vitamin C, also known as ascorbic acid, was discovered in the late 1920s. We all know that fresh fruit and vegetables contain relatively high amounts of Vitamin C, and are good to health. Richard T. Lee and his group screened 880 compounds approved for human use and found that ascorbic acid enhanced ES cell differentiation into cardiomyocytes [Takahashi et al., 2003]. Ascorbic acid is often attributed to its antioxidative properties. However, other antioxidative agents, including NAC, Tiron, and vitamin E, do not have a similar effect on cardiomyocyte differentiation, suggesting that the stimulating effect of ascorbic acid on cardiomyocytes is independent of its antioxidative activity, or that the antioxidative effect is insufficient to induce cardiac differentiation of ES cells. The molecular mechanism of ascorbic acid mediating cardiomyocyte differentiation is still unknown.

Since vitamin C is routine nutriment used for heart injured patients, the potential of vitamin C to promote cardiac stem cells and improve their function in the adult heart [Hughes, 2002] should be worthy for further investigation in stem-cell graft therapy.

Free radicals and reactive oxygen species (ROS). An antioxidative agent often efficiently scavenges toxic free radicals and other ROS formed in cell metabolism. However, ROS may regulate cardiogenesis as a positive factor [Sachinidis et al., 2003a]. When cardiac cells are stimulated by cytokines [Sauer et al., 2004], growth factors, hormones, even mechanical stress [Schmelter et al., 2006], they elicit a small oxidative burst and generate low concentrations of ROS. ROS are important intracellular messengers during cardiogenesis, cell growth, and differentiation [Sauer et al., 2000]. For example, treatment of ES cells with epinephrine, endothelin, or Cardiotrophin-1 significantly stimulates cardiomyogenesis, and ROS signaling pathways confer hypertrophic cell growth [Sachinidis et al., 2003a; Sauer et al., 2004]. During myocardial infarction, cardiac cells generate large amounts of free radicals and ROS, which are involved in the signaling and activation of the intrinsic repair mechanisms of the damaged myocardium [Sorescu and Griendling, 2002; Heng et al., 2004]. In addition, cardiomyogenic differentiation of ES cells is enhanced in the presence of exogenous hydrogen peroxide and menadione [Sauer et al., 1999, 2000]. Conversely, incubation with free radical scavengers trolox, pyrrolidinedithiocarbamate, and N-acetylcysteine inhibit cardiomyogenic differentiation [Sauer et al., 2000]. An NADPH oxidase-like enzyme is involved in the ROS-related cardiogenesis during ES cell differentiation [Sauer et al., 2000].

The free radical NO has also been implicated in cardiomyogenesis [Bloch et al., 1999]. During embryonic development, expressions of two different isoforms of NO synthase (iNOS and eNOS) are detected in atrial and ventricular cardiomyocytes. NOS-inhibitors lead to a pronounced delay of the differentiation of ES cell-derived cardiac precursors.

Further research needs to be carried out to understand the role of free radicals and ROS in regulating the stage-specific cardiac differentiation, and to examine whether exogenous free radicals and ROS are capable of directing

stem cell differentiation into the cardiomyogenic lineage in vitro and in vivo.

Other Cardiogenesis-Related Pathway

As we mentioned, several pathways are involved in cardiogenesis based on in vivo and in vitro studies. In addition, Notch pathway plays an important role in cardiomyocyte differentiation. Notch signaling mediates numerous cell fate decisions during the development of vertebrates and invertebrates, including roles in repression of differentiation and allocation of alternative cell fates [Artavanis-Tsakonas et al., 1999].

Notch signaling plays an important role in the cellularization and epithelial-to-mesenchymal transition of the heart during cardiac development [Timmerman et al., 2004]. Mice, in which the canonical Notch pathway is inhibited, die during embryonic life, in part, due to heart defects [Oka et al., 1995]. Rones et al. [2000] found that Notch-1 and its ligand Serrate have been implicated in the progressive loss of cardiac potency in the Xenopus heart field. Schroeder et al. [2003] found that RBP-J, a key downstream element in the signaling pathway of all four mammalian Notch receptors, alters mesodermal cell fate decisions by suppressing cardiomyogenesis. Activation of Notch1 signaling in cardiogenic mesoderm induces abnormal heart morphogenesis in mice [Watanabe et al., 2006]. These results suggest that Notch signaling and cardiomyocyte differentiation are mutually exclusive.

Notch signaling mediated by the Notch1 receptor is responsible for suppressing cardiogenesis in ES cells [Nemir et al., 2006]. Absence of Notch1 results in increased and/or earlier onset of expression of cardiac specific markers, Nkx2.5, GATA-4, MLC-2a, β-MHC, and α-MHC. Through analysis of the mesodermal markers, brachyury and FGF-8, during an early step in the ES cell differentiation process, Notch pathways were found to inhibit commitment toward the mesodermal lineage, suggesting that Notch signaling could inhibit cardiogenesis. This inhibition may be caused by either blocking mesodermal differentiation or by inhibiting a mesodermal progenitor from adopting its final cardiomyocyte fate [Nemir et al., 2006].

A recent study demonstrated that ventricular Notch1 activity is essential for ventricular chamber development [Grego-Bessa et al., 2007], suggesting that the Notch signaling pathway may play a biphasic role during cardiogenesis: inhibition of early cardiomyocyte differentiation, and promotion of ventricular chamber morphogenesis.

SUMMARY

Despite the large number of studies on the cardiomyogenic differentiation of stem cells in vitro, this area of research is still in its relative infancy. The development of efficient protocols to direct the cardiomyocyte differentiation from ES cells in vitro will not only provide cell sources for cell-based therapy, it will also provide a useful model for molecular studies and genetic manipulation. The combination of various extrinsic factors to direct and control the cardiomyogenic differentiation at different developmental stages should be beneficial for stem cell transplantation therapy to repair damaged myocardium.

ACKNOWLEDGMENTS

We acknowledge our colleagues in the field for their many outstanding studies.

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