Stem Cell Reports
Resource
ISSCR
OPEN ACCESS

Non-genetic Purification of Ventricular Cardiomyocytes from Differentiating Embryonic Stem Cells through Molecular Beacons Targeting IRX-4

Kiwon Ban,[1,6] Brian Wile,[2,6] Kyu-Won Cho,[1,6] Sangsung Kim,[1] Ming-Ke Song,[3] Sang Yoon Kim,[1] Jason Singer,[4] Anum Syed,[2] Shan Ping Yu,[3] Mary Wagner,[4] Gang Bao,[2,*] and Young-sup Yoon[1,5,*]

[1]Department of Medicine, Division of Cardiology, Emory University School of Medicine, Atlanta, GA 30322, USA
[2]Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30322, USA
[3]Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA 30322, USA
[4]Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA 30322, USA
[5]Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul 120-752, Korea
[6]Co-first author
*Correspondence: gang.bao@rice.edu (G.B.), yyoon5@emory.edu (Y.-s.Y.)
http://dx.doi.org/10.1016/j.stemcr.2015.10.021
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

SUMMARY

Isolation of ventricular cardiomyocytes (vCMs) has been challenging due to the lack of specific surface markers. Here we show that vCMs can be purified from differentiating mouse embryonic stem cells (mESCs) using molecular beacons (MBs) targeting specific intracellular mRNAs. We designed MBs (IRX4 MBs) to target mRNA encoding Iroquois homeobox protein 4 (*Irx4*), a transcription factor specific for vCMs. To purify mESC vCMs, IRX4 MBs were delivered into cardiomyogenically differentiating mESCs, and IRX4 MBs-positive cells were FACS-sorted. We found that, of the cells isolated, ~98% displayed vCM-like action potentials by electrophysiological analyses. These MB-purified vCMs continuously maintained their CM characteristics as verified by spontaneous beating, Ca^{2+} transient, and expression of vCM-specific proteins. Our study shows the feasibility of isolating pure vCMs via cell sorting without modifying host genes. The homogeneous and functional ventricular CMs generated via the MB-based method can be useful for disease investigation, drug discovery, and cell-based therapies.

INTRODUCTION

Heart failure is the leading cause of death worldwide; however, current therapies such as surgical interventions are capable only of delaying the progression of this devastating disease (Go et al., 2013). In particular, patients suffering from myocardial infarction (MI), a major cause of heart failure, have cardiac dysfunction due to significant loss of cardiomyocytes (CMs) (Laflamme and Murry, 2011). The adult mammalian heart has very limited ability to regenerate after such a loss.

Due to their self-renewal and multi-lineage differentiation capacity, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), collectively called pluripotent stem cells (PSCs), have emerged as a highly promising and renewable source for generating CMs (Kehat et al., 2001; Laflamme et al., 2007; Yang et al., 2008; Zwi et al., 2009). Studies have shown that cell cultures directed toward differentiation into CMs include three types of CMs, nodal, atrial, and ventricular CMs, in varying ratios as well as other lineage cells (Huber et al., 2007; Lian et al., 2012; Shiba et al., 2012; Tohyama et al., 2013). Each type of cardiac-chamber-specific CM has unique functional, structural, and electrophysiological characteristics (Ng et al., 2010). Thus, transplantation of cardiomyogenically differentiated cells, which include heterogeneous CMs and other lineage cells, into injured myocardium might induce dysrhythmia, asynchronous cardiac contraction, or aberrant tissue formation (Liao et al., 2010). Since ventricular CMs are the most extensively affected cell type in MI and the major source for generating cardiac contractile forces, there has been great interest in producing ventricular CMs from stem cells for treatment of MI (Bizy et al., 2013; Lee et al., 2012; Müller et al., 2000; Zhang et al., 2011). It would therefore be ideal to generate a pure population of ventricular CMs from PSCs for cardiac-cell-based therapies.

Despite the unmet medical need, to date, no studies have demonstrated the feasibility of isolating ventricular CMs without permanently altering their genome. Prior studies used genetic modification for isolating ventricular CMs by inserting a fluorescent reporter gene driven by the MYL2 (or MLC-2v) promoter into mouse ESCs and embryonic carcinoma cell lines (Bizy et al., 2013; Lee et al., 2012; Müller et al., 2000; Zhang et al., 2011). Such genetic modification precludes clinical use of the isolated cells due to concerns of tumorigenicity or adverse reactions. These ventricular CMs would not be appropriate for drug development or disease modeling due to the random and permanent changes in the genome or the use of viral vectors. Further, there are no known surface markers specific for ventricular CMs, disallowing antibody-based cell sorting with flow cytometry, which is the most common method for isolating targeted cells from differentiating PSCs.

CrossMark

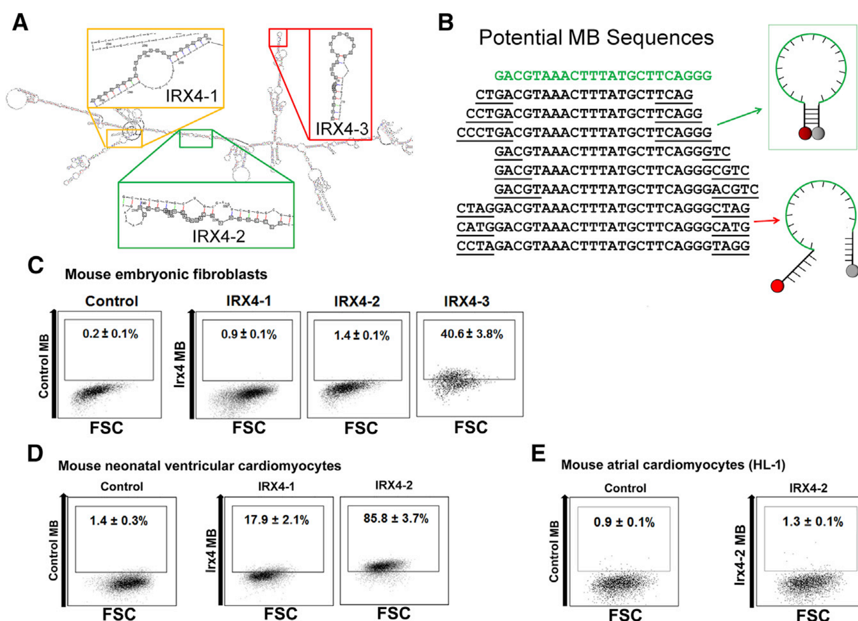

Figure 1. Selection of Optimal Ventricular Cardiomyocyte-Specific IRX4 Molecular Beacons

(A) *Irx4* mRNA structure was predicted using the RNAfold web server. Three unique target sequences were identified in *Irx4* mRNA that maximized the number of predicted unpaired bases as well as the binding affinity of a complementary probe.

(B) Stem sequences were appended to the complementary sequence and evaluated using QUIKFOLD to minimize the free energy that causes the oligonucleotide to assume a hairpin structure in solution.

(C–E) Flow cytometry results after delivering various IRX4 MBs designed to identify Irx4 mRNAs, or control MB, into mouse embryonic fibroblasts (C), eonatal mouse ventricular CMs (D), and HL-1 CMs (E). The number in each panel represents the percentage of fluorescent cells. FSC indicates forward scatter. All experiments were performed on three independent biological replicates (C–E).

Although not surface markers, several genes are known to be specifically expressed in ventricular hearts or CMs. As a ventricular-specific transcription factor, Iroquois homeobox protein 4 (IRX4) has been reported to be exclusively expressed in the ventricular myocardium while absent from both atria and the outflow tract (Bao et al., 1999). IRX4 positively regulates ventricular-chamber-specific gene expression by activating the ventricular myosin heavy chain-1 (VMHC1) gene while suppressing the expression of atrial myosin heavy chain-1 (AMHC1) (Bruneau et al., 2000; Wang et al., 2001). As a structural protein, MYL2 (or MLC-2v), one of the essential MLC-2 isoforms that is important for the contractile function of ventricular CMs, is expressed in ventricular CMs (Marionneau et al., 2005; O'Brien et al., 1993). MYL2 expression is mostly restricted to the ventricular segment of the heart with minimal expression in the outflow track during cardiogenesis (Kubalak et al., 1994; O'Brien et al., 1993).

Accordingly, we have developed a method targeting an intracellular gene to purify ventricular CMs. We used a molecular beacon (MB)-based method for isolating a pure population of ventricular CMs by targeting the mRNA of the ventricular-specific transcription factor IRX4 (Figures 1A and 1B). MBs are 20- to 30-bp oligonucleotide probes with a fluorophore and a quencher at the 5′ and 3′ ends, respectively (Figures 1A and 1B) (Heyduk and Heyduk, 2002). They are designed to form a stem-loop (hairpin) structure so that the fluorophore and quencher are within close proximity and fluorescence is quenched. Hybridization of the MBs with the target mRNA opens the hairpin structure and physically separates the fluorophore from the quencher, allowing a fluorescence signal to be emitted upon excitation (Tsourkas et al., 2002). It has been demonstrated that cellular delivery of MBs does not alter the expression level of the target genes (Rhee and Bao, 2009; Rhee et al., 2008; Santangelo et al., 2006; Tsourkas et al., 2002), and MBs can be used to isolate mESCs by directly targeting specific intracellular mRNAs such as Oct4 (Rhee and Bao, 2009). Further, we demonstrated that MBs enable the enrichment of general CMs from differentiating mouse and human PSCs (Ban et al., 2013).

In the present study, we developed a sophisticated approach using MBs targeting transcription factor mRNAs, which, due to their low copy numbers compared to structural protein mRNAs, are highly challenging and were not previously attempted. By designing specific MBs targeting *Irx4* mRNA, we show here that functional ventricular CMs derived from differentiating mouse ESCs could be isolated with high purity. The MB-based cell isolation method is quite versatile; a wide range of specific intracellular mRNAs could be targeted to achieve high specificity, including mRNAs encoding structural proteins and transcription factors.

RESULTS

Ventricular Cardiomyocyte-Specific Gene Selection

Through an extensive literature search, we selected Irx4 as a target gene for generating ventricular CM-specific MBs (Bao

Download English Version:

https://daneshyari.com/en/article/2093721

Download Persian Version:

https://daneshyari.com/article/2093721

Daneshyari.com