Vascular Pharmacology 60 (2014) 8–16

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Simvastatin-enhanced expression of promyogenic nuclear factors and cardiomyogenesis of murine embryonic stem cells

ChenMin Yang [a,c,e], Rosalinda Madonna [a,e], Yangxin Li [a,e], Qi Zhang [b], Wei-Feng Shen [b], Katharine McNamara [a,e], Yue-Jin Yang [d], Yong-Jian Geng [a,e,*]

[a] The Center for Cardiovascular Biology and Atherosclerosis Research, The University of Texas Medical School at Houston, Houston, TX USA
[b] The Department of Cardiovascular Medicine, Ruijin Hospital, Jiao-Tong University Medical School, Shanghai, China
[c] The Department of Obstetrics and Gynecology, Ruijin Hospital, Jiao-Tong University Medical School, Shanghai, China
[d] FuWai Cardiovascular Hospital, Chinese Academy of Medical Sciences, Beijing, China
[e] Texas Heart Institute, Houston, TX, USA

ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form 30 September 2013
Accepted 28 October 2013

Keywords:
Statin
Myogenesis
Transcription
Cholesterol synthesis
Stem cells

ABSTRACT

A combination of statin and stem cell therapies has been shown to benefit in experimental models of myocardial infarction. This study tests whether treatment with simvastatin has a direct impact on the cardiomyogenic development of murine embryonic stem cells (ESCs) in embryoid bodies. In a concentration-dependent manner, simvastatin treatment enhanced expression of several promyogenic nuclear transcription factors, including GATA4, Nkx2.5, DTEF-1 and myocardin A. The statin-treated cells also displayed higher levels of cardiac proteins, including myosin, α-actinin, Ryanodine receptor-2, and atrial natriuretic peptide, and they developed synchronized contraction. The statin's promyogenic effect was partially diminished by the addition of the two isoprenoids FPP and GGPP, which are intermediates of cholesterol synthesis. Thus, simvastatin treatment enhances ESC myogenesis during early development perhaps via a mechanism inhibiting the mevalonate-FPP/GGPP pathway.

1. Introduction

Replacing damaged or dead cells with stem cells has been a major topic in cardiovascular regenerative medicine, especially those with increased atherosclerotic stress and apoptosis [13]. Although not yet used clinically, embryonic stem cells (ESCs) remain as the primary cell type with the strongest potency (pluripotency) of growth and differentiation than other types of stem cells, including those from adult tissues, such as the bone marrow. Generation of cardiac myogenic cells from ESCs may offer a potential avenue to obtain clinically applicable stem cells for the treatment of various cardiovascular diseases. The capacity of ESC self-renewal and differentiation towards the cardiac lineage makes the cells suitable as an in vitro model to study cardiovascular myogenesis and as a potential source of drug screening [29,48,50].

Cardiomyogenesis represents one of the most fascinating events during early development. This event is driven by a group of promyogenic factors that act as molecular switches to activate or repress specific gene expression programs [34]. The key components that guides ESCs to differentiate into cardiac myogenic cell lineages comprise a cluster of nuclear transcription or transcription-related factors, such as GATA4 [1], myocardin-A [27], Nkx2.5 [24,45], DTEF-1 [2], and Mef2 [5]. These factors are usually expressed at high levels in developing hearts and blood vessels, and they act collaboratively to promote myogenic development. For instance, GATA4 synergizes with myocardin-A and Nkx2.5 to enhance myogenic transcription as many promoters of myogenic genes contain the binding sites for GATA4, myocardin-A and Nkx2.5 [45]. ESCs with a heightened activity of myogenic transcription offer an interesting model of research for determining how the myogenic process is activated or not activated. Also, they can be used as a disease model to test risk factors for cardiovascular disorders. For instance, GATA4-null embryos display heart defects characterized by disrupted looping morphogenesis, and hypoplastic development of ventricular myocardium [43].

Pharmacological interventions offer a useful tool for manipulating myogenesis. For example, 5-azacytidine can induce promyogenic gene expression and myogenesis from bone marrow cells [9,10]. In a matter dependent on its target gene, myogenic transcription factors may interact with other nuclear factors in the regulation of myogenesis [1,14]. In ESCs stressed with reactive oxygen species, there is an induction of the cardiac-specific genes encoding α-actin, β-MHC, MLC2a, MLC2v and ANP as well as several transcription factors, such as GATA4, Nkx2.5, MEF2C, and DTEF-1[5].

Abbreviations: ESCs, embryonic stem cells; EBs, embryoid bodies; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; MHC, myosin heavy chain; MLC, myosin light chain; ANP, atrium natriuretic peptide.

* Corresponding author at: The Center for Cardiovascular Biology and Atherosclerosis Research, Department of Internal Medicine, University of Texas Medical School at Houston, 6431 Fannin Street, MSB 1.246, Houston, Texas 77030, USA. Tel.: +1 713 500 6569; fax: +1 713 500 0637.
E-mail address: yong-jian.geng@uth.tmc.edu (Y.-J. Geng).

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http://dx.doi.org/10.1016/j.vph.2013.10.004

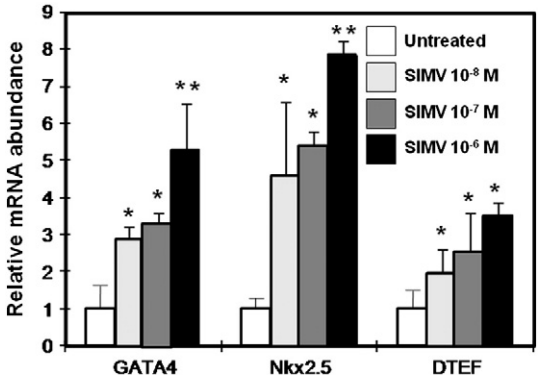

Fig. 1. Expression of GATA4, Nkx2.5 and DTEF-1 mRNAs in murine ESC-derived EBs after treatment with or without simvastatin. Quantitation of GATA4, Nkx2.5 and DTEF-1 mRNAs was conducted by real time PCR in total RNA extracted from murine ESC-derived EBs after treatment with or without simvastatin at different concentrations for 5 days. Data represent relative abundance of genes in the statin-treated vs untreated cells (means ± S.D. of four separate experiments). * $P < 0.05$; ** $P < 0.01$.

Recently, we and others have shown that the cholesterol lowering drug statins can improve the ability of cardiovascular stem cells to resist inflammation [25] and repair and regenerate the heart damaged by ischemia or infarction [22,23,38,47]. Statins selectively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme catalyzing the conversion of HMG-CoA to mevalonic acid and thereby attenuate endogenous biosynthesis of cholesterol. In addition to the control of cholesterol production, statins may regulate other biological activities important for cardiovascular myogenic development from stem cells [4,38]. Our work [25,46] has documented the beneficial effects of simvastatin treatment on post-infarct cardiovascular tissue repair and regeneration. Treatment with statins has been shown to inhibit cellular apoptosis and increase proliferation of progenitor cells [47]. Treatment with statins may confer cardioprotection and reduce myocyte apoptosis by inhibiting expression and function of the nuclear factor NFκB in myocyte progenitors and blocking high output of nitric oxide production induced by inflammatory cytokines [25].

The current study tested the hypothesis that simvastatin treatment promotes cardiovascular myogenic development of undifferentiated embryonic stem cells (ESCs) by inducing expression of promyogenic nuclear factors. Our data illustrate that simvastatin enhances expression of key promyogenic transcription factors and promotes cardiac myogenesis of murine ESCs.

2. Material and methods

2.1. Cell culture and statin treatment

The murine ESC line CCE was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Murine ESCs were cultured without feeder cells in Iscove's medium (Gibco, Live Technologies,

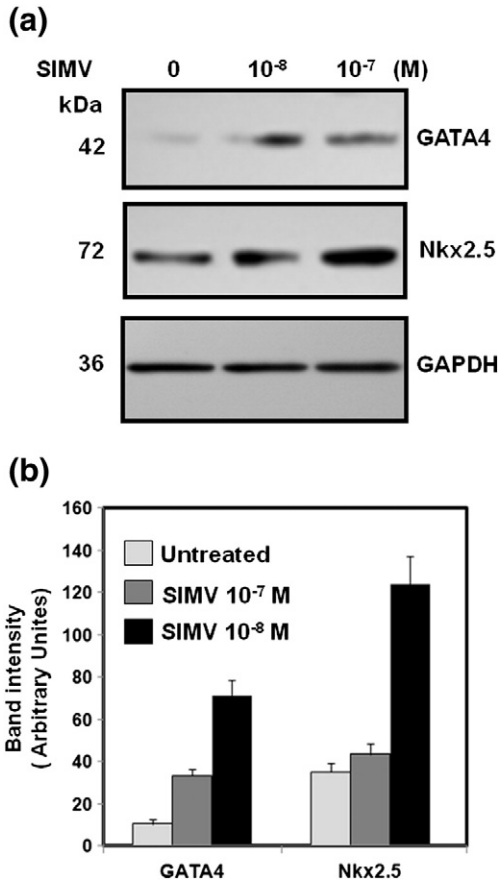

Fig. 2. Expression of GATA4, Nkx2.5, and potassium channel proteins in developing myogenic cells in EBs treated with or without simvastatin. Fig. 2a and b, immunoblotting. Total proteins extracted from ESC-derived EBs after treatment with different concentrations of simvastatin were analyzed by immunoblotting with antibodies to the promyogenic factors GATA4, Nkx2.5, and myocardin-A. a, Immunoblotting with anti-GATA4 Nkx2.5, and GAPDH antibodies; b. densitometry of GATA4 and Nkx2.5 bands (means ± S.D.). from three separate experiments, normalized by GAPDH band intensity. * $P < 0.05$, statistically significant as compared to the untreated control. c-j, immunofluorescence microphotography. c and g, immunofluorescence of KCNQ-1 (green); d, GATA4 (red); e and i, DAPI (blue); f, merging of KCNQ-1/GATA4/DAPI; h, Nkx2.5; and j, Bar shows 20 μm.