available at www.sciencedirect.com

ScienceDirect

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

Heartbeat regulates cardiogenesis by suppressing retinoic acid signaling via expression of *miR-143*

Kota Y. Miyasaka [a], Yasuyuki S. Kida [a,b,c], Toshihiro Banjo [a], Yosuke Ueki [d], Kazuaki Nagayama [e], Takeo Matsumoto [e], Masaaki Sato [d], Toshihiko Ogura [a,b,*]

[a] Graduate School of Life Sciences, Institute of Development, Aging and Cancer, Tohoku University, 4-1, Seiryo, Aoba, Sendai, Miyagi 980-8575, Japan

[b] Department of Developmental Neurobiology, Institute of Development, Aging and Cancer, Tohoku University, 4-1, Seiryo, Aoba, Sendai, Miyagi 980-8575, Japan

[c] Gene Expression Laboratory, The Salk Institute of Biological Studies, 10010 North Torrey Pines Rd., La Jolla, CA 92037, USA

[d] Department of Biomedical Engineering, Graduate School of Biomedical Engineering, Tohoku University, Japan

[e] Department of Mechanical Engineering, Nagoya Institute of Technology, Japan

ARTICLE INFO

Article history:
Received 25 June 2010
Received in revised form
14 September 2010
Accepted 15 September 2010
Available online 30 September 2010

Keywords:
Cardiogenesis
miRNA
Heartbeat
Hemodynamics
Retinoic acid
Mechano-transduction
Zebrafish

ABSTRACT

Cardiogenesis proceeds with concomitant changes in hemodynamics to accommodate the circulatory demands of developing organs and tissues. In adults, circulatory adaptation is critical for the homeostatic regulation of blood circulation. In these hemodynamics-dependent processes of morphogenesis and adaptation, a mechanotransduction pathway, which converts mechanical stimuli into biological outputs, plays an essential role, although its molecular nature is largely unknown. Here, we report that expression of zebrafish *miR-143* is dependent on heartbeat. Knocking-down *miR-143* results in de-repression of retinoic acid signaling, and produces abnormalities in the outflow tracts and ventricles. Our data uncover a novel epigenetic link between heartbeat and cardiac development, with *miR-143* as an essential component of the mechanotransduction cascade.

1. Introduction

The heart is a highly dynamic organ capable of adapting to hemodynamic changes. During embryonic development, the heart undergoes a dynamic morphogenesis, with concomitant changes in blood flow and contraction patterns of heart tubes (Srivastava and Olson, 2000; Stainier, 2001). In the zebrafish heart, occlusion of blood flow results in abnormalities affecting the looping and formation of chambers and valves (Auman et al., 2007; Bartman et al., 2004; Hove et al., 2003), which are phenotypes frequently observed in congenital heart diseases in humans (Combs and Yutzey, 2009; Pasquali et al., 2002). In response to flow-induced shear stress, vascular endothelial cells change their shapes by remodeling their cytoskeletons and altering their gene expression profiles to adapt to physical changes of circulatory conditions (Illi

* Corresponding author at: Graduate School of Life Sciences, Institute of Development, Aging and Cancer, Tohoku University, 4-1, Seiryo, Aoba, Sendai, Miyagi 980-8575, Japan. Tel.: +81 22 717 8564; fax: +81 22 717 8565.
E-mail address: ogura@idac.tohoku.ac.jp (T. Ogura).

0925-4773/$ - see front matter
doi:10.1016/j.mod.2010.09.002

et al., 2003; Resnick and Gimbrone, 1995; Tzima, 2006). These lines of evidence indicate that the heart senses hemodynamic stimuli and responds to them to achieve correct cardiogenesis. This is also evidenced by the fact that the heart will not form normally in the absence of heartbeat and blood flow (Nishii et al., 2008; North et al., 2009; Sehnert et al., 2002). In adulthood, hemodynamics is one of the key factors that maintains circulatory homeostasis and adapts the heart to changes induced by exercise, hypertension, neuroendocrine control, and diseases (de Bold et al., 1996; Ruwhof and van der Laarse, 2000). The complexity of this adaptation system suggests unknown regulatory mechanisms that affect the expression of genes at the transcriptional and post-transcriptional levels. An important feature of this adaptation system is the ability of cells to sense physical forces (mechanical stresses) and convert them into biochemical and genetic responses. This signal conversion system, called mechanotransduction, emerges as an essential epigenetic regulatory program both in embryonic stages and adulthood (Kruger and Linke, 2009; Sawada et al., 2006).

MicroRNAs (miRNAs) are small non-coding RNAs that control gene expression in a sequence-specific way. Primary transcripts of miRNAs are processed by Drosha and Dicer to produce mature double-stranded miRNAs (Chen and Rajewsky, 2007). A single strand of miRNA is incorporated into the RNA-induced silencing complex, where it interacts with its target mRNA. Expression of the target mRNA is negatively regulated by this interaction through degradation or translational inhibition, bypassing transcriptional control of mRNA expression (Jackson and Standart, 2007). We hypothesized that this negative regulation might be active in embryonic development, during which active cardiogenesis proceeds in conjunction with fluctuating changes in hemodynamics.

Here we demonstrate that heartbeat regulates expression of zebrafish *miR-143* in the outflow tracts and ventricles of developing hearts. When the heartbeat was arrested, expression of *miR-143* disappeared, while re-initiation of the heartbeat restored it. In addition, we have found that *miR-143* negatively controls mRNA expression of *retinaldehyde dehydrogenase 2* (*raldh2/aldh1a2*) and *retinoid x receptor alpha b* (*rxrab*), two genes that are important for the correct regionalization of the heart tubes. Hence, *miR-143* and retinoic acid (RA) signaling are targets of heartbeat-dependent physical control, highlighting heartbeat as an essential epigenetic factor during cardiogenesis.

2. Results

2.1. Expression of zebrafish miR-143 and its heartbeat dependency

Expression of *miR-143* commences at 24 h post-fertilization (hpf) in the forebrain, midbrain, and posterior somites. The expression in the brain (blue arrowhead in Fig. 1A) and somites (blue bracket in Fig. 1A) is maintained at 36 hpf. At this stage, faint signals were observed in the developing heart (red arrowhead in Fig. 1A). In a magnified view, ventricular expression was evident (red arrowhead in Fig. 1B), yet no signal was detected in the atrium. In cryosections, *miR-143* was detected both in the endocardium and the myocardium (Fig. 1C and D). Again, atrial compartments showed no detectable signal. At 80 hpf, *miR-143* expression became robust in the outflow tract (OFT) (Fig. 1E) (Wienholds et al., 2005). miR-143 expression was still not observed in the atrium, in contrast to the weak, but distinct expression in the ventricle (Fig. 1E). Overall, *miR-143* is expressed in a graded fashion, with the highest signal in the OFT and no expression in the atrium (Fig. 1G). Expression in the OFT was maintained at 5 days post-fertilization (dpf), as shown in Fig. 1F.

The zebrafish heart initiates contractions around 18 hpf, yet these contractions are not synchronized. Rather, each cardiomyocyte beats in a random manner. After formation of a heart tube, contraction becomes synchronized to commence blood circulation around 22 hpf. The strength of the heartbeat increases enough to support the pulsatile blood flow at 24 hpf. The gradual reinforcement of *miR-143* expression (Fig. 2A–C) and the timing of rhythmic circulation suggest that transcriptional control of *miR-143* might be influenced by blood flow and/or heartbeat.

To confirm this possibility, we stopped the heartbeat with 2,3-butanedione monoxime (BDM) or with an injection of a morpholio antisense oligonucleotide (MO) against *cardiac troponin T2* (*tnnt2*) (Bartman et al., 2004; Sehnert et al., 2002). When the heartbeat was stopped by BDM from 36 to 94 hpf, faint expression of *miR-143* was detected only in the OFT at 96 hpf; ventricular expression was lost (Fig. 2D). After washing out the BDM, the heartbeat restarted after approximately 5 min, and cardiac edema disappeared within 20 min, with a concomitant re-circulation of the pulsatile blood flow (data not shown). Since both the OFT and ventricular expression were not detected when the heartbeat was stopped from 36 to 96 hpf (Fig. 2G), 2 h of heartbeat was enough to activate *miR-143* expression in the OFT, but was unable to activate ventricular expression. When the heartbeat was stopped from 36 to 72 hpf, both the OFT and ventricular expression was observed at 96 hpf, indicating that 24 h of heartbeat is enough to recover the ventricular expression of *miR-143* (Fig. 2E). Heartbeat for 48 h was sufficient to restore the expression in the OFT and the ventricle (Fig. 2F).

When BDM was added from 60 to 72 hpf, the heartbeat was interrupted for 12 h (Fig. 2H). In this case, faint expression of *miR-143* was detected in the OFT at 72 hpf, which is likely a remnant of its initial expression induced by contraction before 60 hpf. Contrary to this, a 36 h interruption of heartbeat cancelled *miR-143* expression completely (Fig. 2I). When heartbeat was arrested by injection of MO against *tnnt2* or continuous BDM treatment, *miR-143* expression was not observed at either 72 or 96 hpf (Fig. 2J and K). These expression data were corroborated by a quantitative RT-PCR analysis of miR-143 mRNA levels (Fig. S1), and the temporal correlation between heartbeat and *miR-143* expression is summarized in Fig. 2L.

Taken together, our data from experiments where we stopped the heartbeat for different periods of time and examined *miR-143* expression, indicate that *miR-143* expression is dependent on heartbeat. Expression levels varied in different regions, with a high sensitivity in the OFT, low sensitivity in the ventricle, and no responsiveness in the atrium. In addition, our data indicate that a 2 h duration of heartbeat