Developmental Biology 365 (2012) 319–327

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Regulation of zebrafish heart regeneration by miR-133

Viravuth P. Yin [a,b], Alexandra Lepilina [a], Ashley Smith [b], Kenneth D. Poss [a,*]

[a] Department of Cell Biology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710, USA
[b] Davis Center for Regenerative Biology and Medicine, Mount Desert Island Biological Labs, Salisbury Cove, ME 04672, USA

ARTICLE INFO

Article history:
Received for publication 27 December 2011
Revised 9 February 2012
Accepted 10 February 2012
Available online 20 February 2012

Keywords:
Zebrafish
Heart
Regeneration
MicroRNAs
miR-133

ABSTRACT

Zebrafish regenerate cardiac muscle after severe injuries through the activation and proliferation of spared cardiomyocytes. Little is known about factors that control these events. Here we investigated the extent to which miRNAs regulate zebrafish heart regeneration. Microarray analysis identified many miRNAs with increased or reduced levels during regeneration. miR-133, a miRNA with known roles in cardiac development and disease, showed diminished expression during regeneration. Induced transgenic elevation of miR-133 levels after injury inhibited myocardial regeneration, while transgenic miR-133 depletion enhanced cardiomyocyte proliferation. Expression analyses indicated that cell cycle factors *mps1*, *cdc37*, and *PA2G4*, and cell junction components *cx43* and *cldn5*, are miR-133 targets during regeneration. Using pharmacological inhibition and EGFP sensor interaction studies, we found that *cx43* is a new miR-133 target and regeneration gene. Our results reveal dynamic regulation of miRNAs during heart regeneration, and indicate that miR-133 restricts injury-induced cardiomyocyte proliferation.

Introduction

Recent evidence indicates that mammals possess a moderate capacity to renew cardiomyocytes (CMs) throughout postnatal life (Bergmann et al., 2009). Yet, there is little or no significant cardiac muscle regeneration after an injury like acute myocardial infarction. Adult zebrafish, on the other hand, robustly regenerate cardiac muscle after major injuries such as resection of the ventricular apex, surface cryoinjury, or genetic ablation of over 60% of CMs (Chablais et al., 2011; Gonzalez-Rosa et al., 2011; Poss et al., 2002b; Schnabel et al., 2011; Wang et al., 2011). Thus, dissecting successful heart regeneration in zebrafish can provide context for understanding, and possibly enhancing, mammalian cardiac regenerative capacity.

Genetic lineage tracing studies recently revealed that existing CMs, not stem cells, are the major source of regenerating cardiac muscle in zebrafish (Jopling et al., 2010; Kikuchi et al., 2010, 2011a). In response to injury, CMs induce cell cycle regulatory genes and proliferate to replace lost myocardium (Jopling et al., 2010; Kikuchi et al., 2010; Poss et al., 2002b). The cardiac environment created by non-muscle cells after injury is believed to be critical in facilitating this regenerative response. Fibroblast growth factor and platelet derived growth factor have been implicated in directing epicardial cells to the injury site, where they can influence muscle regeneration. Furthermore, retinoic acid (RA) synthesis localizes to epicardial cells and endocardial cells at the injury site, where RA signaling is required for CM proliferation (Kikuchi et al., 2011b; Kim et al., 2010; Lepilina et al., 2006). It remains critical to define additional molecular regulators of injury-induced CM proliferation.

Previous studies of zebrafish heart regeneration have suggested that regenerating CMs acquire a less differentiated form after injury, with reduced contractile organization and altered electrical properties (Jopling et al., 2010; Kikuchi et al., 2010). Such a developmental transition suggests potential roles for microRNAs (miRNAs), small, noncoding RNAs that control many cellular processes by binding to mRNA target genes and inhibiting protein translation (He and Hannon, 2004). An abundance of studies have documented indispensable roles for miRNAs during embryonic development, homeostasis, and diseases including cardiomyopathy and cancer (Lin and Friedlander, 2010; Marquez et al., 2010; Sehm et al., 2009; Thatcher et al., 2008; Yin et al., 2008). With respect to tissue regeneration, it was shown recently that many miRNAs show dynamic regulation during fin regeneration and are involved in key regenerative signaling pathways mediated by fibroblast growth factors and Wnts (Thatcher et al., 2008; Yin et al., 2008).

In this study, we used array analysis and new transgenic technology to investigate potential functions for miRNAs during heart regeneration. While many miRNAs showed differential regulation in regenerating versus uninjured cardiac tissue, we focused our work on miR-133, whose family members have been shown to regulate cardiac development and disease (Care et al., 2007; Liu et al., 2008). Our results indicate that miR-133 is an endogenous inhibitor of CM proliferation through modulation of *mps1* and *cx43* activity. Collectively, they support a model in which downregulation of miR-133 in CMs after injury contributes to the regenerative capacity of the zebrafish heart.

* Corresponding author.
E-mail address: kenneth.poss@cellbio.duke.edu (K.D. Poss).

0012-1606/$ – see front matter © 2012 Published by Elsevier Inc.
doi:10.1016/j.ydbio.2012.02.018

Materials and methods

Zebrafish and resection surgery

Zebrafish of the *Ekkwill* (EK) strain or EK/AB hybrid strain 4-6 months old were used for all experiments. Resection surgeries were performed with iridectomy scissors as previously described (Poss et al., 2002b). Transgenic strains were examined as heterozygotes and age-matched clutchmates were used as wildtype controls. For heat-shock experiments, transgenic *hsp70:miR-133*, *hsp70:miR-133sp* and wildtype clutchmates were heat-shocked from 26 °C to 38 °C at either the uninjured state, 6 days post-amputation (dpa), 29 dpa, or once daily for 29 days using experimental conditions previously described (Wills et al., 2008). Following the completion of heat treatment, fish were returned to 26 °C aquaria and hearts were collected 24 h later for analysis.

Gene expression analysis

Total RNA was isolated (Tri-Reagent, Sigma) from whole ventricles at the specified stages of regeneration and used for miRNA microarray hybridizations, northern analysis, and quantitative PCR (Supplemental Methods; (Yin et al., 2008)).

miRNA and mRNA microarrays

Total RNA was isolated from 3 groups each of uninjured and 7 dpa ventricles for miRNA microarray hybridizations using miRBase v.13 miRNAs (www.lcsciences.com). Hybridizations and data filtration were performed by LCSciences in accordance to standard protocols. mRNA microarray hybridizations were performed in triplicate with total RNA isolated from wildtype, *hsp70:miR-133* and *hsp70:miR-133sp* ventricles 5 h following the completion of heat-treatment. MoGene Services performed RNA labeling and hybridizations onto NimbleGene oligo arrays (www.NimbleGen, www.MoGene.com).

Histological methods

Zebrafish hearts were extracted and fixed in 4% paraformaldehyde (PFA) at room temperature for 1 h. Histological analyses were performed on 10 μm cryosections as previously described (Kikuchi et

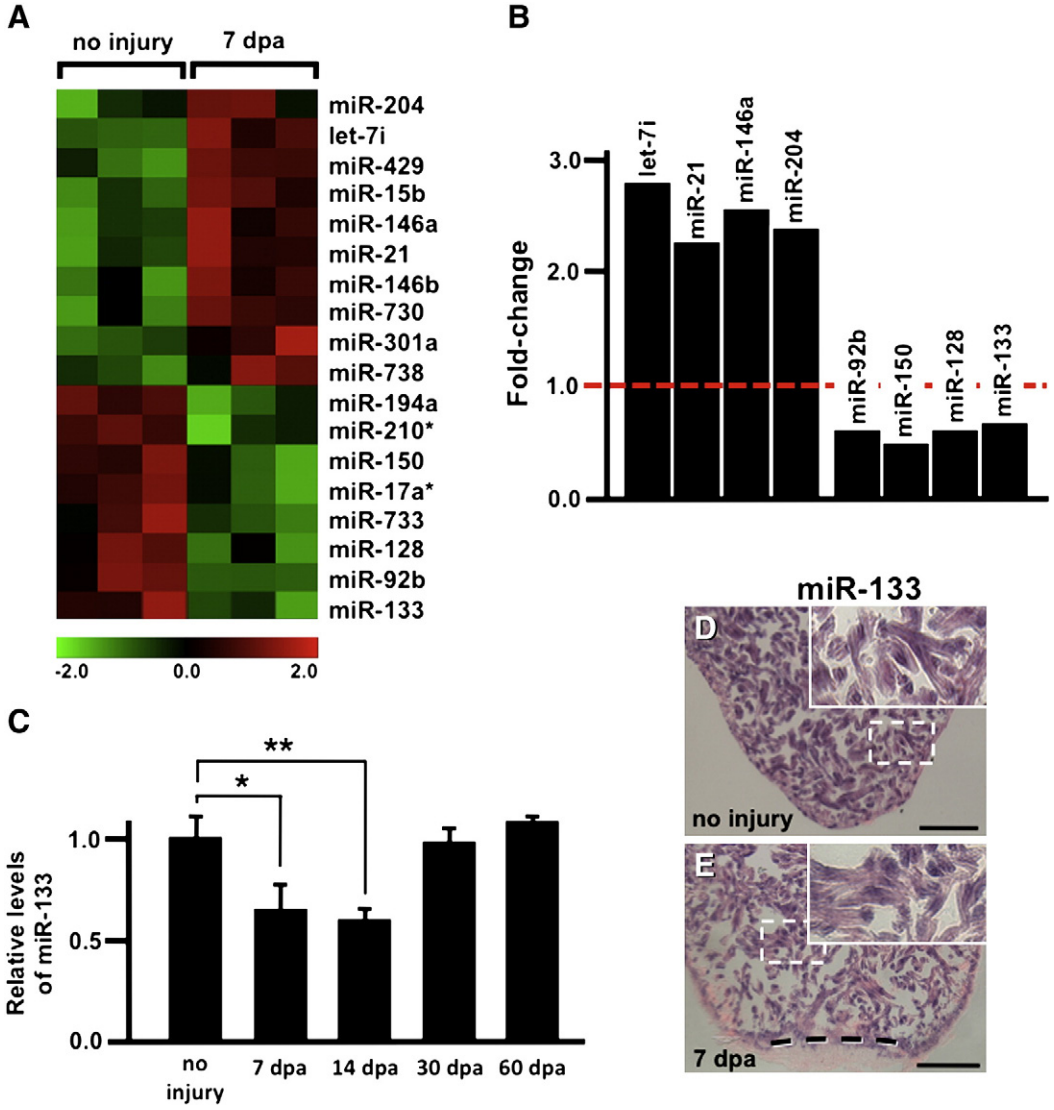

Fig. 1. miRNAs are dynamically regulated during myocardial regeneration. A) A heat-map depicts triplicate microarray hybridizations, revealing a subset of miRNAs that are differentially expressed at 7 dpa when compared to uninjured samples. (Green) lower expression; (red) higher expression. B) Real-time quantitative PCR (QPCR) studies confirm the upregulation of let-7i, miR-21, miR-146A and miR-204 and downregulation of miR-92b, miR-150, miR-128 and miR-133 at 7 dpa when compared to uninjured samples. C) QPCR studies show miR-133 levels are high in the uninjured adult heart and reduced at 7 dpa. Levels return to near uninjured levels by 30–60 dpa. D–E) In situ hybridizations reveal miR-133 is restricted to cardiomyocytes under conditions of no injury and at 7 dpa. Error bars in (C) represent SEM, Student's *t*-test p-value <0.05 for * and **; Insets in (D–E), high zoom images of the white dashed rectangle; dashed line in (E) represents approximate amputation plane; dpa, days post-amputation; scale bar in (D–E) represents 100 μm.