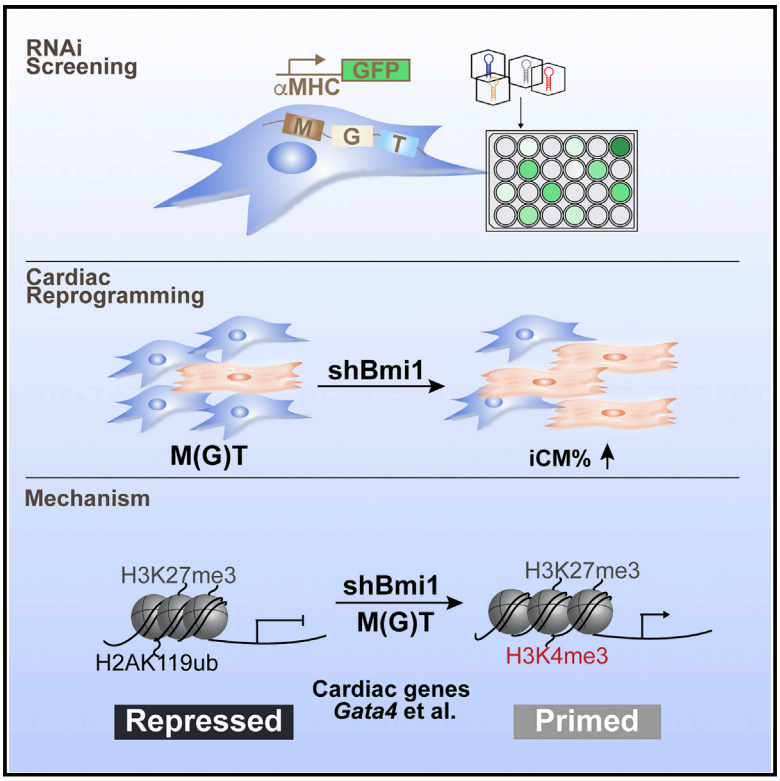
Article

Cell Stem Cell

Bmi1 Is a Key Epigenetic Barrier to Direct Cardiac Reprogramming

Graphical Abstract



Authors

Yang Zhou, Li Wang, Haley Ruth Vaseghi, ..., Greg G. Wang, Jiandong Liu, Li Qian

Correspondence

li_qian@med.unc.edu

In Brief

Qian and colleagues carried out a loss-of-function screen to identify epigenetic factors that modulate reprogramming of fibroblasts into beating cardiomyocytes. They found that repression of Bmi1 enhances reprogramming efficiency and correlates with altered epigenetic status at key cardiogenic loci, resulting in their de-repression.

Highlights

- shRNA screen identified Bmi1 as a major epigenetic barrier to cardiac reprogramming
- Bmi1 downregulation significantly enhanced iCM generation from mouse fibroblasts
- Bmi1 directly binds to and suppresses cardiogenic loci
- Bmi1 depletion can functionally substitute for Gata4 during iCM conversion

Zhou et al., 2016, Cell Stem Cell *18*, 382–395
March 3, 2016 ©2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.stem.2016.02.003

CellPress

Cell Stem Cell
Article

Bmi1 Is a Key Epigenetic Barrier to Direct Cardiac Reprogramming

Yang Zhou,[1,2] Li Wang,[1,2] Haley Ruth Vaseghi,[1,2] Ziqing Liu,[1,2] Rui Lu,[3] Sahar Alimohamadi,[1,2] Chaoying Yin,[1,2] Ji-Dong Fu,[4] Greg G. Wang,[3,5] Jiandong Liu,[1,2] and Li Qian[1,2,*]

[1]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599, USA
[2]McAllister Heart Institute, University of North Carolina, Chapel Hill, NC 27599, USA
[3]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA
[4]Department of Medicine, Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, OH 44109, USA
[5]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599, USA
*Correspondence: li_qian@med.unc.edu
http://dx.doi.org/10.1016/j.stem.2016.02.003

SUMMARY

Direct reprogramming of induced cardiomyocytes (iCMs) suffers from low efficiency and requires extensive epigenetic repatterning, although the underlying mechanisms are largely unknown. To address these issues, we screened for epigenetic regulators of iCM reprogramming and found that reducing levels of the polycomb complex gene *Bmi1* significantly enhanced induction of beating iCMs from neonatal and adult mouse fibroblasts. The inhibitory role of Bmi1 in iCM reprogramming is mediated through direct interactions with regulatory regions of cardiogenic genes, rather than regulation of cell proliferation. Reduced *Bmi1* expression corresponded with increased levels of the active histone mark H3K4me3 and reduced levels of repressive H2AK119ub at cardiogenic loci, and de-repression of cardiogenic gene expression during iCM conversion. Furthermore, *Bmi1* deletion could substitute for Gata4 during iCM reprogramming. Thus, Bmi1 acts as a critical epigenetic barrier to iCM production. Bypassing this barrier simplifies iCM generation and increases yield, potentially streamlining iCM production for therapeutic purposes.

INTRODUCTION

The adult mammalian heart has limited regenerative capacity and is thus an important target for novel regenerative approaches to replenish lost cardiomyocytes (CMs) after cardiac injury (Laflamme and Murry, 2011; Porrello et al., 2011; Ubil et al., 2014; Xin et al., 2013). Cardiac reprogramming that converts fibroblasts to contractile induced cardiomyocytes (iCMs) by overexpression of cardiac-lineage-specific transcription factors holds great promise as an alternative approach for cardiac regeneration and disease modeling (Addis and Epstein, 2013; Addis et al., 2013; Chen et al., 2012; Fu et al., 2013; Hirai et al., 2013; Ieda et al., 2010; Ifkovits et al., 2014; Jayawardena et al., 2012; Muraoka et al., 2014; Nam et al., 2013; Protze et al., 2012; Qian and Srivastava, 2013; Song et al., 2012; Wada et al., 2013; Wang et al., 2015a; Zhao et al., 2015; Zhou et al., 2015). However, our limited understanding of the molecular mechanism underlying cardiac reprogramming has significantly hindered its potential translational applications.

Epigenetic regulation plays a critical role in shaping and maintaining cellular identities during developmental programming and cellular reprogramming. Recent studies on in vitro cardiac differentiation of embryonic stem cells demonstrated that temporal activation of functionally important cardiac genes requires coordinated programmed control of chromatin structure (Paige et al., 2012; Wamstad et al., 2012). Likewise, cellular reprogramming is accompanied by profound changes in the epigenetic landscape (Dhawan et al., 2011; Liang and Zhang, 2013; Luna-Zurita and Bruneau, 2013; Onder et al., 2012; Tursun et al., 2011). This transition in epigenetic status is likely to be involved in suppressing the original cell-type-specific signature and establishing and stabilizing a target cell-type-specific program (Apostolou and Hochedlinger, 2013; Buganim et al., 2013; Papp and Plath, 2013). Epigenetic alterations were also observed at both fibroblast- and CM-specific marker genes during iCM reprogramming (Fu et al., 2013; Ieda et al., 2010). However, how these epigenetic transitions are regulated remains elusive. iCM reprogramming, like other types of cellular reprogramming, is an inefficient and slow process, which is at least in part due to multiple epigenetic barriers that have not been identified. It also remains unclear whether iCM reprogramming shares similar epigenetic mechanisms with induced pluripotent stem cell (iPSC) reprogramming or has its own specific barriers.

Here we report the first shRNA-based loss-of-function screen to explore the role of epigenetic factors in iCM reprogramming. Among the identified epigenetic regulators of iCM reprogramming, the polycomb ring finger oncogene *Bmi1* acted as a major epigenetic barrier during the early phase of iCM reprogramming. Genetic and epistasis analyses suggested that the inhibitory effect of Bmi1 on iCM reprogramming was not completely mediated by its downstream effectors involved in cell proliferation such as $p16^{Ink4a}$, $p19^{Arf}$, and *p53*. Instead, we discovered an uncharacterized role of Bmi1 in directly binding the regulatory regions of several cardiogenic genes including *Gata4*. Knockdown of *Bmi1* caused de-repression of endogenous *Gata4* and could