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Meis1 Regulates Postnatal Cardiomyocyte Cell Cycle Arrest

11

Shalini A. Muralidhar and Hesham A. Sadek

Abstract

The neonatal mammalian heart is capable of substantial regeneration following injury through cardiomyocyte proliferation (Porrello et al, Science 331:1078–1080, 2011; Proc Natl Acad Sci U S A 110:187–92, 2013). However, this regenerative capacity is lost by postnatal day 7 and the mechanisms of cardiomyocyte cell cycle arrest remain unclear. The homeodomain transcription factor Meis1 is required for normal cardiac development but its role in cardiomyocytes is unknown (Paige et al, Cell 151:221–232, 2012; Wamstad et al, Cell 151: 206–220, 2012). Here we identify Meis1 as a critical regulator of the cardiomyocyte cell cycle. Meis1 deletion in mouse cardiomyocytes was sufficient for extension of the postnatal proliferative window of cardiomyocytes and for reactivation of cardiomyocyte mitosis in the adult heart with no deleterious effect on cardiac function. In contrast, overexpression of Meis1 in cardiomyocytes decreased neonatal myocyte proliferation and inhibited neonatal heart regeneration. Finally, we show that Meis1 is required for transcriptional activation of the synergistic CDK inhibitors p15, p16, and p21. These results identify Meis1 as a critical transcriptional regulator of cardiomyocyte proliferation and a potential therapeutic target for heart regeneration.

Keywords

Cardiomyocytes • Heart injury • Regeneration • Meis1 • Cell cycle

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11.1 Introduction

The past decade witnessed a revolution in our understanding of cardiac biology, with groundbreaking research demonstrating that the adult mammalian heart is capable of limited but measurable cardiomyocyte turnover [5–9]. However, the ultimate goal of complete regeneration of the heart remains elusive. In stark contrast to the adult mammalian heart, we recently demonstrate that the mammalian heart is in fact capable of complete regeneration following apical resection of 15 % of the ventricular myocardium [1]. This remarkable regenerative capacity was associated with robust cardiomyocyte proliferation throughout the myocardium. Moreover, lineage-tracing studies demonstrated that the newly formed myocytes were derived from preexisting cardiomyocytes, rather than from a progenitor population. Finally, we showed that cessation of this regenerative phenomenon occurred at postnatal day 7 (P7), which coincides with the developmental window when cardiomyocytes become binucleate and withdraw from the cell cycle [9, 10]. It is unclear whether the loss of this regenerative potential in the adult heart is due to an intrinsic cell cycle block in adult cardiomyocytes or to loss of mitogenic stimuli as the heart ages (or both). Thus, it is important to determine the mechanisms by which the mammalian heart switches off this regenerative capacity in the week after birth.

In an effort to identify genes involved in postnatal regeneration arrest, we performed several gene arrays following MI at multiple postnatal time points. This allowed us to identify Meis1 as one of the few transcription factors that were dysregulated between injury at P1 and injury at P7 and P14. Meis1 has been studied extensively in the hematopoietic system, is required for normal hematopoiesis, and also plays an important role in leukemogenic transformation [11–14]. What little is known about the role of Meis1 in the heart comes from global KO studies resulting in numerous cardiac defects. However, given that global Meis1 deletion results in embryonic lethality by E14.5, full characterization of the role of Meis1 in cardiomyocytes has been difficult [13, 15, 16]. Despite the role of Meis1 in regulation of hematopoiesis and cardiac development, the mechanism of action of Meis1 remains poorly characterized. Our results indicate that Meis1 expression and nuclear localization in cardiomyocytes coincide with cell cycle arrest. Cardiomyocyte cell cycle exit is associated with downregulation of positive cell cycle regulators (CDK2, CDK3, CDK4, CCND1, and CDK cofactors) and induction of cell cycle inhibitors (CDKI, members of the INK4 and CIP/KIP families) [10, 17–20]. We identified conserved Meis1 domains in only two key CDK inhibitors, namely, p16 and p21, which are known to regulate all three cell cycle checkpoints. We demonstrated that Meis1 regulates the pattern of expression of these two cell cycle inhibitors following Meis1 knockdown. These results provide role and mechanism of cell cycle regulation by Meis1.

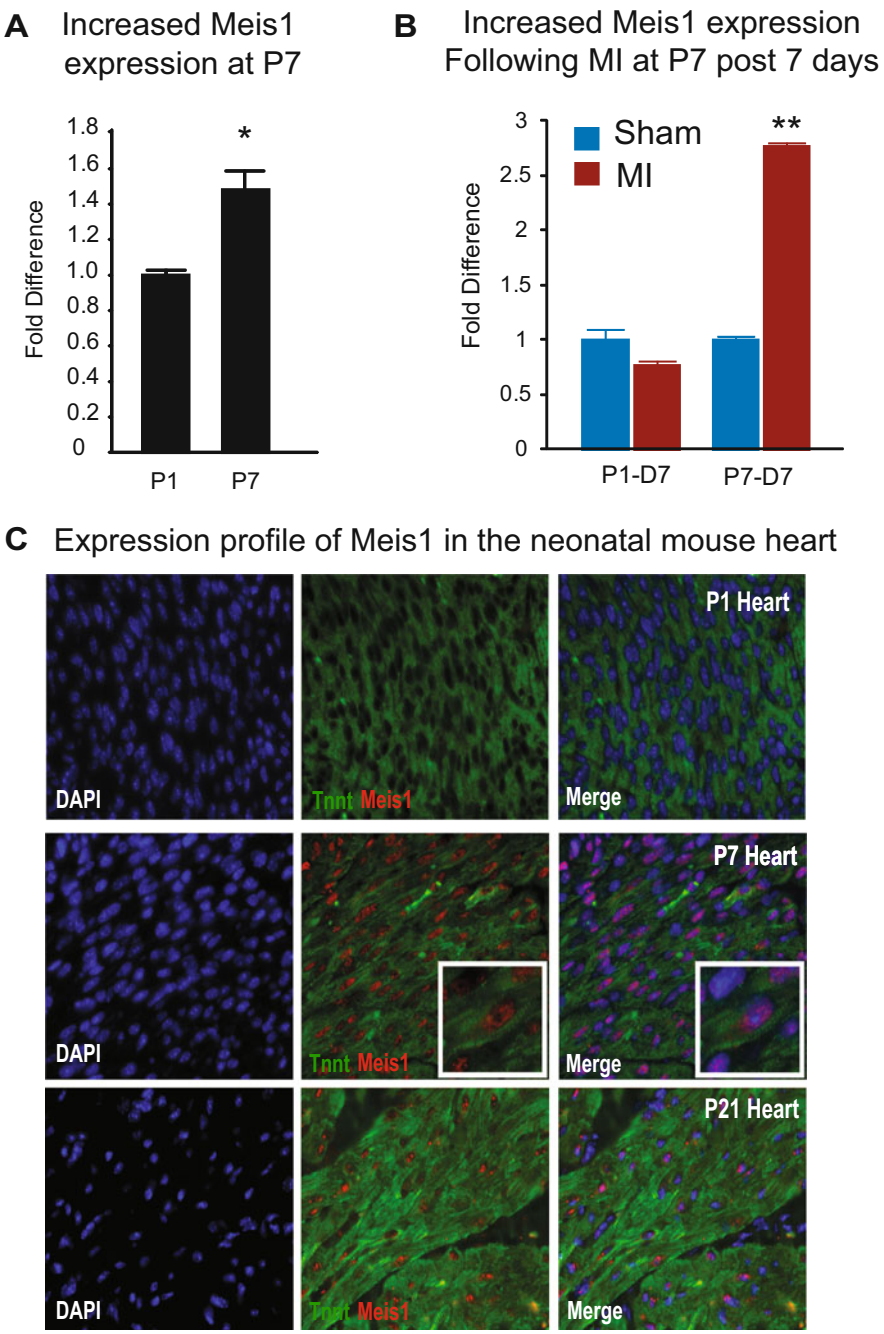

Fig. 11.1 Expression profile of Meis1 in postnatal heart. (**a**) qRT–PCR showing increased expression of Meis1 at postnatal day 7 (P7), a time point that coincides with cell cycle arrest of cardiomyocytes. (**b**) qRT–PCR showing expression levels of Meis1 7 days post Sham or MI at P1 or P7. (**c**) Expression of Meis1 is absent at P1 and nuclear localization of Meis1 in P7 and P21 cardiomyocytes

11.2 Results

11.2.1 Expression of Meis1 During Neonatal Heart Development and Regeneration

Transcriptional regulation of postnatal cardiomyocyte cell cycle arrest is unclear. Our initial screens identified Meis1 as a potential transcriptional regulator of neonatal heart regeneration. Therefore, we conducted this study to determine the role of Meis1 in regulation of cardiomyocyte cell cycle. We first examined the expression pattern of Meis1 during neonatal heart development and regeneration. qPCR showed a modest increase in Meis1 expression between postnatal day 1 (P1) and P7 (Fig. 11.1a). Meis1 was localized to perinuclear regions in neonatal cardiomyocytes at P1 and nuclear localized by P7 and P21 (Fig. 11.1c). Myocardial infarction (MI) at P1, which is associated with an induction of robust cardiomyocyte proliferation at day 7 post-MI, was associated with a modest decrease in the expression of Meis1, whereas Meis1 mRNA expression levels were significantly increased following MI at P7, a time point coinciding with lack of mitotic induction of cardiomyocytes (Fig. 11.1b).

11.2.2 Cardiomyocyte Proliferation Beyond Postnatal Day 7 Following Meis1 Deletion

We generated cardiomyocyte-specific Meis1 knockout (KO) mice by crossing $Meis1^{f/f}$ mice with αMHC-Cre mice (Fig. 11.2a). qRT–PCR of cardiomyocytes from $Meis1^{f/f}$ αMHC-Cre (Meis1KO) compared to αMHC-Cre (control) mouse hearts confirmed a change in gene expression consistent with Meis1 deletion in cardiomyocytes (Fig. 11.2b). Phenotypic characterization of Meis1KO mice at P14 (1 week beyond the normal window of postnatal cardiomyocyte cell cycle arrest) demonstrated that heart size (Fig. 11.2c, d) and cardiac function (Fig. 11.2e) were unaffected by Meis1 deletion. However, Meis1KO cardiomyocytes were smaller compared to control cardiomyocytes (Fig. 11.2f), which may imply that the cardiomyocyte number is increased in Meis1KO (smaller cardiomyocyte size, with no change in heart-to-body weight ratio). Therefore, we examined the Meis1KO hearts for myocyte proliferation using the mitosis marker pH3 (phosphorylated histone H3) and the cytokinesis marker Aurora B kinase. Meis1 deletion resulted in induction of cardiomyocyte proliferation as quantified by an increase in the number of pH3+TnnT2+ (troponin T2) cells (Fig. 11.2g). In addition, we found that Aurora B was markedly expressed in the cleavage furrow between proliferating myocytes in the KO hearts (0.5-fold) (Fig. 11.2h). We also found a significant increase which did not result in an increase in myocyte apoptosis by TdT-mediated dUTP nick end labeling (TUNEL) staining (Fig. 11.2k).

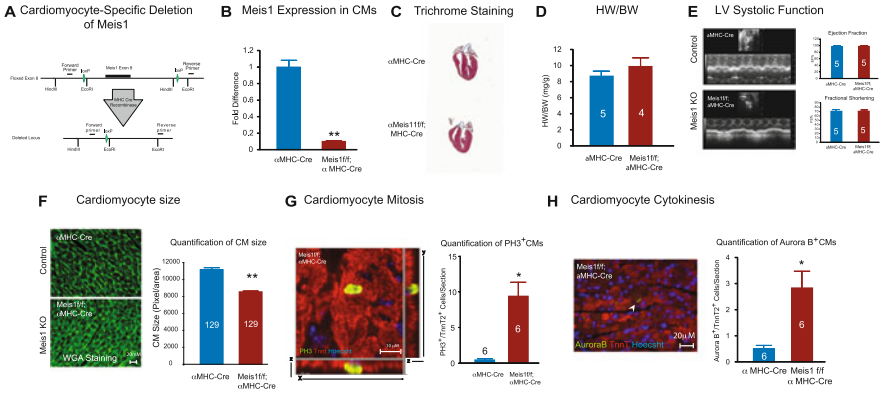

Fig. 11.2 Cardiomyocyte proliferation at P14 following Meis1 deletion. (**a**) Schematic of Meis1 floxed allele. Control mice were αMHC-Cre, Meis1KO mice were Meis1$^{f/f}$αMHC-Cre. (**b**) qRT–PCR demonstrates deletion of Meis1 in cardiomyocytes at P14. (**c**) Trichrome staining of wild-type (WT) and Meis1KO hearts at P14. (**d**) Heart weight (HW)-to-body weight (BW) ratio in WT and Meis1KO hearts. (**e**) Left ventricular (LV) systolic function quantified by ejection fraction (EF) and fractional shortening (FS). (**f**) Wheat germ agglutinin (WGA) staining and cell size quantification. (**g**) Confocal image with z-stacking showing co-localization of pH3, TnnT2, and Hoechst in a Meis1KO heart at P14. Immunostaining showing sarcomere disassembly and normal sarcomeric structure in Meis1KO hearts. Graph shows quantification of the number of pH31+TnnT21+ nuclei. (**h**) Expression of Aurora B in Meis1KO cardiomyocytes at P14 and quantification of the number of Aurora B+TnnT2+ cardiomyocytes

11.2.3 MI in Meis1 Overexpressing Heart Limits Neonatal Heart Regeneration

To determine whether forced Meis1 expression can inhibit neonatal cardiomyocyte proliferation, we generated a cardiac-specific Meis1 overexpressing mouse (Meis1 OE) by crossing pTRE-Meis1 mice with αMHC-tTA mice to allow for specific overexpression of Meis1 in cardiomyocytes (Fig. 11.3a) around birth in the absence of tetracycline. We used a Meis1 line that overexpressed Meis1 by 2.5-fold (Fig. 11.3b). Overexpression of Meis1 did not result in a significant increase in heart-to-body weight ratio (Fig. 11.3c, d), normal systolic function (Fig. 11.3e), and increased cardiomyocyte size (Fig. 11.3f). Despite the increase in cardiomyocyte size in Meis1 (OE) hearts, the lack of decrease in heart-to-body weight ratio most probably reflects a decrease in the number of cardiomyocytes. This is supported by a decrease in the number of mitotic cardiomyocytes in the neonatal Meis1 (OE) hearts (Fig. 11.3g). More notably, Meis1 OE inhibited neonatal heart regeneration following induction of MI at P1 (Fig. 11.3h–j), whereas the wild-type hearts regenerated normally. Finally, Meis1 OE in cardiomyocytes resulted in upregulation of CDK inhibitors, most significantly p21 (Cdkn1a) (Fig. 11.3k). These results indicate that Meis1 overexpression in the neonatal heart results in premature cardiomyocyte cell cycle arrest.

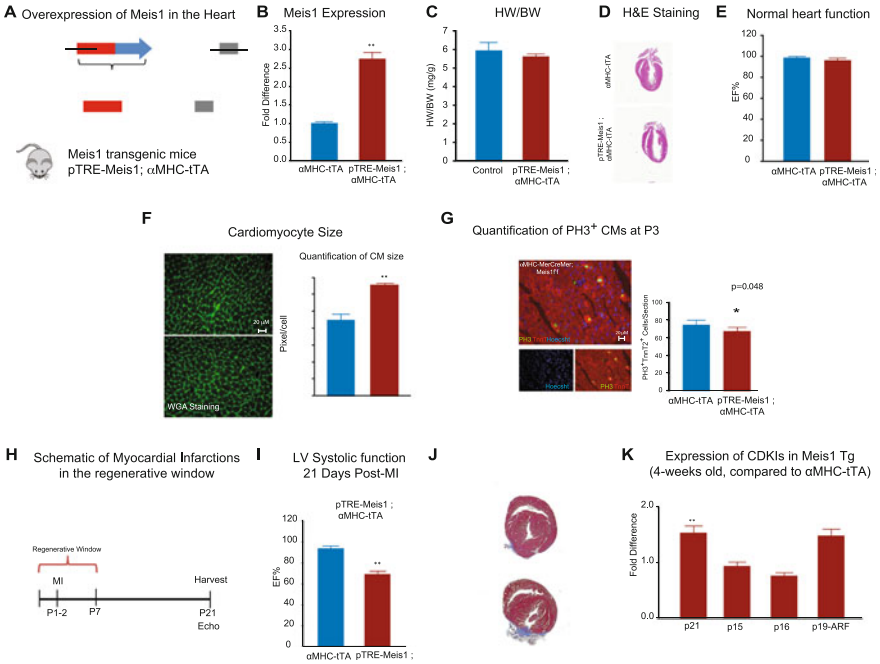

Fig. 11.3 Meis1 overexpression limits neonatal heart regeneration following MI. (**a**) Schematic of Meis1 overexpression (OE) in the heart. Control mice were αMHC-tTA, Meis1 (OE) mice were pTREMeis1-αMHC-tTA. (**b**) qRT–PCR demonstrates overexpression of Meis1. (**c**) HW-to-BW ratio in control and Meis1 (OE) mice. (**d**) H & E staining of WT and Meis1 (OE) hearts. (**e**) LV systolic function quantified by EF. (**f**) WGA staining and cell size quantification. (**g**) Immunostaining image showing co-localization of pH3, TnnT2, and Hoechst in Meis1 (OE) heart at P3. Graph shows quantification of pH3+TnnT2+ nuclei. (**h**) Schematic of neonatal MI during the regenerative window at P1. (**i**) LV systolic function of WT and Meis1 (OE) hearts at 21 days post-MI. (**j**) Trichromes at day 21 post-MI. (**k**) qRT–PCR of CDKIs in hearts of Meis1 (OE) compared to control

11.2.4 Regulation of Cyclin-Dependent Kinase Inhibitors by Meis1

In order to determine the mechanism by which Meis1 regulates cardiomyocyte proliferation, we performed a cell cycle PCR array. We found that Meis1 deletion resulted in downregulation of cyclin-dependent kinase inhibitors in isolated cardiomyocytes, including members of the Ink4b–Arf–Ink4a locus (p16, p15, and p19ARF) and CIP/ KIP family (p21 and p57), as well as upregulation of a number of positive regulators of the cell cycle (Fig. 11.3a, b). qRT–PCR confirmed these results (Fig. 11.4a). Of all the dysregulated cell cycle genes, we identified conserved Meis1 consensus binding sequences in the promoter region of only two loci, namely, the Ink4b–Arf–Ink4a (which includes p16INK4a, p19ARF, and p15INK4b) (Fig. 11.4b) and p21 loci (Fig. 11.4c) using the UCSC genome browser (http://genome.ucsc.edu). To test if Meis1 can transcriptionally activate Ink4b–

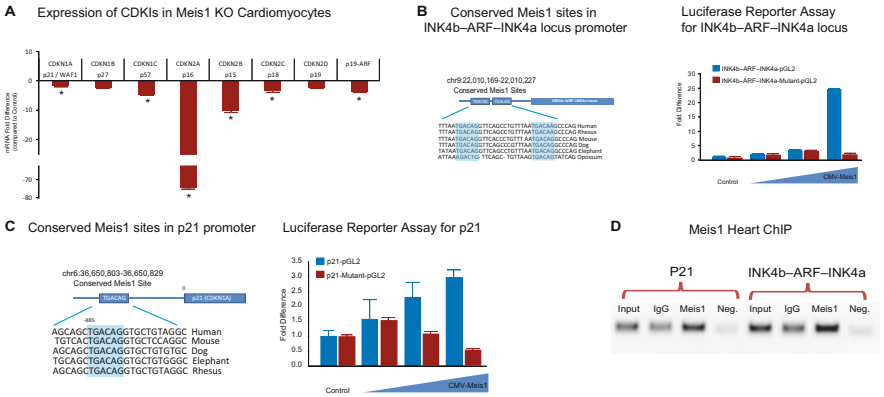

Fig. 11.4 Regulation of cyclin-dependent kinase inhibitors by Meis1. (**a**) qPCR of CDKIs in purified Meis1KO cardiomyocytes demonstrates significant downregulation of CDKN1A (p21), CDKN2B (p15), CDKN2A (p16), p19ARF, CDKN2C (p18), and CDKN1C (p57). (**b**) Highly conserved Meis1 motifs located in p16INK4a/p19ARF/p15INK4b promoter and luciferase reporter assay for INK4b–ARF–INK4a promoter demonstrating induction of INK4b–ARF–INK4a pGL2 reporter by Meis1 and loss of reporter activity following mutation of Meis1 motifs (INK4b–ARF–INK4a-Mutant). (**c**) Highly conserved Meis1 motif located in p21 promoter and luciferase assay for p21 demonstrating induction of p21 pGL2 reporter by Meis1 and loss of reporter activity following mutation of Meis1 motifs (p21 Mutant). (**d**) In vivo confirmation of Meis1 interaction with INK4a/ARF/INK4b and p21 promoters by ChIP assay

Arf–Ink4a and p21, we generated luciferase reporter constructs containing the conserved Meis1 binding motifs. Luciferase reporter assays with INK4b–ARF–INK4a–pGL2 (Fig. 11.4b) and p21–pGL2 reporters (Fig. 11.4c) demonstrated a dose-dependent activation by Meis1. Mutation of the putative Meis1 binding sites abolished the Meis1-dependent activation of the luciferase reporters (Fig. 11.4b, c). Finally, we demonstrated an in vivo interaction between Meis1 and Ink4b–Arf–Ink4a and p21 promoters in the adult mouse heart by ChIP (Figs. 11.4d and 11.5).

11.3 Future Direction and Clinical Implications

The current study identifies Meis1 as a critical transcriptional regulator of cardiomyocyte cell cycle, upstream of two synergistic CDKI inhibitors. Although the mechanism of activation of Meis1 in the postnatal heart is not quite fully understood, results have demonstrated that Meis1 expression coincides with Hox genes that are known to interact with Meis1, stabilize its DNA binding, and enhance its transcriptional activity. Therefore, it would be important for future studies to define the transcriptional network involved in mediating the effect of Meis1 on postnatal cardiomyocytes. Ultimately, we hope to utilize our understanding of the role and mechanism of Meis1 in cardiomyocyte proliferation to uncover new disease mechanisms and therapeutic approaches for cardiovascular diseases.

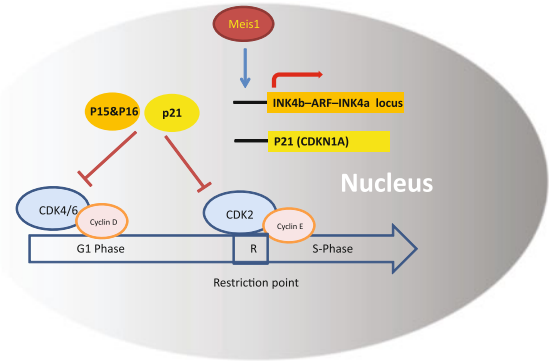

Fig. 11.5 Proposed model of cardiomyocyte cell cycle regulation by Meis1. Meis1 regulates cardiomyocyte cell cycle arrest through transcriptional activation of CDKIs, INK4b–ARF–INK4a locus, and p21, as well as indirectly through a number of other cell cycle regulators. Activation of CDKIs leads to cell cycle arrest by inhibiting CDKs. Statistical significance: Values presented as mean ± SEM. A Student's *t* test was used to determine statistical significance. $*P < 0.05$. $**P < 0.01$

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