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Bone Morphogenetic Protein-10 Induces Cardiomyocyte Proliferation and Improves Cardiac Function After Myocardial Infarction

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ABSTRACT

Heart disease is among the leading causes of death worldwide, and the limited proliferation of mammalian cardiomyocytes prevents heart regeneration in response to injury. Bone morphogenetic protein-10 (BMP10) exerts multiple roles in various developmental events; however, the effect of BMP10 and the underlying mechanism involved in cardiac repair remains unclear. After stimulation with the recombinant BMP10, an obvious dose-dependent cardiomyocyte proliferation and reentry of differentiated mammalian cardiomyocytes into the cell cycle was observed. Furthermore, BMP10 stimulation strikingly enhanced Tbx20 expression. Further analysis demonstrated that T-box 20 (Tbx20) was involved in BMP10-induced proliferation of differentiated cardiomyocytes as preconditioning with Tbx20 siRNA significantly attenuated BMP10-induced DNA synthesis. In vivo, BMP10 induced rat cardiomyocyte DNA synthesis and cytokinesis. After myocardial infarction (MI), BMP10 stimulated cardiomyocyte cell-cycle reentry and mitosis, resulting in the decrease of infarct size and improvement of cardiac repair. Taken together, these data indicated that BMP10 stimulated cardiomyocyte proliferation and repaired cardiac function after heart injury. Consequently, BMP10 may be a potential target for innovative strategies against heart failure. J. Cell. Biochem. 115: 1868–1876, 2014.
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KEY WORDS: BMP10; CARDIOMYOCYTE PROLIFERATION; MYOCARDIAL INFARCTION; CARDIAC REPAIR

Heart disease ranks as the leading cause of morbidity and mortality around the world, resulting in the increasing financial burden [Lloyd-Jones et al., 2010; Kovacic and Fuster, 2011]. It is generally believed that losses of cardiomyocytes are the characteristics of heart disease, such as myocardial infarction (MI), which means irreversible necrosis of heart muscle secondary to prolonged ischemia. Approximately 1.5 million patients of MI are reported annually in the United States [Kociol et al., 2012]. Conventional treatment regimens of heart disease show a limited effectiveness due to modest impact on correcting the loss of cardiomyocytes. Recently, numerous studies have confirmed that inducing cardiomyocyte proliferation can promote heart regeneration, suggesting a potential prospect against heart diseases [Jopling et al., 2010; Laflamme and Murry, 2011; Lam et al., 2012].

Cell-cycle activity triggers cardiomyocytes proliferation during development, its disrupt leads to the deficit of cardiomyocytes, which is the basis of cardiac disease [Zhu et al., 2009; Walsh et al., 2010]. In contrast to most adult cardiomyocytes, fetal cardiomyocytes have a high potential for proliferation, which will be attenuated after birth by up-regulating cell-cycle inhibitor and down-regulating cell-cycle activator [Ahuja et al., 2007; Ikenishi et al., 2012]. Therefore, acquired injuries such as MI, viral myocarditis, ischemia/reperfusion result in cardiomyocyte loss and abrogate cardiac function. Multiple lines of evidence from vitro studies and animal models indicate that the application of extrinsic molecules can promote cardiac repair by enhancing cardiomyocyte proliferation [Bersell et al., 2009; Lam et al., 2012]. However, few molecules that induce cardiomyocyte proliferation are recognized.

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Bone morphogenetic proteins (BMPs) can promote and regulate bone development, growth, remodeling and repair, in both prenatal development and postnatal growth of eye, heart, skin, and other tissues, belonging to the transforming growth factor β (TGF-β) superfamily. Among these, BMP10 is known as a peptide growth factor and can mediate diverse spectrum of developmental events, such as cardiac development [Chen et al., 2004; Somi et al., 2004; Huang et al., 2012]. It has been demonstrated that BMP10 exerts an essential function in regulating cardiac growth, embryonic survival, and chamber maturation. Silencing BMP10 expression in mice shows an elevated expression of p57 (kip2), a cell-cycle inhibitor, and reduces embryonic cardiomyocyte proliferative activity [Chen et al., 2004]. T-box 20 (Tbx20), a cardiac transcription factor, can promote cardiomyocyte proliferation in adult mouse hearts, as well as during embryonic and fetal development [Chakraborty and Yutzey, 2012; Chakraborty et al., 2013]. It is known that Tbx20 is significantly up-regulated in BMP10 transgenic hearts, suggesting as a downstream target for BMP10-regulated cardiac ventricular wall development and function [Zhang et al., 2011]. However, the effect of BMP10 on cardiac repair and the underlying mechanism remains unclear.

In this study, we investigated the role of BMP10 on cardiomyocyte proliferation and cardiac repair after MI, the corresponding molecular mechanism underlying this progress was also discussed. The findings of this research will provide a potential strategy for heart failure.

MATERIALS AND METHODS

ANTIBODIES

Rat anti-BrdU and anti-phospho histone H3 antibodies (anti-H3P) antibodies were obtained from Abcam (Cambridge, MA). Antibodies against troponin T and tropomyosin were purchased from Sigma (St. Louis, MO). Mouse anti-Aurora B and anti-troponin I antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal to p57 Kip2 and ki67 primary antibodies were from Abcam. Alexa-Fluor546 goat anti-mouse and Alexa-Fluor488 goat anti-rabbit antibodies were purchased from Molecular Probes (Eugene, OR).

EXPRESSION OF BMP10 IN VITRO

Total RNA was isolated from rat cardiac tissues using the RNAiso plus kit, followed by the synthesis of cDNA with the cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany). The cDNA was amplified by PCR with gene-specific primers for BMP10 as previously described [Chen et al., 2004]. Then, the obtained cDNA was ligated into the *Eco*RI and *Xho*I cloning site of the pcDNA3.1(+) vector (Invitrogen, Carlsbad, CA). The obtained positive plasmids were transformed into *E. coli* strain DH5α to induce BMP10 expression by the application of IPTG with a final concentration of 1.0 mM for 8 h. To acquire the recombinant BMP10 protein, the MagneHis^{TM} Protein Purification System was used according to the manufacturer's instructions (Promega). The purified protein was analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and high-performance liquid chromatography (HPLC).

CARDIOMYOCYTE ISOLATION AND CULTURE

Ventricular cardiomyocytes were isolated from male (300 g) Lewis rats. Animal experiments were approved by the Institutional Animal Care and Use Committee. Cells were obtained and processed according to the recommendations of the Xijing Hospital, Fourth Military Medical University. Cardiomyocyte were cultured in medium including 5% horse serum and 20 μM cytosine β-D-arabinofuranoside, which was used to abolish the proliferation of non-myocytes. Then, cells were treated with various doses of recombinant BMP10 protein for 6 days, and 25 μM BrdU was added to analyze DNA synthesis for the terminal 3 days. All cells were maintained at 37°C with 5% CO_2.

REAL-TIME PCR

Total RNA from rat ventricles were obtained by TRIzol (Invitrogen). Following synthesis of first strand cDNA with the Oligo (dT) 18 primer, real-time PCR was performed with 10 μmol/L gene-specific primer for Tbx20 (sense: 5′-GGAACCATGGAGTTCACCGCGT-3′; anti-sense: 5′-GCAGAACAAGATCTCATTCC-3′) [Chen et al., 2004]. The reaction conditions followed the instructions provided by the manufacturers of the SYBR Premix Ex Taq^{TM} II Kit (Takara, Otsu, Japan). The mRNA expression levels were normalized based on β-actin levels.

WESTERN BLOTTING

Cells were dissolved in RIPA lysis buffer (Beyotime, Nantong, China). After centrifugation, total protein concentrations were determined using the Bradford assay (Bio-Rad, Hercules, CA). After electrophoresis by SDS–PAGE, proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Pharmacia, Piscataway, NJ) using a commercial semi-dry blotting apparatus. Membranes were blocked by buffer containing 5% nonfat dry milk in Tris-buffered saline at 4°C overnight, and incubated with primary antibodies against BMP10, β-actin, and Tbx20 for 1 h. After washing, HRP-conjugated secondary antibodies were added for 1 h. The bound antibodies were visualized using the LumiGLo reagent (Pierce).

IMMUNOFLUORESCENCE STAINING

After fixing in 4% paraformaldehyde, samples were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS), followed by blocking with 10% goat serum in PBS. Primary antibodies used were rat anti-BrdU (1:200), rabbit anti-phospho histone H3 antibodies (anti-H3P; 1:100), rabbit anti-Troponin I (1:100), mouse anti-Aurora B (1:250), mouse anti-troponin T (1:200), rabbit anti-Tropomyosin (1:250). Immune complexes were visualized with ALEXA 488 or ALEXA 546-conjugated secondary antibodies. The nucleus was stained with 4′,6′-diamidino-2-phenylindole (DAPI). All samples were imaged using fluorescence microscopy.

RNA INTERFERENCE

To specifically silence the expression of TBx20, cells were transfected by Tbx20 siRNA (OriGene #315497). After 48 h in culture, siRNA transfection reagent (GeneTherapy System, San Diego, CA) was introduced to transiently transfect 2 μg/ml Tbx20 and control

siRNA to cardiomyocytes, according to the manufacturer's instructions.

DETERMINATION OF CARDIOMYOCYTE PROLIFERATION AND CELL-CYCLE ACTIVITY IN VIVO

Experiments conformed to the guide for the Care and Use Committee of Xijing Hospital, Fourth Military Medical University. In this study, 20 adult male Lewis rats (300 g) were underwent thoracotomy and received intramyocardial injections of 50 μg BMP10, or 100 mM sodium acetate as a control. Following these treatments, immunofluorescence microscopy and Western blotting were used to assess the presence of injected BMP10 protein. BrdU (70 μmol/kg) was given every 48 h by intraperitoneal injection to evaluation of cell proliferation, and rats were killed 48 h after the third injection. After fixed with formalin and embedded in paraffin, serial 8-mm-thick sections were obtained from each specimen. For cell-cycle analysis of cardiomyocytes, antibodies against Aurora B and H3P were used to visualize cycling cardiomyocytes by laser scanning immunofluorescence microscopy.

MYOCARDIAL INFARCTION AND REGENERATION ANALYSIS

Animal experiments were performed according to the recommendations of the Xijing Hospital, Fourth Military Medical University. Adult male Sprague rats MI models were performed as previously described [Lu et al., 2009]. Briefly, animals were initially anesthetized with pentobarbital (40 mg/kg) by intraperitoneal injection. Then, they were cannulated and artificially ventilated with a small animal ventilator at a rate of 5 ml/min. After penetrating into four limbs of electrodes, the normal electrocardiogram (II) was continuously recorded via an ECG recorder (BL-420F, China). Following the left anterolateral thoracotomy at the third and fourth intercostals space, pericardium was removed in rats and a segment of saline-soaked 5-0 sutures was introduced to encircle the left anterior descending coronary artery, Then, the animals underwent the left coronary artery occlusion and then closed the chest. The successful infarction was assessed by regional cyanosis of myocardial surface and ST-segment elevation. Control animals underwent open chest procedures without coronary artery occlusion. A Gelfoam sponge containing recombinant BMP10 (100 μg) or PBS (used as control) was implanted after MI. To evaluate cardiac function, all the animals were lightly anesthetized with ketamine and xylazine combination, then underwent echocardiography just before the operation (baseline) and on 2, 6, 10, and 12 weeks after MI. The transthoracic echocardiography was performed to determine the cardiac ventricular performance as previously described [Fu et al., 2009]. Intraperitoneal injections of BrdU was administrated with a half-life of 2 h every 48 h. To analyze cardiac regeneration, H3P was introduced to visualize cycling cardiomyocytes. About 12 weeks later, BrdU-positive cardiomyocyte nuclei and cytokines were quantified on 16–20 sections per heart.

STATISTICAL ANALYSIS

All data were analyzed using SPSS 11.0 and presented as mean ± SEM. A typical image from at least three similar experiments was administered. Statistical analysis was determined using *t*-tests. A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

EXPRESSION OF BMP10 IN VITRO

To obtain recombinant rBMP10 protein, BMP10 cDNA was inserted into the pcDNA3.1-(+) expression vector. Abundant recombinant proteins have been successfully purified in a Ni-Particles screening step with 93.4% purity by SDS–PAGE analysis (Fig. 1A). Furthermore, the purity of rBMP10 was 95.3% when evaluating with HPLC assay (Fig. 1B). Following electrophoresis with SDS–PAGE, the expression of purified rBMP10 was determined by western blotting. As shown in Figure 1C, rabbit anti-rat BMP10 antibody exhibited strong reactivity with the rBMP10 protein samples, compared to the control group, indicating that the recombinant rBMP10 protein was successfully obtained.

BMP10 INDUCED CYCLING OF DIFFERENTIATED CARDIOMYOCYTES IN VITRO

To investigate the ability of BMP10 to induce proliferation of differentiated cardiomyocyte, we performed a dose-dependent assay by BrdU incorporation. Following stimulating with BMP10, a dose-dependent increase in neonatal cardiomyocyte BrdU uptake was observed, indicating an important effect of BMP10 on DNA synthesis in neonatal cardiomyocytes (Fig. 2A). Furthermore, more

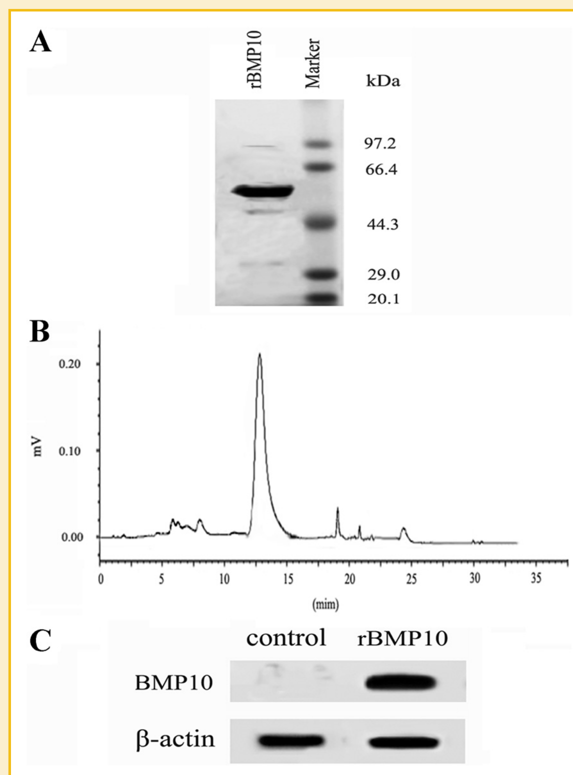

Fig. 1. Western blotting analysis of purified rBMP10 protein. Following transfection with the recombinant pcDNA3.1-BMP10, the recombinant rBMP10 protein was collected. The purity of rBMP10 was evaluated by SDS–PAGE (A) and HPLC (B). After electrophoresis with SDS–PAGE, rabbit anti-rat GPNMB antibodies were used as the primary antibody to identify rBMP10 expression by Western blotting.

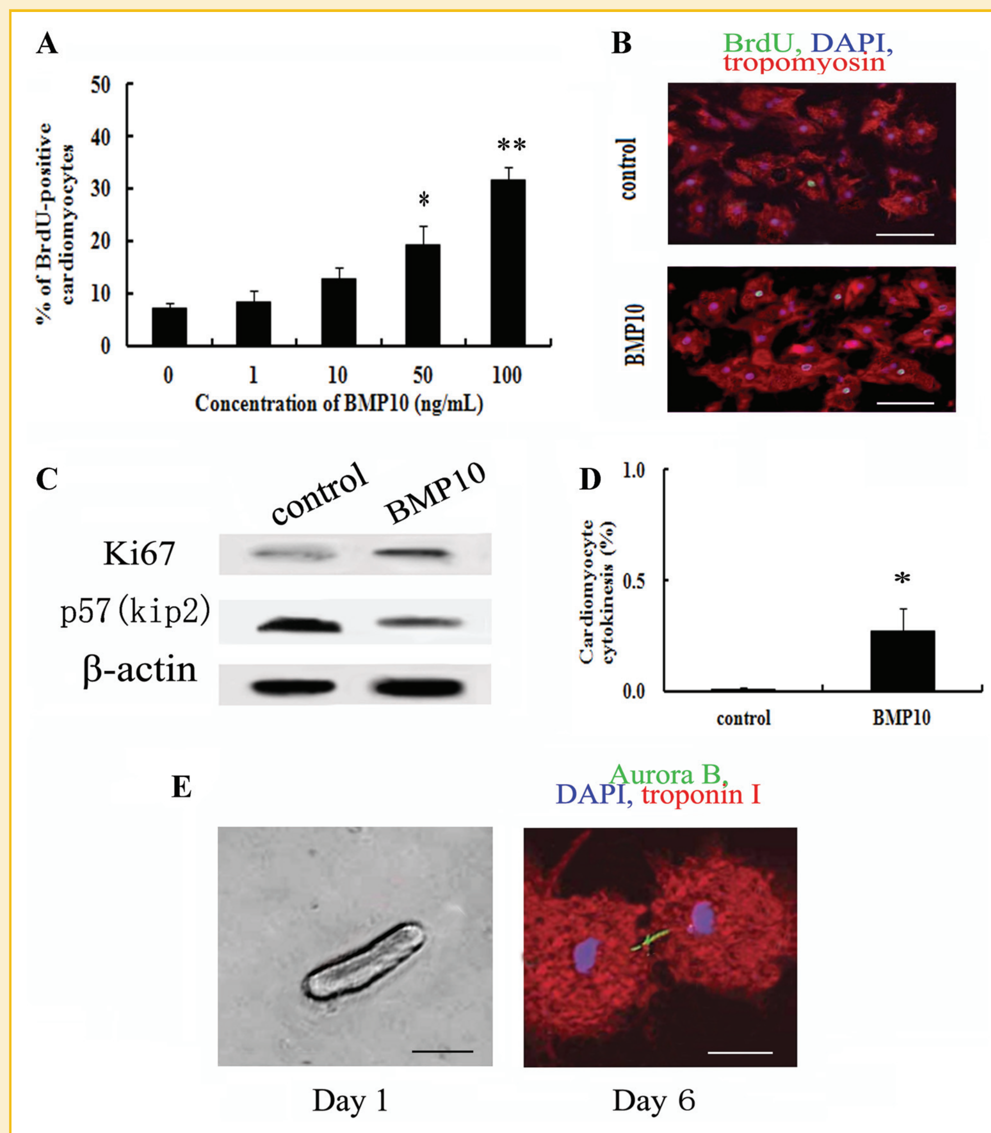

Fig. 2. BMP10-induced proliferation of neonatal cardiomyocytes. A: Primary rat neonatal cardiomyocytes were stimulated with the indicated doses of BMP10 for 6 days, and BrdU was introduced during the last 3 days. B: BMP10 induces BrdU incorporation of neonatal cardiomyocytes. Scale bar 50 μM. C: After treatment with BMP10, the expression of Ki67 and p57 (kip2) was analyzed by Western blotting. D: Aurora B was used to analyze cardiomyocyte cytokinesis by immunofluorescence microscopy, and the corresponding quantified analysis was performed. E: Representative example of neonatal cardiomyocyte stained for Aurora B (green) and Troponin I (red) identifying neonatal cardiomyocytes undergoing cytokinesis. Scale bar 20 μM. $^{*}P < 0.05$ versus control; $^{**}P < 0.01$.

immunofluorescence signals of cardiac contractile protein tropomyosin were also detected (Fig. 2B). Moreover, treatment with 100 ng/ml BMP10 dramatically increased the expression of proliferation marker Ki67 and inhibited the expression of cell-cycle inhibitor p57 (kip2) (Fig. 2C). Aurora B kinase is usually known as a marker for the final step of the mitotic cell cycle. Further analysis demonstrated that exposure to BMP10 for 6 days significantly triggered cytokinesis in 0.29% by visualizing aurora B kinase (Fig. 2D,E). Together, these data indicated BMP10 induced neonatal cardiomyocytes proliferation, which prompted us to further assess the role of BMP10 to induce cell-cycle reentry of differentiated cardiomyocytes.

Following stimulation with BMP10 for 6 days, about 4.6-fold increase in DNA synthesis were confirmed in adult cardiomyocytes, suggesting an obvious up-regulation in cell proliferation in BMP10-induced adult cardiomyocytes (Fig. 3A). To track the fate of individual rod-shaped cardiomyocytes, we found that they undergo DNA synthesis while expressing tropomyosin by immunofluorescence staining (Fig. 3B), and revealed a 5.2-fold increase in DNA synthesis (Fig. 3C). To further investigate whether BMP10 is sufficient to induce mitosis in differentiated cardiomyocytes, we determined the expression of H3P, a marker for mitosis. Immunofluorescence analysis shown that BMP10 promoted cell-cycle progression into mitosis (Fig. 3B), and resulted in a 7.4-fold increase in cell mitosis (Fig. 3C). The similar increase in cytokinesis was also confirmed. Additionally, an obvious up-regulation of Ki67 and down-regulation of p57 (kip2) was also determined in

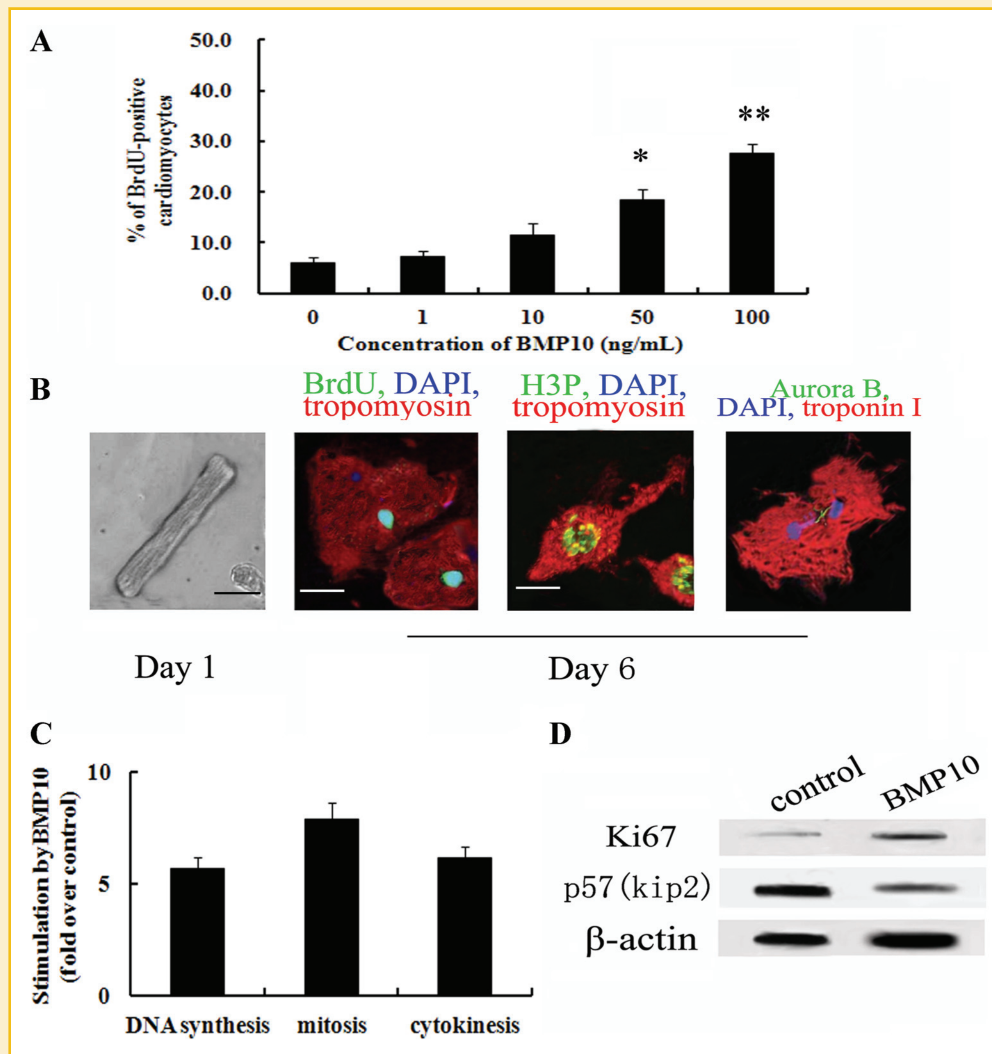

Fig. 3. BMP10 triggered cell cycle reentry in adult cardiomyocytes. A: Quantitative analysis of BMP10-induced BrdU incorporation. BMP10 dose-dependently induced BrdU incorporation of adult cardiomyocytes in vitro. $^{*}P < 0.05$. $^{**}P < 0.01$. B: Primary adult rat ventricular cardiomyocytes were photographed in phase contrast after attachment, followed by stimulation with BMP10 for 6 days. Cell-cycle activity was assessed by immunofluorescence staining. Scale bar 20 μM. C: Quantitative analysis of cell-cycle activity. D: Assay of Ki67 and p57 (kip2) expression levels by Western blotting.

BMP10-treated cells (Fig. 3D). Thus, these results exhibited a potential ability for BMP10 to induce differentiated cardiomyocyte cell-cycle reentry.

BMP10-INDUCED DNA SYNTHESIS WAS TBX20-DEPENDENT

Tbx20 is known to be a cardiac transcription factor, which can induce cardiomyocyte proliferation in hearts as well as fetal development [Chakraborty and Yutzey, 2012; Chakraborty et al., 2013]. To clarify the underlying mechanism of BMP10-induced DNA synthesis, we detected Tbx20 expression in adult cardiomyocytes by western blotting. As shown in Figure 4A, stimulation with BMP10 strikingly enhanced Tbx20 expression compared with control group. Whether Tbx20 was accounted for BMP10-induced proliferation of differentiated cardiomyocytes? To address this question, we silenced Tbx20 expression by specific siRNA, and an obvious reduction in Tbx20 protein levels was observed (Fig. 4B). Simultaneously, preconditioning with Tbx20 siRNA significantly attenuated BMP10-induced BrdU incorporation from 26.8% to 13.4%, implying that Tbx20 mainly regulated the effect of BMP10 on DNA synthesis (Fig. 4C).

BMP10 TRIGGERED CARDIOMYOCYTES DNA SYNTHESIS AND CYTOKINESIS DIVISION IN VIVO

To investigate whether BMP10 induces cardiomyocyte cell-cycle reentry in vivo, we preformed intramyocardial injections of recombinant BMP10 into myocardium. Immunofluorescence staining assay demonstrated the presence of BMP10 near the injected hearts (Fig. 5A). Simultaneously, abundant BMP10 protein was also confirmed by Western blotting assay (Fig. 5B). Six days later, numerous BrdU-positive signals were observed in BMP10-injected group, and cells exhibited differentiated phenotypes by expression of contractile protein (troponin T) (Fig. 5C). Further analysis

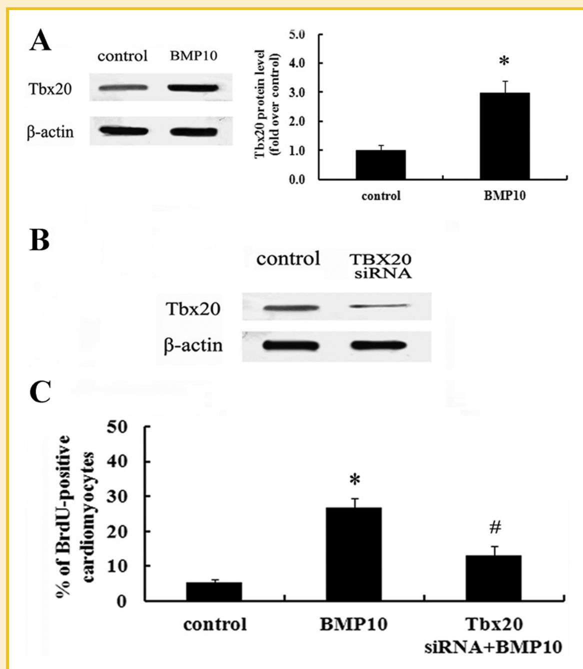

Fig. 4. BMP10-stimulated adult cardiomyocyte DNA synthesis was Tbx20 dependent. A: After stimulation with 100 ng/ml BMP10 for 6 days, the expression level of Tbx20 was detected by Western blotting. B: Primary adult rat ventricular cardiomyocytes were pretreated with Tbx20 siRNA; the corresponding silencing effect on Tbx20 expression was assessed. C: To further determine whether Tbx20 was responsible for BMP10-induced proliferation, analysis of BrdU-incorporation was performed. $^{*}P < 0.05$ versus control; $^{\#}P < 0.05$ versus BMP10-pretreated group.

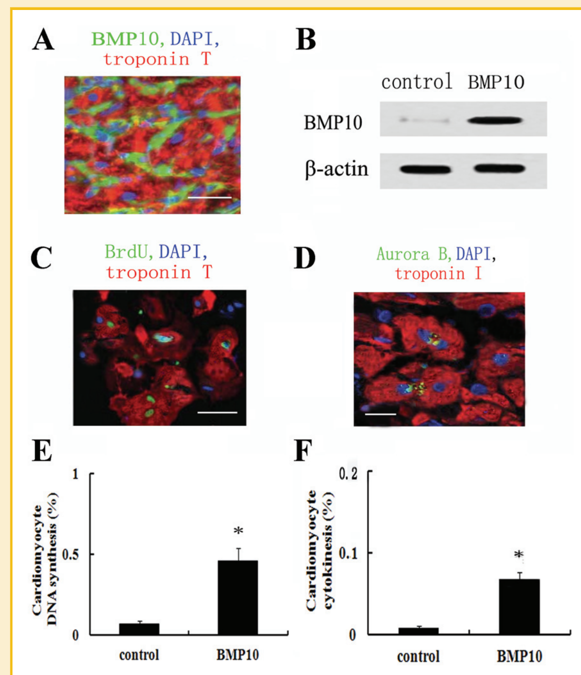

Fig. 5. BMP10-induced cardiomyocyte DNA synthesis cytokinesis division in vivo. A,B: BMP10 was confirmed in the injected hearts by immunofluorescence staining and western blotting assay. C: After three injection of BrdU, BrdU-positive cardiomyocytes were determined by immunofluorescence microscopy. D: Representative example of cardiomyocytes stained for Aurora B and Troponin I to identify cardiomyocyte cytokinesis. E,F: Quantitative analysis of cardiomyocyte DNA synthesis and cytokinesis. Scale bar 20 μM. $^{*}P < 0.05$.

suggested that BMP10 stimulated cardiomyocytes division in vivo by visualizing the formation of cleavage furrow and the midbody (Fig. 5D). In addition, quantified analysis exhibited BMP10 stimulated DNA synthesis in 0.4% (Fig. 5E) and cytokinesis in 0.08% (Fig. 5F) of cardiomyocyte nuclei in the injected area. The above results suggested that BMP10 stimulated cardiomyocyte cycling in vivo.

BMP10 IMPROVED CARDIAC FUNCTION AND CARDIAC REPAIR AFTER MYOCARDIAL INFARCTION

Sustained cardiomyocyte cell-cycle activity can improve cardiac function and reduces infarct size after MI [Hassink et al., 2008]. After constructing a rat MI model, the Gelfoam sponge was used as a scaffold to deliver BMP10 or control buffer into the defective sites. About 12 weeks later, abundant BMP10 protein was released to the ventricles by western blotting (Fig. 6A). Furthermore, over the course of the 12-week experiment after MI, control rats exhibited high ventricular dilatation, which was significantly decreased in BMP10-treated group (Fig. 6B). Moreover, the ejection fraction was also up-regulated in BMP10-injected rats, but not in the control group. Importantly, an approximate 16.3% decrease in scar size was observed after 12 weeks of BMP10 treatment (Fig. 6C).

To address the question about how BMP10 ameliorate infarct size, we investigated cardiomyocyte proliferation as a possible mechanism. As shown in Figure 6D, cardiomyocytes were observed by the expression of troponin T protein. Quantification analysis confirmed that treatment with BMP10 induced DNA synthesis in 0.32% at 12 weeks (Fig. 6E). Furthermore, after 12 weeks, BMP10-treatment induced cardiomyocyte cytokinesis in 0.05% (Fig. 6F) and mitosis in 0.03% (Fig. 6G). Collectively, these results suggested that administration of BMP10 for 12 weeks improved myocardial function, and resulted in the repair of heart injury.

DISCUSSION

Ischemic heart disease is the leading cause of death worldwide, including MI, angina pectoris, and heart failure. Conventional wisdom suggests that mammalian hearts fail to regenerate as the permanent loss of heart cardiomyocyte, resulting in ventricular dysfunction and occurrence of heart disease. Adult cardiomyocytes are known to have a limited ability to proliferate. However, some cardiomyocytes in the adult heart undergo DNA synthesis and mitosis, which has been confirmed during the recent years [Carvalho and de Carvalho, 2010]. Furthermore, subsequent researches have observed that induction of cardiomyocyte proliferation can trigger heart regeneration, suggesting a potential aspect to repair the heart after injury [Jopling et al., 2010; Kikuchi and Poss, 2012; Mollova et al., 2013]. Therefore, it may be possible to apply some molecules to induce cardiomyocyte proliferation with the aim to enhance cardiac regeneration and heart repair after injury.

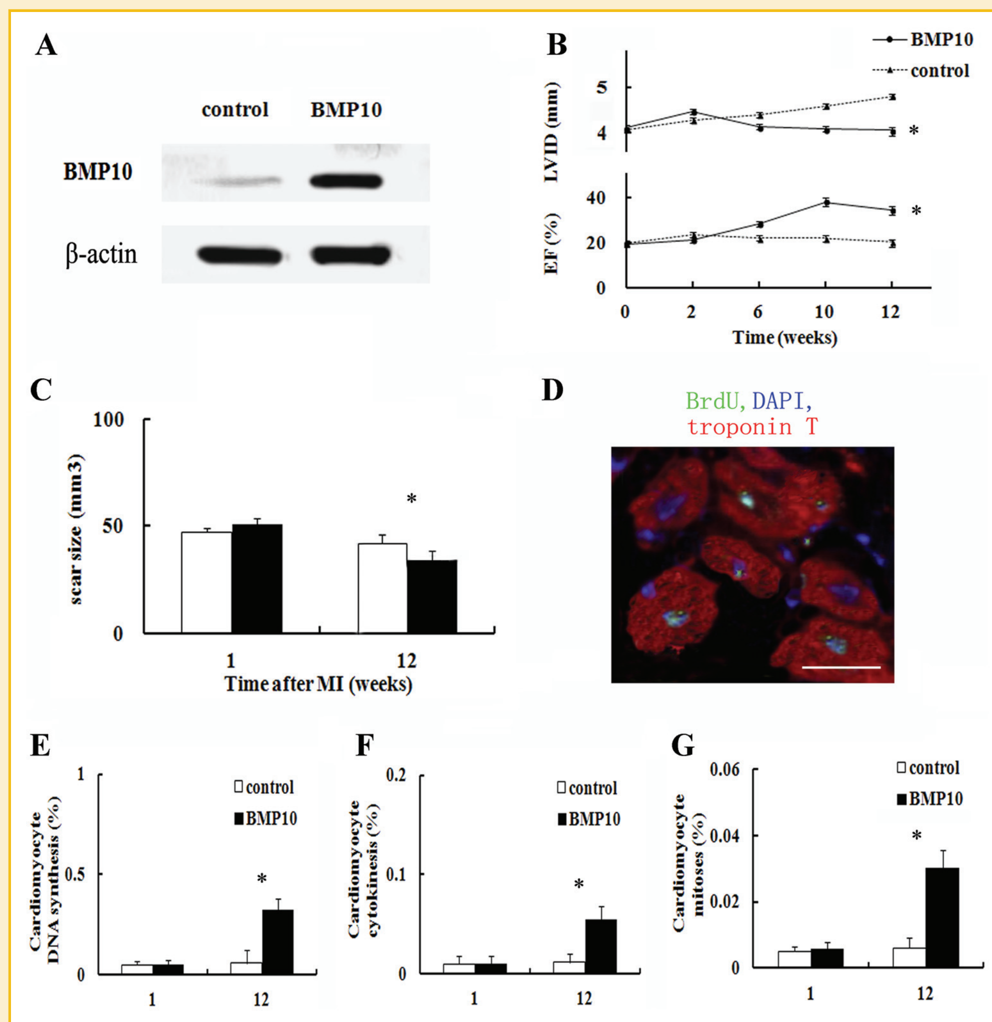

Fig. 6. BMP10 improved cardiac function and cardiac repair after myocardial infarction. A: Confirmation of the presence of recombinant BMP10 in ventricular by Western blotting. B: BMP10 treatment improved ventricular remodeling and myocardial function by echocardiographic analysis of the ejection fraction (EF) and left ventricular internal dimensions (LVID). $^*P < 0.05$. C: Quantification of infarct size after 12 weeks of administration of buffer or BMP10. $^*P < 0.05$. D: Analysis of cardiomyocyte DNA synthesis in BMP10-stimulated heart by detecting BrdU-positive signals. E: Quantification of cardiomyocyte DNA synthesis. F,G: Analysis of cardiomyocyte cytokinesis and mitosis in BMP10-, or buffer-, treated hearts after 1 or 12 weeks. $^*P < 0.05$. Scale bar 20 μM.

BMP10 is an important cardiac cytokine belonging to TGF-β superfamily, and can elicit multiple roles in various developmental events, including cardiac growth, embryonic survival, and chamber maturation [Chen et al., 2004; Somi et al., 2004; Huang et al., 2012]. Furthermore, BMP10 rescues the defect in cell proliferation in Myocd$^{-/-}$ hearts [Huang et al., 2012]. However, the effect of BMP10 on cardiac repair after injury and the underlying mechanism remains unknown. It is well known that cell-cycle activity can induce cardiomyocyte proliferation in cardiac development [Zhu et al., 2009; Walsh et al., 2010; Mercola et al., 2011]. In this study, exposure to BMP10 induced neonatal and adult cardiomyocyte DNA synthesis. Furthermore, treatment with BMP10 decreased cell-cycle inhibitor p57 (kip2) expression and increased the proliferation marker Ki67 expression, resulting in mitosis and cytokinesis in differentiated cardiomyocytes. Hence, exposure to BMP10 induced differentiated cardiomyocyte proliferation, implying a possible potential of BMP10 to repair heart after injury.

As a critical regulator of heart development, Tbx20 is expressed in adult heart and can promote cardiomyocyte proliferation via the PI3K/Akt pathway; its mutation will leads to a wide array of cardiac abnormalities [Chakraborty and Yutzey, 2012; Liu et al., 2012; Chakraborty et al., 2013]. It has been demonstrated that high expression of Tbx20 is detected in BMP10 transgenic hearts, and plays a critical roles in BMP10-regulated cardiac ventricular wall development and function as a downstream mediator for BMP10 [Zhang et al., 2011]. In this study, abundant Tbx20 protein was observed after stimulation with BMP10. Whether BMP10-mediated cardiomyocyte proliferation is correlated with Tbx20 signaling? To clarify this problem, we silenced the expression of Tbx20 by specific siRNA. Further analysis confirmed that BMP10 induced DNA

synthesis in differentiated cardiomyocytes; however, pretreatment with Tbx20 siRNA abated this synthesis, suggesting that BMP10 triggered differentiated cardiomyocyte proliferation majorly through Tbx20 signaling. However, the underlying mechanism involved in this process remains unclear. Dose BMP10 interact with Tbx20 through BMP receptor and its downstream Smads signaling during this process? Which receptors are the function receptors for BMP-regulated effect on cell proliferation? All of these questions need to be further investigation in the future.

Recently, inducing cardiomyocyte proliferation has exhibited a potential to promote heart regeneration, and finally are benefit for repair heart after injury. As mentioned above, BMP10 could induce differentiated cardiomyocyte proliferation in vitro and in vivo. Therefore, we further discussed the function of BMP10 in cardiac repair after MI. As expected, the release of BMP10 from Gelfoam ameliorated ventricular dilatation and increased ejection fraction, which are important predictors of mortality and adverse cardiac events, leading to the improved cardiac function. Moreover, less infarct size was demonstrated in BMP10-treated groups. It is generally believed that sustained cardiomyocyte cell cycle can improve cardiac function and decreased infarct size after MI [Caillava and Baron-Van Evercooren, 2012Caillava and Baron-Van Evercooren, 2012; Li et al., 2013]. To clarify the underlying mechanism involved in BMP10-induced repair of heart injury, we assessed cardiomyocyte proliferation as a possible mechanism. After MI, the delivery of BMP10 enhanced cell proliferation and cell-cycle reentry, which maybe account for the cardiac repair after MI.

In this study, BMP10 induced reentry of differentiated cardiomyocyte into cell cycle and enhanced cardiac function, and eventually promoted cardiac repair after injury. Recently, use of stem cells to generate replacement cells for damaged heart has shown great potential for heart disease [Oldroyd et al., 2012; Leri and Anversa, 2013; Williams et al., 2013]. BMP10 has been reported to induce differentiation of pluripotent stem cells [Piotrowska, 2007]. However, whether BMP10 can induce pluripotent stem cells differentiation to cardiomyocytes and whether the delivery of BMP10 and stem cells together has greater potential for heart repair after injury remain unanswered; these questions will need to be investigated in our next study.

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