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FGF21 attenuates pathological myocardial remodeling following myocardial infarction through the adiponectin-dependent mechanism

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ABSTRACT

Ischemic heart disease is one of the leading causes of death. Fibroblast growth factor 21 (FGF21) is a circulating factor with an anti-diabetic property. Skeletal muscle is an important source of FGF21 production. Here, we investigated whether skeletal muscle-derived FGF21 modulates cardiac remodeling in a murine model of myocardial infarction. Myocardial infarction was produced in C57BL/6J wild-type (WT) mice by the permanent ligation of the left anterior descending coronary artery (LAD). Adenoviral vectors expressing FGF21 (Ad-FGF21) or control β-galactosidase were intramuscularly injected into mice at 3 days before permanent LAD ligation. Intramuscular injection of Ad-FGF21 increased plasma FGF21 levels in WT mice compared with control. Treatment of WT mice with Ad-FGF21 led to improvement of left ventricular systolic dysfunction and dilatation at 2 weeks after LAD ligation. Ad-FGF21 administration to WT mice also led to enhancement of capillary density in the infarct border zone, and reduction of myocyte apoptosis in the remote zone, which were accompanied by decreased expression of pro-inflammatory cytokines. Furthermore, treatment of WT mice with Ad-FGF21 increased plasma levels of adiponectin, which is a cardioprotective adipokine. The beneficial effects of Ad-FGF21 on cardiac dysfunction and inflammatory response after myocardial infarction were diminished in adiponectin-knockout mice. These data suggest that muscle-derived FGF21 ameliorates adverse cardiac remodeling after myocardial infarction, at least in part, through an adiponectin-dependent mechanism.

1. Introduction

Cardiovascular diseases including myocardial infarction (MI) are the major cause of death worldwide [1]. Chronic heart failure after MI is a serious complication, and is closely linked to poor prognosis [1]. Pathological cardiac remodeling, including deterioration of left ventricle morphology and function, and myocardial death contributes to the progression of heart failure after MI [2]. Thus, therapeutic approaches to reduce pathological remodeling of the myocardium are valuable for treatment of MI [2,3].

Fibroblast growth factor (FGF) 21 is a secreted factor that belongs to a family of FGFs [4,5]. Administration of FGF21 protein reduces blood glucose and triglyceride levels in obese mice [5] FGF21 transgenic mice are reported to show improved insulin sensitivity [5]. Furthermore, FGF21-knockout (FGF21-KO) mice develop impaired insulin sensitivity with mild lipodystrophy [6]. Therefore, FGF21 functions as a crucial modulator of insulin sensitivity.

Skeletal muscle secretes a number of bioactive proteins, also known as myokines, which directly affect nearby and remote organs in paracrine or endocrine manners [7,8]. Initial reports demonstrated that FGF21 is preferentially expressed in liver [4,9]. On the other hand, it has been shown that FGF21 is secreted from

Abbreviations: FGF21, fibroblast growth factor 21; MI, myocardial infarction; TNF-α, tumor necrosis factor-α; IL6, interleukin 6; APN-KO mice, adiponectin knockout mice.

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murine muscle cells and upregulated in response to muscle hypertrophy in mice [10]. FGF21 is also induced in human skeletal muscle in response to insulin stimulation [11]. Thus, FGF21 may act as an insulin-sensitizing myokine. A recent report showed that FGF21-KO mice exhibit severer cardiac hypertrophy and dysfunction in response to isoproterenol infusion [12]. FGF21-KO mice also show enhanced cardiac injury following ischemia-reperfusion [13]. Thus, FGF21 may be protective against myocardial injury and dysfunction under pathological conditions. However, nothing is known about the impact of FGF21 on cardiac remodeling in response to chronic myocardial ischemia. Here, we investigated whether skeletal muscle-derived FGF21 influences the adverse myocardial remodeling after myocardial infarction.

2. Material and methods

2.1. Materials

Mouse CD31 antibody was purchased from BD Pharmingen (San Jose, CA). Mouse sarcomeric actinin antibody was purchased from Sigma. Plasma adiponectin levels were determined by ELISA kit (Otsuka Pharmaceutical Co. Ltd.). Plasma FGF21 levels were measured by ELISA kit (R & D system) [14]. Adenoviral vectors expressing mouse full-length FGF21 (Ad-FGF21) were constructed under the control of the CMV promoter [15,16]. Adenoviral vectors expressing β-galactosidase (Ad-β-gal) were used as controls [17].

2.2. Mouse model of myocardial infarction

Male C57BL6 (WT) and adiponectin-knockout (APN-KO) mice [18] at the age of 10 weeks were subjected to myocardial infarction surgery as previously described [19]. Briefly, the left anterior descending coronary artery (LAD) was permanently ligated with 8–0 nylon suture after mice were anesthetized with sodium pentobarbital intraperitoneally (50 mg/kg). Buprenorphine at 0.25 mg/kg was administered before surgery and after surgery for analgesia every 8 h for 48 h. Ad-FGF21 or Ad-β-gal (1×10^9 plaque-forming units (pfu)/mouse) was injected into five different sites of adductor muscle in left hindlimb 3 days prior to the surgery. Study protocols were approved by the Institutional Animal Care and Use Committee at Nagoya University. Our study conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication, 8th Edition, 2011).

2.3. Histological analyses

Mice were sacrificed at 2 weeks post-MI. Optimal cutting temperature (OCT) compound (Sakura, Tokyo, JAPAN)-embedded sections (5 μm thickness) were histologically analyzed. To detect the infarct area, heart sections were stained with Masson's trichrome. Infarct size was calculated as total infarct circumference divided by total LV circumference. Capillary density was assessed by immunohistological staining of CD31 at border zone of infarct heart tissue. Apoptosis in the remote zone was assessed by a terminal deoxynucleotidyltransferase-mediated dUTP-nick end labeling (TUNEL) staining using the In Situ Cell Death detection kit (Roche Diagnostics) [20]. DAPI was used for counter staining. Cardiomyocytes were determined by sarcomeric actinin staining. The mean number of TUNEL-positive cells from five random fields (magnification of ×40) was calculated.

2.4. Echocardiographic analysis

Transthoracic echocardiography was performed to evaluate cardiac function of mice at 2 weeks after MI [20]. Left ventricular diastolic diameter (LVDd) and LV systolic diameter (LVSd) were measured by M-mode images by using an Acuson Sequioa C-256 machine with a 15-MHz probe. LV fractional shortening was calculated as (LVDd − LVSd)/LVDd × 100 (%).

2.5. Measurement of blood pressure and heart rate

Systolic blood pressure (SBP) and heart rate (HR) were measured by tail cuff method with an automatic sphygmomanometer (BP98A; Saffron) at the tail artery while the animals were restrained as previously described [21].

2.6. Quantitative analysis of mRNA

Gene expression levels were quantified by real-time PCR method. Total RNA was extracted with RNeasy-Mini Kit (Qiagen) and reverse-transcribed by using the Revatra Ace (Toyobo) [20]. PCR was performed with a Bio-Rad real-time PCR detection system using THUNDERBIRD SYBR qPCR Mix as a double-standard DNA-specific dye. Primers were 5′-TCACCACCATGGAGAAGGC-3′ and 5′-GCTAAGCAGTTGGTGGTGCA-3′ for mouse GAPDH, 5′-CGGAGTCCGGGCAGGT-3′ and 5′-GCTGGGTAGAGAATGGATGAACA-3′ for mouse TNF-α, 5′-CTTCCATCCAGTTGCCTTCTTG-3′ and 5′-AATTAAGCCTCCGACTTGTGAAG-3′ for mouse IL6. All results were normalized to GAPDH.

2.7. Statistical analysis

Data are shown as mean ± S.E. Differences between groups were evaluated by the Student's *t* test for 2 groups or analysis of variance (ANOVA) for more than 3 groups. A value of $P < 0.05$ was accepted as statistically significant. All statistic calculations were performed by using SPSS for Windows.

3. Results

3.1. Intramuscular injection of Ad-FGF21 improves cardiac function after MI

To investigate the impact of muscle-derived FGF21 on cardiac remodeling post-MI, Ad-FGF21 or control Ad-β-gal was injected into adductor muscle of left hindlimb of WT mice 3 days prior to permanent LAD ligation. Ad-FGF21 treatment increased plasma FGF21 levels in WT mice at 2 weeks after sham or MI operation compared with control Ad-β-gal (sham/control Ad-β-gal; 678 ± 125 pg/ml, sham/Ad-FGF21; 4247 ± 421 pg/ml, MI/control Ad-β-gal; 695 ± 256 pg/ml MI/Ad-FGF21; 3509 ± 774 pg/ml). There were no differences in body weight (BW), systolic blood pressure (BP), heart rate (HR), heart weight (HW) and lung weight (LW) in control and Ad-FGF21-treated WT mice after sham operation (Table 1). MI surgery led to a reduction in systolic BP in WT mice, and this reduction was restored by Ad-FGF21 administration (Table 1). WT mice subjected to MI showed a significant increase in HW, LW, HW to BW

Table 1
Characteristics of WT mice at 2 weeks after sham or MI operation.

	Sham		MI	
	Control	FGF21	Control	FGF21
Body weight (g)	25.2 ± 0.6	24.1 ± 0.4	24.3 ± 1.2	25.4 ± 0.6
Systolic BP (mmHg)	106.6 ± 1.9	105.8 ± 8.0	74.4 ± 3.6**	100.2 ± 3.0##
Heart rate (rpm)	575 ± 11	542 ± 17	535 ± 13	576 ± 31
Heart weight (mg)	121 ± 4	116 ± 4	186 ± 10**	137 ± 6##
Lung weight (mg)	139 ± 5	131 ± 3	184 ± 13**	152 ± 1#

MI: myocardial infarction, WT: wild type, BP: blood pressure.
N = 5 in each group, **P < 0.01 versus Sham/Control group, #P < 0.05 versus MI/Control group, ##P < 0.01 versus MI/Control group.

ratio and LW to BW ratio, which was attenuated by Ad-FGF21 treatment (Fig. 1A, B and Table 1). Ad-FGF21 did not affect the ratios of HW/BW and LW/BW in sham-operated WT mice. Moreover, Ad-FGF21 had no effects on BW and HR in WT mice after MI (Table 1).

To examine the effect of FGF21 on left ventricular function, echocardiography was performed at 2 weeks after sham or MI operation. Administration of Ad-FGF21 to WT mice led to decreased LVDd and increased FS at 2 weeks after MI compared with control treatment, whereas Ad-FGF21 did not affect these parameters in sham-operated mice (Fig. 1C).

3.2. FGF21 increases capillary density and reduces myocyte apoptosis in post-MI hearts

To determine infarct size post-MI, heart tissues were stained with Masson's trichrome. Although Ad-FGF21 treatment minimized LV dilatation, no significant differences were observed in the ratio of total infarct length to total LV circumstance between control and FGF21-treated WT mice (Fig. 2A).

Because impaired angiogenesis contributes to the progression of heart failure after MI [22], capillary density in peri-infarct areas was assessed by staining with CD31. Ad-FGF21 treatment significantly augmented the frequency of CD31-positive cells in the borderline area of infarct hearts (Fig. 2B). To evaluate the extent of myocyte apoptosis, histological sections from the remote areas of infarct hearts were stained with TUNEL. Ad-FGF21 reduced the numbers of TUNEL-positive myocytes (Fig. 2C).

3.3. FGF21 reduces expression of pro-inflammatory cytokines in the heart after MI

Inflammation participates in the development of adverse cardiac remodeling post-MI [23]. To determine the inflammatory

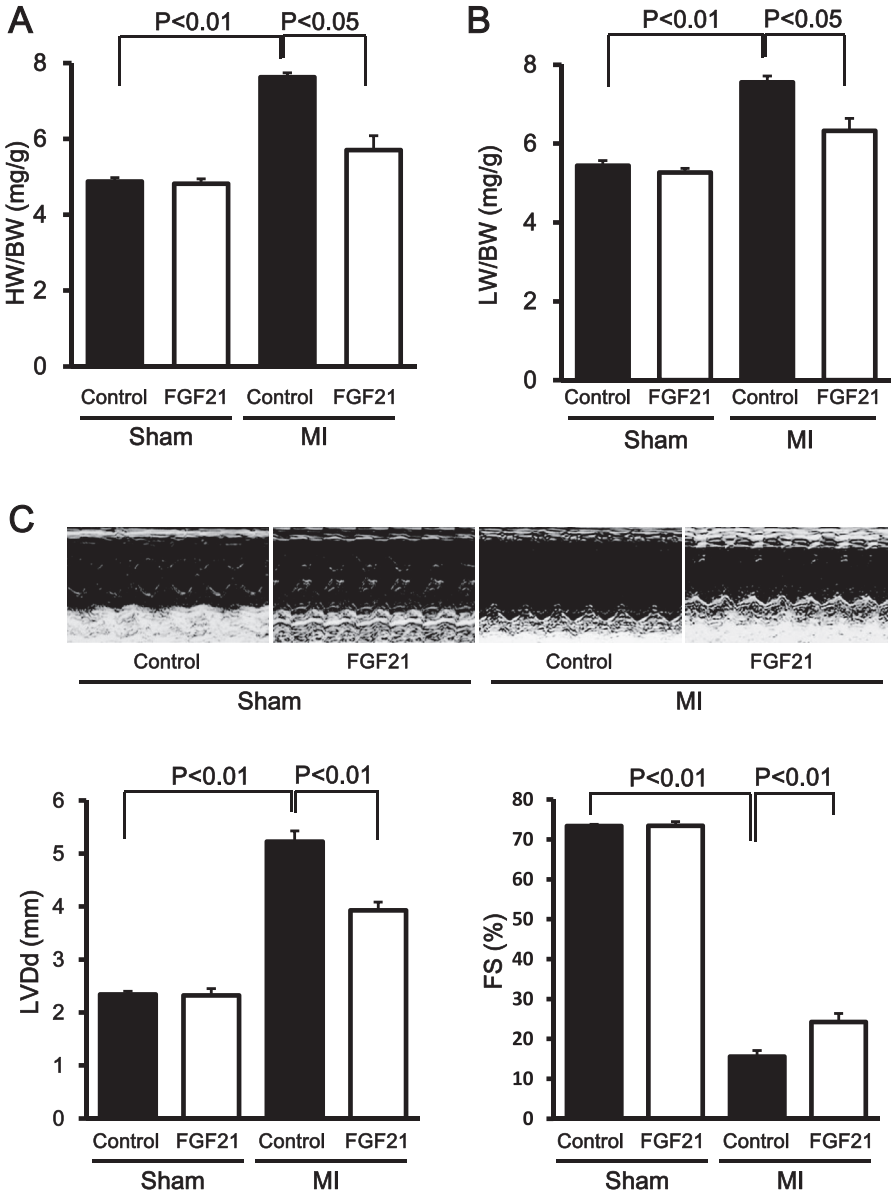

Fig. 1. Intramuscular injection of FGF21 improves cardiac function after myocardial infarction (MI). **A**, Heart weight (HW)/body weight (BW) ratio in Ad-β-gal (control) and Ad-FGF21 (FGF21)-treated WT mice at 2 weeks after sham or MI operation (n = 5 or 6). **B**, Lung weight (LW)/BW ratio of control and FGF21-treated WT mice at 2 weeks after sham or MI operation (n = 5 or 6). **C**, Echocardiographic analyses of control and FGF21-treated WT mice at 2 weeks after sham or MI operation. Left ventricular diastolic diameter (LVDd) and fractional shortening (FS) were analyzed (n = 5 or 6).

response in hearts of WT mice after MI, expression of pro-inflammatory cytokines was quantified by real-time PCR methods. Injection of Ad-FGF21 suppressed mRNA levels of TNF-α and IL6 in heart tissue of WT mice (Fig. 2D).

3.4. Adiponectin is involved in FGF21-mediated improvement of cardiac function

Systemic delivery of FGF21 protein is reported to increase circulating levels of adiponectin, which is a cardioprotective and insulin sensitizing adipokine [24,25]. We assessed plasma adiponectin levels in WT mice that had been intramuscularly treated with Ad-FGF21 or control Ad-β-gal. Ad-FGF21-treated mice showed increased plasma levels of adiponectin at 17 days after intramuscular injection i.e. at 2 weeks after MI (Fig. 3A).

To test the possible involvement of adiponectin in the beneficial actions of FGF21 on cardiac function in response to chronic ischemia, we examined the effect of FGF21 on cardiac parameters of the post-MI hearts in adiponectin-knockout (APN-KO) mice. Ad-FGF21 or control Ad-β-gal was intramuscularly delivered into APN-KO and WT mice at 3 days before permanent LAD ligation. Ad-FGF21 treatment led to an increase in plasma FGF21 level in APN-KO mice at 2 weeks post-MI compared with control Ad-β-gal (Ad-FGF21; 4230 ± 1228 pg/ml, control Ad-β-gal; 661 ± 168 pg/ml, $P < 0.05$) (Fig. 3B), and plasma FGF21 levels in Ad-FGF21-treated APN-KO mice were similar to those in Ad-FGF21-treated WT mice. No differences were observed in BW, systolic BP, HR, HW and

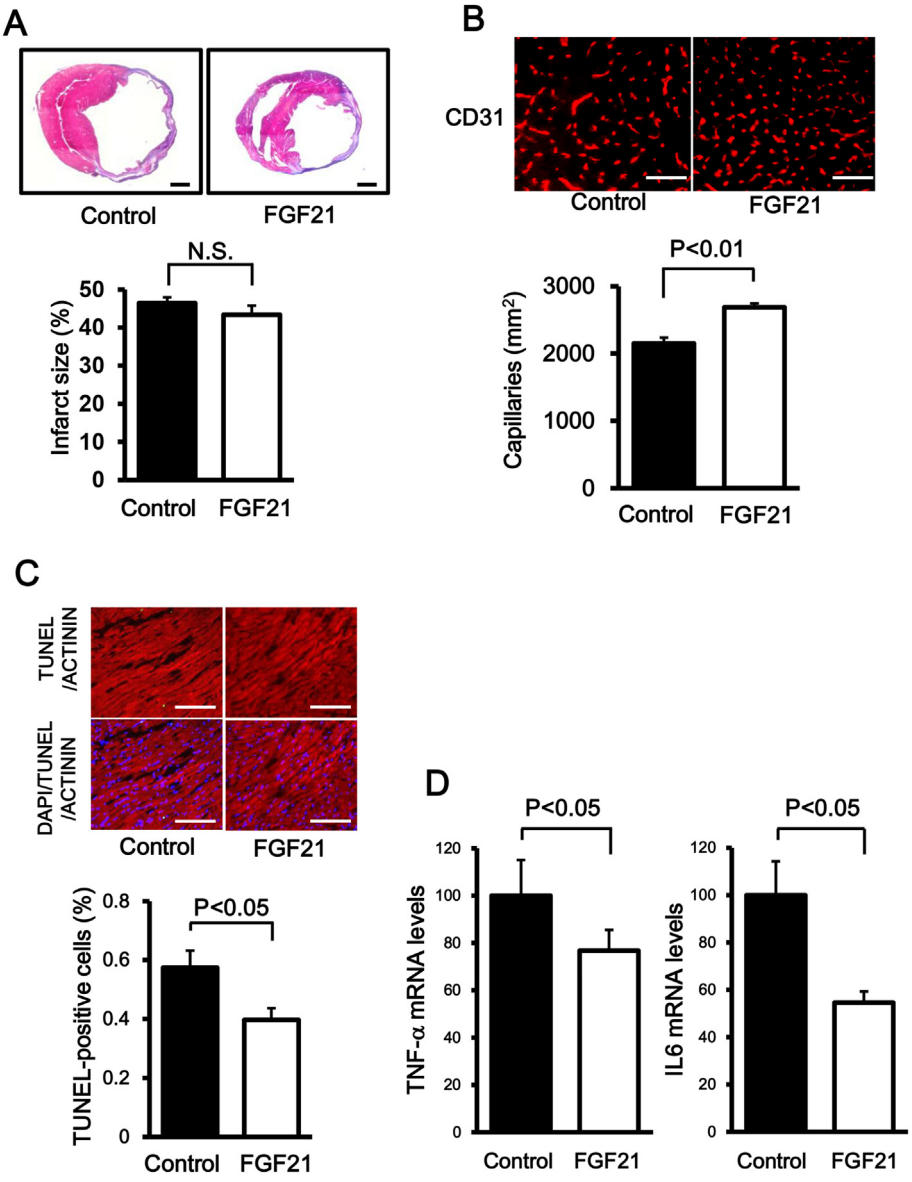

Fig. 2. FGF21 enhances capillary formation and reduces myocyte apoptosis in WT mice after MI. **A**, Infarct size of Ad-β-gal (control) and Ad-FGF21 (FGF21)-treated WT mice at 2 weeks after MI. Upper panels show representative photographs of heart sections stained by Masson's trichrome. Lower panel shows quantitative evaluation of relative infarct size (n = 8). Scale bars = 1 mm. **B**, Capillary density at the border zone of infarct hearts from control and FGF21-treated WT mice at 2 weeks after MI. Upper panels show representative immunohistological images stained with CD31 (red). Lower panel shows quantitative analysis of CD31-positive capillaries (n = 8). Scale bars = 50 μm. **C**, Cardiac myocyte apoptosis at the remote area from infarct hearts of control and FGF21-treated WT mice at 2 weeks post-MI. Upper panels show representative photographs of heart sections stained by TUNEL (green), and with sarcomeric actinin (red) and DAPI (blue). Lower panel shows quantitative analysis of apoptotic cardiomyocytes (n = 8). Scale bars = 50 μm. **D**, mRNA levels of TNF-α and IL6 in the heart tissues from control or FGF21-treated WT mice at 2 weeks after MI (n = 6). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

LW post-MI between control and FGF21-treated APN-KO mice (Table 2). Administration of Ad-FGF21 did not lead to a significant reduction of HW/BW and LW/BW ratios in APN-KO mice at 2 weeks after MI in contrast to the suppression of these parameters by Ad-FGF21 in WT mice (Fig. 3C and D). Similarly, there was a trend toward reduced LVDd and increased FS in Ad-FGF21-treated APN-KO mice relative to control APN-KO mice at 2 weeks after MI, but this was not statistically significant (Fig. 3E). In addition, Ad-FGF21 had no effects on capillary density in peri-infarct areas of APN-KO mice (Fig. 3F). Furthermore, administration of Ad-FGF21 did not reduce the mRNA expression of TNF-α and IL6 in heart tissue in APN-KO mice, although Ad-FGF21 significantly reduced the expression of these genes in the myocardium in WT mice (Fig. 3G).

4. Discussion

The present study demonstrated that administration of FGF21 ameliorates the development of systolic dysfunction after MI.

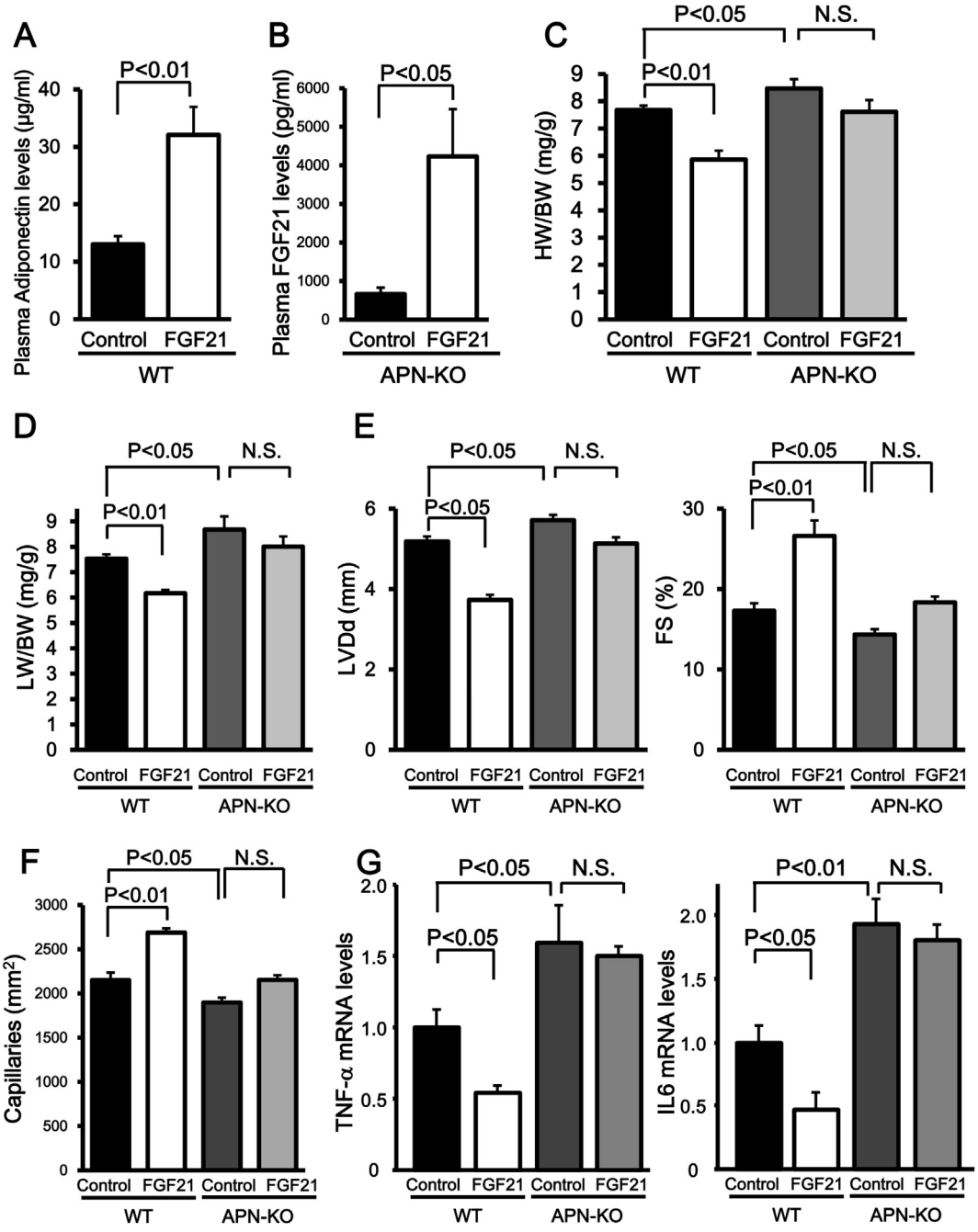

Fig. 3. Involvement of adiponectin in the beneficial effects of FGF21 on cardiac function post-MI. **A**, Plasma adiponectin concentration of WT mice at 2 weeks after MI. Ad-FGF21 (FGF21) or Ad-β-gal (control) was intramuscularly administrated to WT mice 3 days prior to MI operation (n = 8). **B**, Plasma FGF21 levels of APN-KO mice at 2 weeks after MI. APN-KO mice were intramuscularly treated with Ad-FGF21 (FGF21) or Ad-β-gal (control) 3 days prior to MI surgery (n = 6). **C**, Heart weight (HW)/body weight (BW) ratio of WT and APN-KO mice treated with FGF21 or control at 2 weeks after MI (n = 6). **D**, Lung weight (LW)/BW ratio in WT and APN-KO mice treated with FGF21 or control at 2 weeks post-MI (n = 6). **E**, Echocardiographic analyses of WT and APN-KO mice treated with FGF21 or control at 2 weeks post-MI. Left ventricular diastolic diameter (LVDd) and fractional shortening (FS) were analyzed (n = 6). **F**, Capillary density at the border zone of infarct hearts from WT and APN-KO mice treated with FGF21 or control at 2 weeks post-MI (n = 6). **G**, Expression of TNF-α and IL6 in the heart tissues from WT and APN-KO mice treated with FGF21 or control at 2 weeks post-MI (n = 6).

Table 2
Characteristics of APN-KO mice at 2 weeks after MI operation.

	Control	FGF21
Body weight (g)	22.7 ± 0.7	23.8 ± 0.9
Systolic BP (mmHg)	83.3 ± 2.9	79.7 ± 4.3
Heart rate (rpm)	555 ± 19	531 ± 17
Heart weight (mg)	192 ± 6	180 ± 7
Lung weight (mg)	197 ± 14	190 ± 7

MI: myocardial infarction, APN-KO: adiponectin knockout, BP: blood pressure.
N = 6 in each group.

Intramuscular injection of FGF21 using an adenoviral expression system enhances circulating levels of FGF21 in WT mice and reduces LV contractile dysfunction, myocyte apoptosis and capillary loss post-MI with an accompanying increase in plasma adiponectin level. The salutary effects of FGF21 on cardiac function, angiogenesis and inflammatory response following MI were diminished in APN-KO mice. Adiponectin has been shown to exert anti-hypertrophic and pro-angiogenic activities in a model of MI [19]. Approaches to suppress myocardial apoptosis, and enhance neovascularization in the heart are believed to prevent the progression of heart failure after MI [2,3,26]. Thus, it is conceivable that FGF21 improves the pathological remodeling of myocardium in response to chronic ischemia, at least in part, through upregulation of adiponectin. In addition, recent reports show that FGF21 functions as a positive regulator of adiponectin secretion and that the insulin sensitizing actions of FGF21 are dependent on adiponectin [24,25]. Furthermore, adiponectin has protective functions against various obese complications including ischemic heart disease, type 2 diabetes, hypertension, and chronic kidney disease [18,21,27]. These results suggest that the FGF21-adiponectin regulatory axis can contribute to protection against various metabolic and cardiovascular disorders.

FGF21 exerts various cellular functions through FGF receptor (FGFR) 1 and its co-receptor β-klotho [12]. FGF21 promotes glucose uptake in adipocytes via FGFR1/β-klotho system [28]. FGF21 protects against myocardial injury and apoptosis after ischemia-reperfusion via FGFR1/β-Klotho/Akt system in cardiomyocytes [13]. FGF21 has also been reported to prevent cardiomyocyte apoptosis in response to hypoxia/reoxygenation by reducing oxidative stress through Akt-dependent pathway [29]. Consistent with these observations, our study indicates that FGF21 can attenuate myocyte apoptosis in post-MI hearts in vivo. Collectively, these data suggest that FGF21 may protect the hearts from adverse remodeling in vivo through at least two mechanisms; activation of adiponectin-dependent signaling and activation of FGFR1/β-Klotho signaling in the myocardium.

Accumulating evidence indicates that inflammation is involved in the progression of adverse cardiac remodeling after MI [23]. Administration of TNF-α inhibitor improves adverse ventricular remodeling following MI [30]. In addition, TNF-α receptor 1 deficient mice exhibit amelioration of LV function and hypertrophy post-MI [31]. In the present study, administration of FGF21 suppressed expression of pro-inflammatory cytokines such as TNF-α and IL6 in post-MI hearts. Thus, it is plausible that FGF21 exerts anti-inflammatory actions, thereby contributing to protection against pathological myocardial remodeling.

A recent paper demonstrated that FGF21 functions as a myokine that promotes browning of white adipose tissue by communicating with white adipose tissue [32]. Our findings indicate the possibility that muscle-derived FGF21 affects the ischemic myocardium. On the other hand, it has been shown that FGF21 produced by liver and adipose is protective against acute ischemic injury in the heart [13]. Furthermore, FGF21 is expressed in cardiomyocytes and protects against cardiac hypertrophy [12]. Recently, it has been reported that FGF21 is secreted from cardiac myocytes in response to hypoxia and that FGF21 administration restores cardiac function following global ischemia in an ex vivo Langendorff system [33]. Thus, FGF21 secreted from various cells exerts beneficial actions on the myocardium via an autocrine, paracrine or endocrine mechanism.

In conclusion, we found that muscle-derived overproduction of FGF21 improves cardiac dysfunction in response to chronic ischemia by its abilities to suppress myocyte apoptosis, and promote capillary formation. Furthermore, adiponectin mediates the cardioprotective effects of FGF21 in a murine model of MI. Therefore, strategies to increase FGF21 production could be useful for prevention of adverse myocardial remodeling post-MI.

Conflict of interest

None.

Acknowledgments

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