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Effect of adiponectin on cardiac β-catenin signaling pathway under angiotensin II infusion

Yuya Fujishima [a], Norikazu Maeda [a,*], Keisuke Matsuda [a], Noriyuki Komura [a], Ayumu Hirata [a,b], Takuya Mori [a], Ryohei Sekimoto [a], Yu Tsushima [a], Hitoshi Nishizawa [a], Tohru Funahashi [a,b], Iichiro Shimomura [a]

[a] Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2-B5 Yamada-oka, Suita, Osaka 565-0871, Japan
[b] Department of Metabolism and Atherosclerosis, Graduate School of Medicine, Osaka University, 2-2-B5 Yamada-oka, Suita, Osaka 565-0871, Japan

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ABSTRACT

Obesity is associated with heart failure and cardiac hypertrophy. Adiponectin has been shown to play a protective role for cardiovascular diseases. The β-catenin signaling pathway is deeply involved in cardiac hypertrophy. However, the effect of adiponectin on β-catenin signaling has not been investigated in cardiac hypertrophy. Present study aimed to clarify the involvement of adiponectin and β-catenin signaling pathway in the mouse model of angiotensin II (AngII)-induced cardiac hypertrophy. In hearts of Wild type (WT) mice, AngII dose-dependently augmented cytosolic β-catenin protein level. WT and adiponectin knockout (Adipo-KO) mice were administered with AngII at 2.4 mg/kg/day for 14 days and were also injected with adenovirus expressing the adiponectin (Ad-Adipo) or the β-galactosidase (Ad-βgal). Cardiac mRNA levels relating to hypertrophy and β-catenin signaling were increased in Adipo-KO mice and these changes were reversed by Ad-Adipo. Phosphorylation of Akt was increased in Adipo-KO mice and such increases were reversed by Ad-Adipo. Furthermore, the phosphorylation of glycogen synthase kinase 3β (GSK3β) at Ser^9 and cytosolic β-catenin level were increased in Adipo-KO mice and they were significantly reduced by Ad-Adipo treatment. Phosphorylation of mammalian target of rapamycin (mTOR) was reduced by Ad-Adipo-mediated adiponectin supplementation in WT and Adipo-KO mice. The current study suggests that adiponectin attenuates AngII-induced cardiac hypertrophic signals partly through Akt/GSK3β/β-catenin and Akt/mTOR pathways.

1. Introduction

Adiponectin has been shown to play the critical role in the development of metabolic syndrome and atherosclerosis [1]. Evidently, hypoadiponectinemia is closely associated with obesity-related diseases such as coronary artery diseases and type 2 diabetes [1]. A series of in vivo experiments have been demonstrated that adiponectin possesses anti-diabetic [2–4], anti-atherogenic [5,6], and anti-inflammatory [7] properties. Obesity-related diseases are also associated with heart failure and cardiac hypertrophy. Adiponectin exhibits the cardio-protective effect in animal model such the transverse aortic constriction (TAC) [8,9], the ischemia/reperfusion (I/R) injury [10], and the continuous angiotensin II (AngII) infusion [11]. Interestingly, the transplantation of adiponectin knockout (Adipo-KO) mice-derived adipocyte cell sheet to cardiac surface area resulted in higher mortality after myocardial infarction than wild-type (WT) mice-derived adipocyte cell sheet, indicating the direct cardio-protective effect of adiponectin [12]. The 5′ AMP-activated protein kinase (AMPK) is considered as an essential player in cardio-protective action of adiponectin [13], but AMPK does not seem to give a complete account of adiponectin action [14].

Adiponectin is structurally similar to the complement factor C1q [15,16] and resembles the trimetric topology of tumor necrosis factor-α (TNF-α) [17]. Interestingly, adiponectin suppresses phagocytosis via the receptor for C1q [7] and inhibits TNF-α expression in adipose tissues [3]. In the collagen-induced arthritis (CIA) murine model, the adenovirus-mediated overexpression of circulating adiponectin significantly ameliorated arthritis and inhibited complement C1q and C3 deposition in the joints [18]. These observations suggested the molecular interaction of adiponectin and complement C1q. Our group recently demonstrated the direct binding of adiponectin and C1q in human serum by the immunoprecipitation and -blotting [19]. We also generated ELISA system and detected the C1q–adiponectin complex in human serum [19]. Importantly, complement C1q activates Wnt/β-catenin signal pathway and accelerates aging in mice [20]. The significance of β-catenin signaling has been shown in cardiac hypertrophy [21]. The AngII signaling and the excess of reactive oxygen species

* Corresponding author. Fax: +81 6 6879 3739.
E-mail address: norikazu_maeda@endmet.med.osaka-u.ac.jp (N. Maeda).

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(ROS) are also implicated in aging and fibrosis [22]. Increasing evidences suggest that AngII and β-catenin may be associated in various diseases, especially in cardiovascular diseases, but the crosstalk between AngII and β-catenin signaling pathways has not been fully examined. Moreover, the effect of adiponectin on β-catenin signaling pathway has not been clarified. We hypothesized a protective action of adiponectin on cardiac hypertrophy targeting AngII and β-catenin signaling pathways. To test our hypothesis, Adipo-KO mice and AngII-induced cardiac hypertrophic model were introduced.

2. Materials and methods

2.1. Animals

Adiponectin knockout (Adipo-KO) mice were generated and backcrossed as described previously [3]. Experimental protocol of AngII infusion was similar to our previous study [11]. Briefly, AngII (#A2900, Sigma–Aldrich Inc., St. Louis, MO) was dissolved in 0.01 M acetic acid. Wild-type (WT) and Adipo-KO mice were anesthetized and the back body hair was cut and shaved and were implanted osmotic minipumps (Alzet mini-osmotic pump model 2002, Durect Corp., Cupertino, CA) containing 1.2 or 2.4 mg/kg/day of AngII in the midscapular region of mice at 10 weeks of age. Supplementation of adiponectin was performed as described previously [11]. Adenovirus expressing the full-length mouse adiponectin (Ad-Adipo) or the β-galactosidase (Ad-βgal) was prepared using the Adenovirus Expression Vector Kit (Takara, Kyoto, Japan). WT and Adipo-KO mice were injected with adenovirus at 1.6×10^7 plaque-forming units (p.f.u.)/body via the jugular vein at 2 days before AngII infusion at 2.4 mg/kg/day. Blood pressures were measured with an automatic sphygmomanometer (BP98A, Softron Co., Tokyo, Japan) from the tail artery while mice were passive under the same condition (from 17:00 to 19:00). Ten readings were taken for each measurement and the mean value was assigned to each individual animal. On the 14 days after implantation of the osmotic minipump, mice were euthanized by intraperitoneal injection of medetomidine (0.3 mg/kg body wt), midazolam (4 mg/kg body wt), and butorphanol (5 mg/kg body wt). To monitor the adequacy of anesthesia, we carefully tested for no spontaneous movements of mice tail by mild stimulation. Mice were kept in rooms set at 22 °C with a 12:12 h dark–light cycle (lights on from 8:00 to 20:00). All experiments were conducted in male mice fed with normal chow. The experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine. This study also conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. Quantitative RT-PCR

Isolation of total RNA and production of cDNA were performed as described previously [11]. RT-PCR was performed on the ViiATM 7 real-time PCR system (Life Technologies) using the THUNDERBIRDTM qPCR Mix (TOYOBO, Osaka, Japan) according to the instructions provided by the manufacturer. For quantitative precision, the same amount of total RNA was consistently used for each expression analysis and the expression level of each gene was normalized by the mRNA level of a housekeeping gene, ribosomal protein, large, P0 (Rplp0/36B4). The following is a list of the primers used in this study: mouse Collagen I, 5′-GTC CCAACCCCCAAAGAC-3′ and 5′-CAGCTTCTGAGTTTGGTGATA-3′; mouse Collagen III, 5′-TGGTTTCTTCTCACCCTTCTTC-3′ and 5′-TGCA TCCCAATTCATCT ACGT-3′; mouse TGF-β1 5′-GACGTCACTGG AGTTGTACGG-3′; and 5′-GCTGAATCGAAAGCCCTGT-3′ mouse Axin2, 5′-GAGAGTGAG CGGCAGAGC-3′ and 5′-CGGCTGACTCGTTC TCCT-3′; mouse Sfrp4, 5′-AGAGGCAATAGTCACTGACCTTCCAGAAG ATGTGAAG-3′ and 5′-TGTGAATTCAGGTACCCTTCCACAAGCCTTCCC TC-3′; mouse $p22^{phox}$, 5′-GTCCACCATGGAGCGATGTG-3′ and 5′-CA ATGGCCAAGCAGAC GGTC-3′; mouse $p47^{phox}$, 5′-GATGTTCC CCATT GAGGCCG-3′ and 5′-GTTTCAGGTCATCAGGCCGC-3′; mouse F4/80, 5′-CTTTGGCTATGGGCTTGGAGTC-3′ and 5′-GCAAGGAGGACAGAGT TATCGTG-3′; mouse C1qA, 5′-ACAAGGTCCTCACCAACC AG-3′ and 5′-GACAAAGGTCCCACTTGGAG-3′; mouse MCP-1, 5′-CAGCCAGATG CAGTTAACGC-3′ and 5′-CCCTACTCATTGGGATCATCTTG-3′.

2.3. Immunoblotting

Preparation of protein extracts from heart tissues was performed as described previously [8]. To obtain protein in soluble cytosolic fraction, hearts were lysed in a buffer containing 10 mM of sucrose, 5 mmol/L of NA_3VO_4, and protease inhibitor cocktails (complete mini, Roche, USA) by a micro-homogenizer under 4 °C. After homogenate was centrifuged at 14,000*g* for 30 min under 4 °C, the supernatant was centrifuged at 336,000*g* for 10 min under 4 °C. Ten microgram of the ultracentrifuged supernatant protein, as a cytosolic fraction, was subjected to 4–20% gradient SDS–PAGE gel and transferred to a nitrocellulose membrane (GE Healthcare, Little Chalfont, UK). For immunoblotting, the membrane was incubated with 1:1000 dilution of mouse anti-β-catenin (BD Biosciences, San Jose, CA), rabbit anti-GAPDH (Cell signaling technology, Danvers, MA), rabbit anti-phospho(Ser^{473})-AKT (Cell signaling technology, Danvers, MA), rabbit anti-AKT (Cell signaling technology, Danvers, MA), rabbit anti-phospho(Ser^{2448})-mTOR (Cell signaling technology, Danvers, MA), rabbit anti-mTOR (Cell signaling technology, Danvers, MA), rabbit anti-GSK3β (Cell signaling technology, Danvers, MA) or rabbit anti-phospho(Ser^{9})-GSK3β (Cell signaling technology, Danvers, MA). Detection was achieved by using the enhanced chemiluminescence kit (GE Healthcare).

2.4. Statistical analysis

All values were expressed as mean ± SEM. Differences between groups were analyzed by one-factor ANOVA and unpaired Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of angiotensin II infusion on cardiac -catenin signaling

To examine whether AngII affect on β-catenin signal in heart tissue, wild-type (WT) mice were firstly treated with AngII at 0, 1.2, and 2.4 mg/kg/day. Systolic blood pressures (sBP) (Fig. 1A) and the heart weight/body weight (HW/BW) ratios (Fig. 1B) were significantly increased by AngII administration, indicating that mice were systemically and successfully infused with AngII. The mRNA levels of Collagen I and III, markers for cardiac fibrosis, were significantly increased by AngII infusion (Fig. 1C). Interestingly, Axin2 and secreted frizzled-related protein 4 (Sfrp4), those are downstream and inhibitory molecules of Wnt/β-catenin signaling, were significantly elevated in AngII dose-dependent manner (Fig. 1C). AngII administration also increased amounts of cytosolic β-catenin in heart tissues (Fig. 1D).

3.2. Effect of adiponectin on blood pressure and heart weight under Ang II infusion

To investigate the involvement of adiponectin in cardiac β-catenin signaling, adiponectin knockout (Adipo-KO) mice and

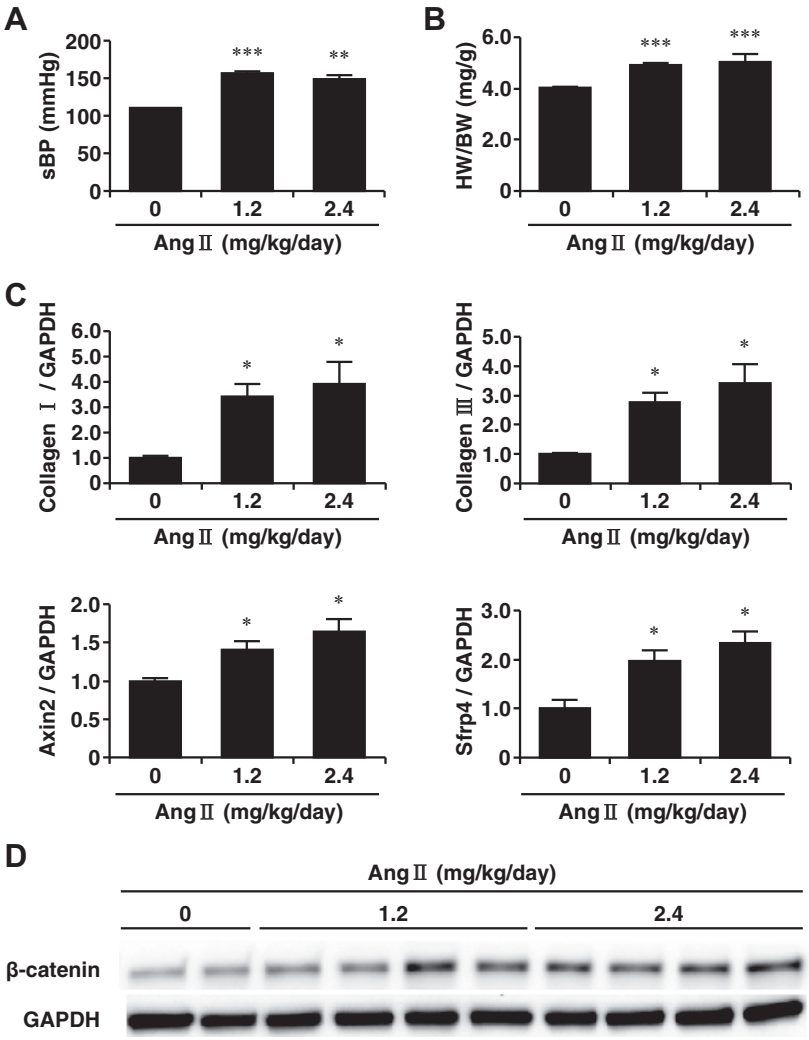

Fig. 1. Effect of angiotensin II infusion on cardiac β-catenin signaling. Wild-type (WT) mice were treated with angiotensin II (AngII) at 0, 1.2, and 2.4 mg/kg/day for 14 days. (A) Systolic blood pressures (sBP) at day 10 after AngII infusion. (B) Heart weight/body weight (HW/BW) ratio (mg/g) at day 14 after AngII infusion. (C) Cardiac relative mRNA levels at day 14 after AngII infusion. Values were normalized to GAPDH mRNA levels. (D) Cytosolic β-catenin protein levels in heart tissues at day 14 after AngII infusion. In panels A–C, data are mean ± SEM; $n = 3–4$ for each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the values of group with AngII at 0 mg/kg/day. Sfrp4, secreted frizzled-related protein 4.

adenovirus expressing adiponectin (Ad-Adipo) were used and mRNA levels were examined in the AngII-administered heart tissues. Similar to Fig. 1A, AngII infusion significantly elevated sBP in both WT and Adipo-KO mice, but there were no differences in sBP of these mice and Ad-Adipo had no effects on sBP (Fig. 2A). However, unlike changes of sBP, HW/BW ratios of Adipo-KO mice were significantly larger than those of WT mice in the control adenovirus expressing β-galactosidase (Ad-βgal) under AngII administration (Fig. 2B, lane 1 versus 3). Such increase of HW/BW ratio in Adipo-KO mice was significantly reduced by the treatment with Ad-Adipo (Fig. 2B, lane 3 versus 4). These results may indicate that adiponectin-mediated amelioration of HW/BW ratio were independent of sBP.

3.3. Effect of adiponectin on various gene expressions relating to cardiac fibrosis, inflammation, and -catenin signaling

The mRNA levels relating to cardiac fibrosis, inflammation, and β-catenin signaling, were next examined in these mice under AngII infusion (Fig. 2C–F). Significant increases of Collagen I and III, and transforming growth factor-β1 (TGF-β1) mRNA levels were observed in Adipo-KO mice than in WT mice in Ad-βgal groups (Fig. 2C, lane 1 versus 3). Ad-Adipo-mediated adiponectin treatment significantly decreased the AngII-induced increases of Collagen I and III, and TGF-β1 mRNAs (Fig. 2C, lane 1 versus 2, lane 3 versus 4). Reactive oxygen species (ROS) are involved in cardiac hypertrophy and fibrosis and NADPH oxidase is located upstream of ROS production [23]. The mRNA levels of NADPH oxidase subunit $p22^{phox}$ and $p47^{phox}$ were significantly higher in Adipo-KO mice relative to those of WT mice in Ad-βgal groups (Fig. 2.D, lane 1 versus 3) and such increases were suppressed by adiponectin supplement with Ad-Adipo (Fig. 2D, lane 3 versus 4).

Inflammatory response is also associated with cardiac hypertrophy and fibrosis and adiponectin has been shown to possess an anti-inflammatory effect [24]. Administration of adiponectin via Ad-Adipo significantly decreased F4/80 mRNA level in Adipo-KO mice (Fig. 2E, lane 3 versus 4). Furthermore, monocyte chemoattractant protein-1 (MCP-1) mRNA level was significantly increased in Adipo-KO mice compared to WT mice in Ad-βgal groups under AngII infusion (Fig. 2E, lane 1 versus 3) and its mRNA level was decreased by Ad-Adipo-mediated adiponectin treatment (Fig. 2E, lane 3 versus 4). Complement C1q has been recently demonstrated to activate Wnt/β-catenin signaling and promote aging [20], and is deeply involved in inflammation. Interestingly, cardiac C1q mRNA

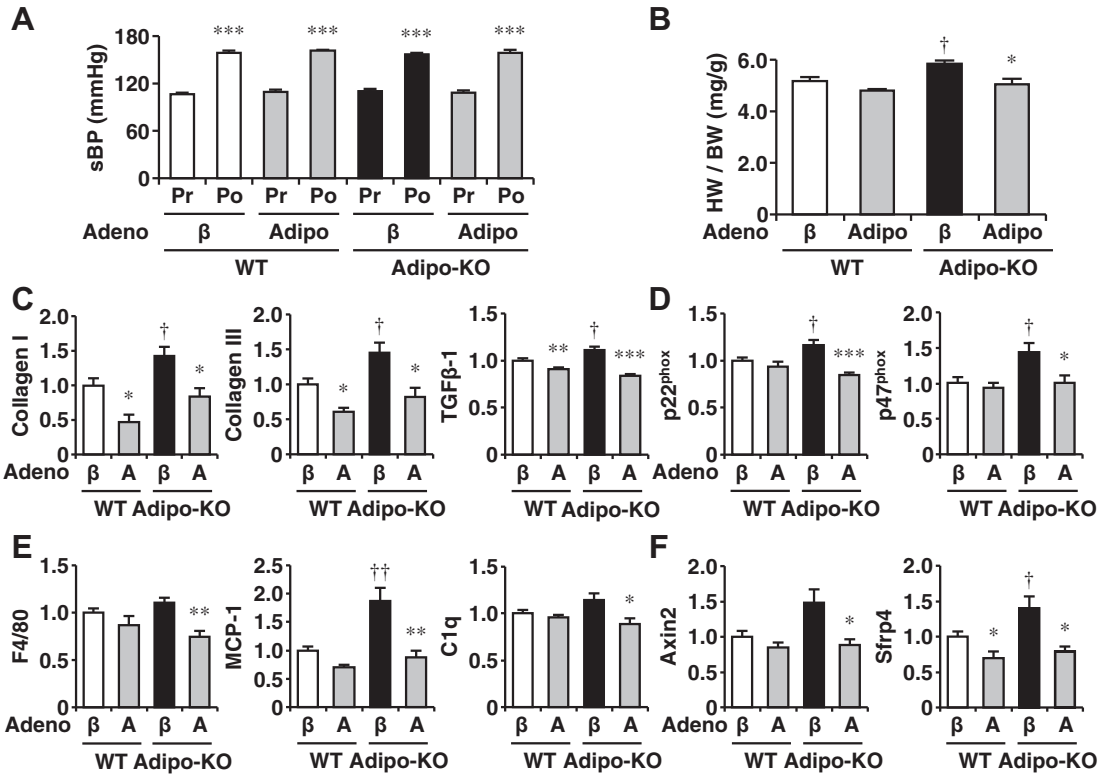

Fig. 2. Effect of adiponectin on various gene expressions relating to cardiac fibrosis, inflammation, and β-catenin signaling. Wild-type (WT) and adiponectin knockout (Adipo-KO) mice were intravenously injected with adenovirus expressing the full-length mouse adiponectin (Ad-Adipo) or the β-galactosidase (Ad-βgal) at 1.6×10^7 plaque-forming units (p.f.u.)/body at 2 days before 2.4 mg/kg/day of angiotensin II (AngII) infusion. (A) Systolic blood pressures (sBP) of pre- and post-AngII infusion. The sBP was measured at day 10 after AngII infusion. (B) Heart weight/body weight (HW/BW) ratio (mg/g) at day 14 after AngII infusion. (C–F) Cardiac relative mRNA levels at day 14 after AngII infusion. Shown are relative ratios to GAPDH mRNA level. Data are mean ± SEM; $n = 6$–9 for each group. In panel A, $^{***}P < 0.001$, compared with the values of pre-AngII infusion group in the same treatment. In panel B–F, $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the values of Ad-βgal group in WT or Adipo-KO mice. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, compared with the values of WT mice with Ad-βgal. Pr, pre-AngII infusion; Po, post-AngII infusion; β, Ad-βgal; A, Ad-Adipo; TGF-β1, transforming growth factor-β1; $p22^{phox}$, NADPH oxidase subunit $p22^{phox}$; $p47^{phox}$, NADPH oxidase subunit $p47^{phox}$; MCP-1, monocyte chemoattractant protein-1; Sfrp4, secreted frizzled-related protein 4.

level tended to increase in Adipo-KO mice compared to WT mice in Ad-βgal groups under AngII infusion (Fig. 2E, lane 1 versus 3; $P = 0.08$). Adiponectin supplement with Ad-Adipo significantly decreased C1q mRNA level in Adipo-KO mice (Fig. 2E, lane 3 versus 4). Axin2 mRNA level tended to increase in Adipo-KO mice compared to WT mice in Ad-βgal groups under AngII infusion (Fig. 2F, lane 1 versus 3; $P = 0.06$) and its level of Adipo-KO mice was significantly reduced by Ad-Adipo-mediated adiponectin supplement (Fig. 2F, lane 3 versus 4). Moreover, Sfrp4 mRNA level of Adipo-KO mice was significantly higher than of WT mice in Ad-βgal groups under AngII infusion (Fig. 2F, lane 1 versus 3) and adiponectin administration with Ad-Adipo significantly decreased Sfrp4 mRNA level in both WT and Adipo-KO mice (Fig. 2F, lane 1 versus 2, lane 3 versus 4). Fig. 2F suggested that adiponectin modulated β-catenin signaling in AngII-infused heart tissues.

3.4. Effect of adiponectin on -catenin signaling pathway under angiotensin II infusion

Finally, cardiac hypertrophic signaling molecules were examined in WT and Adipo-KO mice under AngII infusion (Fig. 3). AngII signaling augments phosphorylation of Akt partly through the increase of intracellular ROS level. Mammalian target of rapamycin (mTOR) is located downstream of Akt signal and accelerate the increases of cardiac hypertrophic gene expressions. Phosphorylation of Akt was significantly increased in Adipo-KO mice than in WT mice of Ad-βgal groups under AngII infusion (Fig. 3A and B, lane 1 versus 3). Strikingly, Ad-Adipo-mediated adiponectin administration significantly ameliorated the increases of phosphorylated Akt and mTOR under AngII infusion (Fig. 3A and B, lane 1 versus 2, 3 versus 4). Glycogen synthase kinase 3β (GSK3β) is a key molecule of β-catenin-dependent signaling pathway. The activated Akt suppresses a kinase activity of GSK3β through the phosphorylation of GSK3β at Ser^9 and it leads to prevent the degradation of β-catenin. Phosphorylated GSK3β was increased in Adipo-KO mice than in WT mice of Ad-βgal groups under AngII infusion (Fig. 3A and B, lane 1 versus 3) and such increase was significantly reduced by adiponectin treatment (Fig. 3A and B, lane 3 versus 4). Finally, amounts of cytosolic β-catenin were examined in this adenovirus study. Significant increase of cytosolic β-catenin was observed in Adipo-KO mice compared to WT mice under AngII infusion (Fig. 3C and D, lane 1 versus 3). AngII-induced increase of cytosolic β-catenin in Adipo-KO mice was significantly suppressed by Ad-Adipo-mediated adiponectin supplementation (Fig. 3A and B, lane 3 versus 4).

4. Discussion

The major findings of the present study were: (1) AngII dose-dependently increased cardiac β-catenin signaling. (2) Adiponectin-deficiency caused changes of several mRNA levels relating to cardiac hypertrophy, fibrosis, ROS, and inflammation under AngII infusion. Such changes were reversed by the supplement of adiponectin. (3) Hypertrophic signals involving β-catenin were augmented in Adipo-KO mice compared to WT mice and these signaling pathways were significantly suppressed by the supplement of adiponectin.

The β-catenin pathway contributes to pathological cardiac hypertrophy and its inhibition will be desired for a novel therapeutic strategy for hypertrophic heart diseases [21]. Cardiac

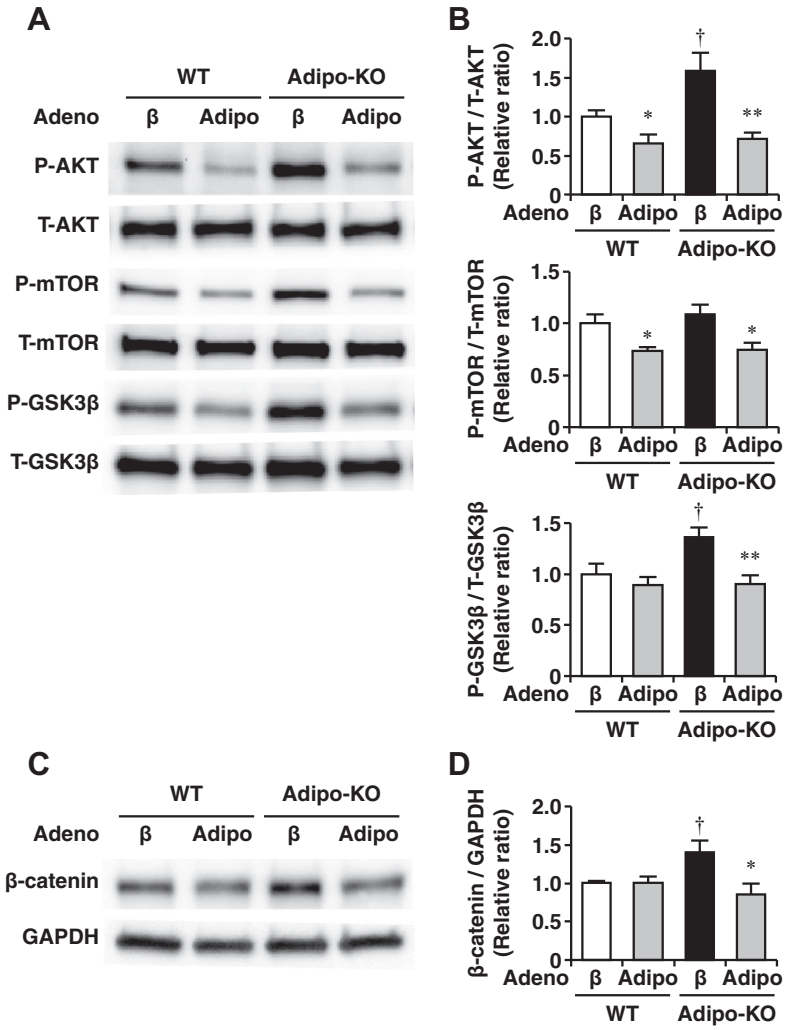

Fig. 3. Effect of adiponectin on cardiac β-catenin signaling pathway under angiotensin II infusion. Protein was extracted from the murine hearts under angiotensin II (AngII) infusion and immunoblottings were performed as described in Section 2. (A and C) Immunoblots for protein relating to β-catenin signaling pathway. In the panel C, cytosolic protein was applied to SDS–PAGE gel. (B and D) Relative protein levels associating with β-catenin signaling pathway. Data are mean ± SEM; $n = 6$ for each group. In panel B and D, $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the values of Ad-βgal group in Wild-type (WT) or adiponectin knockout (Adipo-KO) mice. $^{\dagger}P < 0.05$, compared with the values of WT mice with Ad-βgal. β, Ad-βgal; Adipo, Ad-Adipo; mTOR, mammalian target of rapamycin, GSK3β, glycogen synthase kinase 3β.

hypertrophy is mainly caused by the activation of a neurohumoral pathway involving several molecules such as AngII and catecholamines. AngII not only stimulates ERK signaling pathway, which is known as a major pathway of AngII, but also increases intracellular ROS level [23,25]. In previous [11] and present studies, cardiac ROS and NADPH oxidase subunit mRNA levels were significantly increased in Adipo-KO mice and such increases were reversed by the supplementation of adiponectin, when mice were infused with AngII. Excess of ROS augments phosphorylation of Akt and the Akt activation locates upstream on cardiac hypertrophy. As shown in Fig. 3A and B, a significant increase of Akt phosphorylation in Adipo-KO mice may be accounted for by the higher ROS level induced by AngII in Adipo-KO mice, than WT mice. Active Akt phosphorylates mTOR and GSK3β at the Ser^{9} residue [21]. On the other hand, AMPK negatively regulates mTOR signaling through activation of the tuberous sclerosis complex (TSC) [26] and AMPK is one of target molecules of adiponectin [13]. However, there were no significant changes of AMPK activation in hearts of WT and Adipo-KO mice after AngII infusion for 2 weeks (data not shown), suggesting that the mTOR inactivation by adiponectin treatment may be mediated by Akt signaling, not by AMPK signaling pathway. Strikingly, AngII activated GSK3β/β-catenin pathway and its activation was augmented by adiponectin-deficiency. Therefore, adiponectin may suppress AngII-induced cardiac hypertrophy partly through Akt signaling involving mTOR and GSK3β/β-catenin pathways.

Epidemiological evidence indicates that hypoadiponectinemia is one of risk factors of cancers such as breast cancer, endometrial cancer, colorectal cancer, leukemia, prostate cancer, and pancreatic cancer [27]. The β-catenin controls key developmental gene expression programs as a transcriptional coactivator and may play a significant role of carcinogenesis [28]. Adiponectin has been shown to effect on various molecules implicating in carcinogenesis [27,29], but the association of adiponectin and β-catenin signaling has not been fully elucidated. It was reported that adiponectin suppressed intracellular β-catenin level and its nuclear activity, and inhibited cell proliferation of MDA-MB-231 cells, which are recognized as typical human breast cancer cells [30]. On the other hand, adiponectin stimulated the proliferation of hippocampal neural/progenitor cells by enhancing β-catenin signaling [31]. Adipose mRNA expressions of Wnt ligand family were variously altered in adiponectin-transgenic mice, but amount of β-catenin protein was not changed in adipose tissues compared to WT mice under normal condition [32]. Present study for the first time demonstrated the suppressive effect of adiponectin on cardiac β-catenin

signaling induced by AngII. These results suggest that the effect of adiponectin on β-catenin signaling may be different in cell and tissue types and be dependent on stimulatory conditions affecting on β-catenin signaling.

Our group recently showed that circulating adiponectin binds to complement C1q in human serum [19] and serum ratio of C1q-adiponectin complex/total adiponectin correlates with metabolic diseases [33]. The adiponectin treatment significantly ameliorated murine arthritis and inhibited the accumulation of complement C1q and C3 in the joints [18], suggesting that circulating adiponectin binds to and traps complement C1q in bloodstream and prevents the deposition of C1q in the joints. Naito et al. recently demonstrated that complement C1q activated Wnt/β-catenin signaling and promoted aging in mice and cells [20]. These reports suggest that adiponectin mediates or modulates the biological effect of complement C1q. As shown in Fig. 3, GSK3β/β-catenin signal was augmented by adiponectin-deficiency under AngII infusion, but there were no significant differences in plasma C1q concentrations between WT and Adipo-KO mice (data not shown). However, cardiac C1q mRNA level tended to increase in Adipo-KO mice and was significantly decreased by Ad-Adipo-mediated adiponectin supplementation (Fig. 2E). It remains elucidated whether adiponectin directly or indirectly suppresses cardiac C1q mRNA level under AngII infusion. There is a possibility that such change of C1q mRNA level might be reflected by AngII-induced cardiac inflammation and macrophage infiltration. In addition, the existence of C1q-adiponectin complex in serum has not been demonstrated in mice. Physiological and pathological association of adiponectin and C1q would be biologically important to further analysis.

In summary, adiponectin attenuates AngII-induced cardiac hypertrophic signals partly through Akt/GSK3β/β-catenin and Akt/mTOR pathways.

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Disclosure

None.

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