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Translational Research

Omentin Prevents Myocardial Ischemic Injury Through AMP-Activated Protein Kinase- and Akt-Dependent Mechanisms

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Objectives This study examined the impact of omentin on myocardial injury in a mouse model of ischemia/reperfusion (I/R) and explored its underlying mechanisms.

Background Obesity is a major risk factor for ischemic heart disease. Omentin is a circulating adipokine that is down-regulated by obesity.

Methods In patients who underwent successful reperfusion treatment after acute myocardial infarction, cardiac function and perfusion defect were assessed by using scintigraphic images. Mice were subjected to myocardial ischemia followed by reperfusion.

Results This study found that high levels of plasma omentin were associated with improvement of heart damage and function after reperfusion therapy in patients with acute myocardial infarction. Systemic administration of human omentin to mice led to a reduction in myocardial infarct size and apoptosis after I/R, which was accompanied by enhanced phosphorylation of AMP-activated protein kinase (AMPK) and Akt in the ischemic heart. Fat-specific overexpression of human omentin also resulted in reduction of infarct size after I/R. Blockade of AMPK or Akt activity reversed omentin-induced inhibition of myocardial ischemic damage and apoptosis in mice. In cultured cardiomyocytes, omentin suppressed hypoxia/reoxygenation-induced apoptosis, which was blocked by inactivation of AMPK or Akt.

Conclusions Our data indicate that omentin functions as an adipokine that ameliorates acute ischemic injury in the heart by suppressing myocyte apoptosis through both AMPK- and Akt-dependent mechanisms. (J Am Coll Cardiol 2014;63:2722–33) © 2014 by the American College of Cardiology Foundation

Ischemic heart disease, including myocardial infarction, is the major cause of mortality worldwide (1). Although complications of obesity are associated with the severity and adverse outcome of ischemic heart disease (2–4), the molecular mechanisms by which obesity contributes to the development of heart diseases are incompletely understood. Adipose tissue functions as an endocrine organ by secreting various bioactive molecules, also referred to as adipokines, which can directly affect the nearby or remote tissues (5). Omentin/intelectin-1 has been identified as an adipokine that is detected abundantly in human visceral fat tissue (6,7). Plasma omentin levels are down-regulated in patients with obesity-related disorders, including type 2 diabetes, metabolic syndrome, and atherosclerosis (8–11). Furthermore, low levels of circulating omentin are associated with the prevalence of coronary heart disease in male patients (12–14). Decreased levels of circulating omentin are also associated with the presence and severity of coronary artery disease in postmenopausal women (15). An experimental

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study found that omentin stimulates glucose uptake in cultured adipocytes (6).

Omentin also reportedly enhances vasodilation in isolated blood vessels and reduces inflammatory responses in cultured endothelial cells (16,17). We have reported that administration of omentin promotes revascularization processes of ischemic muscle (18). A recent report demonstrated that exposure of cardiomyocytes to conditioned media derived from epicardial adipose tissue from patients with type 2 diabetes leads to induction of contractile dysfunction and insulin resistance, which is prevented by recombinant omentin protein (19). Thus, it is plausible that omentin participates in obesity-related metabolic and cardiovascular complications. However, nothing is known about the role of omentin in regulation of ischemic heart disease. In the present article, we assessed the clinical significance of omentin in acute myocardial infarction (AMI) and investigated whether human omentin modulates acute ischemic injury in the heart in vivo and in vitro.

Methods

For an expanded Methods section, please see the Online Appendix.

Clinical study. A total of 20 patients with ST-segment–elevated AMI of the left anterior descending coronary artery alone that was treated successfully by coronary stenting (bare metal stents) were enrolled in this study. Medication use included 200 mg of ticlopidine, 160 to 200 mg of aspirin, an angiotensin type I receptor antagonist, and statins. The percutaneous coronary intervention was performed within 6 h from the onset of chest pain by experienced interventionists according to the guidelines for coronary angiography and percutaneous coronary intervention of the American Heart Association. We excluded patients with adverse cardiovascular events, including angiographic restenosis during the observation period, major bleeding requiring blood transfusion during the percutaneous coronary intervention, a history of hepatic or renal dysfunction, malignant neoplasia, and an unwillingness to participate. This protocol was approved by the ethics committee of the Nagoya University Graduate School of Medicine (Nagoya, Japan), and all subjects enrolled provided written informed consent.

At 7 days after AMI, all patients received an injection of 111 MBq of iodine-123-beta-methyl iodophenyl pentadecanoic acid (^{123}I-BMIPP). The single-photon emission computed tomography (SPECT) image acquisition for the early BMIPP image began at 20 minutes after injection. A second BMIPP SPECT acquisition was started at 3 h after injection. At 6 months after AMI, patients also received an injection of 147 MBq of ^{99m}Tc-tetrofosmin, and SPECT images were then acquired within 2 h. These scintigraphic examinations were used to evaluate myocardial salvage index and ejection fraction. The myocardial salvage index was calculated as [(percent initial perfusion defect size − percent final infarct size)/percent initial perfusion defect size], as described previously (20). The initial perfusion defect size was obtained by using BMIPP SPECT imaging, and the final infarct size was obtained by using tetrofosmin SPECT imaging. Blood samples were obtained in a fasting state at day 7 after AMI to determine plasma omentin levels.

Mouse model of ischemia/reperfusion injury. Male and female C57BL/6 mice were purchased from Oriental BioService, Inc. (Kyoto, Japan). We subjected mice at the age of 10 to 12 weeks to myocardial ischemia/reperfusion (I/R), as described previously (21,22). Briefly, after anesthetization (pentobarbital 50 mg/kg intraperitoneally) and intubation, the left anterior descending artery was ligated for 60 min with a suture by using a snare occluder and then loosened. At 24 h after reperfusion, the suture was re-tied, and Evans blue dye was systemically injected into the mice to determine the nonischemic tissue. The heart was excised, cut, and incubated with 2,3,5-triphenyltetrazolium chloride to determine the infarcted region. Left ventricular area, the area at risk, and infarct area were assessed by computerized planimetry using ImageJ software. Adenoviral vector expressing human omentin (Ad-omentin) or adenoviral vector expressing beta-galactosidase (Ad-β-gal) were injected into the jugular vein of mice 3 days before the I/R injury (4.0×10^7 PFU per mouse). In some experiments, LY294002 (40 mg/kg) or compound C (20 mg/kg) dissolved in dimethyl sulfoxide or dimethyl sulfoxide was intraperitoneally injected into mice before the operation and after reperfusion.

Our initial experiment demonstrated that plasma human omentin was detected at the mean concentration of 274 ± 41.9 ng/ml at 30 min after intravenous injection of recombinant human omentin protein (0.1 μg/g per mouse). Because this concentration of omentin in the bloodstream was similar to the level of omentin that was observed in healthy subjects (8,10,14), we injected recombinant human omentin protein (0.1 μg/g per mouse) or vehicle (phosphate-buffered saline) through the right jugular vein before the induction of ischemia or 5 min after reperfusion. All protocols were approved by the Institutional Animal Care and Use Committee of Nagoya University.

Generation of transgenic mice overexpressing human omentin in a fat-specific manner. Omentin-transgenic (OMT-TG) mice were generated by subcloning the

Abbreviations and Acronyms

^{123}I-BMIPP = iodine-123-beta-methyl iodophenyl pentadecanoic acid

Ad-β-gal = adenoviral vector expressing beta-galactosidase

Ad-dnAMPK = c-Myc–tagged dominant-negative mutant of AMPK

Ad-dnAkt = hemagglutinin-tagged dominant-negative mutant of Akt

Ad-omentin = adenoviral vector expressing human omentin

AMI = acute myocardial infarction

AMPK = AMP-activated protein kinase

I/R = ischemia/reperfusion

OMT-TG = omentin–transgenic

SPECT = single-photon emission computed tomography

TUNEL = deoxynucleotidyl transferase–mediated dUTP nick end labeling

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Table 1 Clinical Characteristics (N = 20)

Age, yrs	62.5 ± 1.6
Male/female	13/7
Body mass index, kg/m^2	24.3 ± 0.64
Systolic blood pressure, mm Hg	116.4 ± 2.1
Glucose, mg/dl	115.5 ± 6.1
LDL cholesterol, mg/dl	123.8 ± 7.9
HDL cholesterol, mg/dl	46.3 ± 4.1
Triglyceride, mg/dl	104 ± 8.1
Peak creatine phosphokinase, IU/ml	112.5 ± 8.6
Omentin, ng/ml	241.5 ± 27.0
^{123}I-BMIPP	
Initial perfusion defect size, %	39.1 ± 2.0
EF in acute phase, %	43.7 ± 1.6
^{99m}TC-TF	
Final infarct size, %	15.8 ± 1.6
EF in chronic phase, %	56.7 ± 2.2
Myocardial salvage index	0.59 ± 0.03
Changes in EF	13.1 ± 2.0

Values are n or mean ± SEM.
^{123}I-BMIPP = iodine-123-beta-methyl iodophenyl pentadecanoic acid; ^{99m}TC-TF = technetium-99m-tetrofosmin; EF = ejection fraction; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

full-length human omentin complementary deoxyribonucleic acid into pBluescript (Stratagene, La Jolla, California) vector with the 5.4 kb murine aP2 promoter. The deoxyribonucleic acid fragment containing aP2 gene promoter, the human omentin, and polyadenylation sequences were excised with ClaI and SacII restriction enzymes and used for pronuclear microinjection. OMT-TG mice in a C57BL/6J genetic background were generated by Oriental BioService. OMT-TG founders were determined by polymerase chain reaction analysis and bred to C57BL/6J mice to generate stable lines.

Statistical analysis. Data are presented as mean ± SEM. The Student *t* test was performed for comparison between 2 independent groups. The one-way analysis of variance test was performed for comparison of 3 or more independent groups. The Fisher protected least significant difference test was used only if the overall comparison by one-way analysis of variance test was statistically significant. All continuous variables were assumed to be normally distributed. The correlations between omentin levels and the indicated parameters were examined by using single logistic regression analyses. A value of $p < 0.05$ denoted the presence of a statistically significant difference.

Results

Association of omentin with myocardial salvage and function in patients with myocardial infarction. First, we examined whether the circulating omentin level is associated with myocardial function and injury in patients with AMI. A total of 20 patients who underwent successful reperfusion treatment after AMI were enrolled (Table 1). Cardiac function and perfusion defect were assessed by using scintigraphic images of ^{123}I-BMIPP in the acute phase and ^{99m}Tc-tetrofosmin in the chronic phase. Plasma omentin levels at 7 days' post-AMI were positively associated with myocardial salvage index, representing the proportion of initial perfusion defect rescued by reperfusion and recovery of ejection fraction in the chronic phase (Figs. 1A and 1B).

Systemic delivery of adenoviral vectors encoding human omentin reduces myocardial infarct size and apoptosis in mice after I/R. To test whether increased production of circulating omentin affects acute cardiac ischemic injury in mice, male wild-type C57BL/6J mice were intravenously treated with Ad-omentin or Ad-β-gal as a control, followed by subjection to 60 min of myocardial ischemia and 24 h of reperfusion. Murine omentin messenger ribonucleic acid in heart and adipose tissue of wild-type mice after the surgical induction of I/R could not be detected (data not shown). Although circulating human omentin could not be detected in the control wild-type mice, mean plasma human omentin

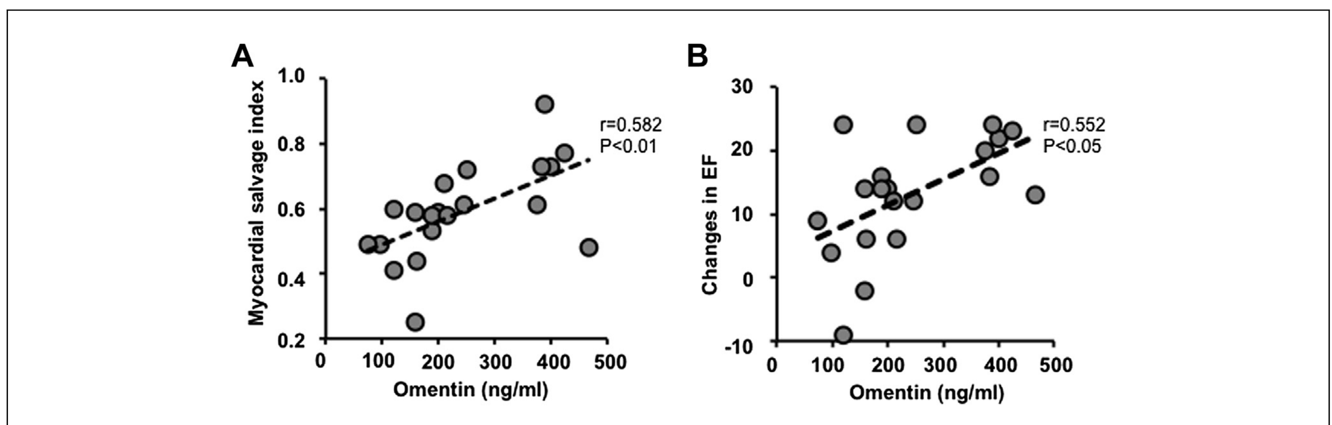

Figure 1 Association of Plasma Omentin Levels at 7 Days After AMI With Myocardial Salvage Index and Changes in EF

Association of plasma omentin levels at 7 days after acute myocardial infarction (AMI) with **(A)** myocardial salvage index and **(B)** changes in ejection fraction (EF). A total of 20 patients who underwent successful reperfusion treatment after AMI were enrolled. Values are mean ± SEM.

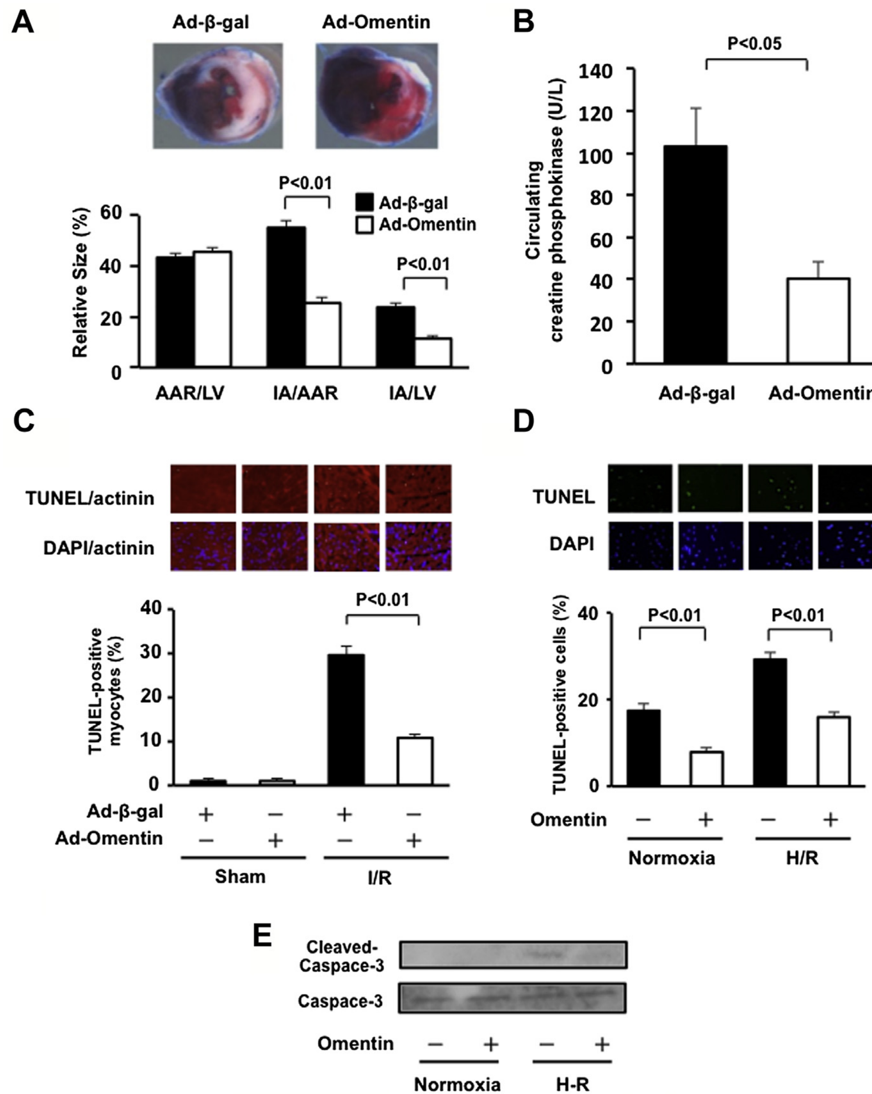

Figure 2 **Systemic Delivery of Omentin Improves Myocardial Ischemic Injury in Mice**

(A) Adenovirus-mediated administration of omentin reduces infarct size after myocardial ischemia/reperfusion (I/R). Wild-type mice were systemically treated with an adenoviral vector expressing human omentin (Ad-omentin) or adenoviral vector expressing beta-galactosidase (Ad-β-gal) as control (4.0×10^7 PFU total) and subjected to I/R injury. Representative pictures of the heart sections stained with Evans blue dye and 2,3,5-triphenyltetrazolium chloride at 24 h after I/R are shown in the **upper panels**. Left ventricular (LV) area, the area at risk (AAR) **(red)**, and infarct area (IA) **(white)** were measured. Quantitative analysis of infarct size is shown in the **lower panel** (n = 6 in each group). **(B)** Ad-omentin treatment reduced plasma creatine phosphokinase at 24 h after reperfusion (n = 6 in each group). **(C)** Ad-omentin reduced myocyte apoptosis in ischemic heart in mice. Representative photographs of heart sections stained with deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) **(green)**, sarcomeric actinin **(red)**, and 4′,6-diamidino-2-phenylindole (DAPI) **(blue)** are shown in the **upper panels**. Quantitative analyses of TUNEL-positive myocytes are shown in the **lower panel** (n = 6 in each group). **(D)** Omentin attenuated apoptosis of cultured cardiac myocytes. Neonatal rat cardiac myocytes were treated with omentin protein (300 ng/ml) or vehicle under conditions of normoxia or hypoxia/reoxygenation (H/R). **Upper panels** show representative photographs of cardiac myocytes stained with TUNEL **(green)** and DAPI **(blue)**. **Lower panel** shows quantitative analysis of TUNEL-positive cells (n = 8 in each group). **(E)** Omentin inhibited the degree of cleaved caspase-3 of cardiac myocytes under conditions of H/R (n = 4 in each group).

levels increased to 555.9 ± 141.7 ng/ml in Ad-omentin–treated wild-type mice at 5 days after adenoviral injection. All mice survived after the surgical induction of I/R. Body weight and blood pressure did not differ between the 2 experimental groups (data not shown).

Figure 2A shows representative photographs of heart tissues stained with Evans blue dye to delineate the area at risk and 2,3,5-triphenyl tetrazolium chloride to delineate the infarct area. Systemic delivery of Ad-omentin significantly reduced the mean infarct area/area at risk and infarct area/left ventricular ratios by 53.9 ± 7.1% and 51.5 ± 9.6%, respectively, compared with control mice. In contrast, the area at risk/left ventricular ratio did not differ between the 2 experimental groups. Furthermore, circulating levels of

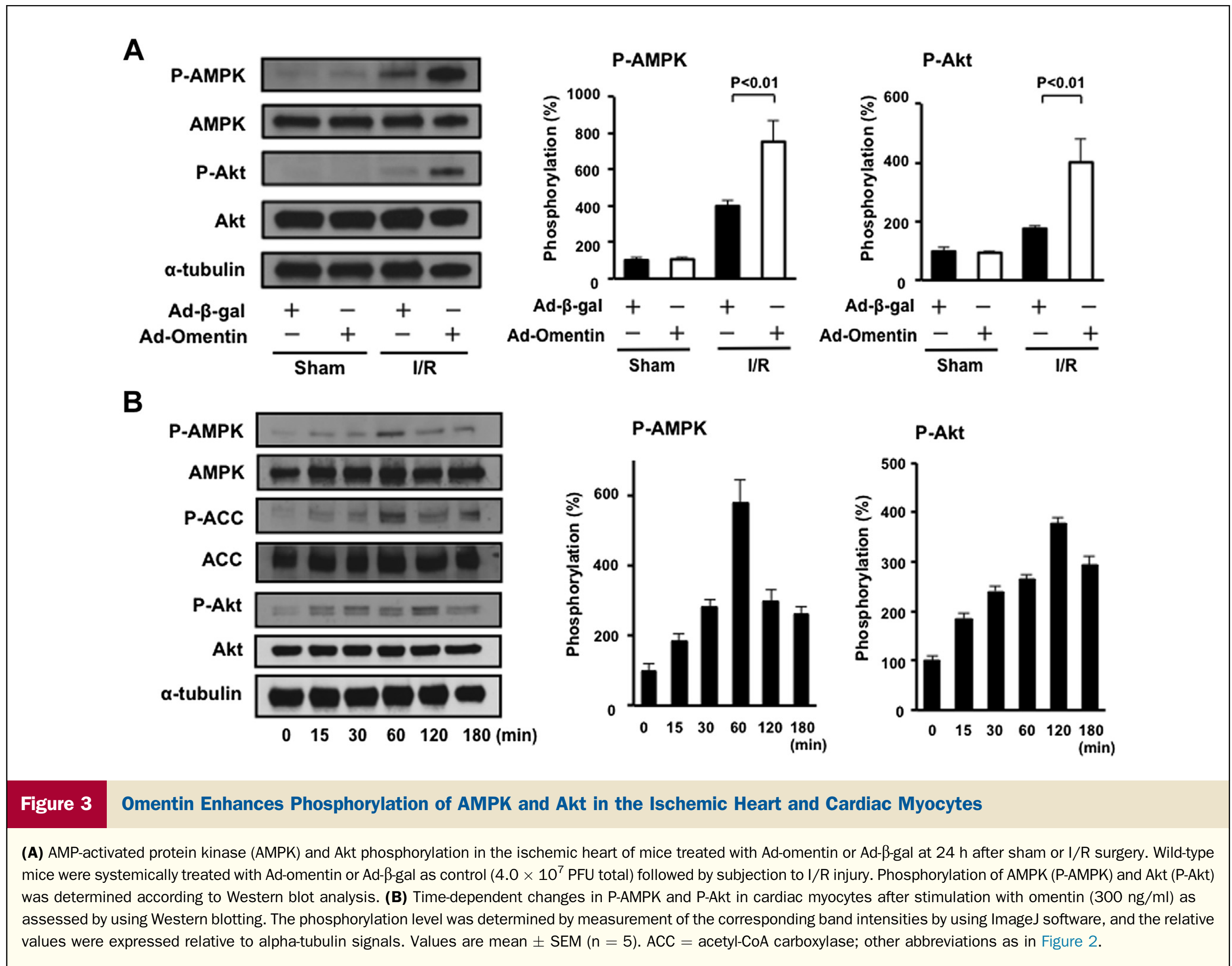

Figure 3 **Omentin Enhances Phosphorylation of AMPK and Akt in the Ischemic Heart and Cardiac Myocytes**

(A) AMP-activated protein kinase (AMPK) and Akt phosphorylation in the ischemic heart of mice treated with Ad-omentin or Ad-β-gal at 24 h after sham or I/R surgery. Wild-type mice were systemically treated with Ad-omentin or Ad-β-gal as control (4.0×10^7 PFU total) followed by subjection to I/R injury. Phosphorylation of AMPK (P-AMPK) and Akt (P-Akt) was determined according to Western blot analysis. **(B)** Time-dependent changes in P-AMPK and P-Akt in cardiac myocytes after stimulation with omentin (300 ng/ml) as assessed by using Western blotting. The phosphorylation level was determined by measurement of the corresponding band intensities by using ImageJ software, and the relative values were expressed relative to alpha-tubulin signals. Values are mean ± SEM (n = 5). ACC = acetyl-CoA carboxylase; other abbreviations as in Figure 2.

creatine phosphokinase, a marker of heart injury, were significantly lower in Ad-omentin–treated mice compared with control mice at 24 h after reperfusion (Fig. 2B). In addition, we subjected female wild-type C57BL/6J mice to myocardial ischemia followed by reperfusion. Systemic administration of Ad-omentin to female mice significantly reduced myocardial infarct size (Online Fig. 1). Thus, omentin could improve acute ischemic injury in the heart regardless of sex.

To investigate the impact of omentin on myocyte apoptosis in the heart, we stained heart sections with deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) and sarcomeric actinin at 24 h after I/R or sham operation. Figure 2C shows representative fluorescent photographs of TUNEL-positive nuclei in the area at risk. Quantitative analysis revealed that Ad-omentin significantly reduced the frequencies of TUNEL-positive myocytes in the ischemic heart compared with controls. In contrast, little or no TUNEL-positive myocytes were observed in the heart of control or omentin-treated mice after the sham operation.

To examine the effect of omentin on apoptosis at a cellular level, neonatal rat cardiac myocytes were subjected to normoxia or hypoxia/reoxygenation under conditions of serum deprivation in the presence of recombinant human omentin protein or vehicle. Treatment with a physiological concentration of omentin protein significantly suppressed the frequencies of TUNEL-positive cells under conditions of normoxia and hypoxia/reoxygenation (Fig. 2D). Furthermore, the induction in cleaved caspase-3 expression in response to hypoxia/reoxygenation in cardiac myocytes was suppressed by omentin treatment (Fig. 2E).

Omentin activates AMP-activated protein kinase and Akt in the ischemic heart and cardiac myocytes. Because AMP-activated protein kinase (AMPK) and Akt reportedly protect myocytes from apoptosis and ischemic injury (21,23–26), we assessed the activating phosphorylation of AMPK at Thr-172 and Akt at Thr-308 in the heart after the sham or I/R operation. I/R led to an increase in phosphorylation of AMPK and Akt in the control heart, but this induction was enhanced in the heart of Ad-omentin–treated mice (Fig. 3A). We next assessed the

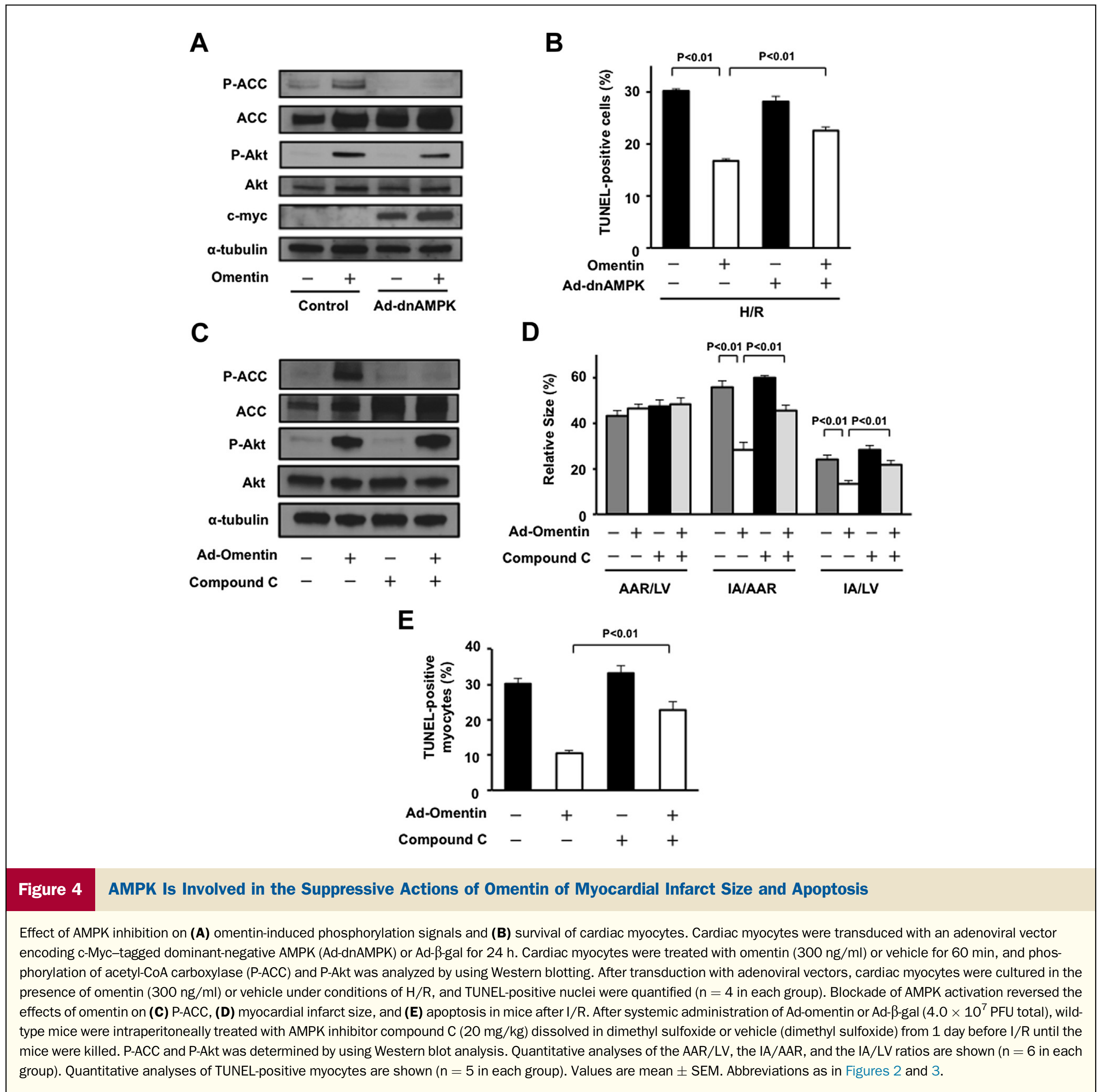

Figure 4 **AMPK Is Involved in the Suppressive Actions of Omentin of Myocardial Infarct Size and Apoptosis**

Effect of AMPK inhibition on **(A)** omentin-induced phosphorylation signals and **(B)** survival of cardiac myocytes. Cardiac myocytes were transduced with an adenoviral vector encoding c-Myc–tagged dominant-negative AMPK (Ad-dnAMPK) or Ad-β-gal for 24 h. Cardiac myocytes were treated with omentin (300 ng/ml) or vehicle for 60 min, and phosphorylation of acetyl-CoA carboxylase (P-ACC) and P-Akt was analyzed by using Western blotting. After transduction with adenoviral vectors, cardiac myocytes were cultured in the presence of omentin (300 ng/ml) or vehicle under conditions of H/R, and TUNEL-positive nuclei were quantified (n = 4 in each group). Blockade of AMPK activation reversed the effects of omentin on **(C)** P-ACC, **(D)** myocardial infarct size, and **(E)** apoptosis in mice after I/R. After systemic administration of Ad-omentin or Ad-β-gal (4.0×10^7 PFU total), wild-type mice were intraperitoneally treated with AMPK inhibitor compound C (20 mg/kg) dissolved in dimethyl sulfoxide or vehicle (dimethyl sulfoxide) from 1 day before I/R until the mice were killed. P-ACC and P-Akt was determined by using Western blot analysis. Quantitative analyses of the AAR/LV, the IA/AAR, and the IA/LV ratios are shown (n = 6 in each group). Quantitative analyses of TUNEL-positive myocytes are shown (n = 5 in each group). Values are mean ± SEM. Abbreviations as in Figures 2 and 3.

phosphorylation of AMPK and Akt in cardiac myocytes after treatment with human omentin protein or vehicle. Omentin stimulated the phosphorylation of AMPK in a time-dependent manner, with maximal levels occurring at 60 min (Fig. 3B). Similarly, omentin time-dependently enhanced the phosphorylation of acetyl-CoA carboxylase, a downstream target of AMPK in cardiac myocytes. Omentin also promoted Akt phosphorylation in a time-dependent manner, with maximal induction at 120 min.

AMPK participates in the suppressive effects of omentin on myocardial injury. To test whether AMPK signaling is involved in the antiapoptotic actions of omentin, cultured cardiac myocytes were transduced with c-Myc–tagged dominant-negative mutant of AMPK (Ad-dnAMPK) or Ad-β-gal as a control. Transduction with Ad-dnAMPK suppressed omentin-stimulated phosphorylation of acetyl-CoA carboxylase in cardiac myocytes (Fig. 4A). In contrast, Ad-dnAMPK did not affect Akt phosphorylation in response to omentin. Furthermore, transduction with Ad-dnAMPK reversed the inhibitory effects of omentin on apoptosis under conditions of hypoxia/reoxygenation (Fig. 4B).

To further examine whether AMPK is involved in omentin-mediated protection against I/R damage in vivo, we intraperitoneally injected AMPK inhibitor compound C or vehicle into mice. Administration of compound C

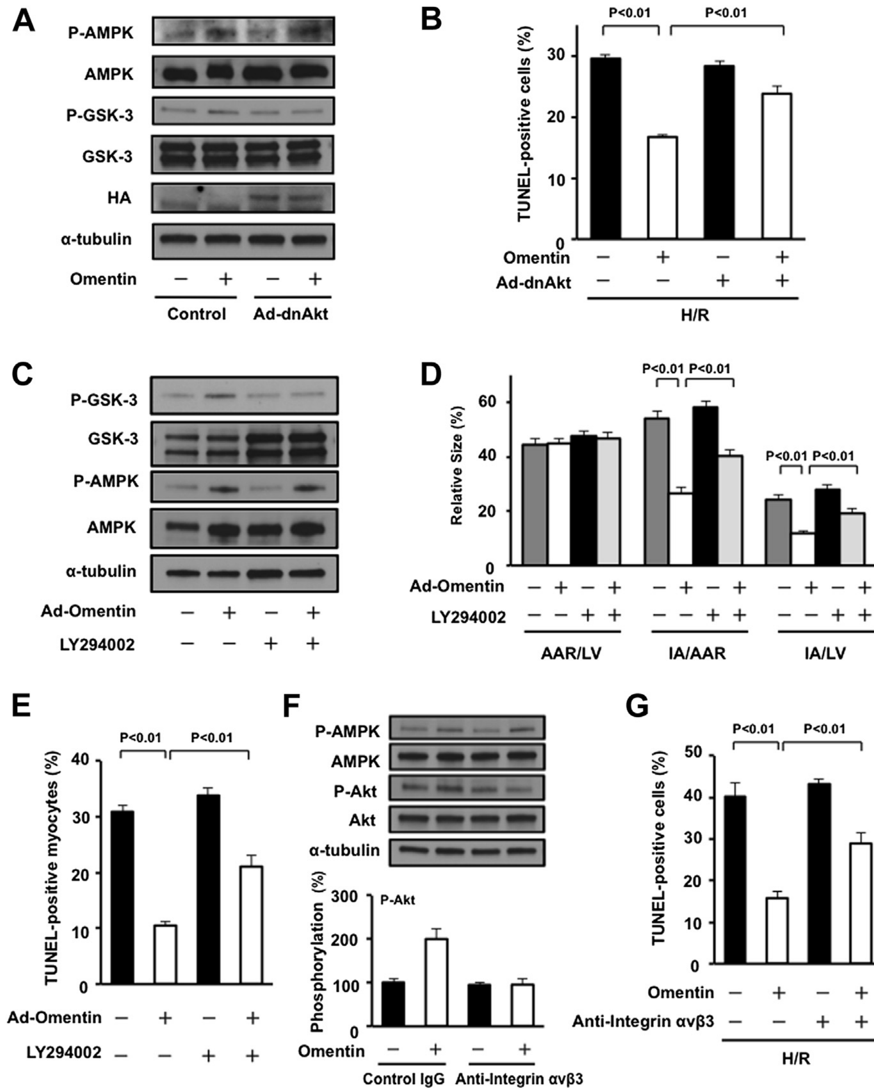

Figure 5 Akt Signaling Contributes to the Protective Actions of Omentin on Myocardial Injury

Effect of Akt inactivation on **(A)** omentin-stimulated phosphorylation signals and **(B)** survival of cardiac myocytes. Cardiac myocytes were transduced with an adenoviral vector producing hemagglutinin-tagged dominant-negative Akt (Ad-dnAkt) or Ad-β-gal for 24 h. Cells were treated with omentin (300 ng/ml) or vehicle for 60 min for determination of phosphorylation of glycogen synthase kinase–3 (P-GSK-3) and P-AMPK. TUNEL-positive nuclei were quantified after treatment of myocytes with omentin (300 ng/ml) or vehicle under conditions of H/R (n = 4 in each group). Effect of PI3-kinase inhibitor on **(C)** phosphorylation signals, **(D)**, infarct size and **(E)** apoptosis in response to I/R after systemic administration of omentin. After systemic delivery of Ad-omentin or Ad-β-gal (4.0×10^7 PFU total), wild-type mice were intraperitoneally treated with PI3-kinase inhibitor LY294002 (40 mg/kg) dissolved in dimethyl sulfoxide or vehicle (dimethyl sulfoxide) from 1 day before I/R until the mice were killed. Quantitative analyses of infarct size (AAR/LV, IA/AAR, and IA/LV ratios) (n = 6 in each group) and TUNEL-positive myocytes (n = 5 in each group) are shown. Involvement of integrin αvβ3 in omentin-stimulated **(F)** P-Akt and **(G)** survival of cardiac myocytes. Cardiac myocytes were pre-treated with anti-integrin αvβ3 antibody (25 μg/ml) or control immunoglobulin G (IgG) (25 μg/ml) for 60 min. Cells were treated with omentin (300 ng/ml) or vehicle for 60 min. P-Akt and P-AMPK were determined by using Western blot analysis. TUNEL-positive myocytes in response to H/R were quantified after treatment with omentin (300 ng/ml) or vehicle (n = 5 in each group). Values are mean ± SEM. Abbreviations as in Figures 2 and 4.

reduced the omentin-induced increase in acetyl-CoA carboxylase phosphorylation in the ischemic heart without altering the Akt phosphorylation (Fig. 4C). Although compound C had no effects on myocardial infarct size in control-treated mice, it significantly blocked the inhibitory actions of omentin on myocardial infarct area of mice after I/R (Fig. 4D). Compound C also reversed the omentin-induced decrease in TUNEL-positive myocytes in the ischemic heart (Fig. 4E). Thus, omentin can reduce myocardial infarct size and apoptosis in response to I/R injury partly via an AMPK-mediated signaling pathway.

Akt is involved in the inhibitory effects of omentin on cardiac ischemic injury. To assess whether Akt activation participates in the antiapoptotic effects of omentin, cardiac myocytes were transduced with hemagglutinin-tagged

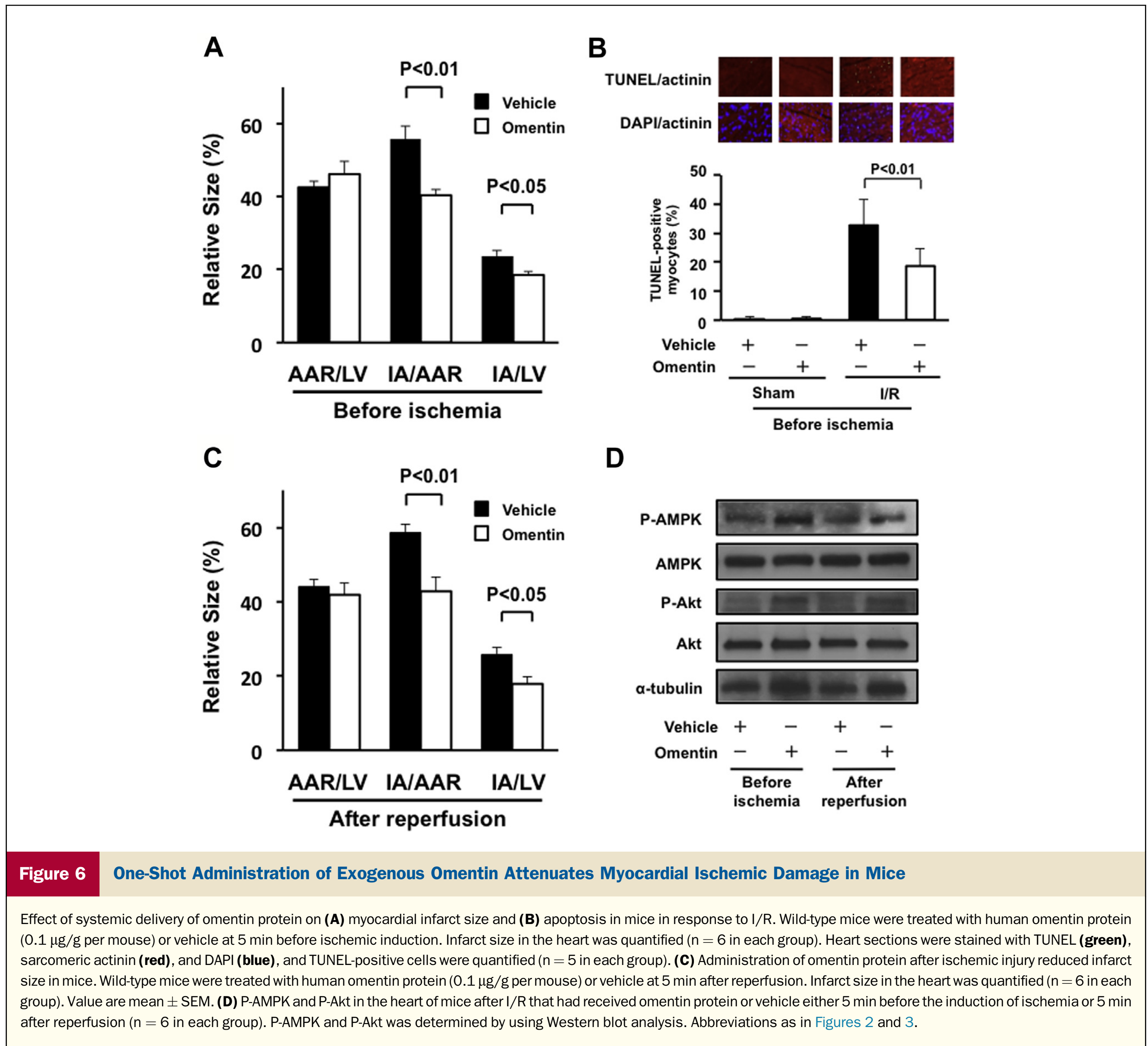

Figure 6 **One-Shot Administration of Exogenous Omentin Attenuates Myocardial Ischemic Damage in Mice**

Effect of systemic delivery of omentin protein on **(A)** myocardial infarct size and **(B)** apoptosis in mice in response to I/R. Wild-type mice were treated with human omentin protein (0.1 μg/g per mouse) or vehicle at 5 min before ischemic induction. Infarct size in the heart was quantified (n = 6 in each group). Heart sections were stained with TUNEL **(green)**, sarcomeric actinin **(red)**, and DAPI **(blue)**, and TUNEL-positive cells were quantified (n = 5 in each group). **(C)** Administration of omentin protein after ischemic injury reduced infarct size in mice. Wild-type mice were treated with human omentin protein (0.1 μg/g per mouse) or vehicle at 5 min after reperfusion. Infarct size in the heart was quantified (n = 6 in each group). Value are mean ± SEM. **(D)** P-AMPK and P-Akt in the heart of mice after I/R that had received omentin protein or vehicle either 5 min before the induction of ischemia or 5 min after reperfusion (n = 6 in each group). P-AMPK and P-Akt was determined by using Western blot analysis. Abbreviations as in Figures 2 and 3.

dominant-negative mutant of Akt (Ad-dnAkt) or control Ad-β-gal. Transduction with Ad-dnAkt suppressed omentin-induced phosphorylation of glycogen synthase kinase–3, a downstream target of Akt in cardiac myocytes (Fig. 5A). However, Ad-dnAkt treatment had no effects on AMPK phosphorylation after omentin stimulation. Moreover, Ad-dnAkt canceled the omentin-mediated inhibition of myocyte apoptosis in response to hypoxia/reoxygenation (Fig. 5B).

To investigate whether Akt signaling mediates omentin-induced protection from cardiac ischemic injury in vivo, we intraperitoneally injected PI3-kinase inhibitor LY294002 or vehicle into mice. Administration of LY294002 diminished omentin-induced phosphorylation of glycogen synthase kinase–3 in the ischemic heart without influencing AMPK phosphorylation (Fig. 5C). Although LY294002 did not affect myocardial infarct size and apoptosis in control-treated mice, it reversed the suppressive actions of omentin on infarct size and myocyte apoptosis after myocardial I/R (Figs. 5D and 5E). Thus, omentin attenuates myocardial I/R injury partly through activation of Akt signaling that is independent of AMPK. Furthermore, it is unlikely that AMPK and Akt mutually affect each other's response to omentin in ischemic heart and cardiac myocytes.

Integrin αvβ3 is one of the major integrins that are expressed in cardiac myocytes (27), and it activates Akt-dependent survival pathways in various cells (28). To determine whether integrin αvβ3 is involved in omentin-stimulated Akt activation in vitro, cardiac myocytes were preincubated with a neutralizing antibody against integrin αvβ3 or control immunoglobulin G followed by treatment

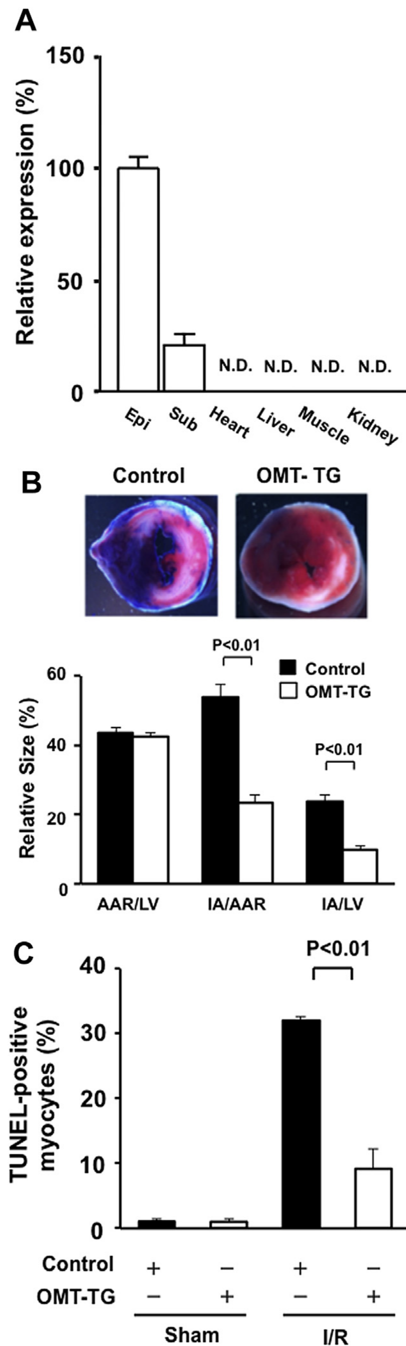

Figure 7 **Overproduction of Fat-Derived Omentin Protects the Heart From Myocardial Ischemic Damage**

(A) Restrictive expression of human omentin gene in fat tissue of human omentin transgenic (OMT-TG) mice. Transcript expression of human omentin in various tissues of OMT-TG mice was determined by real-time polymerase chain reaction methods (n = 4). Fat-specific OMT-TG mice show a reduction in **(B)** infarct size and **(C)** apoptosis after myocardial I/R. Representative pictures of heart tissues from OMT-TG and control mice are shown in the **upper panels**. Quantitative analysis of infarct size is shown in the **lower panel** (n = 5 in each group). Heart sections were stained with TUNEL **(green)**, sarcomeric actinin **(red)**, and DAPI **(blue)**, and TUNEL-positive cells were quantified (n = 5 in each group). Results are shown as mean ± SEM. Epi = epididymal adipose tissue; ND = not detectable; Sub = subcutaneous adipose tissue; other abbreviations as in Figure 2.

with omentin or vehicle. Pretreatment with anti-integrin αvβ3 antibody suppressed the omentin-induced increase in Akt phosphorylation, whereas it did not affect omentin-induced AMPK activation (Fig. 5F). Pre-treatment with anti-integrin αvβ3 antibody also reversed the inhibitory effects of omentin on apoptotic response to hypoxia/reoxygenation (Fig. 5G). In contrast, pre-treatment with anti-integrin αvβ5 antibody did not affect omentin-induced Akt activation and survival of cardiac myocytes (data not shown). These data suggest that omentin suppresses apoptosis partly through an integrin αvβ3-Akt–mediated pathway that is independent of AMPK.

Omentin modulates the phosphorylation of endothelial nitric oxide synthase and nuclear factor–κB in ischemic heart. The key regulator of endothelial function, endothelial nitric oxide synthase, protects myocytes from I/R injury (29,30), and the inflammatory mediator nuclear factor (NF)-κB contributes to myocardial injury after I/R (31,32). Therefore, we assessed the effect of omentin on phosphorylation status of endothelial nitric oxide synthase and NF-κB in the heart after the sham or I/R operation. I/R led to an increase in phosphorylation of endothelial nitric oxide synthase in the control heart, but this induction was enhanced in the heart of omentin-treated mice (Online Fig. 2). Phosphorylation of NF-κB in myocardium was increased in response to I/R, but the increase in NF-κB phosphorylation was suppressed by omentin treatment.

Recombinant omentin protein minimizes myocardial infarct size in mice after I/R. To test whether systemic administration of omentin protein could minimize infarct area before or after myocardial ischemia, we intravenously injected a single dose of recombinant human omentin protein or vehicle to wild-type mice 5 min before the induction of ischemia or 5 min after reperfusion. Plasma glucose and insulin levels at 24 h after reperfusion did not differ between the 4 experimental groups (data not shown). Systemic administration of omentin protein to mice, before ischemia, significantly attenuated infarct size after I/R relative to control mice (Fig. 6A). Administration of omentin protein before ischemia reduced the frequencies of apoptotic myocytes in the ischemic heart compared with control hearts (Fig. 6B). Systemic delivery of omentin protein after reperfusion also led to a significant reduction in myocardial infarct size in mice after I/R (Fig. 6C). In addition, we assessed the phosphorylation of AMPK and Akt in the heart of mice after I/R that had received omentin protein or vehicle either 5 min before the induction of ischemia or 5 min after reperfusion. Omentin administration, either before ischemia or after reperfusion, led to increases in AMPK and Akt phosphorylation in the ischemic heart (Fig. 6D).

Fat-specific OMT-TG mice are protected from myocardial I/R injury. Although human omentin is abundantly detected in visceral fat tissue, murine omentin transcript is expressed exclusively in the small intestine (33). Thus, to test whether fat-derived omentin could

minimize myocardial infarct size, we generated transgenic mice expressing the human omentin gene under the control of the aP2 promoter (fat-specific OMT-TG mice) in the background of C57BL/6J. Human omentin transcript was specifically expressed in subcutaneous fat and epididymal fat but not in other tissues examined in OMT-TG mice at 9 weeks of age (Fig. 7A). Plasma human omentin levels increased to 1,293.1 ± 207.9 ng/ml in OMT-TG mice, whereas human omentin protein was undetectable in littermate control mice. Morphometric and hemodynamic parameters of fat-specific OMT-TG mice seemed indistinguishable from littermate mice under basal/physiological conditions (Online Table 1). Of note, fat-specific OMT-TG mice showed a marked reduction in mean infarct area/area at risk and infarct area/left ventricular ratios by 56.8 ± 7.9% and 57.8 ± 9.9%, respectively, compared with control mice (Fig. 7B). OMT-TG mice also exhibited a lower frequency of apoptotic myocytes in the ischemic heart compared with control mice (Fig. 7C). In contrast, little or no TUNEL-positive myocytes were observed in the heart of control or OMT-TG mice after the sham operation.

Discussion

The present study provides the first evidence that the adipokine omentin confers resistance to acute ischemic damage in the heart. Clinically, increased levels of plasma omentin in post-AMI patients were associated with improvement in myocardial damage and function after successful reperfusion therapy. Increase in circulating human omentin by adenoviral or transgenic overexpression systems before the induction of ischemia led to a reduction in cardiac injury after reperfusion in mice. Supplementation of exogenous human omentin protein, either before ischemia or after reperfusion, was also effective in limiting the myocardial I/R damage in mice. Thus, these data suggest that the therapeutic approaches to enhance circulating omentin levels before or after ischemic insult can be valuable for prevention or treatment of acute myocardial injury.

Myocyte apoptosis is a key feature in various heart disorders (34,35). A limitation of apoptosis represents an important therapeutic target for ischemic heart disease (36,37). In the present study, omentin attenuated myocyte apoptosis in response to I/R in a murine model. Omentin also suppressed stress-inducible cardiomyocyte apoptosis in vitro. Similarly, omentin reportedly suppresses apoptosis of endothelial cells under conditions of serum starvation (18). Therefore, the ability of omentin to attenuate myocardial infarct size after I/R is dependent, at least in part, on its ability to reduce apoptosis in the heart.

It has been shown that both AMPK and Akt function as signaling molecules that regulate cellular apoptosis and survival (21,24,25,38). We have demonstrated that omentin stimulates the phosphorylation of AMPK or Akt in cultured endothelial cells (17,18). Consistent with these findings, omentin promoted activation of AMPK and Akt in cultured myocytes and ischemic hearts of mice. Importantly, inhibition of AMPK activity blocked the suppressive effects of omentin on cardiac injury and myocyte apoptosis in an Akt-independent manner. Blockade of Akt activity reversed omentin-induced inhibition of myocardial ischemic damage and apoptosis independently of AMPK signaling. Furthermore, integrin αvβ3 seemed to participate in omentin-inducible Akt survival signaling that was independent of AMPK activation. Taken together, these data suggest that omentin protects the heart from ischemic injury through its ability to promote 2 independent pro-survival pathways involving AMPK and Akt within cardiac myocytes.

We have shown previously that omentin enhances the activating phosphorylation of endothelial nitric oxide synthase in both ischemic muscles and cultured endothelial cells (18). Omentin also promotes vasodilation of isolated aorta, which is prevented by nitric oxide synthase inhibition (16). In addition, omentin reduces tumor necrosis factor-alpha–stimulated expression of adhesion molecules in endothelial cells via suppression of the NF-κB pathway (39). In agreement with these findings, the present study found that treatment of mice with omentin led to enhancement of endothelial nitric oxide synthase phosphorylation and suppression of NF-κB phosphorylation in the ischemic heart. Collectively, omentin can modulate endothelial function and inflammatory response in the heart in addition to its pro-survival properties, thereby contributing to protection against the myocardial ischemic injury.

Omentin was originally identified as a soluble galactofuranose-binding lectin (7). It has been shown that differential saccharide-binding specificities are observed between human and mouse omentin (40). In this context, human omentin forms a disulfide-linked and N-glycosylated trimer, whereas mouse homologue is present in a monomeric form. It has been reported that murine omentin is specifically expressed in the small intestine despite the abundant expression of human omentin in visceral fat depots (6,7,33). Thus, it is possible that human omentin has a distinct function from murine homologue in the setting of cardiovascular disease. Our present study found that transgenic overexpression of human omentin in a fat-specific manner effectively prevented myocardial ischemic damage and apoptosis in the heart in mice. Of importance, the circulating levels of human omentin derived from adipose tissue of mice are similar to the levels observed in healthy human subjects (8,10). Furthermore, human omentin at the physiological concentration was effective in reducing myocyte apoptosis in vitro. Taken together, these data suggest that human omentin acts as a crucial adipokine that exerts beneficial actions on the injured myocardium and that decreased levels of circulating omentin under conditions of obesity contribute to the exacerbation of ischemic injury in the heart.

Study limitations. In our clinical study, the sample size was small. Future studies in a larger population will be required to elucidate the relationship of circulating omentin levels with clinical outcomes after AMI.

Conclusions

We found that omentin can protect cardiac myocytes from apoptosis during I/R through both AMPK- and Akt-dependent mechanisms, thereby leading to protection against acute cardiac injury. We also demonstrated the effectiveness of 1-shot administration of human omentin for myocardial damage in response to ischemia in a murine model, indicating the potential clinical utility of omentin. We and other groups have previously demonstrated that circulating omentin levels are reduced in patients with coronary heart diseases, including AMI and angina pectoris (12–14,41). Collectively, these data suggest that omentin represents a novel target molecule for the prevention and/or treatment of ischemic heart disease.

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For an expanded Methods section, as well as supplemental figures and a table, please see the online version of this article.