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Transgenic MMP-2 expression induces latent cardiac mitochondrial dysfunction

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Abstract

Matrix metalloproteinases (MMPs) are central to the development and progression of dysfunctional ventricular remodeling after tissue injury. We studied 6 month old heterozygous mice with cardiac-specific transgenic expression of active MMP-2 (MMP-2 Tg). MMP-2 Tg hearts showed no substantial gross alteration of cardiac phenotype compared to age-matched wild-type littermates. However, buffer perfused MMP-2 Tg hearts subjected to 30 min of global ischemia followed by 30 min of reperfusion had a larger infarct size and greater depression in contractile performance compared to wild-type hearts. Importantly, cardioprotection mediated by ischemic preconditioning (IPC) was completely abolished in MMP-2 Tg hearts, as shown by abnormalities in mitochondrial ultrastructure and impaired respiration, increased lipid peroxidation, cell necrosis and persistently reduced recovery of contractile performance during post-ischemic reperfusion. We conclude that MMP-2 functions not only as a proteolytic enzyme but also as a previously unrecognized active negative regulator of mitochondrial function during superimposed oxidative stress.

Keywords: Matrix metalloproteinase-2; Ischemia; Reperfusion; Mitochondria; Preconditioning

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes encompassing more than 20 members. Under normal physiological conditions, MMPs are regulated by circulating protease inhibitors and a series of endogenous tissue inhibitors [1]. MMP-2 (EC 3.4.24.24, gelatinase A) and MMP-9 (EC 3.4.24.35, gelatinase B) have been implicated in the response to acute and chronic cellular injury and in chronic ventricular remodeling after myocardial infarction [2–6]. Activated MMP-2 degrades many major components of the myocardial extracellular matrix including types I–V collagen, gelatins, laminin, fibronectin and elastin, thereby impairing the normal structural support of cardiomyocytes [1]. MMP-2 is also synthesized by both cardiac myocytes and fibroblasts, and is colocalized with troponin I within myofilaments [5], sarcomeres [6], and nuclei [7]. Acute activation of MMP-2 leads to a reduction of contractile performance following ischemia/reperfusion (I/R) injury [8].

We have generated a transgenic mouse model expressing constitutively active MMP-2 promoter that is governed by hypoxia [9–11], and have shown that acute I/R in isolated murine heart activates both the MMP-2 promoter and MMP-2 protein synthesis [12]. In the present study, we used the heterozygous MMP-2 Tg mice to ask several questions concerning the effects of chronic activation of MMP-2 in the pathogenesis of acute myocardial injury: (1) Are MMP-2 Tg hearts more vulnerable to I/R injury? (2) Is ische-

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mic preconditioning (IPC)-mediated cardioprotection against I/R injury preserved in MMP-2 Tg mice? and (3) Does chronic MMP-2 overexpression disrupt normal mitochondrial function? Our study demonstrates that compared to their WT littermates, MMP-2 Tg hearts are more vulnerable to I/R injury during oxidative stress. These hearts exhibit disturbed mitochondrial respiration and excessive lipid peroxidation that may contribute to the deleterious effects of myocardial injury and to the abolition of IPC-mediated cardioprotection during post-ischemic reperfusion.

Methods

Materials. Generation of cardiac-specific expression MMP-2 transgenic mice has been described in detail previously [9,10]. Transgenic animals were maintained as heterozygotes within the outbred CD-1 background. In this study, 6 month old, male transgenic mice and age-matched wild-type littermates (WT) were used. At this time, transgenic mice exhibited significant elevation of MMP-2 expression whereas MMP-9, MMP-13 and MMP-14 were unchanged [9,10]. Animals were acclimated in a quiet quarantine room for at least 3 days before starting experiments. The study was approved by the Animal Care Subcommittee of the SF VA Medical Center. All protocols conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the "Guiding Principles in the Care and Use of Animals" of the American Physiological Society. All chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise stated.

In vivo hemodynamics. Transthoracic echocardiography was performed on conscious mice using a 15-MHz linear array transducer coupled to an Acuson Sequoia c256 echocardiograph [14]. Systolic blood pressure was measured in trained, awake mice using a non-invasive computerized tail cuff system. Results were the means of two-independent daily measurements [14].

Ex vivo hemodynamics. Mice were anesthetized with sodium pentobarbital (60 mg/kg, IP) and anticoagulated with heparin sodium (5000 USP U/kg, IP). Excised hearts were cannulated via the aorta and perfused in retrograde fashion at constant pressure (70 mmHg) and temperature (37 °C) with Krebs–Henseleit buffer containing (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, glucose 11, and EDTA 0.5. Hearts were paced at 6 Hz, and hemodynamic measurements were recorded throughout the experiment using a micromanometer (Millar Instruments, Houston, TX) passed into a polyvinylchloride balloon in the left ventricular cavity. Coronary sinus effluent (CF) was collected as a measure of coronary flow and expressed as ml/min.

Ischemia–reperfusion protocol. After baseline hemodynamics were recorded during a 20 min equilibration period, all mouse hearts were subjected to 30 min of global ischemia and 30 min of reperfusion. Ischemic preconditioning was performed using 2 min of single cycle no-flow global ischemia followed by a 5 min washout prior to index ischemia.

Creatine kinase assays. Creatine kinase (CK) release was measured from the coronary effluent collected during reperfusion using a commercially available kit (Stanbio CK, Stanbio Laboratory, Boerne, TX) and corrected for flow rate and wet heart weight. The results are expressed as U/min/mL/g wet heart weight.

Infarct size measurement. Upon completion of reperfusion, hearts were perfused with 1% 2,3,5-triphenyltetrazolium chloride solution and fixed in 10% neutral buffered formalin [15]. The left ventricle was sliced into transverse sections and each section was weighed. Both sides of each section were imaged with a color digital videocamera (Leica, COHU Y/C 460 HTYL, 768 × 494 array, San Diego CA) connected to a microscope (Leica, Stereo Zoom 6 photo, San Diego CA). Images of the viable areas (red-stained) and necrotic areas (unstained) were analyzed using NIH Scion Image software in a blinded fashion. Infarct size was adjusted to the weight of each section and expressed as a percentage of total left ventricular mass [14].

Mitochondrial respiratory activity. Intact cardiac mitochondria were isolated as previously described in our laboratory [13]. Mitochondrial oxygen consumption rate (OCR) was estimated polarographically at 25 °C using a Clark-type oxygen electrode connected to a mitochondrial respiration chamber (YSI Incorporated, Yellow Springs, OH) and a linear chart recorder. State 3 respiration was measured by the addition of ADP (0.5 mM) to respiration buffer (20 mM Hepes, 5 mM KH_2PO_4, 0.2 mM EDTA, 2.5 mM $MgCl_2$, 10 mM KCl, 0.25 M sucrose, and 1 mg/mL fatty acid free BSA, pH 7.4) with NADH-linked substrates (glutamate/malate). Mitochondrial state 4 respiration was measured in the absence of ADP. The result was expressed as ng-atoms of oxygen per min per mg of protein. The respiratory control ratio (RCR) was calculated as the ratio of the state 3 to state 4 respiration [13].

Lipid peroxidation. Lipid peroxidation in individual hearts was determined by the appearance of thiobarbituric acid reactive substances measured spectrophotometrically at an absorbance of 532 nm as previously described [16]. The concentration of malondialdehyde (MDA) was calculated using an extinction coefficient of 1.56×10^5/M cm and was expressed as nmol/g wet weight heart.

Transmission electron microscopy (EM). EM examinations of tissue and mitochondrial ultrastructure were performed as previously described [17]. Briefly, heart tissue was fixed in cold 2.5% glutaraldehyde with 2% paraformaldehyde in 0.1 mol/L cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon resin. Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined by EM (Philips Tecna I, Holland). Micrographs were taken systematically at 6700× magnification and were analyzed for specific ischemic alterations as described in Results.

Statistics. Values are mean ± SEM. Measurements during reperfusion were compared with the baseline of each heart by Student's *t*-test. Comparisons among groups were by one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls post hoc test. $P < 0.05$ was considered significant.

Results

Morphology and baseline cardiac function

MMP-2 Tg mice showed normal behavior and general appearance at 6 months of age. As shown in Table 1, body weight, the ratio of heart weight to body weight, systolic

Table 1
Morphometry and baseline hemodynamics in wild-type littermate (WT) and MMP-2 transgenic mice (MMP-2 Tg) at age 6 months

	WT	MMP-2 Tg
In vivo (no anesthesia, $n = 6$) BW (g)	44.5 ± 2.2	43.8 ± 1.6
BP (systolic, mmHg)	104 ± 5	101 ± 8
HR (beat/min)	600 ± 10	595 ± 12
ECHO EF (%)	86.4 ± 1.2	80.7 ± 2.2
FS (%)	61 ± 1.7	54.8 ± 2.7
LV mass (mg)	110.9 ± 2.9	125.3 ± 7.2
Ex vivo ($n = 13$) BW (g)	42.9 ± 0.8	43.5 ± 1
PP (mmHg)	70 ± 0.5	70 ± 0.1
CF (ml/min)	3.8 ± 0.3	3.9 ± 0.2
HW (mg)	214 ± 8	211 ± 7
HW/BW ($\times 10^{-3}$)	4.8 ± 0.13	4.9 ± 0.15
LVDP (mmHg)	98 ± 3.9	95 ± 4.2
LVEDP (mmHg)	6 ± 0.7	7 ± 0.5

All values are mean ± SEM. BW, body weight; HW, heart weight; HW/BW, heart weight/body weight ratio; CF, coronary flow; PP, perfusion pressure; LVDP, left ventricular developed pressure (LVDP = LV systolic pressure−diastolic pressure); LVEDP, LV end-diastolic pressure; ECHO, echocardiography; EF, ejection fraction; FS, fractional shortening. There were no significant differences in any parameters between WT and MMP-2 Tg mice.

blood pressure and baseline hemodynamic parameters as measured both *in vivo* and *ex vivo* showed no significant differences between MMP-2 Tg and age-matched WT littermates. Previous studies from our laboratory confirmed a fourfold increase in the level of MMP-2 protein in the transgenic mice associated with a similar increase in enzyme activity [9,10].

Hemodynamics

Myocardial contractility was assessed by measurement of left ventricular developed pressure (LVDP = LV systolic pressure−diastolic pressure) and LV end-diastolic pressure (LVEDP). As shown in Fig. 1, LVDP recovery after I/R in WT hearts declined to 34% of baseline (panel A) and LVEDP was elevated to 33 mmHg (B), whereas in MMP-2 Tg hearts, outcomes were even worse. Thus, LVDP recovery was reduced to 21% of baseline and LVEDP increased to 44 mmHg after reperfusion (panel B) ($P < 0.05$, $n = 13$, respectively). However, IPC significantly improved LVDP recovery (panel C) and reduced LVEDP elevation (panel D) in WT hearts, but not in MMP-2 Tg hearts ($P < 0.01$, $n = 13$, respectively).

Myocardial infarct size and creatine kinase (CK) release

As shown in Fig. 2, MMP-2 Tg hearts showed a significantly larger average infarct size than WT hearts (39.2 ± 2.3 vs. 31 ± 2.5 %, $P < 0.05$, $n = 8$) (panel A). In preconditioned WT hearts, infarct size after I/R was markedly reduced to 20%, whereas in MMP-2 Tg mice, infarct size averaged 35% of LV mass ($P < 0.01$, $n = 6$, panel C). CK assays in these hearts paralleled infarct size measurements (panels B and D).

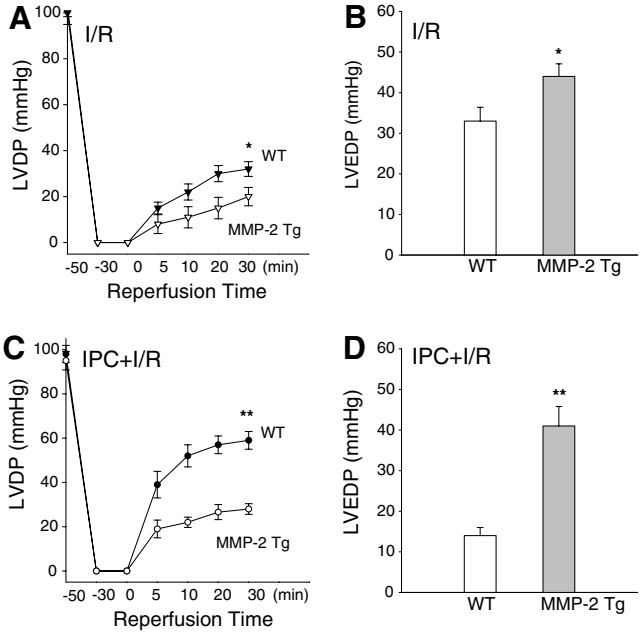

Fig. 1. Measurement in MMP-2 Tg and WT hearts of myocardial contractile performance before and after ischemia/reperfusion (I/R) or in hearts subjected to ischemic preconditioning followed by ischemia–reperfusion (IPC+I/R). ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. WT, respectively, $n = 13$.)

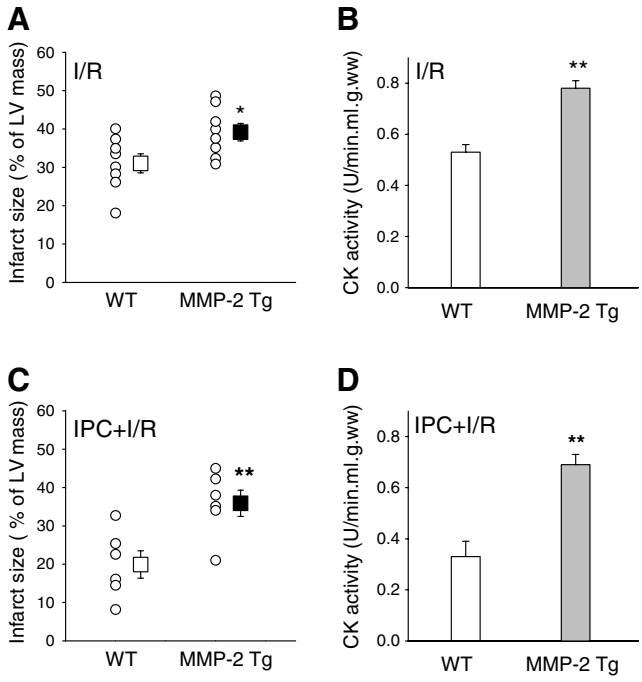

Fig. 2. Measurement of infarct size and creatine kinase (CK) release in MMP-2 Tg and WT hearts after ischemia reperfusion (I/R) or in the hearts subjected to ischemic preconditioning followed by I/R (IPC+I/R). ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. WT, $n = 6$–8.)

Mitochondrial respiration

Results of mitochondrial oxygen consumption rate (OCR) and respiratory control ratio (RCR) are shown in Fig. 3. OCR (panel A) was significantly reduced by an average of 59% and 52%, and RCR (panel B) was similarly reduced by an average of 63% and 54% in WT and MMP-2 Tg hearts, respectively, after I/R compared to normoxic hearts. Preconditioning increased OCR and RCR significantly in WT hearts to an average of 97% and 88% of normoxic heart levels, respectively, but there was no improvement in MMP-2 Tg hearts treated with IPC.

Myocardial malondialdehyde (MDA)

Malondialdehyde (MDA) level is an index of the extent of cellular lipid peroxidation injury caused by toxic free radicals. As depicted in Fig. 3C, MDA levels were significantly increased in hearts subjected to I/R, particularly in MMP-2 Tg hearts ($P < 0.05$, $n = 7$). IPC reduced MDA production in WT hearts but not in MMP-2 Tg hearts ($P < 0.01$, $n = 6$).

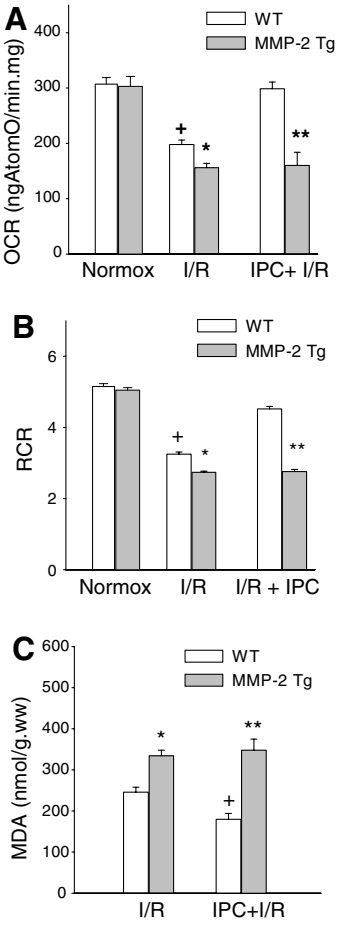

Fig. 3. Mitochondrial oxygen consumption rates (OCR, A) and respiratory control ratios (RCR, B) from normoxic and ischemia–reperfusion (I/R) hearts, and hearts subjected to ischemic preconditioning (IPC) followed by I/R. ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. respective WT controls; $^{+}P < 0.05$ vs. both normoxic and IPC+I/R WT, $n = 7$.) (C) Myocardial malondialdehyde (MDA) content in MMP-2 Tg and WT heart tissue after ischemia–reperfusion (I/R) and in hearts subjected to ischemic preconditioning before I/R (IPC+I/R) ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. respective WT controls; $^{+}P < 0.05$ vs. WT I/R, $n = 6$–7).

Myocardial ultrastructure

Fig. 4 shows representative electron microscopic images of myocardial sections from age-matched WT and MMP-2 Tg hearts before and after I/R. As shown in panel C, I/R caused myofibrillar and mitochondrial deformation in WT associated with mitochondrial swelling and loss of integrity of membranes and cristae, These abnormalities were more extreme in MMP-Tg (panel D) hearts compared to normoxic heart tissue (panels A and B). IPC attenuated mitochondrial ultrastructural deformation after I/R in WT hearts (panel E), but not in MMP-2 Tg hearts (panel F).

Discussion

The major finding of this study is that transgenic expression of active MMP-2 impairs the cardiac response to acute reperfusion injury and IPC-induced cardioprotection compared to WT littermates even though gross structural and functional myocardial alterations are not evident at baseline. These adverse responses are the result of a major disruption in mitochondrial function that remains latent until the advent of oxidative stress.

MMP-2 is known to be transiently activated by acute myocardial injury [8,18–22]. Pharmacological inhibition or targeted deletion of the MMP-2 gene attenuates left ventricular dilation and contractile depression early after murine myocardial infarction [18]. Cheung et al. showed that during reperfusion following ischemia, MMP-2 was increased and correlated inversely with the recovery of myocardial contractility [8]. However, this increase was transient, lasting only 10–20 min, and was associated with a marked reduction of tissue MMP-2 activity [8]. A small transient increase in MMP-2 activity of 50% also was reported during reperfusion by Giricz et al. [22]. These observations contrast with the persistent 4-fold increase in MMP-2 activity seen in our transgenic mice.

To determine whether chronic expression of MMP-2 could prevent preconditioning protection through modulation of mitochondrial respiration [23,24], we treated hearts from MMP-2 Tg and WT mice with IPC followed by ischemia/reperfusion injury. We were surprised to find that mitochondria were more seriously damaged in the MMP-2 Tg hearts compared with their age-matched WT littermate controls after myocardial reperfusion. The severity of ultrastructural deformation, shown by EM, and functional impairment, assessed by OCR and RCR, suggest a causal connection with the depressed recovery of myocardial contraction in MMP-2 Tg hearts after I/R injury. Of particular importance, our data also indicate that IPC-induced mitochondria-dependent salvage pathways are interrupted in MMP-2 Tg hearts.

In a previous study from our laboratory, Wang et al. demonstrated that 8 month-old transgenic mice expressing MMP-2 also showed a normal cardiac phenotype, but the response of isolated right ventricular papillary muscle preparations to inotropic stimulation was diminished even in the absence of oxidative stress [9]. Both the present study and the report of Wang et al. [9] indicate that MMP-2 overexpression impairs cardiac performance even though gross structural alterations are not evident. Wang et al. [9] attributed these abnormalities to a defect in calcium sensitivity. The present data suggest in addition that MMP-2 impairs the cardioprotective response to oxidative stress via disturbed mitochondrial respiration and excessive lipid peroxidation.

Although there is little information regarding the molecular mechanisms by which MMP-2 disrupts mitochondria, it is well-recognized that reactive oxygen species generated by mitochondria can drive both MMP-2 expression and activation [25]. Such activation could result in a negative feedback cycle that degrades mitochondrial membrane potential and impairs mitochondrial function [26]. Thus, Menon et al. have recently reported that MMP-2 interferes

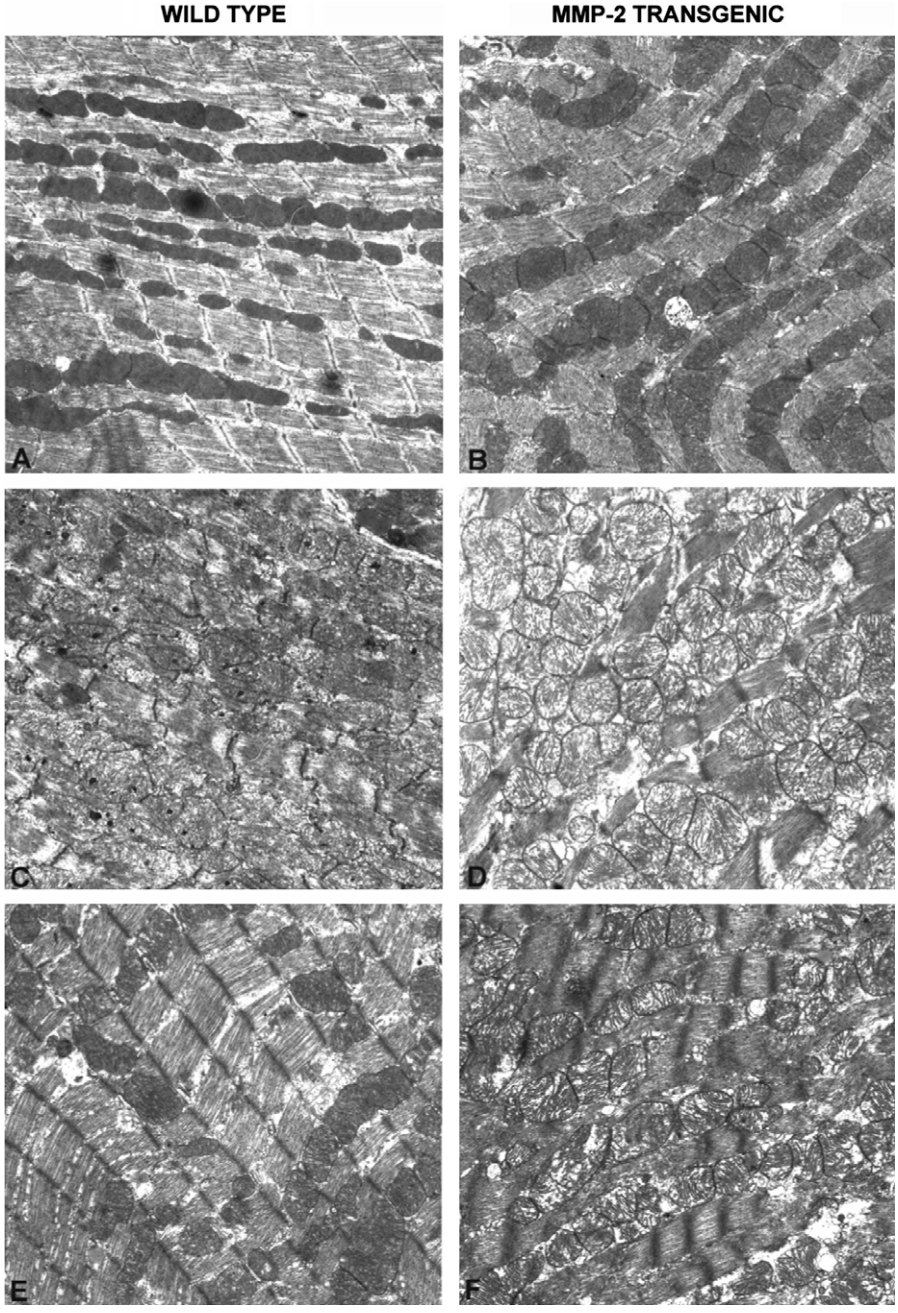

Fig. 4. Representative electron micrographs of ultrastructure in WT and MMP-2 Tg hearts tissue before and after I/R injury. Samples were from the non-infarcted zone of the LV myocardium. (A) Normoxic WT heart (NX-WT): the sarcoplasm is filled with branching myofibrils. Mitochondria are precisely located between myofibrils and Z-lines at the level of the A-band. Both outer and inner membranes are intact. (B) Normoxic transgenic heart (NX-MMP-2 Tg) showed similar baseline morphology and electron density of mitochondria as seen in WT except for a slight increase in collagen. (C) WT heart subjected to ischemia–reperfusion (I/R-WT, ischemic zone): mitochondria are severely edematous with disorganized cristae. They are detached from myofibrils, clustered and swollen with clearly broken membranes. (D) MMP-2 Tg heart subjected to ischemia–reperfusion: (I/R-MMP-2 Tg, ischemic zone): mitochondria are edematous and detached from myofibrils as shown in I/R-WT hearts. (E) Ischemic preconditioned WT heart after ischemia–reperfusion (IPC+I/R, ischemic zone): although some myofibrils are distorted, the baseline morphology of mitochondria and organelle contents are substantially preserved, indicating that IPC reduces the severity of mitochondrial impairment after I/R. (F) Ischemic preconditioned MMP-2 Tg heart (IPC+I/R, ischemic zone): characteristics are similar to those in the MMP-2 Tg heart subjected to I/R without IPC (D), indicating preconditioning did not attenuate damage to the myocardial ultrastructure (EM amplification 6700×).

with β_1 integrin survival signals and activates the JNK-dependent mitochondrial death pathway in adult rat ventricular myocytes [27]. Our observations and those of Menon et al. [27] support a previously unrecognized adverse effect of increased MMP-2 activity on mitochondrial function. The loss of IPC-induced protection in MMP-2 Tg hearts suggests that MMP-2 functions not only as a proteolytic enzyme but also as an active negative reg-

ulator of mitochondrial respiration and cardiac performance which remains latent until the advent of superimposed oxidative stress.

Myocardial ischemic injury evokes dysfunctional ventricular remodeling which constitutes an anatomic substrate for developing congestive heart failure and sudden cardiac death [28]. In contrast to acute ischemic events, patients with chronic heart failure exhibit persistently elevated serum levels of MMP-2 [29]. Based on our data, it can be predicted that such patients would exhibit greater susceptibility to recurrent ischemia/reperfusion injury. Our observations, which are the first to link chronic MMP-2 overexpression to latent mitochondrial dysfunction, also identify MMP-2 as a potential target for therapeutic intervention.

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