Cardiac overexpression of 8-oxoguanine DNA glycosylase 1 protects mitochondrial DNA and reduces cardiac fibrosis following transaortic constriction

Jianxun Wang,1 Qianwen Wang,2 Lewis J. Watson,2,4 Steven P. Jones,2,4 and Paul N. Epstein3

1Department of Pharmacology and Toxicology, 2Department of Physiology, 3Department of Pediatrics and 4Institute of Molecular Cardiology, University of Louisville School of Medicine, Louisville, Kentucky

Submitted 15 February 2011; accepted in final form 23 August 2011

Oxidation of DNA by reactive oxygen species (ROS) can produce nucleotide mutations potentially involved in carcinogenesis and aging (40). Guanine is the nucleic acid base with the lowest oxidation potential, rendering it the most easily oxidizable by hydroxyl radicals and singlet oxygen (31). Therefore, oxidized guanine [7,8-dihydro-8-oxoguanine (8-oxo-dG)] is the most abundant DNA lesion upon oxidative exposure. During DNA replication, 8-oxo-dG will frequently mispair with adenine, leading to formation of G:C to T:A transversions (4, 10, 25). DNA base excision is a major pathway to repair oxidized nucleotides, and the enzyme 8-oxoguanine DNA glycosylase 1 (OGG1) has a major role in removal of 8-oxo-dG (7). For example, OGG1 deficiency in Saccharomyces cerevisiae gives rise to a spontaneous mutator phenotype (11); a 10-fold increase of G:C to T:A transversion frequency in DNA from liver cells was found in homozygous OGG1 knockout (OGG−/−) mice (19); and human OGG1 gene mutations and polymorphisms were found to be associated with head, neck, lung, and kidney cancers (29, 30).

Mitochondrial DNA (mtDNA) damage is more abundant and longer lasting than nuclear DNA (nDNA) damage following exposure to oxidative stress (40, 43). A case in point is the aging rat where levels of 8-oxo-dG in nDNA do not change from 6 to 23 mo of age while 8-oxo-dG increases 2.5-fold in mtDNA with age (15) and 8-oxo-dG levels in mtDNA are an order of magnitude higher than in nDNA (13). One of the reasons mtDNA is more sensitive to ROS mediated damage than nDNA is that mtDNA has no histone protection that can serve as a barrier against ROS. Secondly, mitochondria are the major sources for superoxide production from the electron transport chain (ETC), thereby placing mtDNA in close proximity to ROS production. Lastly, mitochondria have low capacity to repair mutagenesis. The level of 8-oxo-dG in mtDNA correlates with mitochondria dysfunction in many disorders associated with oxidative stress such as aging and neurodegeneration (23), and levels of 8-oxo-dG are inversely correlated to mammalian life span (3). Therefore, 8-oxo-dG in mtDNA is an important lesion that is at least a marker and possibly a cause for development of various diseases associated with oxidative stress.

Oxidative stress is increased in heart failure patients (16) and is frequently associated with quantitative and qualitative defects in mtDNA (17). In heart failure, mitochondria produce more superoxide than normal (38). A vicious cycle can develop for mitochondrial damage in which leakage of superoxide from the ETC promotes mtDNA damage resulting in more ETC damage that then produces more ROS and mtDNA damage. We hypothesized that this destructive cycle in the heart could be disrupted by enhancing mtDNA repair via overexpression of the repair enzyme OGG1. To test this hypothesis, we produced transgenic mice with cardiomyocyte overexpression of OGG1 and subjected them to transaortic constriction (TAC).

Address for reprint requests and other correspondence: P. N. Epstein, 570 S Preston St., Univ. of Louisville, Louisville, KY 40202 (e-mail: paul.epstein@louisville.edu).

Materials and Methods

Construction of the OGG1 transgenic mouse model with cardiomyocyte-specific overexpression of OGG1. First-strand cDNA was synthesized from human fibroblast total RNA by superscript II and oligo dT primers (Invitrogen). The cDNA was amplified by PCR using primers designed for the human mitochondrial isotype 2a of OGG1 (5, 26). The sense primer extends from nucleotide 78 of the OGG1–2a mRNA in the 5′-untranslated region and included an additional Sal I restriction site (gaatttgccagccgagataagctcgca). The antisense primer
extended to position 1,782 in the OGG1–2a mRNA 3′-untranslated region and contained an additional Hind III restriction site (actata-agctccgaggccataagt). This PCR reaction produced a fragment containing all of the coding sequence of the human OGG1–2a isoform. The OGG1–2a isoform is predominantly localized in mitochondria (26) due to the presence of a putative mitochondrial targeting sequence and absence of a nuclear localization signal from exon 7 of the OGG1 gene. The purified OGG1 cDNA fragment was subcloned behind a 5.5-kb DNA fragment from the α-myosin heavy chain (MHC) promoter (12) that produces transcription exclusively in cardiac myocytes and in front of a 500 bp of poly(A) DNA sequence derived from the rat insulin II gene. The sequence of the entire subcloned OGG1 cDNA fragment was confirmed. The transgene was released from the plasmid by Not I digestion and microinjected into single cell fertilized FVB mouse embryos by standard embryo microinjection procedures. Male mice between the ages of 90 and 120 days were used for subsequent experiments. This age was chosen so that animals were large enough for surgery and to reduce variation between mice. All animal procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and were approved by the United States Department of Agriculture-Certified Institutional Animal Care Committee.

**TAC surgery.** TAC surgery was performed by a modification of a previously published technique (2). Mice were anesthetized with avertin (0.4 g/kg), maintained on a 37°C pad, and ventilated with 100% oxygen. An incision at the left second intercostal space was made to open the chest. A chest retractor was applied to facilitate the view. The thymus was pulled away, and transverse aorta was dissected from surrounding tissues. A 6–0 silk suture was passed around the transverse aorta and tightened against a 26-G needle. The needle was immediately removed to provide a lumen with a stenotic aorta. Lungs were inflated, and the chest cavity, muscles, and skin were closed layer by layer with 6–0 silk sutures. The whole procedure lasted 20–30 min. After surgery, mice were warmed with a heating lamp and received an intraperitoneal injection of 0.5 mL of saline at 37°C. For 48 h, mice were given subcutaneous injections of buprenorphine, 0.1 mg/kg every 12 h. Sham mice were done same as TAC animals except that banding of the transverse aorta was omitted.

**Echocardiographic assessment of cardiac function.** Transthoracic echocardiography of the left ventricle (LV) was performed using a 15-MHz linear array transducer (15L8) interfaced with a Sequoia AJP-Heart Circ Physiol • VOL 301 • NOVEMBER 2011 • www.ajpheart.org

**Histological experiments.** Crystall sections (5 μm) were fixed in 10% formalin for 15 min and washed three times with PBS. The cryostat slides were incubated with a saturated solution of picric acid containing 0.1% Sirius red for staining collagen and 0.1% fast green for staining noncollagen proteins. Staining was performed in the dark for 2 h. The slides then were rinsed with distilled water, dehydrated with alcohol, and mounted with permount. The sections were visualized and photographed by a blinded observer. Interstitial fibrosis in the sections was scored by a blinded observer against reference images using a scale of 1 to 4 based on the severity of fibrosis with scores of 1 for low, 2 for mild, 3 for moderate, and 4 for severe.

Quantitative RT-PCR. Cardiac RNA was extracted with Trizol reagent. The total RNA was transcribed to cDNA with Superscript II enzyme and random oligonucleotide primers (Invitrogen). The primers, probes, and reaction buffer for RT-PCR were purchased from AB (Applied Biosystems, Carlsbad, CA) including hOGG1 (Hs00213454_m1), α-MHC (Mm01313844_mH), β-MHC (Mm00600555_m1), atrial natriuretic peptide (ANP) (Mm01355770_g1), procollagen 1 peptide (ANP) (Mm01254476_m1), brain natriuretic peptide (BNP; Mm01255770_g1), procollagen 1(Mm01302043_g1), procollagen 3(Mm01254476_m1), 18S RNA (Hs99999901_s1), and 2 1(Mm01313844_mH), 18S RNA (Hs99999901_s1), and 2

Histological experiments. Crystall sections (5 μm) were fixed in 10% formalin for 15 min and washed three times with PBS. The cryostat slides were incubated with a saturated solution of picric acid containing 0.1% Sirius red for staining collagen and 0.1% fast green for staining noncollagen proteins. Staining was performed in the dark for 2 h. The slides then were rinsed with distilled water, dehydrated with alcohol, and mounted with permount. The sections were visualized and photographed by a blinded observer. Interstitial fibrosis in the sections was scored by a blinded observer against reference images using a scale of 1 to 4 based on the severity of fibrosis with scores of 1 for low, 2 for mild, 3 for moderate, and 4 for severe.
Measurement of 8-oxo-dG content. Content of 8-oxo-dG was measured using a commercial enzyme-linked immunosorbent assay kit (Trevigen, Gaitherburg MD) as described by Gao et al. (9) with modification. In brief, 0.5 μg of sample DNA or 8-oxo-dG standards from 0 to 60 ng/ml were mixed with anti-8-oxo-dG antibody overnight at 4°C, and the sample DNA and 8-oxo-dG standards were transferred to a 96-well microplate previously coated with albumin:8-oxo-dG adduct and kept in the dark at room temperature for 2 h. The secondary antibody coupled with peroxidase was added to each well and incubation followed for 1 h in the dark, and the plate was washed. After six rounds of washing, the substrate tetramethylbenzidine was added and the reaction continued for 15 min in the dark at room temperature before a stop solution was used to terminate the reaction. The wavelength of 450 nm was set to measure the absorbance of the final solution in each well. Results were calculated according to the standard curve.

RESULTS

Characterization of OGG1 transgenic mice. Transgenic mice carrying a cardiac targeted transgene for overexpression of human OGG1 were produced as described in the MATERIALS AND METHODS. A total of four positive founders were obtained from 22 pups. The founder lines were named OGG1–3, OGG1–6, OGG1–7, and OGG1–10. Lines OGG1–3 and OGG1–10 were fertile and passed on the transgene to ~50% of offspring. These lines were tested for expression of human OGG1 mRNA and protein and OGG1 activity. The expression of human OGG1 mRNA in heart was significantly elevated (Fig. 1A; *P < 0.01 vs. FVB control). Western blots showed that human OGG1 protein was markedly in-

![Fig. 1. Elevation of 8-oxoguanine DNA glycosylase 1 (OGG1) mRNA, protein, and enzyme activity in OGG1 transgenic mice.](image-url)
increased in mitochondria of both transgenic lines (Fig. 1B). A possible explanation for the two immunoreactive bands in mitochondria is that the larger band (~45 kDa) derives from the full human OGG1 protein and the smaller band (~42 kDa) comes from the OGG1 protein after removal of the mitochondrial targeting sequence. OGG1 activity measured in extracts of cardiac mitochondria was over twofold elevated over FVB and OGG1 mice (Fig. 1C). A protein extracts from hearts of control and OGG1–10 transgenic mice (Fig. 1C). A

**Table 1. Measurement of heart weight, body weight, tibia length, and the ratios of heart weight to body weight and to tibia length in FVB and OGG1 mice 13 wk after TAC or sham surgery**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FVB-Sham</th>
<th>FVB-TAC</th>
<th>OGG1-Sham</th>
<th>OGG1-TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, mg</td>
<td>124.8 ± 2.9</td>
<td>186.9 ± 9.1*</td>
<td>128.9 ± 3.0</td>
<td>168.8 ± 8.0*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>30.8 ± 0.9</td>
<td>31.8 ± 0.7</td>
<td>31.8 ± 0.7</td>
<td>33.7 ± 1.0</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>18.14 ± 0.16</td>
<td>18.29 ± 0.07</td>
<td>18.24 ± 0.07</td>
<td>18.32 ± 0.07</td>
</tr>
<tr>
<td>HW/BW</td>
<td>4.09 ± 0.15</td>
<td>5.91 ± 0.31*</td>
<td>4.01 ± 0.14</td>
<td>5.09 ± 0.32‡</td>
</tr>
<tr>
<td>HW/TL</td>
<td>6.88 ± 0.14</td>
<td>10.22 ± 0.50*</td>
<td>7.07 ± 0.16</td>
<td>9.22 ± 0.45*</td>
</tr>
</tbody>
</table>

Values are means ± SE. OGG1, 8-oxoguanine DNA glycosylase 1; TAC, transaortic constriction; HW/BW, heart weight-to-body weight ratio; HW/TL, heart weight-to-tibia length ratio. *P < 0.05, FVB-sham vs. FVB-TAC or OGG1-sham vs. OGG1-TAC. †P < 0.05, OGG1-TAC vs. FVB-TAC by two-way ANOVA analysis (n = 11 for sham and n = 14 for TAC groups).

Echocardiography was used to assess the effect of TAC and the OGG1 transgene on cardiac function 13 wk after TAC. The data reported in Table 2 show that TAC mice had significantly impaired cardiac function compared with sham mice. This was evident by several parameters including increased left ventricular end diastolic and systolic diameter as well as reduced fractional shortening. However, there were no significant differences between FVB and OGG1 mice, indicating that OGG1 overexpression did not alter cardiac function nor protect it from the effect of TAC.

Hypertrophic mRNA markers β-MHC, α-MHC, ANP, and BNP were measured to determine whether OGG1 could reduce the fetal pattern of RNA expression induced by pressure overload and hypertrophy (Fig. 2). In sham-treated mice, there were no differences between OGG1 and FVB RNA expression. In both OGG1 and FVB mice, TAC produced the expected increases in expression for β-MHC, ANP, and BNP as well as the expected reduction in the expression of α-MHC. Compared with FVB-TAC mice, OGG1-TAC mice had 41% lower expression of β-MHC (P < 0.05) and OGG1-TAC mice tended toward lower BNP expression (35% lower than FVB-TAC; P = 0.12).

**TAC induction of cardiac fibrosis.** Semiquantitative analysis showed that 13 wk after TAC surgery, fibrosis was significantly increased in both OGG1 and FVB mice (Fig. 3, A and B; P < 0.05). This analysis also showed that interstitial fibrosis staining was lower in OGG1-TAC mice compared with FVB-TAC mice (P < 0.05). The staining analysis was supported by quantitative RT-PCR assays of cardiac collagen mRNA expression (Fig. 3, C and D). Compared with FVB-TAC mice, expression of collagen 1α1 mRNA and collagen 3α1 mRNA was 49 and 48% lower in OGG1-TAC mice, respectively (P < 0.05 for both mRNAs).

**8-oxo-dG content in DNA after TAC.** Thirteen weeks after surgery, we tested whether TAC increased 8-oxo-dG content in nDNA and mtDNA and whether OGG1 overexpression could reduce 8-oxo-dG content. In mitochondria (Fig. 4A), TAC

**Table 2. Cardiac function measured by echocardiography in FVB and OGG1 mice 13 wk after TAC or sham surgery**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FVB-Sham</th>
<th>FVB-TAC</th>
<th>OGG1-Sham</th>
<th>OGG1-TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD, mm</td>
<td>3.81 ± 0.08</td>
<td>4.15 ± 0.05*</td>
<td>3.81 ± 0.10</td>
<td>4.03 ± 0.08*</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.36 ± 0.10</td>
<td>2.76 ± 0.08*</td>
<td>2.27 ± 0.09</td>
<td>2.59 ± 0.08*</td>
</tr>
<tr>
<td>IVS(d), mm</td>
<td>0.91 ± 0.02</td>
<td>1.02 ± 0.03*</td>
<td>0.89 ± 0.03</td>
<td>1.0 ± 0.02*</td>
</tr>
<tr>
<td>IVS(s), mm</td>
<td>1.18 ± 0.03</td>
<td>1.29 ± 0.03*</td>
<td>1.19 ± 0.03</td>
<td>1.24 ± 0.02</td>
</tr>
<tr>
<td>PWTh(d), mm</td>
<td>0.80 ± 0.04</td>
<td>0.89 ± 0.03*</td>
<td>0.76 ± 0.03</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>PWTh(s), mm</td>
<td>1.16 ± 0.04</td>
<td>1.25 ± 0.02</td>
<td>1.17 ± 0.05</td>
<td>1.25 ± 0.03</td>
</tr>
<tr>
<td>IVS/Th</td>
<td>30.87 ± 2.78</td>
<td>29.26 ± 3.50</td>
<td>33.74 ± 2.67</td>
<td>24.72 ± 2.73</td>
</tr>
<tr>
<td>PW/Th</td>
<td>40.67 ± 5.27</td>
<td>40.32 ± 3.08</td>
<td>48.51 ± 3.31</td>
<td>49.03 ± 4.08</td>
</tr>
<tr>
<td>IVS/SPW</td>
<td>1.16 ± 0.05</td>
<td>1.13 ± 0.03</td>
<td>2.2 ± 1.01</td>
<td>1.19 ± 0.04</td>
</tr>
<tr>
<td>%FS</td>
<td>38.6 ± 1.7</td>
<td>33.8 ± 1.1*</td>
<td>40.59 ± 1.40</td>
<td>34.74 ± 1.06*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>487 ± 10</td>
<td>516 ± 11</td>
<td>513 ± 12</td>
<td>535 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; IVS(d), interventricular septum thickness at diastole; IVS(s), interventricular septum thickness at systole; PWTh(d), postwall thickness at diastole; PWTh(s), postwall thickness at systole; IVS%Th, interventricular septum % thickening; PW%Th, posterior wall % thickening; IVS/SPW, interventricular septum to posterior wall thickness ratio; %FS, percent fractional shortening; HR, heart rate. *P < 0.05, FVB-sham vs. FVB-TAC or OGG1-sham vs. OGG1-TAC by two-way ANOVA analysis (n = 12 for FVB-sham, n = 10 for OGG1-sham, n = 14 for FVB-TAC, and n = 13 for OGG1-TAC group).
produced a 30% increase in 8-oxo-dG ($P < 0.05$) of FVB mice. This TAC-induced increase in mtDNA 8-oxo-dG content was completely prevented by overexpression of OGG1. In addition, OGG1-sham mice had 25% lower 8-oxo-dG content in mtDNA than FVB-sham mice ($P < 0.05$). In nuclear DNA 8-oxo-dG levels were much lower than in mtDNA (Fig. 4B). TAC produced a significant 10% increase in 8-oxo-dG content in both types of mice but there were no significant differences for nuclear DNA 8-oxo-dG content between OGG1 and FVB mice. Overall, these results show that OGG1 provides protection against some components of cardiac dysfunction in the TAC model including reductions in fibrosis, β-MHC expression, and 8-oxo-dG content in mtDNA.

**DISCUSSION**

Damage to cardiac DNA, especially mtDNA by reactive oxygen has been proposed to be a contributing factor in the development of heart failure (3, 37). Human studies (17) and animal models (8, 21, 27, 33) have demonstrated that there is a strong association between cardiac injury and oxidative damage to mtDNA. This relationship has been shown following various treatments including exposure to doxorubicin (21, 33), angiotensin (32), or isoproterenol (18), the process of aging (6, 15), and knockout of mitochondrial SOD2 (24). However, no cardiac-specific models have been created to determine if reducing mtDNA damage can protect the heart, thereby testing whether the association between oxidative mtDNA damage and heart failure is actually a causal relationship. In this study, we describe successful production of OGG1 transgenic mice that have cardiac-specific overexpression of active OGG1 enzyme, which catalyzes the first essential step in the repair pathway of oxidized DNA. OGG1 transgenic mice were shown to have increased hOGG1 mRNA, elevated hOGG1 protein, 28% increased nuclear OGG1 activity, and a 115% increase of OGG1 enzyme activity in mitochondria. In vivo efficacy of the transgene was demonstrated by reduced levels of mitochondrial 8-oxo-dG content in transgenic mice under basal conditions, after doxorubicin treatment, and after TAC surgery. Overexpression of OGG1 produced no measurable detrimental effects on the heart. Thus this transgenic line provides a suitable model for performing direct tests for a causal role of 8-oxo-dG mutations in cardiac pathology.

The efficacy of OGG1 overexpression was tested by challenge with TAC surgery. Transgenic mice demonstrated essentially complete protection from TAC-induced elevation of 8-oxo-dG, but despite this protection, we saw little benefit of the OGG1 transgene either to reduce cardiac hypertrophy or to improve cardiac function measured by echocardiography 13 wk after surgery. There are multiple potential explanations for this lack of effect. One explanation may be that mechanisms mediating stress-induced cardiac hypertrophy such as growth factor pathways (14) or kinase-mediated actin rearrangements (20, 22) are not closely linked to the condition of mtDNA. If these pathways are not linked to mtDNA damage, then the reduction in mtDNA mutations produced by the OGG1 transgene cannot protect from TAC-induced dysfunction. However,
the fact that OGG1 transgenic mice had reduced β-MHC mRNA expression after TAC suggests that mtDNA mutations have at least a modest impact on the cardiac hypertrophy response or that decreasing fibrosis limited the elevation in β-MHC expression. An alternative reason for the lack of a larger OGG1 effect may be that we did not wait long enough after surgery. The 13-wk time point after TAC may not be long enough for sufficient mtDNA mutation to accumulate and disrupt mitochondrial function in many cardiomyocytes. Over a longer period mutated mtDNA may spread within individual myocytes from heteroplasmy towards homoplasmy through relaxed replication (6). Accumulation of mtDNA mutations appears to have a high threshold before essential mitochondrial functions are compromised: in OGG1<sup>−/−</sup> mice elevation of 8-oxo-dG content in mtDNA did not produce a measurable impairment of mitochondrial function including respiratory rate and ATP synthetic activity in heart or liver (35) despite the fact that some threshold of 8-oxo-dG accumulation can be expected to impair mitochondrial respiration. Also, our unpublished studies revealed that the OGG1 transgene had no effect on the amount of hydrogen peroxide generated by isolated mitochondria using substrates for Complex I or II and in the presence or absence of the respiratory chain inhibitors rotenone, antimycin, and myxothiazol. This finding is consistent with the previous report of Stuart et al. (35) that protein oxidation is not increased in cardiac mitochondria of OGG1<sup>−/−</sup> despite elevated 8-oxo-dG content. Sufficient oxidative damage to mtDNA is associated with increased apoptosis (28). Therefore, additional study of these transgenic mice may reveal that OGG1 can reduce apoptosis after TAC treatment and that this

---

Fig. 3. Fibrosis in FVB and OGG1 hearts 13 wk after TAC. A: representative Sirius red staining for fibrosis in FVB and OGG1 hearts. B: semiquantitative scores for fibrosis staining performed as described in MATERIALS AND METHODS by a blinded observer. Staining of fibrosis in OGG1-TAC hearts was significantly lower than that in FVB-TAC hearts (*P < 0.05, OGG1-TAC vs. FVB-TAC; *P < 0.05, sham vs. TAC by two-way ANOVA; n = 5 for each group). Expression of collagen 1α1 mRNA (C) and collagen 3α1 mRNA (D) measured by RT-PCR was significantly lower in OGG1-TAC hearts than in FVB-TAC hearts (*P < 0.05, OGG1-TAC vs. FVB-TAC; *P < 0.05, sham vs. TAC by two-way ANOVA; n = 5 for FVB-sham, FVB-TAC, and OGG1-sham; n = 8 for OGG1-TAC).

---

Fig. 4. Content of 8-oxo-dG content in mitochondrial and nuclear DNA 13 wk after TAC. A: in FVB mitochondria TAC increased 8-oxo-dG content (*P < 0.05, for FVB-sham vs. FVB-TAC). OGG1 lowered sham levels of 8-oxo-dG and prevented the TAC-induced increase in 8-oxo-dG content (*P < 0.05, for OGG1-sham vs. FVB-sham and for OGG1-TAC vs. FVB-TAC; n = 6 for each group). B: in nuclei TAC increased 8-oxo-dG content in FVB and OGG1 mice (*P < 0.05, for sham vs. TAC; n = 5 per group, all statistics by two-way ANOVA). OGG1 transgene did not alter 8-oxo-dG content in nuclear DNA of sham or TAC mice.
could be responsible for the lower level of cardiac fibrosis. Potentially more time after TAC surgery or long-term aging studies may reveal greater benefit of the OGG1 transgene.

Apart from decreased mitochondrial 8-oxo-dG, the principle benefit of OGG1 overexpression was a significant decrease in TAC-induced cardiac fibrosis. This protection was first indicated by reduced Sirius red staining on OGG1 cardiac sections. The OGG1 effect on fibrosis was confirmed by measuring decreased induction of collagen 1 and 3 mRNA expression in OGG1 hearts after TAC surgery. Cardiac fibrosis is primarily mediated by collagen secreting myofibroblasts (39). Myofibroblasts cannot express the OGG1 transgene due to the transgene’s regulation by the cardiomyocyte-specific α-MHC promoter (1). Therefore, cardiomyocyte overexpression of OGG1 apparently decreased a profibrotic signal that originated in cardiomyocytes and then acted on myofibroblasts to stimulate production of collagen matrix. It is also surprising that the OGG1 transgene had only a small effect on cardiac hypertrophy but a larger effect on fibrosis. This may imply that the profibrotic-signaling process is more sensitive to mtDNA oxidation than the hypertrophic response. Alternatively, the fact that many myofibroblasts can be stimulated by a diffusible molecule may result in amplification of the profibrotic signal produced by the most damaged cardiomyocytes. The large antifibrotic effects of the OGG1 transgene may be due to production of fewer severely injured cardiomyocytes or less profibrotic signaling from these cardiomyocytes.

A limitation of this study is that OGG1 levels were increased either condition. In summary, cardiac overexpression of OGG1 reduced mtDNA content of 8-oxo-dG in normal and stressed hearts. After TAC, the protection of mtDNA was associated with a slightly reduced hypertrophic response and clearly reduced cardiac fibrosis. These findings support the concept that mtDNA oxidation contributes to LV remodeling during pressure overload hypertrophy.

GRANTS
This work was supported by National Institutes of Health Grants DK-073586 (to P. N. Epstein), HL-094419 and HL-083320 (to S. P. Jones), and NCRR COBRE P20-RR-024489.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES