Ogg1-Dependent DNA Repair Regulates NLRP3 Inflammasome and Prevents Atherosclerosis

Gantsetseg Tumurkhuu, Kenichi Shimada, Jargalsaikhan Dagvadorj, Timothy R. Crother, Wenhuan Zhang, Daniel Luthringer, Roberta A. Gottlieb, Shuang Chen,* Moshe Arditi*

Rationale: Activation of NLRP3 (nucleotide-binding domain and leucine-rich repeat pyrin domain containing 3) inflammasome-mediating interleukin (IL)-1β secretion has emerged as an important component of inflammatory processes in atherosclerosis. Mitochondrial DNA (mtDNA) damage is detrimental in atherosclerosis, and mitochondria are central regulators of the nucleotide-binding domain and leucine-rich repeat pyrin domain containing 3 inflammasome. Human atherosclerotic plaques express increased mtDNA damage. The major DNA glycosylase, 8-oxoguanine glycosylase (OGG1), is responsible for removing the most abundant form of oxidative DNA damage.

Objective: To test the role of OGG1 in the development of atherosclerosis in mouse.

Methods and Results: We observed that Ogg1 expression decreases over time in atherosclerotic lesion macrophages of low-density lipoprotein receptor (Ldlr) knockout mice fed a Western diet. Ogg1−/−Ldlr−/− mice fed a Western diet resulted in an increase in plaque size and lipid content. We found increased oxidized mtDNA, inflammasome activation, and apoptosis in atherosclerotic lesions and also higher serum IL-1β and IL-18 in Ogg1−/−Ldlr−/− mice than in Ldlr−/−. Transplantation with Ogg1−/− bone marrow into Ldlr−/− mice led to larger atherosclerotic lesions and increased IL-1β production. However, transplantation of Ogg1−/−Nlrp3−/− bone marrow reversed the Ogg1−/− phenotype of increased plaque size. Ogg1−/− macrophages showed increased oxidized mtDNA and had greater amounts of cytosolic mtDNA and cytochrome c, increased apoptosis, and more IL-1β secretion. Finally, we found that proatherogenic miR-33 can directly inhibit human OGG1 expression and indirectly suppress both mouse and human OGG1 via AMP-activated protein kinase.

Conclusions: OGG1 plays a protective role in atherogenesis by preventing excessive inflammasome activation. Our study provides insight into a new target for therapeutic intervention based on a link between oxidative mtDNA damage, OGG1, and atherosclerosis via NLRP3 inflammasome. (Circ Res. 2016;119:e76-e90. DOI: 10.1161/CIRCRESAHA.116.308362.)

Key Words: atherosclerosis ■ DNA repair ■ inflammasomes ■ macrophages ■ mitochondria

Atherosclerosis is a chronic inflammatory disease that arises from an imbalance in lipid metabolism and a maladaptive immune response driven by the accumulation of cholesterol-laden macrophages in the artery wall. Rupture of atherosclerotic lesions results in thrombotic occlusion of coronary and cerebral vessels, producing the clinical complications of atherosclerosis.1,2 There is a growing understanding that blood monocytes are recruited to the inflamed vascular wall develop into inflammatory macrophages (M1-like phenotype) and foam cells, which contribute to pathogenesis at many stages of this disease and, therefore, represent a target for therapeutic interventions.1,4 In addition to hyperlipidemia and other genetic factors, inflammation is now known as a critical contributor to atherogenesis. The activation of NLRP3 (nucleotide-binding domain and leucine-rich repeat pyrin domain containing 3) inflammasome-mediating interleukin (IL)-1β secretion has recently emerged as an important component of inflammatory processes underlying atherosclerosis.5,7 The NLRP3 inflammasome can be activated by many diverse danger signals and...
mitochondrial (mt) function as the central hub for integrating these various signals.5,6,9 We recently showed that during apoptosis/pyroptosis, mitochondrial DNA (mtDNA) damaged by oxidative stress activates the NLRP3 inflammasome, releasing active IL-1β.10 Mitochondria are a major site of reactive oxygen species (ROS) production, and mtDNA is a vulnerable target for ROS-mediated oxidative damage because of its close proximity and the lack of protective histones. Human atherosclerotic plaques showed increased mtDNA damage compared with normal vessels, and leukocyte mtDNA damage was associated with higher-risk plaques.11,12

Oxidative damage in DNA is repaired primarily via the enzymes of the base excision repair pathway. Among these, 8-oxoguanine glycosylase (OGG1), the major DNA glycosylase, is responsible for removal of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-OH-dG), one of the most frequent endogenous base lesions formed in the DNA of aerobic organisms.13 OGG1 deficiency leads to elevated levels of 8-OH-dG in mtDNA.14,15 In contrast, mitochondrial overexpression of Ogg1 improves mitochondrial function, cell survival, and fewer mtDNA deletions through increased repair of 8-OH-dG under oxidative stress conditions in vitro.16 A recent study reported that the loss of OGG1 led to increased apoptosis and larger fatty streaks in mice fed on Western diet (WD) and overexpression of Ogg1 reversed this phenotype, suggesting that Ogg1 may play a protective role in atherosclerosis.17

Micro-RNAs are critical post-transcriptional regulators of their target genes. miR-33 targets many genes involved in regulating cellular cholesterol export and fatty acid oxidation, including Prkaal (protein kinase, AMP-activated, alpha 1 catalytic subunit) whose gene product is commonly referred to as AMPKα1 (5′ AMP-activated protein kinase alpha subunit 1), which is a metabolic master switch and a target of several drugs used for metabolic diseases in patients.18–20 Interestingly, AMPK also regulates Ogg1 expression.21 Antagonizing miR-33a/b may be an effective strategy for raising plasma high-density lipoprotein levels and protecting against atherosclerosis in mice and nonhuman primates.22,23

In this work, we show the atheroprotective role of OGG1 in a WD-induced model of atherosclerosis using Ldlr−/− mice. The loss of OGG1 resulted in increased mtDNA damage, increased NLRP3 inflammasome activation in macrophages, increased IL-1β production, and more apoptosis in atherosclerotic plaques, which led to accelerated atherogenesis. NLRP3 deficiency counteracted the loss of OGG1, evidenced by smaller atheromatous plaque lesions.

**Methods**

**Animals and Diets**

Ogg1+/− mice were kindly provided from Dr Christi A. Walter (University of Texas Health Science Center at San Antonio). Nlrp3−/− mice were provided by Dr K.A. Fitzgerald (University of Massachusetts Medical School, Worcester, MA). We have crossed for at least 8 generations and established Ogg1+/−Ldlr−/− and Ogg1−/−Nlrp3−/− double knockout (DKO) mice. Starting at 8 weeks of age, mice were fed a WD (TD88137, Harlan Teklad) for 16 weeks. For bone marrow (BM) transplantation, BM from Ogg1−/−, wild-type (WT), and Ogg1−/−Nlrp3−/− mice was transplanted into irradiated Ldlr−/− mice. After recovery (8 weeks), chimeric mice were placed on a WD for 12 weeks. Whole blood was collected to confirm the efficiency of the BM transplant. All animal experimental procedures were conducted in strict compliance with the policies on animal welfare of the National Institute of Health. The protocol was approved by the Animal Care and Use Committee at Cedars-Sinai Medical Center.

**Assessment of Atherosclerotic Lesions in the Aorta and Aortic Sinus**

The aortas were dissected, and the adherent (adventitial) fat was gently removed. Whole aortas were opened longitudinally from the aortic arch to the iliac bifurcation, mounted en face, and stained for lipids with Oil Red O. Hearts were embedded in optimum cutting temperature compound (Tissue-Tek; Sakura, Torrance, CA), and serial 7-μm-thick cryosections from the aortic sinus were mounted and stained with Oil Red O and hematoxylin. Image analysis was performed by a trained observer blinded to the genotype of the mice. Representative images were obtained, and lesion areas were quantified with Image analysis software using a BZ-9000 microscope (Keyence, Itasca, IL). The lesion area in the aorta en face preparations was expressed as a percent of the aortic surface area, as previously reported. The lesion area in the aortic sinus was measured.24

**Immunofluorescent Analysis of Cryosections of the Aortic Sinus**

Aortic plaques in mice were detected in TUNEL (TdT-mediated dUTP nick end labeling) after proteinase K and EDTA treatment, using the In Situ Cell Death Detection Kit (Millipore). Caspase-1 activity was detected by fluorochrome-labeled inhibitors of caspases assay staining. For Immunohistochemical staining for frozen sections, fixing and antigen blocking were performed using immunoglobulin from the species of the secondary antibodies. Next, the sections were incubated with primary antibodies overnight at 4°C, followed by incubation with an appropriate secondary antibodies conjugated with fluorescent dyes. For assessment of macrophage colocalization, macrophages were detected using anti-F4/80 antibody (eBioscience) and anti-CD68 (eBioscience); nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole). For some analysis, anti-8OH-dG (Bioss) was used, along with anti-TOM20 (Santa Cruz, CA). Images were detected using BZ-9000 microscope and analyzed by BZ analyzer software.

**Laser-Capture Microdissection**

Laser-capture microdissection was performed using an LMD instrument (Leica LMD 700; Leica Biosystems) as previously described.25 To visualize F4/80+ cells, a guide slide was prepared by staining for F4/80 as described above. Cells corresponding to F4/80+ area in serial sections were collected, and RNA was extracted using the RNA Isolation Kit (Clontech). cDNA was synthesized and SYBR Green 2x polymerase chain reaction mixture (Clontech) was used in quantitative polymerase chain reaction according to the manufacturer’s instructions. Ogg1 primers: Fw: 5′-tgtgacgcgagacgcacta-3′, Rv: 5′-ctggtcagcaggcatcacta-3′.
BMDMs produced less IL-1β compared to WT cells. Ogg1−/− macrophages secreted significantly more IL-1β, but not tumor necrosis factor-α, compared with WT cells (Figure 1E). We next investigated whether we could rescue this phenotype by adding mitochondrial-specific Ogg1 back into Ogg1−/− BMDMs. Retrovirus-expressing mtOgg1 introduced into the Ogg1−/− BMDMs produced less IL-1β in response to LPS+ATP stimulation compared with control virus transduced cells (Figure 1F). The efficiency of retroviral infection was checked by eGFP (enhanced green fluorescent protein) expression under fluorescent microscope (Online Figure IA). Taken together, these results indicate that macrophages deficient in OGG1 exhibit greater mitochondrial dysfunction, increased inflammasome activity, and more apoptosis when exposed to 7-ke tocholesterol than to WT cells.

### Results

#### OGG1 Deficiency Promotes Apoptotic and Inflammatory Responses in Macrophages

Somatic mtDNA damage has been reported to accumulate with aging-associated coronary atherosclerotic heart disease. OGG1 removes the most common oxidative DNA lesion, 8-OH-dG, which we have previously linked to NLRP3 inflammasome activation. We wanted to determine the effect of OGG1 deficiency in oxidized mtDNA damage in macrophages. Ogg1−/− bone marrow–derived macrophages (BMDMs) treated with menadione, an ROS inducer, had greater amounts of oxidative damage in their mtDNA as measured by 8-OH-dG dot blot analysis compared with WT BMDMs (Figure 1A). Failure to repair oxidative DNA damage in OGG1-deficient mice can lead to further mt dysfunction and apoptosis. Therefore, we examined the effect of the cytotoxic oxysterol, 7-ketocholesterol (7-KC), which is found in oxidized low-density lipoprotein and atherosclerotic lesions, on apoptosis.

7-KC treatment resulted in more cytochrome c and mtDNA in the cytosol of OGG1-deficient macrophages compared with WT cells (Figure 1B and 1C). 7-KC can activate the NLRP3 inflammasome. To assess whether an increase in oxidized mtDNA could promote an inflammatory phenotype caused by 7-KC, we examined caspase-1 activation by fluorochrome-labeled inhibitors of caspases assay. 7-KC treatment elicited more caspase-1 activation in Ogg1−/− macrophages than in WT controls (data not shown). We assessed cell viability by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after incubating with 7-KC for 24 hours. Exposure to 7-KC resulted in a concentration-dependent increase in cell death that was significantly greater in Ogg1−/− cells (Figure 1D). The NLRP3 inflammasome is activated by many diverse danger signals, and mt function is the central hub for integrating these signals, resulting in inflammation. We previously showed that oxidized mtDNA binds to and activates the NLRP3 inflammasome. Thus, in Ogg1−/− BMDMs, where there is more oxidized mtDNA damage, there should be greater NLRP3 inflammasome activation. BMDMs were pretreated with lipopolysaccharide (LPS) and then stimulated with various NLRP3 activators, such as ATP, 7-KC, and cholesterol crystals. Ogg1−/− macrophages secreted significantly more IL-1β, but not tumor necrosis factor-α, compared with WT cells (Figure 1E). We next investigated whether we could rescue this phenotype by adding mitochondrial-specific Ogg1 back into Ogg1−/− BMDMs. Retrovirus-expressing mtOgg1 introduced into the Ogg1−/− BMDMs produced less IL-1β in response to LPS+ATP stimulation compared with control virus transduced cells (Figure 1F). The efficiency of retroviral infection was checked by eGFP (enhanced green fluorescent protein) expression under fluorescent microscope (Online Figure IA). Taken together, these results indicate that macrophages deficient in OGG1 exhibit greater mitochondrial dysfunction, increased inflammasome activity, and more apoptosis when exposed to 7-ke tocholesterol than to WT cells.

#### Expression of OGG1 Diminishes as Atherosclerotic Lesions Progress

mtDNA damage has been correlated with the extent of atherosclerosis and usually precedes atherogenesis. Oxidative damage to mtDNA can be detected by strong immunoreactivity for 8-OH-dG, is found in all cell types of the plaque, and correlates with atherosclerosis progression. However, the expression of Ogg1 in the lesion during diet-induced atherogenesis is not known. At 8 weeks of age, Ldlr−/− mice were fed WD, and blood and tissue were harvested at the indicated time points. As expected, serum total cholesterol and aortic root plaque size increased over time (Figure 2A and 2B). OGG1 was detected in the lesions by immunofluorescence and was readily visible in the lesion after 4 weeks on WD (Figure 2C and 2D). However, OGG1 protein decreased as the lesion progressed, with staining in few cells by 16 weeks of WD. The reduction in OGG1 staining was also specifically observed in F4/80-positive macrophages (Figure 2C and 2D). Recent studies have found that some smooth muscle cells may upregulate F4/80 on their surface as they become more macrophage like. However, in our system, the majority of F4/80-positive cells coexpressed CD45, indicative of their hematopoietic origin (Online Figure IIA). We confirmed our immunofluorescence results using laser-capture microdissection. F4/80+ macrophages were isolated from atherosclerotic plaques of Ldlr−/− mice fed WD for 4 and 16 weeks. Total RNA was extracted and mRNA expression analysis performed by real-time polymerase chain reaction (RT-PCR). Notably, Ogg1 mRNA expression levels decreased in lesional macrophages at 16 weeks compared with 4 weeks (Figure 2E). Taken together, these data suggest that Ogg1 expression diminishes in the plaque lesions generally, and in macrophages specifically, as the atheroma progresses.

#### OGG1 Deficiency Leads to Accelerated Atherosclerosis

OGG1 expression diminishes in the plaque lesions generally, and in macrophages specifically, as the atheroma progresses. To understand the significance of OGG1 in the development and progression of atheromas, we generated Ogg1−/−Ldlr−/− DKO mice and fed them a WD diet for 16 weeks starting at 8 weeks of age. Representative images of the aortic root lesions are shown in Figure 3A. We found that the atherosclerotic lesion area and its macrophage content in cross sections of the aortic root were increased significantly in Ogg1−/−Ldlr−/− mice compared with Ldlr−/− controls (Figure 3B and 3C). The critical features of advanced atheromatous lesions are increases in TUNEL-positive apoptotic cells and necrotic area and a corresponding decrease in collagen content. OGG1 deficiency resulted in a significant increase in both TUNEL-positive cells and necrotic area, as well as a decrease in collagen content in their lesions (Figure 3D through 3F; Online Figure IIIB). En face analyses of the aorta showed an increase in the total surface area occupied by lesions in DKO mice compared with controls.
Figure 1. 8-Oxoguanine glycosylase (OGG1)–deficient macrophages are more apoptotic and inflammatory. A, The level of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-OH-dG) in mitochondrial DNA (mtDNA). Bone marrow–derived macrophages (BMDMs) derived from wild-type (WT) or Ogg1−/− mice were stimulated with reactive oxygen species inducer (menadione 25 μmol/L for 30 min), followed by 2-h incubation to allow for DNA repair. mtDNA was extracted, 8-OH-dG was measured by dot blot analysis, and COX-1 (cytochrome c oxidase subunit I) polymerase chain reaction (PCR) served as a loading control. Densitometry is shown in the right. Similar results were observed in 3 independent experiments. B and C, BMDM derived from WT or Ogg1−/− mice were stimulated with 40 μg/mL 7-ketocholesterol (7-KC) or control for 4 h. B, Cytochrome c release into the cytosol was assessed by Western blot analysis. Similar results were observed in 3 independent experiments. C, mtDNA content in the cytosol was assessed by RT-PCR. D, Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after indicated doses of 7-KC treatment for 24 h. E, Four-hour LPS-primed BMDM from WT or Ogg1−/− mice were stimulated with 5 mmol/L ATP (1 h), 20 μg/mL 7-KC (6 h), or 2 mg/mL cholesterol crystals (16 h). IL-1β and tumor necrosis factor (TNF)-α concentrations in the culture supernatant were determined by ELISA. F, Ogg1 KO BMDM cells were transduced with control and mtOgg1 expressing retrovirus for 48 h before the treatment of 400 ng/mL of LPS for 4 h plus 5 mmol/L of ATP in the last hour. IL-1β and TNF-α concentrations in the culture supernatant were determined by ELISA. C–F, All data are means±SD and representative of 3 independent experiments in triplicate. Significance was determined using Student’s t test *P<0.05, **P<0.01, ***P<0.001.
(Figure 3G and 3H), suggesting the loss of OGG1 accelerates diet-induced atheroma development in Ldlr−/− mice. We did observe a gene dose effect as Ogg1 +/- Ldlr−/− mice displayed an intermediate phenotype. Thus, OGG1 deficiency leads to larger more advanced atheromatous lesions on the Ldlr−/− background.

OGG1 Deficiency Leads to Increased Damaged mtDNA (8-OH-dG) and Inflammasome Activity in Atherosclerotic Lesions
Because OGG1 removes 8-OH-dG from DNA, we expected that we would find increased accumulation of 8-OH-dG modifications of DNA in the plaques in our Ogg1−/− Ldlr−/− mice. Immunofluorescence revealed a ≈4-fold greater accumulation of 8-OH-dG in the plaques of DKO mice compared with controls (Figure 4A and 4B). Using Tom20 as a mitochondrial marker, colocalization analysis indicated that >80% of the 8-OH-dG staining was mitochondrial, implicating mtDNA as the likely target (Figure 4B; Online Figure IIC). In addition, F4/80-positive macrophages were observed to have increased 8-OH-dG staining in the lesions as well (Figure 4C).

Because we found an increase in 8-OH-dG in the lesions, and concomitantly an increase in apoptotic cells, we reasoned...
that we would also find an increase in IL-1β–producing cells as apoptotic signals along with oxidized mtDNA can activate the NLRP3 inflammasome. To elucidate the contribution of NLRP3 inflammasome in the accelerated atherogenesis that we observed in OGG1-deficient Ldlr−/− mice (DKO mice), caspase-1 activation was investigated by fluorochrome-labeled inhibitors of caspases assay in lesions of the aortic roots of both groups of mice. Nearly 2-fold more fluorochrome-labeled inhibitors of caspases assay–positive F4/80+ macrophages were detected in the plaques of DKO mice compared with controls (Figure 4D and 4E). Taken together, these results indicate that the loss of OGG1 results in advanced plaque formation characterized by increased oxidized DNA damage (8-OH-dG), more apoptotic cells, and more cells with active caspase-1, which can produce IL-1 and IL-18, cytokines that are both strongly proatherogenic.

Lipid accumulation and cell death within the lesions contribute to the activation of inflammatory cells that release proinflammatory and proatherogenic mediators into the serum. In addition, our data suggest increased inflammasome activity in OGG1-deficient mice. Analysis of key cytokines and chemokines in the plasma revealed significantly higher concentrations of IL-1β, IL-18, and MCP-1 (monocyte chemoatractant protein-1) in DKO mice, whereas the concentrations of IL-12p40, IL-6, and tumor necrosis factor-α were indistinguishable between the 2 groups (Figure 5). Both IL-1β and IL-18 are dependent on the inflammasome for their secretion. A previous study reported that Ogg1−/− mice may develop metabolic syndrome. To determine whether the differences we observed were because of possible metabolic changes, we tested the mice after 16 weeks of WD for glucose tolerance and insulin resistance and found no difference between the genotypes (Online Figure IIIA). The body weight gains and plasma cholesterol levels (triglycerides and lipoprotein profiles) were also similar (Online Figure IIIIB and IIIC). Thus, the accelerated atheroma development in the OGG1-deficient mice in
this study cannot be attributed to metabolic changes such as more severe dyslipidemia or altered glucose homeostasis.

NLRP3 Significantly Contributes to OGG1 Deficiency–Induced Acceleration of Atherosclerosis in Hematopoietic Cells

We hypothesized that OGG1 deficiency and the consequent accumulation of 8-OH-dG in mtDNA would result in more NLRP3 inflammasome activation as a critical driver of the accelerated atherosclerosis that we observed. Therefore, we next investigated whether hematopoietic NLRP3 is required for the acceleration of atherosclerosis in Ogg1−/− mice.

We generated Ogg1−/− Nlrp3−/− mice, created bone marrow (BM) chimeric mice using BM cells from WT, Ogg1−/−, or Nlrp3−/− into Ldlr−/−-recipient mice and placed them on WD for 12 weeks. Mice that received Ogg1−/− BM developed significantly larger, more complex lesions in aortic root and aorta than mice receiving WT BM (Figure 6A–6E). In agreement with our hypothesis, OGG1 deficiency–accelerated lesion size was reduced in Ldlr−/− mice that received OGG1 and NLRP3 doubly deficient BM cells (Figure 6A through 6E). Indeed, although the lesion size was significantly reduced in the Ogg1−/−Nlrp3−/− chimeras compared with Ogg1−/− alone, there was no significant difference between Ogg1−/−Nlrp3−/− and Nlrp3−/−, highlighting the importance of the NLRP3 pathway in the Ogg1−/− mice for atherosclerotic lesion size (Figure 6B and 6E). Finally, Nlrp3−/− chimeras alone did not

Figure 4. 8-Oxoguanine glycosylase (OGG1) deficiency leads to increased oxidative DNA damage (7,8-dihydro-8-oxo-2′-deoxyguanosine [8-OH-dG]) dominantly in mitochondria and caspase-1 activity in macrophages atherosclerotic lesions.

Ogg1−/−Ldlr−/− and control Ogg1+/+Ldlr−/− mice were fed Western diet for 16 wk. Aortic root plaques were analyzed by immunofluorescence. A, DNA damage was measured by immunostaining for 8-OH-dG (red) and nuclei (blue). B, Quantitative analysis of 8-OH-dG+ cells in selected area. Increased 8-OH-dG found mainly in mitochondria (TOM20). C, 8-OH-dG positive macrophages in lesion. D, Quantification of active caspase-1+ cells in lesional macrophages. E, Caspase-1 activity was assessed by fluorochrome-labeled inhibitors of caspases assay (FLICA; green) and by labeling macrophages with anti-F4/80 (red) and nuclei (blue). All data (in B–D) are mean±SD and representative of 2 independent experiments (n=10). Significance was determined using Student t test. **P<0.01.
have significantly smaller lesions than WT mice although they did trend toward a reduction (Figure 6B, 6C, and 6E), consistent with a recent study.\(^5\)

Consistent with the role of OGG1 to remove oxidized guanine residues, we noted a greater accumulation of 8-OH-dG in the plaques of Ogg1\(^{-/-}\) and Ogg1\(^{-/-}\)Nlrp3\(^{-/-}\) BM chimeras (Figure 6F). The accumulation of 8-OH-dG was not affected by the presence or absence of NLRP3. We then investigated whether we could detect increased amounts of mature IL-1\(\beta\) in the lesions. Ldlr\(^{-/-}\) mice that received Ogg1\(^{-/-}\) BM indeed expressed more mature cleaved IL-1\(\beta\) as compared with WT BM (Figure 6G). Importantly, atheroma development was substantially reduced in the chimeric mice that received Ogg1\(^{-/-}\)Nlrp3\(^{-/-}\) BM, despite no difference in glucose tolerance, insulin resistance, and lipid metabolism compared with the WT chimeras (Online Figure IIID and IIIE). Collectively, these data suggest that OGG1 deficiency in hematopoietic cells accelerates plaque progression at least partially through the Nlrp3 inflammasome.

miR-33 Downregulates OGG1 in Human and Mouse Cells by Different Mechanisms, Leading to Increased 8-OH-dG Accumulation and IL-1\(\beta\) Production

OGG1 content in the aortic root diminished significantly as atherosclerosis progressed, suggesting that atherosclerosis inversely affected Ogg1 expression or stability. Therefore, we examined the role of AMPK, an upstream regulator of Ogg1.\(^{21}\) We confirmed that AMPK activation induced Ogg1 transcription by treating mouse BMDM cells with AICAR (5-aminomidazole-4-carboxamide ribonucleotide) and metformin, both potent AMPK activators. Indeed, both treatments led to increased Ogg1 mRNA (Figure 7A). We next assessed whether activated AMPK levels changed during atherogenesis. Consistent with the decreased expression of Ogg1 during atherogenesis, the amount of phospho- and AMPK in aortic arch lysate of Ldlr\(^{-/-}\) mice that had a 16-week WD was also diminished (Figure 7B). Thus, it is likely that the decrease in AMPK leads to reduced Ogg1 expression during atherosclerosis.

Analysis of the 3’-untranslated region regions of human OGG1 identified putative binding sites for miR-33 at nucleotides 312 to 318 and 367 to 373, but not in mouse Ogg1 (Online Figure IV A). miR-33 is known to play an important proatherogenic role, negatively affecting lipid metabolism and cholesterol export, as well as the master regulatory switch AMPK.\(^{40-42}\) Therefore, we asked whether these miR-33–binding sites were functional and regulated OGG1 levels in the cell either directly or through AMPK. miR-33 mimics and their specific inhibitors were transfected into both human and mouse cells to test for their effects on OGG1 expression. As predicted, densitometric quantification of OGG1 protein amounts normalized by GAPDH amounts showed that the miR-33 mimics were able to inhibit OGG1 expression in HeLa cells, but not in B6-MCL (immortalized BMDM) cells (Figure 7C; Online Figure IVB), suggesting that miR-33a could directly regulate human OGG1, but not mouse Ogg1. This was especially the case for miR-33-5p, whereas miR-33-3p was less effective in its inhibition. Inclusion of the mimic inhibitors abrogated the effects of the miR-33 mimics (Figure 7C and 7D). Next, we assessed protein expression by treating the cells with 1 mmol/L AICAR (AMPK activator) for 2 hours and observed that this led to increased phosphorylation of AMPK\(\alpha\) and increased expression of Ogg1, but the addition of miR-33-3p and miR-33-5p mimics significantly inhibited them in both human and mouse cells (Figure 7C and 7D). As before, cotransfection of
miR-33-3p– or miR-33-5p–specific inhibitors reversed the effect of miR-33-3p and miR-33-5p mimics in these cells (Figure 7C and 7D). To assess the functionality of these sequences, we transfected HEK293 (human embryonic kidney 293) cells with a reporter construct, which expressed a luciferase coding sequence fused to the 3′-untranslated region of OGG1 (human). Cotransfection of miR-33-3p and miR-33-5p mimics markedly suppressed the activity of the OGG1 3′-untranslated region luciferase construct, compared with empty vector control (Figure 7E). Next, we examined the effect of miR-33 inhibition on OGG1 activity to repair oxidized DNA damage. HeLa cells were transfected with miR-33-3p and miR-33-5p mimic, and 48 hours later, they were treated with 25 µmol/L of menadione to induce mtROS. The level of 8-OH-dG was determined by flow cytometry. We found that menadione treatment led to more 8-OH-dG oxidation in OGG1−/− mice compared with WT mice, and this was reversed by miR-33 inhibitors (Figure 7F).
Figure 7. The regulation of Ogg1 expression. 

A. mRNA Ogg1 expression was examined by RT-polymerase chain reaction. The cells were treated with AMP-activated protein kinase (AMPK) activators (1 mmol/L AICAR for 4 h and 7.5 μmol/L metformin for 8 h). B. p-AMPK and AMPKs protein in the aortic arch. Each lane represents the pooled aortic arch lysates from 2 Ldlr−/− mice and analyzed by Western blot. GAPDH used as a loading control. Band densitometry is shown in the right. C and D. Western blot analysis of Ogg1, p-AMPK, AMPK, and GAPDH protein expression transfected with the control mimic (control), miR-33-3p and -5p mimics, and mimic inhibitors for 48 h treated with or without 1 mmol/L AICAR for 2 h. E. Luciferase reporter activity in HEK293 cotransfected with the miR control, miR-33a-3p, and miR-33a -5p with Luciferase reporter (contains 3′ untranslated region of OGG1 [human]) plasmid for 48 h. F. HEK293 cells were transfected with miR-33-3p and miR-33-5p mimic and empty vector (EV) control miRNA before 25 μmol/L menadione (reactive oxygen species inducer) treatment for 30 min, followed by 4-h incubation to allow for DNA repair. DNA damage (Continued)
in miR-33 mimic transfected cells, compared with control cells (Figure 7F). Finally, similar to Ogg1−/− macrophages, B6-MCL transfected with miR-33 mimics produced more IL-1β in response to LPS+ATP stimulation (Figure 7G), but produced similar amounts of tumor necrosis factor-α. Taken together, these data suggest that miR-33a downregulates Ogg1 expression through AMPK in both mice and humans and also directly targets human but not mouse Ogg1. Thus, reduction in OGG1 by miR-33 leads to increased 8-OH-dG accumulation and greater IL-1β production.

It was recently reported that miR-33 is upregulated in human atherosclerotic plaques.43 As our data showed that OGG1 diminishes over time during atherogenesis in mice, and its expression can be regulated miR-33 and AMPK, we next investigated whether OGG1 was also reduced in human atherosclerotic plaques. Indeed, in agreement with our mouse data, we found that OGG1 transcript was indeed reduced in human coronary and carotid plaques compared with normal vessels (Online Figure V).

**Discussion**

Atherosclerosis is a disease characterized by lipid accumulation in the vessel wall, cell death, and chronic inflammation. In our study, we found that WD-fed Ogg1−/−Ldlr−/− mice and Ogg1−/− BM chimeric mice developed bigger, more complex lesions with larger necrotic cores. However, this increase was abrogated in Ogg1−/−Nlrp3−/− BM chimeric mice, suggesting that the mechanism by which Ogg1 deficiency leads to accelerated atherogenesis is at least, in part, through the NLRP3 inflammasome. Although some human Ogg1 polymorphisms have been linked with various pathologies such as large artery atherosclerotic stroke in smokers44 and systemic lupus erythematosus severity,45,46 no studies have yet demonstrated its role in atherosclerosis.

Apoptosis in both vascular smooth muscle cells and macrophages may promote inflammation and alter plaque composition although the significance of apoptosis in atherosclerosis depends on the stage of the plaque, localization, and the cell types involved.47 Previously, Ogg1−/− mice showed decreased intracellular ATP content48 and were more susceptible to apoptosis in response to oxidative stress.49,50 Here, we show that the loss of OGG1 in macrophages results in increased mtDNA damage, greater cytochrome c release, increased cytosolic mtDNA, and caspase-1 activation, leading to increased apoptosis and increased IL-1β secretion and inflammation. Because intrinsic apoptosis is regulated by mitochondria, mitochondrial dysfunction caused by mtDNA damage can lead to more cell death.51,52 In the case of atheromas, macrophage apoptosis contributes to necrotic core formation, which can trigger acute thrombotic events.53,54 Apoptotic cells display a variety of recognition signals that lead to their swift removal by professional phagocytes. However, improper removal of these cells can lead to secondary necrosis and subsequent inflammation.54 As Ogg1 expression diminishes over time as atherosclerosis progresses, more cell death may occur, resulting in progressively larger necrotic cores.

OGG1 is responsible for repairing 8-OH-dG lesions in DNA and is particularly important for clearing these lesions from mtDNA. Although there is more nuclear DNA in the cell compared with mtDNA, mtDNA is much more susceptible to oxidative damage caused by its lack of protective histones and its close proximity to mtROS. In addition, mitochondria only have base excision repair, of which OGG1 is a member, whereas the nucleus possesses both base excision repair and nucleotide excision repair.55,56 Thus, OGG1 deficiency would likely have a much greater effect on mtDNA than nuclear DNA, with which our data were in agreement. Ogg1 deficiency resulted in a substantial increase in oxidized mtDNA, which resulted in NLRP3 activation, IL-1β secretion, and accelerated atherosclerosis. Several reports have investigated the causes and pathological consequences of excessive mtROS in atherosclerosis in both humans and mouse models.36,57 It is reported that 8-OH-dG accumulation in mtDNA is increased 20-fold in livers of Ogg1−/− mice compared with WT mice.14 The human Ser326Cys (C8055G) OGG1 polymorphism is associated with decreased repair activity59 and is linked to large artery atherosclerotic stroke in smokers42; the C1245G polymorphism correlates with increased 8-OH-dG in leukocyte DNA and more severe manifestations of systemic lupus erythematosus.45,46 Atherosclerosis is a well-recognized complication of systemic lupus erythematosus, but underlying mechanisms are complex and remain areas of active investigation. Thus, growing evidence supports the potential role of Ogg1 in modifying atherosclerosis. Oxidation of mtDNA is directly attributed to ROS derived from mitochondrial respiration, making mitochondria both the source and the target of oxidative stress. Several reports have linked mitochondrial damage to atherosclerosis in both humans and mouse models.36,57 Although mtDNA damage might be expected to result in mitochondrial dysfunction including decreased ATP production and increased generation of ROS, OGG1-null mice up to the age of 14 months had normal mitochondrial function.50 We suggest that the proatherogenic effect of OGG1 deficiency is more likely to be a consequence of activation of NLRP3 by overabundant 8-OH-dG. Our finding that loss of NLRP3 mitigated the proatherogenic effect of OGG1 deficiency on plaque size supports this notion. The NLRP3 inflammasome is activated by many diverse stimuli making NLRP3 the most versatile and importantly, also the most clinically implicated inflammasome.60,61 Furthermore, IL-1β is known as a critical component of inflammation that accelerates atherosclerosis,62,64 and our data are in agreement with this concept.

It was previously reported that Ogg1−/− mice on a WD develop obesity and metabolic syndrome.39 However, in our study, the genetic loss of Ogg1 did not significantly exacerbate

Figure 7 Continued. was measured by intracellular immunostaining for 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-OH-dG) and measured by flow cytometry. B–D and F. Similar results were observed in 3 independent experiments. G, MCL cells were transfected with control miR, miR-33-3p, and miR-33-5p mimic for 48 h before the treatment of 400 ng/mL of LPS for 4 h plus 5 mmol/L of ATP in the last hour. A, E, and G. All data are mean±SD and representative of 3 experiments in duplicate. Significance was determined using 1-way ANOVA with Tukey post hoc test. **P<0.01.
metabolic alterations after 16 weeks on WD, compared with Ldlr−/− control mice. Importantly, adoptive transfer of Ogg1−/− BM cells into irradiated Ldlr−/− mice also resulted in increased atherogenesis, increased apoptosis, and increased inflammatory activity, with no changes in metabolic readouts. Although our data suggest a strong link between OGG1 and the NLRP3 inflammasome during atherosclerosis, it is also possible that Ogg1 acts through other pathways. Indeed, although lesion size (aortic sinus and aorta en face) in Ogg1−/− mice seemed to mainly be driven by the NLRP3 pathway, the lipid content of the lesions was increased in Ogg1−/− mice, but was not affected by NLRP3 deletion at 12 weeks WD (data not shown), suggesting that OGG1 may affect lipid accumulation in the plaque through a non-NLRP3 inflammasome-related pathways. Any new mechanisms by which OGG1 may affect the progress of atherosclerosis that is NLRP3 independent will require further studies.

It is intriguing that an another study using an acute injury model, LPS-activated organ failure model, suggested a proinflammatory role for OGG1 as Ogg1−/− mice had reduced inflammation. The authors proposed that single-strand breaks made by OGG1 in the DNA may serve as a danger signal that induces inflammation in their model of acute inflammation. However, other studies have shown that OGG1 has an anti-inflammatory effect in different disease models, in agreement with our findings. It could be that the pro- or anti-inflammatory effects of OGG1 are disease dependent with OGG1 being anti-inflammatory in more chronic disorders, such as atherosclerosis, whereas OGG1 may be proinflammatory in certain acute diseases. Further research is required to distinguish between these 2 possibilities.

The role of miR-33 in atherogenesis has increasingly been recognized. Treatment with miR-33 antagonist oligonucleotide in mouse and nonhuman primates, as well as studies in miR-33 KO mice, showed protection against atherosclerosis by increasing reverse cholesterol transport, leading to higher plasma high-density lipoprotein levels. In addition, miR-33−/− was found to be markedly increased in human carotid atherosclerotic plaques compared with normal arteries. Furthermore, miR-33 controls energy metabolism via repression of key mitochondrial genes to limit ATP production and dampen cholesterol efflux. Also, miR-33 was shown to skew toward M1 polarization of macrophages, which is proinflammatory and proatherogenic, whereas antagonism of miR-33 leads to M2 polarization, which is atheroprotective. Mitochondrial activity and macrophage polarization are linked, with miR-33 influencing both processes. OGG1, which is required for mtDNA repair, may therefore play a regulating role in both these pathways. Finally, miR-33 can also regulate IIβ expression levels in atherosclerotic lesions. However, Goedeke et al reported that long-term anti-miR-33 therapy in mice might be associated with side effects such as hypertriglyceridemia and moderate hepatic steatosis, but overall, miR-33 is regarded as a potential therapeutic target for atherosclerosis. Therefore, it is of great potential interest that in this study, we discovered that miR-33 also targets OGG1 and downregulates its expression directly in human cells, and indirectly in both human and mice.

However, although the complete repertoire of miR-33 target genes is unknown, miR-33 targets many metabolic pathways, including AMPKα. The expression of AMPKα is diminished during atherogenesis, which leads to decreased autophagy and paradoxical upregulation of SREBP (sterol regulatory element-binding transcription factor)-1c and SREBP-2, as well as further progression of atherosclerosis. Restoration of AMPKα expression protects from atherogenesis. Importantly, AMPKα acts upstream of Ogg1, thus miR-33 can also act on Ogg1 indirectly, and this pathway is conserved in both humans and mice. Indeed, we also found that hOGG1 expression was also reduced in human atherosclerotic plaques in diseased arteries compared with normal controls.

Several publications have reported that stress responsive transcription factors, Nrf-2 (nuclear factor [NF] erythroid 2-related factor 2) and NF-YA (nuclear transcription Y subunit alpha), also regulate Ogg1 expression. Coincidentally, they are also regulated by AMPK. Furthermore, NF-Y is a transcriptional complex composed of 3 subunits, NF-YA, NF-YB, and NF-YC. A recent study showed that NF-YC is also a miR-33 target gene and it was markedly increased in mice administered with miR-33 antisense oligonucleotide compared with control micro-RNA–treated mice. Thus, it is likely that miR-33 plays an important role in vivo controlling Ogg1 during atherogenesis, and further studies are required to understand the complex interaction with miR-33 and OGG1 during plaque progression.

There is a growing understanding on the key roles of macrophages in the initiation, progression, and resolution of atherosclerotic inflammation. Our study further highlights the importance of macrophages as OGG1 deficiency caused a significant increase in oxidative damage in mtDNA leading to increased caspase 1 activation, most notably in macrophages, leading to greater IL-1β production and accelerated atherogenesis. Two isoforms of OGG1 protein exist (mitochondrial and nuclear), but the importance of mitochondrial OGG1 in the protection of oxidative stress is clearly demonstrated in this study. Furthermore, mtDNA damage is significantly more abundant and persists longer than nuclear DNA damage after exposure to oxidative stress. Human atheromatous plaques show increased mtDNA damage, particularly associated with higher-risk plaques. In conclusion, this work provides direct evidence for a causal link between oxidative DNA damage repair enzyme, OGG1, and atherosclerotic plaque development, in part, via the NLRP3 inflammasome and IL-1β. Our study reveals a novel therapeutic target to limit oxidative mtDNA damage, which then downregulates the NLRP3/IL-1β axis and prevent or treat atherosclerosis.

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Disclosures
None.
References


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**Novelty and Significance**

**What Is Known?**

- mtDNA damage has been implicated in NLRP3 inflammasome activation and in atherogenesis and is increased in human atherosclerotic plaques.
- OGG1 is the main enzyme responsible for repairing mitochondrial oxidative DNA damage.
- miR-33 negatively regulates several antiatherosclerotic pathways and is considered to be a potential antiatherosclerosis therapeutic target.

**What New Information Does This Article Contribute?**

- Loss of OGG1 leads to increased mtDNA damage and NLRP3 inflammasome activation in plaque macrophages.
- This study shows that OGG1 plays a protective role in atherogenesis by preventing excessive inflammasome activation.
- OGG1 is reduced in plaque macrophages as the lesion progresses and is negatively regulated by the proatherogenic miR-33.

Recent evidence suggests that oxidative stress and mtDNA damage is present in atherosclerotic plaques, directly promoting NLRP3 inflammasome activation and multiple proatherogenic processes through mitochondrial dysfunction. Using mice deficient in both OGG1 and the LDLR we found that (1) genetic deletion of OGG1 leads to larger and more advanced plaques, (2) increased accumulation of the oxidative DNA damage product 8-OH-dG localized to the mitochondria of plaque macrophages, and (3) increased caspase-1 activation. By using OGG1/NLRP3 double knockout mice as donors for bone marrow chimera experiments, we found that the OGG1 deficiency–mediated increase in atherosclerotic plaque progression was predominately NLRP3-dependent. We observed that OGG1 expression diminishes in atherosclerotic plaque macrophages in mice as the lesion progresses and in human atherosclerotic plaques. Finally, we discovered that miR-33, a proatherogenic micro-RNA, which is known to be increased in atherosclerotic lesions, downregulates OGG1 expression. These findings demonstrate that OGG1 is atheroprotective and that targeting mtDNA repair may provide a new strategy for reducing plaque development by harnessing preexisting protective processes.
Ogg1-Dependent DNA Repair Regulates NLRP3 Inflammasome and Prevents Atherosclerosis
Gantsetseg Tumurkhuu, Kenichi Shimada, Jargalsai Khan Dadvadorj, Timothy R. Crother, Wenxuan Zhang, Daniel Luthringer, Roberta A. Gottlieb, Shuang Chen and Moshe Arditi

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*Ogg1-dependent DNA repair regulates NLRP3 inflammasome and prevents atherosclerosis*

Gantsetseg Tumurkhuu Ph.D.,¹ Kenichi Shimada, Ph.D.¹, Jargalsaikhan Dagvadorj, Ph.D.¹, Timothy R. Crother, Ph.D.¹, Wenxuan Zhang¹, Daniel Luthringer, M.D.², Roberta A. Gottlieb, M.D.³, Shuang Chen, M.D. Ph.D.¹,† and Moshe Arditi, M.D.¹,4,†,*

¹ Departments of Pediatrics, Biomedical Sciences, and Infectious and Immunologic Diseases Research Center (IIDRC), Cedars-Sinai Medical Center, Los Angeles, CA 90048.
² Department of Pathology, Cedars-Sinai Medical Center, Los Angeles, CA 90048.
³ Department of Medicine, Barbra Streisand Women's Heart Center, Heart Institute of Cedars-Sinai, Cedars-Sinai Medical Center, Los Angeles, CA 90048.
⁴ David Geffen School of Medicine, UCLA, Los Angeles, CA 90048, USA.

† These authors contributed equally to this work.

**Supplementary methods.**

**Materials.**

Antibodies: anti-Ogg1 (Proteintech group, Rosemont, Illinois), anti-cytochrome c and anti-GAPDH (Santa Cruz Biotechnologies, Dallas, Texas), and anti-8-OHdG (Bioss, Atlanta, Georgia). The FAM-FLICA kit was purchased from Immunochemistry Technologies and the TUNEL staining kit from Millipore. Secondary fluorescently labeled antibodies were from Molecular Probes (Invitrogen, Carlsbad, California) and the total RNA isolation kit was from Qiagen (Valencia, California). AICAR was purchased from Abcam (Cambridge, MA).

**Cell culture.**

Bone marrow derived macrophages (BMDM) were prepared as previously described¹.

**Western blot analysis.**

As described earlier ², cells were lysed in lysis buffer with protease inhibitor cocktail (Roche). Cell lysates were rotated at 4°C for 0.5 h before the insoluble material was removed by centrifugation at 12,000 × g for 10 min. After normalization for equal protein concentration, cell lysates were resuspended in SDS sample buffer before separation by SDS-PAGE and transferred onto to PVDF membrane. Membranes were then incubated in blocking buffer and primary antibodies were applied in blocking buffer overnight at 4°C. Membranes were washed and incubated with a secondary antibody conjugated with horseradish peroxidase (GE Healthcare) for 2h at room temperature. After washing the membranes, a signal was detected by chemiluminescence using ECL Plus Western Blotting Detection kit (GE Healthcare Life Sciences, Piscataway, NJ) and a BioSpectrum UVP Imaging system (Bio-Rad, City, CA).

**Plasma Lipid profiles.**

Blood was obtained by retro-orbital puncture. Total cholesterol, high- and low density lipoprotein and triglyceride in the plasma were measured by colorimetric assay: Cholesterol E, L-Type Triglyceride M, L-Type LDL-C, HDL-Cholesterol E (Wako Diagnostics, Richmond, Virginia) per the manufacturer's instructions.
Glucose tolerance test.
Mice were fasted overnight (16 hours). Glucose dose was calculated based upon 2.5 g/kg body weight. Zero time point blood glucose was measured by glucometer with tail vein blood for each mouse. Injection volume i.p. was calculated using BW(g)X10 ul of 250mg/ml glucose solution. Blood glucose was measured at 30, 60, and 120 minutes after the glucose injection.

Insulin tolerance test
Mice were fasted for four hours. Insulin dose was calculated based upon 0.75 U/kg body weight. Zero time point blood glucose was measured by glucometer with tail vein blood for each mouse. Injection volume i.p. was calculated using BW(g)X7.5 ul of 0.1U/ml insulin solution. Blood glucose was measured at 30, 60, and 120 minutes after the glucose injection.

Creation of Bone-marrow chimeric mice.
8-week old recipient Ldlr KO mice were irradiated 9.5 Gy (Gammacell 40 Cs γ-irradiation) to eliminate endogenous BM stem cells and most of the BM-derived cells. Bone marrow from donor mice was harvested by flushing the femurs and tibias with PBS (pH 7.4). Single-cell suspensions were prepared by passing the cells through a 70 μm cell strainer (BD, Breda, The Netherlands). Next, 2x10⁶ donor BM cells were injected into the tail vein of recipient irradiated mice. The mice were kept in microisolator cages for 8 weeks to allow full BM reconstitution. After recovery, chimeric mice were placed on western diet for additional 12 wks. At the termination of each experiment, bone marrow was harvested and genotyping PCR analysis for the corresponding genes (Ogg1or Nlrp3) was performed on BM derived macrophages, peritoneal macrophages, blood PBMCs (donor) ECs (recipient) to confirm chimerism.

Serum levels of chemokines and cytokines.
Serum concentrations of MCP-1, IL-6, IL-18, TNF-α and IL-12-P40 (eBiosciences, San Diego, California) were detected by ELISA according to the manufacturer’s instructions.

Meso Scale Discovery (MSD).
IL-1β in mouse plasma samples was measured using the U-PLEX Mouse IL-1β Assay (Meso Scale Diagnostics, Rockville, Maryland) per the manufacturer’s instructions. The samples were read and analyzed by MSD QuickPlex SQ120 instrumentation and Workbench 4.0 Software (Meso Scale Diagnostics, Rockville, Maryland).

miR-33 binding site identification.
The online target prediction programs www.targetscanhuman.com and www.microRNA.org were used to find potential targets for miR-33a and -b.

miRNA transfection.
Hela and immortalized BDMN B6-MCL cells were transfected with 20 nM mirVana miRNA mimic (miR-33a -3p and -5p) or with 30 nM mirVana miRNA inhibitors (Inh-miR-33a-3p and -5p) (Life technologies) utilizing Xfect™ (Clontech). 48h after transfection, select cells were treated with 1mM AICAR for 2h (Abcam).

3′-UTR luciferase reporter assays.
HEK-293 cells were cotransfected with 0.75 μg of OGG1 3′-UTR luciferase reporter and empty control vectors (abm) and 20 nM miR-33a-3p and -5p mimics or negative-control mimic using Xfect transfection reagent (Clontech). Luciferase activity was measured after 48h using the luciferase assay and was quantified as fold increase compared to the empty control vector transfected cells. Experiments were performed in duplicate and repeated at least three times.
Retroviral transduction
MIGR1-GFP and MIGR1-mtOGG1-GFP retroviruses were generated according to the manufacturer's instructions (Clontech Laboratories, Mountain View, California) and transduced into Ogg1 KO BMDM cells for 48h. The expression of eGFP were examined by fluorescent microscopy.

Human atherosclerotic lesion analysis
Human cases for study were identified by search of the pathology patient database at Cedars Sinai Medical Center (IRB# 00044050). The large-bore coronary arteries of explanted heart specimens (transplantation) and carotid artery specimens (endarterectomy) removed for atherosclerotic disease were identified. Reports and glass slides were reviewed, and the appropriate tissue in paraffin blocks were utilized for this study. The large-bore coronary arteries of explanted heart specimens of non-atherosclerotic resection specimens served as normal control tissue. The tissue within the paraffin blocks was homogenized with a TissueLyser II (Qiagen) and used for RNA isolated (Clontech). Total RNA was reverse transcribed using the QuantiTect kit (Qiagen) following the manufacturer's protocol. Quantitative real-time PCR was performed in triplicate using SYBR green mix (Takara, Clontech) using the following primers: 18S RNA FP: 5'-GGC CCT GTA ATT GGA ATG AGT 3' and 18S RP: 5' CCA AGA TCC AAC TAC GAG CTT 3'; OGG1 FP: 5'-CAC ACT GGA GTG GTG TAC TAG-3' and OGG1 RP: 5'CCA GGG TAA CAT CTA GCT GGA A -3'. The mRNA level was normalized to 18S RNA as a housekeeping gene.

References:


Supplemental Figure 1. (A) Representative images of eGFP expression in retrovirus infected BMDMs. Similar results were observed in 3 independent experiments.
Supplemental Figure 2.
(A) F4/80 (marker of macrophage) is predominantly expressed in hematopoietic cells (CD45 positive) in aortic root plaques. Immunostaining for CD45 (green), F4/80 (red), and nuclei (blue).
(B) Representative pictures of in-situ TUNEL staining (green) and nuclei (blue) for apoptosis.
(C) Representative pictures of mitochondria (TOM20; green) and 8-OH-dG (red) in lesion.
Glucose tolerance test

Insulin resistance test

C

D

E

Supplemental Figure 3.
Supplemental Figure 3.

*Ogg1*<sup>−/−</sup>*Ldlr*<sup>−/−</sup> mice develop similar degree of glucose tolerance and insulin resistance as *Ldlr*<sup>−/−</sup> mice. *Ldlr*<sup>−/−</sup> and *Ogg1*<sup>−/−</sup>*Ldlr*<sup>−/−</sup> mice were fed WD for 16 weeks (n=12). *(A)* Glucose tolerance and Insulin resistance test. *(B)* Body weight gain during WD. *(C)* Plasma lipid profile. Total cholesterol, HDL, LDL and TG concentration in plasma. *(D)* *Ogg1*<sup>−/−</sup>BM chimeric mice on *Ldlr*<sup>−/−</sup> develop glucose tolerance and insulin resistance similarly to control chimeric *Ldlr*<sup>−/−</sup> mice. BM from WT or *Ogg1*<sup>−/−</sup> mice transferred into irradiated *Ldlr*<sup>−/−</sup> mice and after 8 week reconstitution, mice were fed a WD for 12 weeks. Fasted glucose tolerance test (n=5). Insulin resistance (n=5). *(E)* Plasma lipid profile (n=5). *(A and D)* Significance was determined by calculating the area under the curve for each replicate (mice), and then groups were compared by One-Way ANOVA with Tukey’s post-hoc test. *(B, C and E)* All data are means±SD. Significance was determined by Students t test. NS=not significant. *(C and E)* The experiments performed in triplicates.
Supplemental Figure 4. (A) miR-33 specifically targets the 3' UTR of human Ogg1. Annealing of 3'-UTR of OGG1 is complementary to miR-33a and -b. hsa indicates homo sapiens. (B) Densitometric quantification of OGG1 protein amounts normalized by GAPDH amounts in HeLa (human) and B6-MCL (mouse) cells by Western blots shown in Figure 7C and D. The data is representative of 2-3 independent experiments.
Supplemental Figure 5. OGG1 mRNA in human atherosclerotic plaques (A) The expression of OGG1 mRNA in healthy arteries (Normal) (n=8) or coronary (n=8) and carotids (n=6) from patients with atherosclerosis (Plaque) from the CSMC. The PCR was performed in triplicates. Significance was determined using One-Way ANOVA with Tukey’s post-hoc test. (**P≤0.01, ***P≤0.001).