Original Article

IL-6 Mobilizes Bone Marrow-Derived Cells to the Vascular Wall, Resulting in Neointima Formation via Inflammatory Effects

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Aim: Among the many factors related to bone marrow cell mobilization, local inflammation induced by cytokines may drive bone marrow cells to the vascular wall, resulting in a thickened neointima. However, the relationship between inflammatory reactions and bone marrow cell invasion has not yet been fully clarified.
Methods: We inserted a large wire into the femoral artery in male balb/c (WT), interleukin (IL)-6-knockout (KO) and bone marrow-transplanted (BMT) mice that had received bone marrow cells from KO mice. Immunohistochemistry was performed to evaluate the degree of intimal hyperplasia and inflammation following vascular injury.
Results: Three days after the vascular injury, the number of CD34/Sca-1-positive cells in the blood was higher in the KO mice. The numbers of apoptotic cells in the neointima was lower in the KO and BMT mice at two hours after injury. The morphometric analysis performed at one and four weeks after injury showed that the intima/media ratio was significantly lower in the KO and BMT mice, while CD34-positive cells were much more frequent in the WT mice. Furthermore, re-endothelialization appeared earlier in the KO and BMT mice than in the WT mice. No differences in the levels of vascular endothelial growth factor or hepatocyte growth factor were observed in the mice sera between the WT, KO and BMT mice after injury. The *in vitro* culture of bone marrow cells showed more differentiated smooth muscle-like cells in the WT mice than in the KO mice.
Conclusions: IL-6 is involved in neointimal formation following vascular injury, possibly acting through inflammatory effects inducing the production of bone marrow cells.

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Key words: IL-6, Bone marrow-derived cells, Arterial remodeling

Introduction

With respect to the treatment of restenosis after percutaneous coronary intervention (PCI), clarifying the detailed mechanisms underlying neointimal hyperplasia is very important. Ross *et al.*[1] indicated that smooth muscle cells present in the neointima after PCI are derived from the media of the injured artery. However, various other studies using models of post-PCI restenosis have suggested that bone marrow cells give rise to progenitors and potentially contribute to pathological vascular remodeling[2, 3].

Among the many factors related to the mobilization of bone marrow cells, local inflammation induced by cytokines may drive bone marrow cells to the vascular wall, resulting in a thickened neointima. As an inflammatory cytokine, interleukin (IL)-6 is associated with atherosclerosis, inducing Intercellular Adhesion Molecule-1 (ICAM-1), which accumulates leukocytes and inflammatory cells in the vessel lumina and modulates the proliferation of smooth muscle cells[4-6]. A

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recent clinical study indicated that the IL-6 level is strongly associated with the risk of coronary heart disease[7]. However, the relationship between the inflammatory reactions associated with IL-6 and bone marrow cell invasion has not been fully clarified.

We herein created models of vascular injury using IL-6-knockout (KO) mice and bone marrow transplantation model (BMT) mice in which bone marrow cells from KO mice were transplanted into wild-type (WT) mice as recipients to investigate the involvement of inflammation in the mobilization of bone marrow cells in the process of neointimal formation after vascular injury.

Methods

Animal Experimental Protocol

All experiments conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health in the United States, and all animal protocols were approved by the Animal Care and Use Committee of Showa University. Adult male WT mice (balb/c), IL-6-KO mice (balb/c background)[8] and BMT mice weighing between 25 and 35 g were used. The mice were anesthetized for all surgical procedures. Transluminal mechanical injury of the femoral artery was induced by the insertion of a large wire (diameter, 0.38 mm), as previously described[9, 10]. The mice were euthanized via the intraperitoneal administration of an overdose of pentobarbital at either two hours, three days or one or four weeks after injury. At the time of sacrifice, blood was collected from the right cardiac ventricle for fluorescence-activated cell sorting (FACS) and enzyme-linked immunosorbent assay (ELISA) analyses. The mice were perfused with 0.9% NaCl solution followed by perfusion fixation with 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4). The femoral artery was carefully excised, further fixed in 4% paraformaldehyde overnight and embedded in paraffin. Cross-sections (5 μm) were stained with hematoxylin and eosin.

Bone Marrow Transplantation

Bone marrow transplantation was performed as previously described[11]. Bone marrow cells were harvested from the femora and tibiae of donor mice. Recipient WT mice were lethally X-irradiated with a total dose of 9.5 Gy (MBR-1520; Hitachi, Tokyo, Japan). One day later, the recipient mice received unfractionated bone marrow cells (3×10^6) from IL-6-KO mice suspended in 0.3 mL of PBS via tail vein injection.

FACS Analysis

Peripheral blood was collected three days after injury in order to perform an immunofluorocytometric analysis using anti-mouse CD34 (BD Bioscience; San Jose, CA) and Sca-1 (Dainippon Pharma, Osaka, Japan) antibodies. A total of $2\text{-}3 \times 10^5$ cells were resuspended with 200 μL of Dulbecco's PBS containing 10% fetal bovine serum and 0.01% NaN_3 and incubated for 20 minutes at 4℃ with phycoerythrin-conjugated monoclonal antibodies against CD34 and Sca-1. Immunofluorocytometry was performed with a flow cytometer (Becton Dickinson, Franklin Lakes, NJ). All groups were studied in at least triplicate.

Immunohistochemistry

Paraffin-embedded sections (thickness, 5 μm) were deparaffinized, blocked with 0.5% goat serum and incubated with anti-mouse CD34 antibodies (clone H-140; Santa Cruz Biotechnology, Dallas, TX) or von Willebrand factor (vWF) antibodies (clone C-20; Santa Cruz Biotechnology). The antibody distribution was visualized using the avidin-biotin complex technique and Vector Red substrate (Vector Laboratories; Burlingame, CA). The sections were counterstained with hematoxylin.

TUNEL Analysis

Apoptotic cells were analyzed using a TUNEL assay two hours after vascular injury. The TUNEL assay was performed using an apoptosis detection kit (Takara Syuzo, Ohtsu, Japan).

Morphometric Analysis

A morphometric analysis of the sections was performed as previously described[12, 13]. Transverse sections were created every 100 μm. Five different sections from each of 10 animals in each group were digitized to trace the lumen, internal elastic lamina, and external elastic lamina using the WIN ROOF image analysis software program (Mitani, Fukui, Japan). The intima/media ratio was averaged for each artery. The ratio of TUNEL-positive cells at two hours after vascular injury was measured by counting the number of neointimal cells with DAPI-stained nuclei and calculated as the number of TUNEL-positive cells to the total number of DAPI-positive cells in the neointima. The cells were counted in five TUNEL-stained sections from each of the 10 animals in each group. Re-endothelialization was morphologically assessed using immunofluorescent staining with anti-vWF antibodies. Re-endothelialization was calculated as the ratio of the surface covered by vWF-positive cells to the total luminal surface.

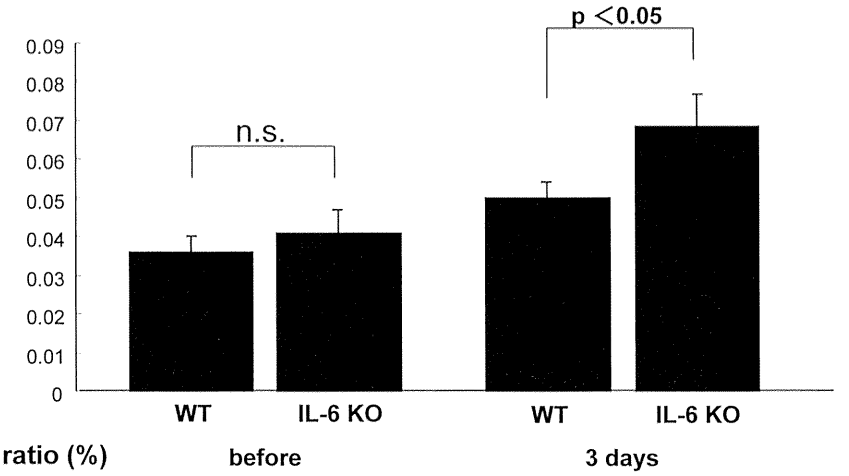

Fig. 1. Progenitor cells in the blood were more abundant in the KO mice than in the WT mice at three days after vascular injury.

In the FACS analysis, CD34/Sca-1-positive progenitor cells in the blood were more abundant in the KO group than in the WT group at three days after injury ($p<0.05$). The data are reported as the mean ± SE.

The ratio of CD34-positive cells was measured by counting the number of neointimal cells with hematoxylin-stained nuclei and calculated as the number of CD34-positive cells to the total number of hematoxylin-positive cells in the neointima. The number of CD34-positive cells was manually counted in randomly selected sections. The measurements were obtained from five sections of the injured arteries in each of the 10 animals in each group.

ELISA

The serum was assayed using ELISAs specific for murine vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) (R&D Systems, Minneapolis, MN). Quantification was performed by measuring the absorption at 450 nm, then subtracting out the baseline absorption at 540 nm using an automated plate reader (Fluostar Optima; BMG Labtech, Ortenberg, Germany). Standard curves were generated using recombinant mouse VEGF and HGF (R&D Systems).

Cell Culture

Total bone marrow cells were isolated from WT and IL-6-KO mice as previously described[13]. The cells (4×10^6) were cultured in wells of a 24-well dish in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS), 10 ng/mL of platelet-derived growth factor BB (PDGF-BB) and 10 ng/mL of basic fibroblast growth factor (FGF). At three days, murine recombinant IL-6 (Sigma Aldrich) was added at a concentration of 0.05 μg/mL to the culture medium of bone marrow cells obtained from the IL-6 KO mice.

For immunocytochemistry, the cells were fixed in 4% paraformaldehyde. Following permeabilization with 0.5% NP40 in PBS, the cells were stained with Cy-3-conjugated monoclonal antibodies to alpha smooth muscle actin (alpha-SMA) (clone 1A4; Sigma-Aldrich) and DAPI (1:10,000).

Statistical Analysis

All values are expressed as the mean ± SE. Means were compared using unpaired Student's t-test. Values of $p<0.05$ were considered to be statistically significant.

Results

FACS Analysis

In the FACS analysis, CD34/Sca-1-positive progenitor cells[14] in peripheral blood were more abundant in the KO mice (0.069 ± 0.008%) than in the WT mice (0.050 ± 0.004%; $p<0.05$) at three days after injury (**Fig. 1**). No differences were evident between the groups before vascular injury (WT, 0.036 ± 0.004%; KO, 0.041 ± 0.007%, p=N.S.).

These results indicate that endogenous progenitor cells are more abundant in KO mice than in WT mice after vascular injury.

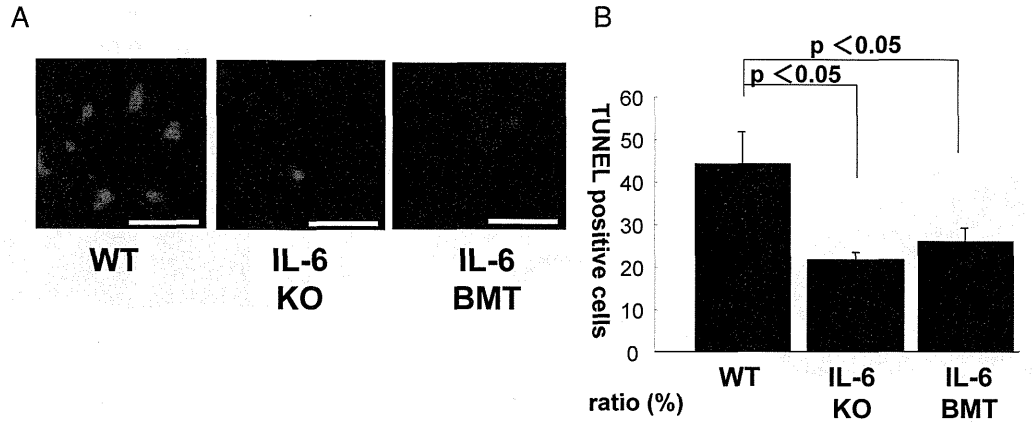

Fig. 2. Apoptotic cells were less abundant in the KO and BMT mice than in the WT mice at two hours after vascular injury.

Apoptotic cells were detected using TUNEL staining. The graph demonstrates a decrease in the percentage of apoptotic cells in the KO and BMT groups ($p<0.05$ each).
Bar, 20 m. The data are reported as the mean ± SE.

Histochemical and Immunohistochemical Analyses

The number of apoptotic cells, and possibly inflammatory cells, in the neointima was much lower in the KO and BMT groups than in the WT group at two hours after injury (percent of apoptotic cells: WT, 43.9 ± 7.7%; KO, 21.5 ± 1.8%; BMT, 25.7 ± 3.2%; $p<$ 0.05 each, $n=10$ each) (**Fig. 2**). In the morphometric analysis conducted one week after injury, the neointimal area was smaller in the KO and BMT groups than in the WT group (intima/media ratio: WT, 1.18 ± 0.11; KO, 0.60 ± 0.06; BMT, 0.56 ± 0.07; $p<0.01$ each, $n=10$ each) (**Fig. 3**).

In the immunohistochemistry analysis using CD34 performed at one week, positive cells possibly containing bone marrow-derived cells were less frequent in the KO and BMT groups than in the WT group (percent of CD34-positive cells: WT, 3.89 ± 0.25%; KO, 1.81 ± 0.24%; BMT, 1.72 ± 0.25; $p<0.01$ each, $n=10$ each) (**Fig. 3**).

At four weeks, no CD34-positive cells were observed in any of the WT, KO or BMT groups. Neointimal hyperplasia was significantly reduced in the KO and BMT groups compared to that observed in the WT group (intima/media ratio: WT, 3.29 ± 0.34; KO, 1.85 ± 0.22; BMT, 1.97 ± 0.23; $p<0.01$ each, $n=$ 10 each) (**Fig. 4**).

Re-endothelialization detected using vWF antibody staining was more extensive in the KO and BMT groups than in the WT group (percentage luminal coverage: WT, 60.5 ± 3.1%; KO, 82.6 ± 2.9%; BMT, 83.6 ± 2.6%; $p<0.01$ each, $n=10$ each, respectively) (**Fig. 5A, B**).

ELISA

In the ELISA, the concentrations of VEGF (before: WT, 27.2 ± 4.3 pg/mL; KO, 21.7 ± 4.6 pg/mL; BMT, 23.0 ± 4.5 pg/mL; after 1 week: WT, 19.9 ± 2.7 pg/mL; KO, 22.3 ± 2.4 pg/mL; BMT, 22.6 ± 2.3 pg/mL; $n=10$ each) and HGF (before: WT, 1.13 ± 0.14 ng/mL; KO, 0.89 ± 0.15 ng/mL; BMT, 1.01 ± 0.16 ng/mL; after 1 week: WT, 1.12 ± 0.20 ng/mL; KO, 1.06 ± 0.14 ng/mL; BMT, 1.08 ± 0.13 ng/mL; $n=10$ each) in the mice sera did not differ significantly between the groups before or one week after vascular injury.

These results indicate that vascular injury did not affect the concentrations of systemic growth factors in any of the three groups.

Cell Culture

Total bone marrow cells were plated on a plastic dish in the presence of 10% serum. At four days, floating cells were washed and PDGF-BB (10 ng/mL) was added to the adherent cells. In the presence of PDGF-BB (10 ng/mL) and basic FGF (10 ng/mL), the adherent cells differentiated into smooth muscle-like cells. Immunohistochemistry revealed that these cells expressed alpha-SMA, which was not expressed in the bone marrow cells before the culture. The KO mice showed significantly fewer alpha-SMA-positive cells than the WT mice (number of alpha-SMA-positive cells: WT, 114.3 ± 5.34/HPF; KO, 51.6 ± 2.55/HPF; $p<0.01$, $n=10$ each). In the presence of murine recombinant IL-6 in the bone marrow cells obtained from the KO mice, the number of alpha-SMA-posi-

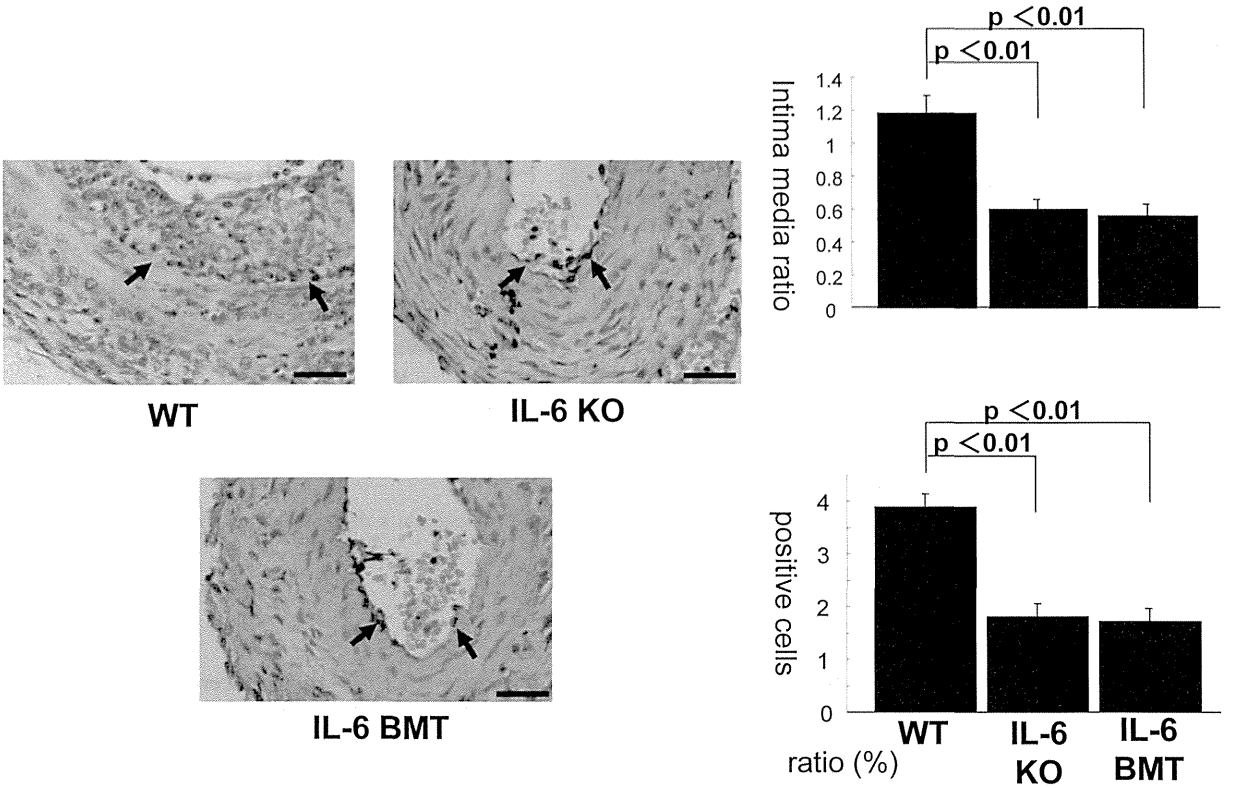

Fig. 3. Neointimal hyperplasia was inhibited in the KO and BMT mice one week after vascular injury.

The injured arteries were embedded in paraffin and stained with hematoxylin and eosin. The graph indicates that neointimal areas and CD34-positive, bone marrow-derived cells were less frequent in the IL-6 KO and BMT mice ($p<0.01$ each, respectively).
Cross-sections were obtained for immunohistochemistry of CD34 antibodies.
The arrowheads indicate internal elastic lumina.
The data are reported as the mean ± SE.

tive cells was increased, as observed in the WT mice (number of alpha-SMA-positive cells: 107.8 ± 4.12/HPF; $p<0.01$, $n=10$) (**Fig. 6**).

Discussion

We investigated the involvement of inflammation in bone marrow cell mobilization in the process of neointimal formation using IL-6, an inflammatory cytokine, and KO, WT and BMT mice. *In vivo*, CD34/Sca-1-positive progenitor cells in the peripheral blood were more abundant in the KO mice at three days after vascular injury. The neointimal portion after vascular injury was smaller in both the KO and BMT groups. TUNEL-positive apoptotic cells and CD34-positive bone marrow-derived cells were less frequent in the KO and BMT groups. The intima/media ratio was decreased in the KO and BMT groups at one and four weeks after vascular injury. Re-endothelialization was more extensive in the KO and BMT groups. No differences were observed in the VEGF and HGF levels before or after vascular injury between the three groups. *In vitro*, bone marrow-derived cells were able to transdifferentiate into smooth muscle marker-positive cells and were less frequent in the KO mice.

IL-6 itself is known to be involved in atherosclerosis and modulates the proliferation of smooth muscle cells[5, 6]. As another inflammatory cytokine, tumor necrosis factor alpha (TNF-α) has been reported to exhibit a relationship with inflammatory effects in the bone marrow in the process of atherosclerosis[15]; however, the relationships between the IL-6 level, bone marrow cell invasion and the induction of neointimal proliferation have not been fully clarified. In addition to KO mice, we used BMT model mice in an *in vivo* study in order to clarify the relationships between bone marrow-derived cells and inflammatory cytokines. Bone marrow-derived cells have the ability to

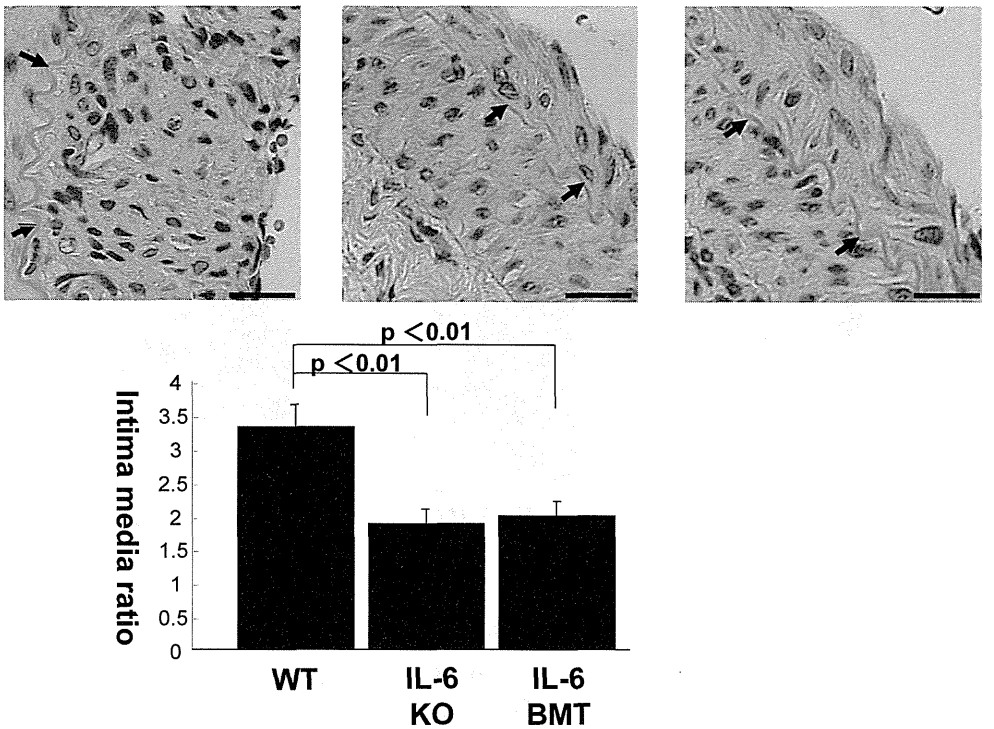

Fig. 4. Neointimal hyperplasia was inhibited in the KO and BMT mice four weeks after vascular injury.

Four weeks after vascular injury, neointimal hyperplasia was significantly inhibited in the KO and BMT groups ($p < 0.01$ each).
The arrowheads indicate internal elastic lumina.
The data are reported as the mean ± SE.

transdifferentiate into various cell types required for the repair of injured areas[3]. Bone marrow may therefore be a source of vascular cells in the neointima that contribute to the repair and remodeling of the vessel wall[16-19]. Our immunohistological data obtained from the BMT model mice were similar to those obtained for the KO mice, meaning that the IL-6 level may signal the need for repair, mobilizing endogenous bone marrow cells and playing an important role in the process of neointimal hyperplasia and re-endothelialization after vascular injury. Moreover, our FACS data demonstrated that CD34/sca-1-positive progenitor cells[14] may be involved in the process of re-endothelialization detected on immunohistological staining, especially in KO and BMT mice.

In the present study, apoptosis and inflammatory reactions were much more apparent in the vessel wall after vascular injury in the WT mice. Furthermore, CD34-positive cells were observed among inflammatory cells in the neointimal portion, especially in the WT mice. These findings suggest that inflammatory cell invasion may induce the mobilization of bone marrow-derived cells and affect the process of neointimal formation, resulting in elevation of the intima/media ratio after vascular injury[20].

Our *in vitro* experiment showed that IL-6 accelerates the differentiation of bone marrow cells into alpha actin-positive cells. Iwata *et al.*[21] indicated that bone marrow-derived cells are unlikely to form a definitive smooth muscle cell lineage, instead inducing monocyte/macrophage lineages. Our immunohistological findings also indicated that the neointimal portion after injury does not show positive staining for definitive smooth muscle cell lineage markers (data not shown) as Iwata *et al.* described. This indicates that the neointimal portion contains immature smooth muscle lineages, thereby increasing the intimal/media ratio mobilized by the inflammatory effects of IL-6. Such cells may be unlikely to function as smooth muscle cells.

The kind of stimuli that promote the differentiation of bone marrow-derived progenitors into imma-

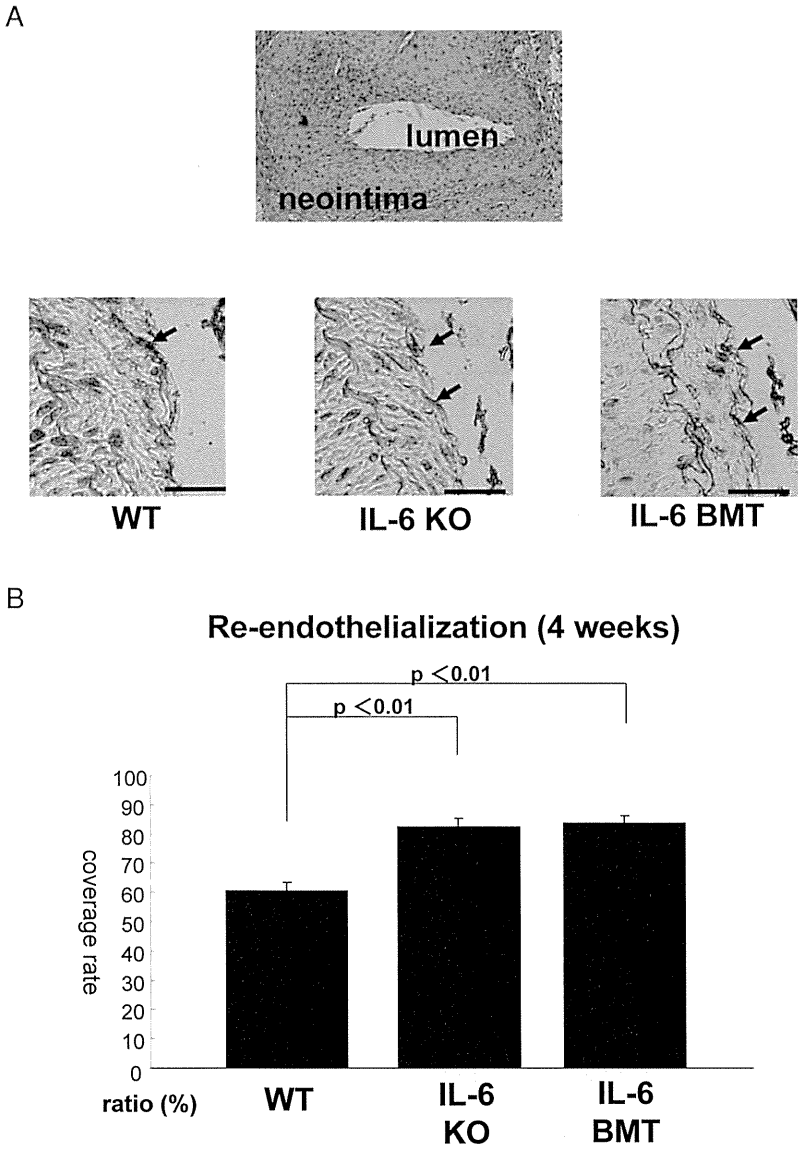

Fig. 5A, B. Re-endothelialization occurred earlier in the KO and BMT mice at four weeks after vascular injury.

Re-endothelialization detected with vWF antibody staining was more extensive in the KO and BMT groups ($p < 0.01$ each).
The arrowheads indicate marker-positive cells.
The data are reported as the mean ± SE.

ture alpha-SM actin-positive cells following the activation of IL-6 remains to be elucidated. Inflammatory cytokines, such as TNF-α- and IL-6, induce the expression of biologically active molecules, such as ICAM-1, that may accentuate the fibroproliferation response to injury[22-25]. In the early stage after injury, the denudated vessel wall is coated with platelets and leukocytes, as we previously indicated[9]. Monocytes and leukocytes accumulate in the injured artery. Endothelial injury and the subsequent deposition of leukocytes may plausibly provide optimal conditions for homing of bone marrow-derived smooth muscle progenitor cells.

Our results demonstrated that neointimal hyper-

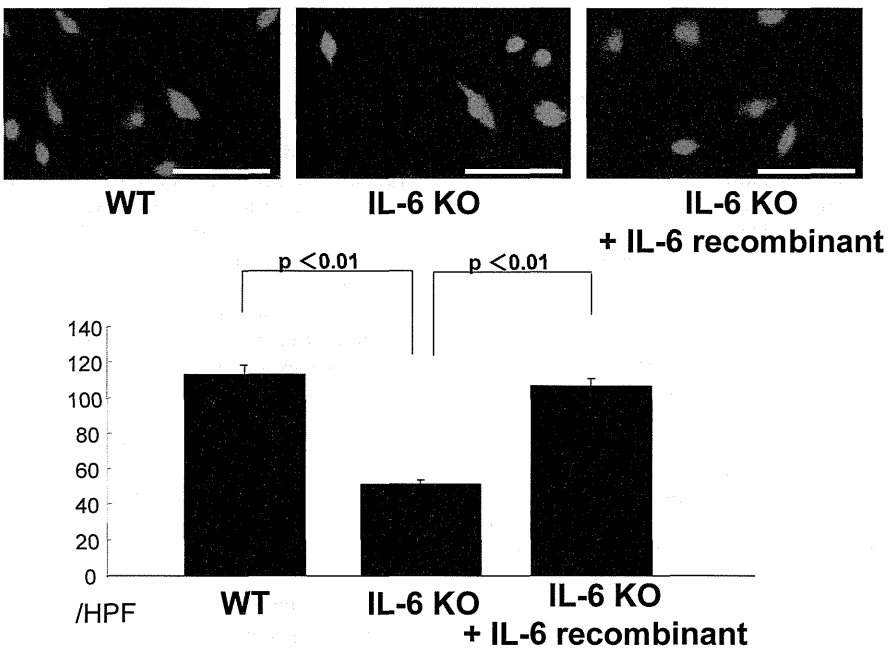

Fig. 6. Differentiation of bone marrow cells into alpha actin-positive cells.

Alpha-SMA-positive cells were significantly less frequent in the KO mice than in the WT mice ($p<0.01$).
In the presence of murine recombinant IL-6, the number of alpha-SMA-positive cells was increased, as also observed in the WT mice ($p<0.01$).
The data are reported as the mean ± SE.

plasia after vascular injury is decreased in KO and BMT mice via the inhibition of inflammatory actions and a reduction in the accumulation of bone marrow-derived cells. In the process of neointimal hyperplasia, no changes in the growth factors VEGF or HGF in mice sera were observed in this study. In the *in vitro* study, bone marrow-derived transdifferentiated alpha smooth muscle cells were less obvious in the KO mice. Overall, the activation of inflammation induced by the inflammatory cytokine IL-6 may be involved in the process of neointimal hypertrophy after vascular injury.

Conclusion

IL-6 is involved in the formation of the neointima after vascular injury, possibly via its inflammatory effects, to induce bone marrow cells.

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Cell Metabolism
Review

DNA Damage Response and Metabolic Disease

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Accumulation of DNA damage has been linked to the process of aging and to the onset of age-related diseases including diabetes. Studies on progeroid syndromes have suggested that the DNA damage response is involved in regulation of metabolic homeostasis. DNA damage could impair metabolic organ functions by causing cell death or senescence. DNA damage also could induce tissue inflammation that disturbs the homeostasis of systemic metabolism. Various roles of molecules related to DNA repair in cellular metabolism are being uncovered, and such molecules could also have an impact on systemic metabolism. This review explores mechanisms by which the DNA damage response could contribute to metabolic dysfunction.

Introduction

Accumulation of DNA damage has been implicated in the phenotypic manifestations of aging in rodents and humans. DNA damage can be caused by various endogenous or exogenous stresses, including oxidative stress, telomere erosion, oncogenic mutations, genotoxic stress, and metabolic stress (López-Otín et al., 2013) (Figure 1). p53 is a key player in the intrinsic cellular responses to DNA damage, and activation of p53 leads to cell-cycle arrest, apoptosis, and senescence (Stewart and Weinberg, 2006). Cellular senescence is defined as a state of irreversible growth arrest accompanied by changes of both cell morphology and gene expression (Hayflick and Moorhead, 1961). Accumulation of senescent cells, particularly senescent stem cells, could impair tissue regeneration and homeostasis, leading to metabolic dysfunction. In addition, accumulation of senescent cells in the tissues leads to chronic inflammation mediated by various proinflammatory cytokines and chemokines (Rodier et al., 2009). There is accumulating evidence that chronic inflammation associated with senescence has a pivotal role in the progression of age-related diseases such as diabetes, cardiovascular disease, and cancer (Tchkonia et al., 2013).

The number of people with obesity has increased dramatically in the modern world, and this has become a major healthcare problem for many societies. Obesity is known to be involved in the development of diabetes and atherosclerotic disease and has also been reported to increase the mortality rate, particularly deaths from cardiovascular disease (Van Gaal et al., 2006). Systemic insulin resistance is one of the crucial underlying molecular mechanisms that accelerates various disease states in individuals with obesity. It is widely accepted that an inflammatory response in visceral fat plays an important role in the development of systemic insulin resistance associated with obesity (Rajah et al., 2001). Excessive energy intake induces the hypertrophy of adipocytes by increasing the influx of fatty acids and promotes a proinflammatory phenotype in adipose tissue. These changes upregulate the production of chemoattractants, including the chemokine (C-C motif) ligand 2 (CCL2), which is also known as monocyte chemoattractant protein-1 (MCP-1). Subsequent recruitment of inflammatory cells results in a vicious cycle of adipose tissue inflammation that leads to impairment of glucose and lipid metabolism (Hotamisligil, 2006; Hotamisligil et al., 1993; Kanda et al., 2006; Weisberg et al., 2003; Xu et al., 2003). Tumor necrosis factor-alpha (TNF-α) is the best-characterized proinflammatory cytokine secreted by inflammatory macrophages that contributes to the development of systemic insulin resistance via activation of c-Jun N-terminal kinase (JNK) and IκB kinase (IKK), which inhibit insulin signaling in metabolic organs by serine phosphorylation of insulin receptor substrates (Hotamisligil, 2006) (Figure 2). It has been reported that inhibition of TNF-α by administration of a neutralizing antibody improves insulin sensitivity in rodents (Araújo et al., 2007; Borst et al., 2004).

The DNA repair system has evolved in eukaryotes to overcome DNA damage, and it includes processes such as homologous recombination (HR), nonhomologous end joining (NHEJ), base excision repair (BER), and nucleotide excision repair (NER) (Lombard et al., 2005). Double-strand DNA breaks are repaired by HR or NHEJ, whereas single-strand breaks are repaired by BER or NER. The efficiency of the DNA repair system appears to decrease with advancing age, leading to accumulation of DNA damage in tissues (Lombard et al., 2005). In mice and humans, mutations of genes related to the DNA repair machinery result in phenotypic changes that share features with age-associated pathological conditions, including metabolic and cardiovascular abnormalities, as well as being associated with an increased incidence of malignancies and a shortened lifespan (Hasty et al., 2003; Lombard et al., 2005). Recent studies have shown that factors involved in DNA repair also regulate cellular metabolism in response to DNA damage to avoid further genomic instability (Berkers et al., 2013; Imai and Guarente, 2014; Jeong et al., 2013). Evidence has also been obtained that suggests the cellular response to DNA damage is critically involved in the processes leading to impairment of glucose metabolism. This review focuses on the role of the DNA damage response, particularly activation of p53, in obesity and diabetes, and also explores the mechanisms that may be involved.

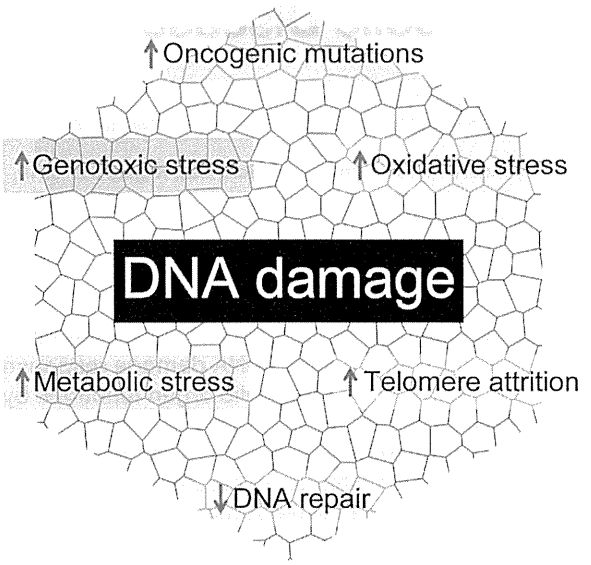

Figure 1. Stresses that Provoke the DNA Damage Response

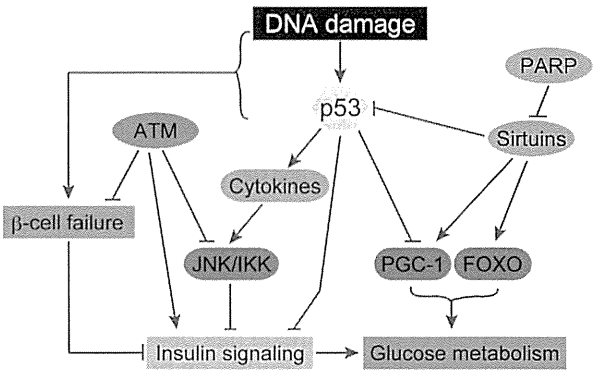

Figure 2. Signaling Molecules that Promote Insulin Resistance via DNA Damage

DNA damage activates p53 that directly or indirectly inhibits insulin signaling via JNK/IKK-mediated pathways. DNA damage also induces β cell failure that impairs insulin secretion. Expression of glucose transporters and enzymes is directly regulated by p53, and it also influences glucose homeostasis by modulating key metabolic regulators such as PGC-1. ATM, PARP, and sirtuins have dual roles in DNA repair and metabolic processes, linking both in a coordinated fashion.

Link between the DNA Damage Response and Chronological Aging

Aging is defined as a progressive decline of physiological function, leading to an increase in vulnerability and eventually to death. DNA damage and mutations accumulate with age, and this has been suggested to contribute to aging phenotypes and to the onset of age-associated diseases (Lombard et al., 2005). DNA damage can cause cells to enter an irreversible state of cell-cycle arrest known as cellular senescence. Although cellular senescence was initially discovered through in vitro studies (Hayflick and Moorhead, 1961), it was subsequently observed in vivo, and it was found that the number of senescent cells increases with advancing age (Dimri et al., 1995; Herbig et al., 2006). Cellular senescence has been suggested to have a potent anticancer effect, but evidence has emerged that links cellular senescence to age-related pathology (Campisi, 2005; Collado et al., 2007).

The p53/p21 and p16/Rb signaling pathways are known to be involved in regulation of cellular senescence, and p53 protein is the best-characterized transcriptional factor mediating the DNA damage response that is involved in preserving genomic stability and inhibiting tumorigenesis. However, p53 has recently been shown to have undesirable effects on aging and age-associated diseases (Vousden and Lane, 2007). p53-mediated transcriptional activity increases with age, suggesting a role of DNA damage-induced p53 activation in the development of aging phenotypes. Initial studies demonstrated that mice with one truncated p53 mutant allele and constitutive p53 activation develop premature aging, as well as showing resistance to cancer (Tyner et al., 2002) (Table 1). Likewise, mice with overexpression of a naturally occurring truncated p53 isoform have a short lifespan and display premature aging (Maier et al., 2004) (Table 1). Activation of p53 signaling is also found in aged vessels, failing hearts, and the visceral fat of obese persons, and it reportedly contributes to negative remodeling in atherosclerosis, as well as to progression of heart failure and the onset of diabetes (Minamino and Komuro, 2008; Minamino et al., 2009; Sano et al., 2007). Other studies have demonstrated that normal regulation of p53 may increase longevity in mice (García-Cao et al., 2002; Matheu et al., 2007) (Table 1), suggesting that abnormal chronic activation of p53 confers protection against cancer at the expense of reducing the lifespan. Expression of p16 is also known to increase with aging in mice and humans and has been found to contribute to aging phenotypes in the regenerative organs of mice (Kim and Sharpless, 2006; Krishnamurthy et al., 2004).

Association of Inflammation with Cellular Senescence

Chronic low-grade sterile inflammation is associated with age-related diseases, such as diabetes, cancer, and cardiovascular disease, and has been postulated to have a central role in accelerating these disease processes (Hu et al., 2004; Pai et al., 2004; Schetter et al., 2010; Spranger et al., 2003). Interestingly, proinflammatory cytokines are elevated in the vascular cells (Donato et al., 2008) and serum (Seidler et al., 2010) of elderly persons with no overt disease, suggesting that inflammation accompanying the natural aging process may contribute to the onset of age-related diseases. Cellular senescence provides a possible link between inflammation and aging (Freund et al., 2010). An important feature shared by several types of senescent cells is persistent upregulation of inflammatory molecules, including cytokines and adhesion molecules that recruit inflammatory cells (Tchkonia et al., 2013). Proinflammatory phenotypic changes of senescent cells may be triggered by the DNA damage response, which leads to activation of NF-κB and increased production of inflammatory cytokines (Freund et al., 2010; Liu et al., 2011; Rodier et al., 2009). A number of reports have demonstrated that senescence-associated inflammation acts as a two-edged sword. It is involved in maintaining tissue homeostasis as well as inhibiting malignancy, since proinflammatory signals emitted by senescent cells may help to prevent the development of

cancer by promoting the elimination of cells with elevated oncogene expression that have malignant potential (Kang et al., 2011; Lujambio et al., 2013). However, senescence-associated chronic inflammation could also promote tumor progression by disrupting tissue architecture and inducing cellular damage (Campisi et al., 2011; Yoshimoto et al., 2013). In a similar way, senescence-associated inflammation may be beneficial by contributing to the clearance of damaged cells and limiting fibrosis during tissue repair, but persistent inflammation may have a detrimental effect on tissue homeostasis that promotes age-related diseases including diabetes (Tchkonia et al., 2013).

Association of Oxidative DNA Damage and Telomere Shortening with Diabetes

A high level of oxidative DNA damage associated with an increase of reactive oxygen species is found in rodents and humans with diabetes. An increased serum level of 8-hydroxy 2′-deoxy-guanosine, a sensitive biomarker for oxidative DNA damage, has been found in obesity and diabetes and has also been reported to show a positive correlation with the body mass index of diabetic subjects (Al-Aubaidy and Jelinek, 2011). Telomeres are repeating DNA sequences located at the ends of chromosomes that have a critical role in maintaining genomic integrity (Stewart and Weinberg, 2006). As a consequence of semiconservative DNA replication, the extreme terminals of chromosomes are not completely duplicated, resulting in successive shortening of the telomeres with each cell division. When the telomeres reach a critically short length, they become dysfunctional and the p53-dependent DNA damage response is activated. Telomeres are known to become shorter with advancing age in humans, and telomere shortening is thought to provoke age-associated pathology (Armanios, 2013). Progressive telomere shortening is associated with obesity and insulin resistance (Gardner et al., 2005). Among subjects with type 2 diabetes, those with atherosclerotic plaques have greater shortening of telomere length compared to those without plaques (Adaikalakoteswari et al., 2007). Since oxidative stress is known to accelerate telomere shortening, it is conceivable that the diabetic state further exacerbates impaired glucose homeostasis by promoting telomere dysfunction.

Telomerase is an enzyme that adds telomeres to the terminals of chromosomes. Telomerase-deficient mice have a normal phenotype in the first generation, presumably because mice possess very long telomeres (Blasco et al., 1997; Lee et al., 1998). However, their telomeres become shorter with successive generations, and these mice then exhibit a shortened lifespan as well as various types of age-related pathology, including a reduced capacity to respond to stresses such as wound healing and hematopoietic ablation (Rudolph et al., 1999) (Table 1). When fed a high-calorie diet, these mice develop glucose intolerance and insulin resistance without enhancement of obesity (Minamino et al., 2009) (Table 1). Telomere dysfunction promotes senescence of adipocytes by activating p53-dependent signaling, thus inducing chronic inflammation in adipose tissue and systemic insulin resistance (Figure 2). Telomere dysfunction is also associated with impaired mitochondrial biogenesis and function, decreased gluconeogenesis, and an increase of reactive oxygen species (Sahin et al., 2011). Mechanistically, telomere dysfunction leads to p53 activation, thereby downregulating the expression of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α and β, which are transcriptional cofactors that modulate expression of various genes involved in mitochondrial function and glucose metabolism (Sahin et al., 2011) (Figure 2). Insulin secretion is impaired in mice with short telomeres, leading to glucose intolerance despite their β cell mass being normal (Guo et al., 2011) (Table 1). Impaired insulin secretion seems to be multifactorial and may be mediated by β cell-autonomous defects of mitochondrial function as well as aberrant Ca^{2+} handling. Taken together, these reports suggest that age-associated telomere shortening can lead to impairment of glucose homeostasis by inducing tissue inflammation and disturbing cellular metabolism, as well as by reducing tissue regeneration.

Role of p53 Activation in Metabolic Disorders

White adipose tissue was initially thought to be mainly involved in energy storage, but it is now also recognized to be an endocrine organ that secretes various cytokines and chemokines, which are called adipokines (Hotamisligil, 2006; Ouchi et al., 2011). It has been shown that inflammation of adipose tissue, characterized by infiltration of inflammatory cells and increased production of inflammatory cytokines, induces systemic insulin resistance and accelerates the processes underlying the development of diabetes (Johnson and Olefsky, 2013). DNA damage has been linked to adipose tissue inflammation and systemic insulin resistance (Shimizu et al., 2012). A high-calorie diet promotes hypertrophy of adipocytes by increasing the influx of fatty acids, leading to overproduction of reactive oxygen species, accumulation of DNA damage, and cellular senescence in adipose tissue. Under such conditions, p53 in adipose tissue plays a pivotal role in inducing inflammation and systemic insulin resistance (Minamino et al., 2009) (Figure 2 and Table 1). In mice fed a chow diet, overexpression of adipose tissue p53 also provokes inflammation and impairs glucose metabolism. Semaphorins and their receptors (plexins) were originally identified as molecules involved in axon guidance (Luo et al., 1993; Tamagnone et al., 1999), but have been shown to also contribute to development of the cardiovascular system during embryogenesis (Gitler et al., 2004; Torres-Vázquez et al., 2004). Semaphorin 3E (Sema3E) is one of the class 3 semaphorins, and its receptor is plexinD1 (Christensen et al., 1998). It was recently reported that Sema3E is upregulated in obese visceral fat via a p53-dependent mechanism and acts as a chemoattractant for plexinD1-positive inflammatory macrophages, thus contributing to adipose tissue inflammation and systemic metabolic dysfunction through production of inflammatory cytokines (Shimizu et al., 2013). It has been demonstrated that the p53/p21 signaling pathway is critically involved in adipocyte differentiation and hypertrophy, which links it to obesity and insulin resistance (Inoue et al., 2008). There is also evidence that pancreatic β cell senescence contributes to the pathogenesis of diabetes (Tavana and Zhu, 2011). One recent study demonstrated that elevation of glucose metabolism by β cells causes DNA double-strand breaks and activation of p53, leading to β cell failure in mice with type 2 diabetes (Tornovsky-Babeay et al., 2014) (Figure 2). Likewise, mice expressing a truncated isoform of p53 show hypoinsulinemia and glucose intolerance with increased p21 expression in islets (Hinault et al., 2011) (Table 1). In contrast to

Table 1. Mutant Mice that Exhibit or Are Protected against Aging Phenotypes with Metabolic Dysfunction

Molecules	Mouse models	Aging phenotypes	Metabolic phenotypes	References
p53	*Trp53*$^{+/m}$ (m: truncated *Trp53* mutant allele)	Accelerated, short lifespan, tumor resistant	ND	Tyner et al., 2002
p53 (p44)	p44 transgenic (p44: short isoform of p53)	Accelerated, short lifespan, tumor resistant	β cell failure, glucose intolerance	Hinault et al., 2011; Maier et al., 2004
p53 (Ser18 mutant)	*Trp53* (S18A) mutation knockin	Normal lifespan, not tumorigenic	Glucose intolerance, insulin resistance	Armata et al., 2010; Sluss et al., 2004
p53	*Trp53* transgenic	Normal lifespan, tumor resistant	Better glucose tolerance on a normal diet	Franck et al., 2012; García-Cao et al., 2002
p53/Arf	*Trp53/Cdkn2a* transgenic	Extended median lifespan, tumor resistant	ND	Matheu et al., 2007
p53 (adipocytes)	*Fabp4*-Cre; *Trp53*$^{flox/flox}$	ND	Improved insulin resistance on a high-calorie diet	Minamino et al., 2009; Shimizu et al., 2013
p53 (endothelial cells)	*Tie2*-Cre; *Trp53*$^{flox/flox}$	ND	Improved insulin resistance on a high-calorie diet	Yokoyama et al., 2014
Telomerase	*Tert*$^{-/-}$, *Terc*$^{-/-}$	Accelerated (late generation)	Insulin resistance, β cell failure, glucose intolerance	Guo et al., 2010; Minamino et al., 2009; Rudolph et al., 1999; Sahin et al., 2011
Sirt1	*Sirt1*$^{-/-}$	Embryonic lethal, genomic instability	ND	Wang et al., 2008
Sirt6	*Sirt6*$^{-/-}$	Accelerated, premature death	Hypoglycemic phenotype	Mostoslavsky et al., 2006
Sirt6	*Sirt6* transgenic	Extended maximum lifespan	Protection against age-associated insulin resistance and glucose intolerance	Kanfi et al., 2010, 2012
Parp1	*Parp1*$^{-/-}$	ND	Better glucose tolerance, increased energy expenditure	Bai et al., 2011
Parp1	Human *PARP1* knockin	Accelerated, short median lifespan	Glucose intolerance	Mangerich et al., 2010
Wrn	*Wrn*$^{-/-}$	No phenotype of premature aging	Insulin resistance and glucose intolerance on a high-calorie diet	Lombard et al., 2000; Moore et al., 2008
Wrn	*Wrn*$^{-/-}$*Terc*$^{-/-}$	Accelerated, short lifespan	Glucose intolerance	Chang et al., 2004
Bub1b	*Bub1b*$^{H/H}$ (H: hypomorphic allele)	Accelerated, short lifespan	ND	Baker et al., 2004
Bub1b	*Bub1b* transgenic	Extended maximum lifespan, tumor resistant	ND	Baker et al., 2013a
Ercc1	*Ercc1*$^{-/-}$	Accelerated, premature death	ND	Niedernhofer et al., 2006
Ercc1	*Fabp4*-Cre; *Ercc1*$^{flox/-}$	ND	Glucose intolerance	Karakasilioti et al., 2013
Atm	*Atm*$^{-/-}$	Mild aging phenotype	Impaired insulin secretion	Miles et al., 2007
Atm	*Atm*$^{-/-}$*Apoe*$^{-/-}$, *Atm*$^{+/-}$*Apoe*$^{-/-}$	ND	Insulin resistance, glucose intolerance, atherosclerosis	Schneider et al., 2006

ND, not determined.

these negative metabolic impacts of p53 activation, it has been reported that defects in p53 Ser18 phosphorylation result in glucose intolerance and insulin resistance (Armata et al., 2010; Sluss et al., 2004), whereas an additional copy of normally regulated p53 improves glucose tolerance (Franck et al., 2012) (Table 1), suggesting that physiological p53 activity is required to maintain metabolic homeostasis.

Recently, information on the metabolic role of p53 at a cellular level has emerged with regard to regulation of tumor development as well as normal cellular homeostasis (Berkers et al., 2013; Vousden and Ryan, 2009). For example, p53 downregulates the expression of glucose transporters and glycolytic enzymes (Kondoh et al., 2005; Schwartzenberg-Bar-Yoseph et al., 2004), while it upregulates *TP53*-induced glycolysis and apoptosis regulator (Bensaad et al., 2006), thereby inhibiting glycolysis (which is the preferred metabolic pathway for tumor cells). p53 could negatively regulate the insulin signaling pathway by upregulating PTEN (Stambolic et al., 2001) (Figure 2). Under mild stress, p53 drives oxidative phosphorylation and helps to maintain mitochondrial integrity (Lebedeva et al., 2009; Matoba et al., 2006). In contrast, severe stress causes p53 to repress expression of the transcriptional cofactors PGC-1α and PGC-1β that are critical regulators of mitochondrial biogenesis (Sahin et al., 2011) (Figure 2). Moreover, p53 functions as a negative regulator of lipid synthesis by activating fatty acid oxidation and by inhibiting fatty acid synthesis (Goldstein

and Rotter, 2012). These metabolic functions of p53 may be related to regulation of systemic metabolic homeostasis, but its precise role remains to be determined.

The *TP53* gene has a common polymorphism at codon 72, resulting in two variants (Arg and Pro) that differ with respect to their efficiency for inducing apoptosis and inhibiting tumorigenesis. Genetic analysis has demonstrated that p53 polymorphism (Arg72Pro) influences insulin resistance in patients with type 2 diabetes (Burgdorf et al., 2011). Interestingly, single-nucleotide polymorphism of a noncoding region near *CDKN2A* and *CDKN2B* shows a strong association with diabetes (Saxena et al., 2007). Variations of genes involved in DNA damage and repair such as CHEK2 have been implicated in the onset of type 2 diabetes (North et al., 2010), further emphasizing the crucial role of the DNA damage response in human glucose metabolism.

Metabolic and Cardiovascular Effects of the DNA Damage Response

Heart failure develops when myocardial injury reduces cardiac pump function. There is evidence of a close link between heart failure and diabetes, and clinical studies have shown that systemic metabolic dysfunction is prevalent among patients with cardiac dysfunction (Witteles and Fowler, 2008). In addition, systemic insulin resistance has been shown to be a risk factor for the development of heart failure (Ingelsson et al., 2005). Heart failure has been reported to promote the accumulation of DNA damage in visceral fat and cardiac tissue that provokes adipose tissue inflammation and systemic insulin resistance (Shimizu et al., 2012). Excessive lipolysis related to activation of the sympathetic nervous system leads to an increase of oxidative stress that promotes DNA damage and adipose tissue inflammation via p53-dependent signaling. Interestingly, inhibition of adipose tissue inflammation by deletion of p53 can suppress metabolic abnormalities and improve cardiac dysfunction, indicating that a vicious feedback loop may exist between the heart and fat tissue. Another line of evidence has suggested a close relationship between vascular function and diabetes (Yokoyama et al., 2014). Endothelial expression of p53 is markedly upregulated when mice are fed a high-calorie diet, presumably due to an increase of DNA damage related to metabolic stress. Inhibition of endothelial p53 activation leads to improvement of insulin resistance and obesity, while upregulation of endothelial p53 precipitates metabolic abnormalities by inhibiting glucose homeostasis in skeletal muscle (Table 1). These findings raise the possibility that inhibiting the DNA damage response in certain tissues could be a therapeutic target for blocking the vicious cycle between metabolic abnormalities and cardiovascular dysfunction.

Role of Sirtuins in Metabolic Regulation and DNA Repair

Silencing information regulator 2 (Sir2) was originally identified as a transcriptional silencer of mating foci and then was found to mediate calorie restriction-induced longevity in the budding yeast *Saccharomyces cerevisiae* (Lin et al., 2000). A number of Sir2-related proteins (collectively known as sirtuins) have been identified in many species ranging from yeasts to mammals (Longo and Kennedy, 2006). Sirtuins are NAD^+-dependent enzymes that display a conserved role in age-related pathology and longevity (Finkel et al., 2009; Longo and Kennedy, 2006). Mammalian sirtuins have seven isoforms, and each isoform shows distinctive localization (Haigis and Guarente, 2006). SIRT1 and SIRT2 are found in both the nucleus and the cytoplasm, while SIRT3, SIRT4, and SIRT5 are localized to the mitochondria. SIRT6 and SIRT7 show nuclear localization. Because of being NAD^+ dependent, it has been suggested that sirtuins play a critical role in regulating energy metabolism (Haigis and Guarente, 2006). It is now widely accepted that sirtuins are important regulators of various metabolic pathways, including those involved in gluconeogenesis, glycolysis, oxidative phosphorylation, and fatty acid oxidation (Schwer and Verdin, 2008).

SIRT1 has been studied most extensively and is known to have a role in both gluconeogenesis and glycolysis by regulating various key modulators, such as PGC-1α and forkhead transcription factors (FOXO) (Brooks and Gu, 2009) (Figure 2). SIRT1 also regulates lipid metabolism by modulating various transcriptional factors, including peroxisome proliferator-activated receptor (PPAR)-α and sterol-response element-binding protein (SREBP) 1c (Rodgers and Puigserver, 2007; Walker et al., 2010). With regard to DNA metabolism, sirtuins have been reported to be involved in the DNA repair system, regulation of chromatin structure, and maintenance of telomere integrity (Lombard et al., 2005; Palacios et al., 2010). A critical role of SIRT1 in DNA repair is strongly suggested by the observation that most *Sirt1*-deficient mice undergo embryonic death due to impairment of the DNA damage response and chromosomal abnormalities (Wang et al., 2008) (Table 1). SIRT6 is another nuclear sirtuin like SIRT1, and it has also been shown to have a role both in DNA repair and cellular metabolism. *Sirt6*-deficient mice develop fatal hypoglycemia due to enhanced glucose uptake by muscle and brown adipose tissue with loss of subcutaneous fat, indicating a crucial role of SIRT6 in glucose and lipid homeostasis (Mostoslavsky et al., 2006) (Table 1). *Sirt6* deficiency also results in hypersensitivity to genotoxic stress and genomic instability, thereby leading to various abnormalities that overlap with age-associated pathology in mice (Mostoslavsky et al., 2006). In contrast, mice that overexpress *Sirt6* are protected against both dietary and age-associated metabolic abnormalities, and also have a longer lifespan (Kanfi et al., 2010, 2012) (Table 1). It has been reported that SIRT6 is involved in repair of double-strand DNA breaks by stabilizing DNA-dependent protein kinase at sites of NHEJ repair (McCord et al., 2009). SIRT6 is also known to contribute to maintenance of telomere integrity (Michishita et al., 2008) and activates poly-ADP ribose polymerase (PARP) 1, thereby enhancing the repair of double-strand breaks by NHEJ and HR (Mao et al., 2011).

Although PARPs have long been considered to be DNA damage repair enzymes, recent evidence has suggested that these enzymes have an important role in metabolic regulation by influencing mitochondrial function and oxidative metabolism (Krishnakumar and Kraus, 2010). PARP1 and PARP2 interact with a large number of nuclear receptor transcriptional factors (such as PPARγ and FOXO) to regulate mitochondrial and lipid oxidation genes (Bai et al., 2007; Sakamaki et al., 2009). PARP1 is responsible for the majority of cellular PARP activity (Krishnakumar and Kraus, 2010). Given that SIRT1 and PARP1 are both NAD^+ dependent, these enzymes compete for the NAD^+ pool, and PARP1 probably influences Sirt1 activity by reducing the bioavailability of NAD^+ (Imai and Guarente, 2014) (Figure 2). In

fact, *Parp1*-deficient mice display increased energy expenditure along with a reduced fat mass and increased glucose clearance due to increased activity of SIRT1 in skeletal muscle and brown adipose tissue and are therefore protected against metabolic disease (Bai et al., 2011) (Table 1). Consistent with these findings, a gain-of-function mouse model with overexpression of human PARP1 shows premature onset of age-associated pathology, enhanced adiposity (Mangerich et al., 2010), and glucose intolerance (Table 1), indicating an essential role of this enzyme in metabolic regulation and age-related diseases.

Since sirtuins and PARP are involved in both DNA repair and cellular metabolism, they could function as a convergence point in the regulation of both processes. It has been shown that DNA damage directly activates metabolic responses in a coordinated fashion. SIRT4 is best known for its role in glutamine metabolism (Haigis and Guarente, 2006). Recently, it was reported that SIRT4 inhibits entry of glutamine into the TCA cycle under genotoxic stress, thus preventing dysregulated cell proliferation and genomic instability (Jeong et al., 2013). PARP shows chronic activation with aging, and this is thought to contribute to an age-associated decline of NAD^+ (Imai and Guarente, 2014). NAD^+ supplementation has a protective effect against age-induced impairment of glucose metabolism (Yoshino et al., 2011), which further suggests a pathological influence of DNA damage on metabolic regulation.

Relationship between DNA Damage and Metabolic Dysfunction in Progeroid Syndromes

Progeroid syndromes are heritable human disorders that cause premature aging. All of the known progeroid syndromes involve defects of the DNA repair and DNA damage responses, suggesting that maintenance of genomic stability has a central role in the aging process, although these syndromes only partially mimic normal human aging (Martin, 2005). Among the various human progeroid syndromes, Werner syndrome (WRN) and Hutchinson-Gilford progeria syndrome (HGPS) are two of the best-characterized disorders that most closely reproduce the features of normal aging. The mutation causing WRN has been identified as affecting a member of the RecQ family of helicases (Yu et al., 1996). WRN protein has helicase, exonuclease, and single-stranded DNA annealing activities and is involved in DNA recombination, replication, repair, and transcription, as well as in maintaining the integrity of telomeres (Opresko et al., 2004). WRN is characterized by short stature, early graying and hair loss, increased susceptibility to relatively uncommon cancers (such as sarcomas, lymphomas, thyroid neoplasms, malignant melanoma, and meningioma), scleroderma-like skin changes, type 2 diabetes, and atherosclerosis. Death usually occurs in middle life due to myocardial infarction or stroke. HGPS is referred to as "childhood progeria" to differentiate it from WRN, which is referred to as "adult progeria." Patients with HGPS appear normal at birth, but soon show premature aging that is characterized by alopecia, atherosclerosis, osteolysis, scleroderma, hyperpigmentation, and systemic insulin resistance (Hennekam, 2006). Their mean lifespan is around 13 years, and the main causes of death are coronary artery disease and stroke. The genetic basis of HGPS was discovered in 2003, when it was found that most persons with this disease have a single nucleotide substitution that leads to aberrant splicing of the *LMNA* gene encoding A-type nuclear lamins (Eriksson et al., 2003). Lamin A protein is synthesized as a precursor protein (prelamin A), and the mutation creates an abnormal prelamin A protein termed progerin. Enhancement of the DNA damage response and resulting p53-dependent senescence have been postulated to contribute to the pathogenesis of these progeroid syndromes (Gordon et al., 2014; Lombard et al., 2005).

Since patients with WRN and HGPS share common age-related features, including type 2 diabetes, it has been assumed that DNA damage per se can lead to impaired glucose homeostasis in the general population. WRN protein null mice develop normally and do not exhibit premature aging (Lombard et al., 2000) (Table 1). In contrast, WRN protein-deficient mice with shorter telomeres like humans show a variety of changes similar to those seen in WRN patients, including diabetes, graying and loss of hair, osteoporosis, and cataracts (Chang et al., 2004) (Table 1), suggesting that telomere shortening is a key element in the pathology of WRN. When fed a high-calorie diet, Wrn null mice also develop weight gain, insulin resistance, and impaired glucose tolerance (Moore et al., 2008) (Table 1). It seems likely that enhancement of genomic instability by additional stresses leads to exacerbation of metabolic dysfunction in progeroid syndromes, although the precise mechanisms leading to development of type 2 diabetes in patients with WRN and HGPS have not been fully elucidated.

The *BUB1B* (also known as BUBR1) gene encodes a mitotic regulator that ensures accurate segregation of chromosomes. *BUB1B* mutations are known to cause mosaic variegated aneuploidy syndrome, which is a rare condition characterized by a short lifespan, increased incidence of cancer, and developmental delay (Hanks et al., 2004). It has been reported that Bub1b expression decreases with age in various murine tissues, while sustained Bub1b overexpression protects against aneuploidy and cancer, delays the onset of age-related dysfunction, and extends the lifespan of mice (Baker et al., 2013a) (Table 1), suggesting a potential role of Bub1b in chronological aging. Mutant mice carrying Bub1b hypomorphic alleles develop various phenotypic features of premature aging that include a short lifespan, fat loss, sarcopenia, and cataracts (Table 1). These mice show selective accumulation of p16-positive cells in tissues that develop age-associated pathological changes, including adipose tissue, skeletal muscle, and the eye (Baker et al., 2004). The contribution of cellular senescence to aging in this mouse model has been demonstrated through an elegant transgenic system that involved elimination of p16-positive cells by a drug-inducible caspase. The inducible deletion of p16-positive senescent cells delays age-related tissue changes in mice with a Bub1b progeroid background (Baker et al., 2011), indicating that removal of senescent cells can inhibit tissue dysfunction associated with aging. In contrast to other mouse models of premature aging, loss of p53 or p21 has been shown to accelerate cellular senescence in the adipose tissue and skeletal muscles of Bub1b progeroid mice (Baker et al., 2013b), suggesting that the p53/p21 pathways regulate cellular senescence in a context-dependent manner.

ERCC1-ERCC4 (also known as XPF) is a structure-specific heterodimeric endonuclease required for NER as well as for repair of DNA interstrand crosslinks. Mutations in both of its subunits have been found to cause segmental progeroid syndromes

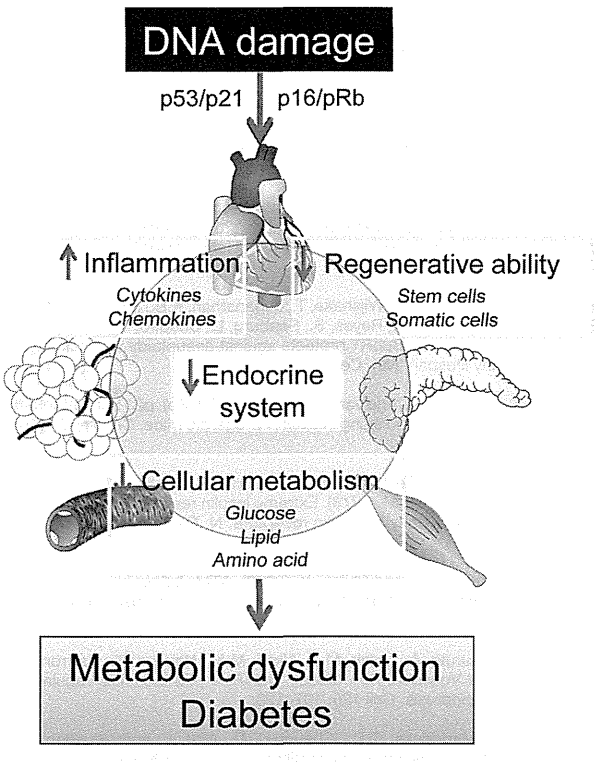

Figure 3. Potential Mechanisms by which the DNA Damage Response Contributes to Metabolic Dysfunction
Various stresses provoke the DNA damage response, leading to an increase of inflammation, a decrease of regeneration, impairment of cellular metabolism, and suppression of endocrine function. These changes can disturb systemic metabolic homeostasis and contribute to the onset of diabetes.

in humans (Grillari et al., 2007), while mouse models of *Ercc1* or *Ercc4* deficiency show a severe progeroid phenotype (McWhir et al., 1993; Tian et al., 2004) (Table 1). It was recently reported that DNA damage triggers adipose tissue inflammation in a progeroid mouse model of *Ercc1* deficiency, resulting in fat loss and insulin resistance (Table 1). Deletion of *Ercc1* leads to persistent DNA damage and increases the expression of proinflammatory cytokines in adipocytes via histone modifications associated with transcriptional activation and dissociation of nuclear receptor corepressor complexes from the promoters of proinflammatory cytokines (Karakasilioti et al., 2013). These findings clearly indicate that DNA damage itself is sufficient to trigger inflammation that leads to impaired glucose metabolism. Investigation of *Ercc1*-deficient mice has also revealed a shift toward anabolism and reduced signaling via the growth hormone/insulin-like growth factor (IGF)-1 pathway (Niedernhofer et al., 2006). Since similar changes occur in response to chronic genotoxic stress and calorie restriction or with aging, it is assumed that DNA damage induces a metabolic shift from growth to preservation of the organism in order to minimize further damage. Consistent with this hypothesis, genetic disruption of *Sirt6* in mice increases genomic instability and shortens the lifespan along with suppression of the IGF axis (Mostoslavsky et al., 2006; Schwer et al., 2010). There is evidence that p53 may be one of the regulators linking the DNA damage response to the IGF axis (Maier et al., 2004), but it remains unclear how the metabolic shift induced by DNA damage contributes to age-related pathology such as effects on glucose homeostasis and the lifespan in progeroid syndromes and during the normal aging process.

Ataxia telangiectasia (A-T) is an autosomal recessive disease caused by mutations of the ataxia telangiectasia mutated (ATM) gene (Martin, 2005). ATM is a protein kinase that has a critical role in the DNA damage response to double-stranded DNA breaks (Lombard et al., 2005). When ATM is activated, it phosphorylates p53 at a site that regulates transcriptional activity. Patients with A-T are characterized by growth retardation, an increased incidence of cancer, and neuronal degeneration. They also have an elevated risk of insulin resistance and type 2 diabetes (Bar et al., 1978; Schalch et al., 1970). Against an *Apoe* null background, *Atm* deficiency in mice causes exacerbation of various features of metabolic syndrome, including increased adiposity, insulin resistance, hypertension, and atherosclerosis (Schneider et al., 2006) (Table 1). ATM seems to play a protective role by inhibiting JNK, a kinase involved in inflammation and insulin resistance (Aguirre et al., 2000; Hirosumi et al., 2002) (Figure 2). In addition, it was reported that *Atm* deficiency does not affect fasting glucose levels and insulin sensitivity, but significantly impairs glucose tolerance related to delayed insulin secretion by pancreatic β cells (Miles et al., 2007) (Figure 2 and Table 1). Furthermore, Atm activation is required for insulin-induced phosphorylation of Akt and for glucose transport in mouse skeletal muscle (Ching et al., 2013) (Figure 2). Interestingly, the effects of *Atm* deficiency are also observed in the absence of DNA damage, suggesting that it can mediate the response to metabolic stress via a mechanism independent of the DNA damage response. Mammalian cells that lack ATM are known to have high ROS levels and show hypersensitivity to oxidative stress (Barzilai et al., 2002). ATM is the sensor for reactive oxygen species in human fibroblasts (Guo et al., 2010), and it has also been reported to mediate mitochondrial ROS signaling and to extend the lifespan of yeast (Schroeder et al., 2013). A more recent study demonstrated that ATM promotes antioxidant defenses and the repair of double-strand DNA breaks by activating the pentose phosphate pathway, which represents a link between DNA repair processes and cellular metabolism (Cosentino et al., 2011).

Conclusions

Accumulation of DNA damage can promote metabolic dysfunction in two ways, which are cell-autonomous and non-cell-autonomous mechanisms (Figure 3). Tissue regeneration is impaired by DNA damage-induced senescence and/or apoptosis of stem cells and somatic cells, leading to metabolic dysfunction such as pancreatic β cell loss. DNA damage induces non-cell-autonomous tissue inflammation through upregulation of cytokines and chemokines, resulting in interference with systemic insulin signaling. DNA damage can also affect systemic metabolic homeostasis through influences on cellular metabolism and the endocrine system. In particular, activation of the DNA damage response in certain tissues could influence the function of vital metabolic organs, thereby provoking systemic insulin resistance. Thus, better understanding of the systemic DNA damage response may help us to develop novel therapeutic strategies for metabolic disorders.

Cells have evolved various systems to actively regulate nutrient availability in order to maintain homeostasis, and have also developed active DNA repair machinery to avoid detrimental genomic instability. Evidence has been obtained that suggests that these two distinct cellular activities are highly coordinated. In this review, we have described some of the key regulatory molecules with dual roles in regulating DNA repair and cellular metabolism, including p53, sirtuins, PARP, and ATM. Future studies are expected to identify additional factors that modulate both processes and shed further light on the role of DNA damage in metabolic homeostasis.

Another intriguing aspect of DNA damage is that cellular responses are determined by the extent of the damage. For example, mild DNA damage induces reversible cell-cycle arrest to allow repair, whereas moderate to severe DNA damage leads to senescence or death that prevents the accumulation of potentially tumorigenic damaged cells. However, an excessively prolonged DNA damage response has a detrimental impact on metabolic homeostasis as well as on tumorigenesis via cell-autonomous and/or non-cell-autonomous mechanisms. Likewise, normal p53 activity is required for the physiological regulation of glucose metabolism, but inhibiting dysregulated p53 activation is beneficial for attenuating insulin resistance associated with dietary obesity, suggesting that fine-tuning of the DNA damage response is critical for the prevention and treatment of metabolic diseases.

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