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Histopathology on Atherosclerosis to Metabolic Syndrome Using Genetically Modified Animal Models

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Abstract: Since atherosclerosis and its complications account for approximately up to 30% of cause of human death in the whole world, it is very critical to overcome this issue from the economical as well as medical aspects. I first focused on the histopathological viewpoints that the initiation to progression of atherosclerosis basically depends on inflammatory response. In order to elucidate its mechanism, I have thoroughly performed the *in vivo* experiments together with the extensively morphological to biochemical and molecular analyses from the wide view, by using various genetically modified animal models of mice or rabbits. Furthermore, I recently aim to multidimensionally and comprehensively re-evaluate these chronic inflammatory diseases, via overlooking the entity of metabolic syndrome, manifesting as not only atherosclerosis but diabetes mellitus (DM) or non-alcoholic steatohepatitis (NASH).

In the atherosclerotic lesions, besides vascular wall-comprising cells including smooth muscle cells (SMCs) and endothelial cells (ECs), we can histologically see various types of inflammatory cells, such as macrophages (MΦ), whose complicated reactions consider to canonically regulate the promotion of atherosclerosis. I have figured out the mechanism(s) of metabolic syndrome including atherosclerosis, via extensively investigating the production and regulation of histamine, a classic inflammatory low-molecular-weight amine, apoptotic activities, oxidative stress and the regulation of lipid/bile acid metabolism, especially in those inflammatory lesions, at least in part. Based on a growing body of evidences using variable target genes-knockout/transgenic mice/rabbit, my ultimate aim is to contribute understanding the pathophysiology of life style-related diseases, through careful investigation of those unique animal models.

Key Words: atherosclerosis, chronic inflammatory disease, metabolic syndrome, animal model

Introduction

Since atherosclerosis and its complications including myocardial/cerebral infarction account for approximately up to 30% of cause of human death in the whole world, it is very critical to overcome those issues from the economical as well as medical viewpoints (1). Moreover, accumulating evidence has revealed that atherosclerosis is an extremely complicated and multifactorial disease, orchestrated variably by diet type affecting lipid/bile acid (BA)/glucose metabolism, inflammatory cell and cytokine levels, apoptotic activities, oxidative stressors, insulin sensitivity in various organs, translocation of either intestinal bacteria or microbial cell components, a background of hepatic/intestinal function, and other factors (1-3). Our group has considered atherosclerosis as one aspect of chronic inflammatory diseases manifested by metabolic syndrome, and thus, I first focused on the histopathological viewpoints that the initiation to progression of atherosclerosis basically depends on inflammatory response. Following that, in order to elucidate its complex mechanism(s), I have thoroughly performed the *in vivo* experiments together with the extensively morphological to biochemical and molecular analyses from the broad viewpoints

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ranging not only inflammation but also apoptosis and oxidative stressors, by using various genetically modified animal models of mice or rabbits, as summarized in Figure 1 and Table 1. Overall, our laboratory ultimately aims to contribute understanding the pathophysiology of those life style-related diseases, through careful investigation of several unique animal models of metabolic syndrome. Indeed, I have more recently re-evaluated multidimensionally and comprehensively, via overlooking the entity of metabolic syndrome, manifesting as not only atherosclerosis but diabetes mellitus (DM) or non-alcoholic steatohepatitis (NASH). I herein reviewed briefly our useful animal models for studying the pathogenesis and key molecules or factors on chronic inflammatory human diseases, i.e., metabolic syndrome including atherosclerosis.

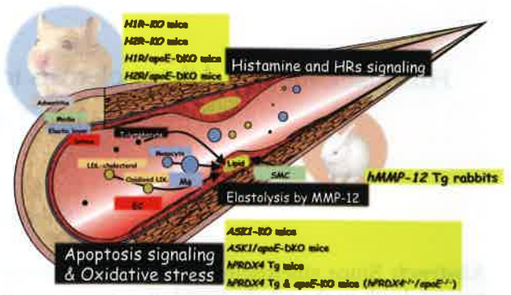

Figure 1. Schematic presentation of various genetically modified mice or rabbits on metabolic syndrome, representing as atherosclerosis. ApoE, apolipoprotein E; ASK1, apoptosis signal-regulating kinase; DKO, double knockout; EC, endothelial cell; hMMP-12, human matrix metalloproteinase-12; hPRDX4, human peroxiredoxin 4; HR, histamine receptor; KO, knockout; LDL, low-density lipoprotein; MΦ, macrophage; SMC, smooth muscle cell; Tg, transgenic.

Table 1. Summary of the phenotypes in each genetically modified animal and each animal model of metabolic syndrome.

Genetically modified animals	Phenotypes	References
hMMP-12 Tg rabbits	Pro-atherosclerotic/arteriosclerotic HcD-induced atheroma ↑ Vascular injury-induced neointima ↑	Yamada *et al.*, 2008 [ref. 4]
H1R-KO or H1R/apoE-DKO mice	Pro-atherosclerotic / Anti-arteriosclerotic HcD-induced atheroma ↑ Vascular injury-induced neointima ↓ HcD- or CA-induced NAFLD → insulin resistance → ~ ↓ Obesity Allocation of glucose	Yamada & Wang *et al.*, 2010,2013,2015,2016 [refs. 9,10,11,12]
H2R-KO or H2R/apoE-DKO mice	Anti-atherosclerotic / Pro-arteriosclerotic HcD-induced atheroma ↓ Vascular injury-induced neointima ↑ HcD- or CA-induced NAFLD ↑ ↑ Insulin resistance ↑ ↑ Weight loss	Yamada & Wang *et al.*, 2010,2013,2015,2016 [refs. 9,10,11,12]
ASK1-KO or *ASK1/apoE*-DKO mice	Pro-atherosclerotic / Anti-arteriosclerotic HcD-induced atheroma ↑ Vascular injury-induced neointima ↓ Stable plaque ↑ Anti-inflammatory (BDL-induced liver injury (acute phase)) Anti-fibrogenic (BDL-induced liver injury (subacute to chronic phase))	Yamada & Tasaki & Noguchi *et al.*, 2011,2013,2014 [ref. 13,14,15]
hPRDX4 Tg or hPRDX4 Tg & *apoE*-KO mice	Anti-atherosclerotic HcD-induced atheroma ↓ Stable plaque ↑ Oxidative stress (ROS) ↓ & Apoptosis ↓ & Inflammation ↓ STZ-induced Type 1 DM ↓ HFrD+STZ- & MCD+HF-induced NAFLD ↓ ↓ & Type 2 DM ↓ & Obesity ↓ Intestinal function ↑	Yamada & Ding & Guo & Nabeshima & Nawata *et al.*, 2010,2012,2013,2016 [ref. 17,18,19,20]

ApoE, apolipoprotein E; ASK1, apoptosis signal-regulating kinase 1; CA, cholic acid; DKO, double knockout; DM, diabetes mellitus; HcD, high-cholesterol diet; HF, high fat diet; HFrD, high fructose diet; hMMP-12, human matrix metalloproteinase 12; hPRDX4, human peroxiredoxin 4; HR, histamine receptor; KO, knockout; MCD+HF, methionine- and choline-deficient high fat diet; NAFLD, nonalcoholic fatty liver disease; ROS, reactive oxygen species; STZ, streptozotocin; Tg, transgenic.

Various unique animal models of atherosclerosis to metabolic syndrome

1. *Atherosclerosis in Human Matrix Metalloproteinase 12 (hMMP-12) Transgenic (Tg) Rabbit*

Matrix metalloproteinase 12 (MMP-12) can degrade a broad spectrum of substrates, extracellular matrix (ECM), including elastin synthesized by smooth muscle cells (SMCs), which is one of main and critical components of the arterial media, maintaining the wall structure and function by its elastic nature (4). According to the classically established hypothesis known as the 'Response to Injury Theory' by Russel Ross, after migration of monocytes with subsequent differentiation into macrophages (MΦ), from the peripheral blood into the intima via degradation of the endothelial cells (ECs) to basement membrane, early atherosclerotic lesions (mainly fatty streaks) occur (5). Its appearance is followed by a microscopic event, i.e., the migration of SMCs from the media to the intima, potentially resulting in the progression from fatty streaks to modestly advanced atherosclerosis, i.e., fibrous plaques (5). Considering these processes, elastolysis of the ECM including internal elastic lamina would need to be accelerated for such a microscopic/macroscopic transition. My laboratory has investigated the details of elastolysis of the elastic layer within a relatively short period of time by using *human MMP-12 (hMMP-12)* Tg rabbits in 3 different types of experimental models of atherosclerosis, i.e., high cholesterol diet (HcD)- and vascular injury-induced (ligation- or cuff-induced) atherosclerotic initiation/progression, as previously described in more detail (1, 4). The hMMP-12 transgene was under the control of the human scavenger receptor-A enhancer/promoter, which is a MΦ-specific promoter. but not the promoter for the MMP-12 gene. My aim is to discuss the role of the disruption of the basement membrane and elastolysis of the internal elastic layer by MMP-12 in the early stage of atherosclerosis. Fatty streaks, i.e., early atherosclerotic lesion, in the *hMMP-12* Tg rabbit fed a 1% HcD for 6 wk were more accelerated, along with more significant degradation of the internal elastic layer, compared with those of wild type (WT) Japanese white rabbit. In the *hMMP-12* Tg rabbit, infiltrating MΦ and SMCs in the lesion were larger in number than those in the WT. Similarly, in vascular injury-induced atherosclerotic models, SMC-predominant neointimal lesions were more pronounced, associated with more significant elastolysis of the internal elastic lamina, in the Tg than in the WT rabbits, wherein 'micro-elastolytic sites' were recognized prior to formation of the neointima especially in the cuff model. In conclusion, those obtained data can speculate that the microenvironmental change in the MMP-12-induced ECM and inflammatory response should be critical factors for the initiation and progression of atherosclerosis via degradation of the elastic layers and/or basement membrane.

2. *Atherosclerosis to Non-Alcoholic Steatohepatitis (NASH) in Histamine-Specific Membrane H1 and H2 Receptors (H1/2R) Gene-Knockout (H1R-KO and H2R-KO) Mice*

Histamine, 2-(4-imidazole)-ethylamine, is one of the most well-known molecules in the field of medicine, and this classic low-molecular-weight amine is synthesized from L-histidine by histidine decarboxylase (HDC) (1, 6). Histamine is produced by various cells, including mast cells, gastric enterochromaffin-like cells, basophils and central nervous system neurons (1, 6). Histamine-specific membrane receptors (HRs), which are now classified into four subclasses (H1R-H4R), specifically transmit the pleiotropic effects of histamine. Among them, the H1 receptor (H1R) couples with Gq and leads to the phosphoinositol hydrolysis pathway, whereas the H2 receptor (H2R) links to Gs to activate the adenylate cyclase pathway (1, 6). It is well known that H1R antagonists still serve as a critical therapy for a number of allergic conditions, such as rhinitis/cystitis, and H2R antagonists have revolutionized the treatment of various gastric acid-related ulcer (1). In contrast, our previous studies have uniquely focused on the pivotal roles in the pathogenesis of atherosclerosis, in which H1R and H2R are expressed in peripheral blood cells (e.g., MΦ) and in arterial wall cells, and the transcription of *H1R* mRNA is augmented as atherosclerosis initiates and progresses in the human aorta and coronary arteries (1, 6, 7). Indeed, the expression profile of the main receptors (H1/2R) has been shown to be switched from H2R to H1R during monocyte to MΦ differentiation, and H1R is also predominant in SMCs and ECs of atheromatous plaque (8). Our laboratory has aimed to figure out how H1R and/or H2R are strongly involved in not only the promotion of atherosclerosis but also the development of NASH, leading to metabolic syndrome.

We herein review our findings based on serial studies of various *in vivo* animal models using *H1R* and *H2R* gene-knockout mice (*H1R*-KO and *H2R*-KO mice) (1), as summarized in Table 1. First, on the HcD-induced atherosclerosis model, in which *HRs* and *apolipoprotein E (apoE)*- double KO (DKO) mice, pronounced hyperlipidemia-induced atherosclerotic progression occurred in *H1R/apoE*-DKO mice, but in *H2R/apoE*-DKO mice less atherosclerosis (9). Accordingly, the increased expressions of scavenger receptors (SRs), such as SR-A, CD36 and lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1),

nuclear factor-kappa B (NFκB), monocyte chemoattractant protein (MCP-1), MMPs or liver X receptor (LXR)-related inflammatory signaling factors, were consistent with the pro-atherogenic phenotype of *H1R/apoE*-DKO mice. Taken together, histamine/H1R signaling especially protects against atheromatous formation, and of particular note, the above H1R switch during MΦ differentiation may be a hallmark of *in vivo* inherent protective mechanisms against the progression of HcD-induced atherosclerosis.

In marked contrast, upon the ligated carotid arteries of *H1R*-KO or *H2R*-KO mice during the vascular injury-induced model, *H1R*-KO mice had significantly less arteriosclerosis consistent with anti-atherogenic phenotype, whereas $H2R^{-/-}$ mice had more, compared with WT mice (10). In line with those results, the decreased expressions of MCP-1, platelet-derived growth factor (PDGF), adhesion molecules and LXR-related inflammatory signaling factors, including Toll-like receptor (TLR3), interleukin-1 receptor (IL-1R) and tumor necrosis factor receptor (TNF-R), was consistent with the anti-arteriosclerotic phenotype of *H1R*-KO mice. Based on those conflicting data, each HR would have distinct and opposite roles in the development of respective experimental atherosclerosis model. We have proposed that the mechanisms responsible for atherosclerosis must be fundamentally different between these two models of HcD- and vascular injury-induced atherosclerosis, from the aspects of predominant cell types in its lesions, at least in part (1, 2, 4, 9, 10). Indeed, the injury model shows the SMC-rich neointimal hyperplasia, very likely reminiscent of human restenosis after angioplasty or shoulder lesions of vulnerable atheroma, manifesting as acute to subacute inflammatory disease (1, 2). On the contrary, the HcD-induced atherosclerotic model displays the progression of MΦ-rich neointimal lesions with necrotic lipid cores and fibrous cap formation, reminiscent of human atheromatous plaques, manifesting as chronic inflammatory disease (1, 2).

Intriguingly, after feeding mice an HcD for 14 weeks, our laboratory has found that *H2R*-KO mice were exclusively affected by severe liver damage caused by lipid (i.e. triglyceride [TG]) accumulation and inflammation, representing a NASH phenotype, despite showing severe body weight loss (11). In marked contrast, *H1R*-KO mice showed prominent obesity without NASH along with visceral adiposity and hyperleptinemia. Furthermore, *H1R*-KO mice revealed mildly increased insulin resistance and markedly augmented glucose uptake in various organs, including the brain, kidney, and colon, but not the liver. However, *H2R*-KO mice unexpectedly manifested more severe insulin resistance and glucose intolerance during progression of NASH. In this context, liver cells, including hepatocytes and Kupffer cells, might have dominant H2R and not H1R expression, unlike arterial wall cells (11). According to the additional experiment of short-term HcD, histamine/H2R signaling suppressed cholesterol absorption in the jejunum and the liver (12). Alternatively, with the high-cholic acid (CA) diet, decreased serum BA levels and increased fecal BA levels were regulated by histamine/H2R signaling. Hence, the critical roles played by histamine/H2R signaling significantly reduce the liver damage during initiation and progression of NASH through these orchestrated effects, related closely to enterohepatic cholesterol and BA metabolism. In brief, histamine signaling via H1R and H2R might reciprocally regulate each *in vivo* metabolic and inflammatory process of each target organ or each animal model of metabolic syndrome, reminiscent of the potential but critical cross-talk function between H1R and H2R.

3. *Atherosclerosis to Cholestatic Liver Injury in Apoptosis Signal-Regulating Kinase 1 (ASK1)-KO Mice*

Apoptotic cell death is considered not only vital processes, including normal cell turnover, immune system functioning and embryonic development, but also an important factor in many human detrimental conditions, such as inflammatory and/or autoimmune disorders, neurodegenerative diseases and many types of cancer (2). However, the *in vivo* roles of the apoptosis upon the vascular and hepatic cells in the respective initiation/progression of atherosclerosis and cholestatic liver injury, representing the majority of human inflammatory disorders, remain to be elucidated. Metabolic syndrome, representing atherosclerosis and liver injury in a background of disordered lipid/BA metabolism, is an extremely complex disease orchestrated by multiple molecular and histopathologic factors, including not only elevated inflammatory cytokines or oxidative stressors but also apoptosis. Our aim is to figure out the key and central factors and signaling pathways closely related to apoptosis, critically controlling the responses to injury in not only artery but also liver. Our laboratory has focused on apoptosis signal-regulating kinase 1 (ASK1), which is a mitogen-activated protein kinase kinase kinase (MAPKKK) family member activated through distinct mechanisms in response to various cytotoxic stressors, including immune system mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β or Fas ligands, and oxidative stressors mediated by hydrogen peroxide (H_2O_2) or endoplasmic reticulum (ER) stress (2). It is also well known that ASK1 is ubiquitously expressed by multiple cell types and situated upstream of many signal transduction pathways, such as the c-Jun N-terminal kinase (JNK) and p38 MAP kinase

(MAPK) pathways, which subsequently induce not only intrinsic apoptotic signaling via mitochondria-dependent caspase activation, but also inflammation or cell proliferation and differentiation (2). I have hypothesized that ASK1 signaling can play diverse, key roles in the pathogenesis of atherosclerosis and cholestasis.

In that vein, we herein review our collective findings based on serial studies of several *in vivo* animal models using *ASK1*-knockout mice (*ASK1*-KO mice) (2), as summarized in Table 1. First, in the HcD-induced atherosclerosis model, as compared to the *apoE*-KO mice, the *ASK1/apoE*-DKO mice demonstrated pro-atherogenic profiles, including the manifestations of more MΦ-rich atheroma, smaller necrotic cores, and faster atherosclerotic progression, in addition to the suppression of apoptosis in MΦ (13). In other words, ASK1 signaling reduced MΦ-rich atherosclerosis but accelerated necrotic core formation via the stimulation of MΦ apoptosis, which might simultaneously cause plaque vulnerability. In marked contrast, according to the ligation-induced vascular injury model, as compared to the WT mice, the *ASK1*-KO mice showed anti-atherosclerotic/arteriosclerotic profiles, including manifestations of less-SMC-predominant intimal lesions and smaller numbers of neointimal microvessels, associated with the repression of apoptosis in SMCs and ECs (14). In brief, ASK1 signaling exacerbates arteriosclerotic development in ligation injury-induced neointimal thickening by enhancing the apoptosis of ECs and SMCs, at least in part. Taken together, it is suggested that ASK1 expression and its signaling along with apoptotic activities, especially in SMCs and MΦ, could be not only critically but diversely responsible for various potentially pro-atherosclerotic and anti-atherosclerotic effects, depending on the predominant cell types in the thickened intima and disease stage/phase, from acute to chronic status. Ultimately, we emphasize that the fundamental mechanisms responsible for atherosclerosis in a broad sense differ completely between the atheromatous plaques in the aorta (i.e., chronic phase) and the injured arteriosclerotic arteries (i.e., acute/subacute phase).

Finally, I herein review the crucial roles of ASK1 in bile duct ligation (BDL)-induced cholestatic liver injury using the above *ASK1*-KO mice with the ultimate aim of determining the net effects of ASK1 in the progression of liver necro-inflammation (i.e. acute inflammatory phase) to subsequent fibrogenesis/fibrosis (i.e. subacute-to-chronic inflammatory phase) (15). In this BDL model, as compared with WT mice that expressed elevated levels of phosphorylated ASK1, *ASK1*-KO mice displayed potential anti-inflammatory profiles, especially in the acute phase (around day 3) of liver injury, including manifestations of reduced hepatocellular necrotic foci, suppressed extensive inflammatory reactions in lobules and portal areas and decreased proliferation of hepatocytes and large cholangiocytes (15), as summarized in Table 1. Unexpectedly, the morphological characteristics of apoptosis were not completely recognized during the BDL experiments. Indeed, the numbers of apoptotic liver cells after BDL were negligible based on the histological morphology, such as cell shrinkage, chromatin condensation and margination and formation of apoptotic bodies, and intriguingly, these numbers were not markedly increased compared to sham-operated controls. Next, the above effects of *ASK1* deficiency in the acute phase improved the subsequent peribiliary fibrosis/fibrogenesis in *ASK1*-KO mice with anti-fibrogenic profiles in the subacute-to-chronic phase at day 14 post-BDL injury (15). In line with that, *ASK1*-KO mice demonstrated significantly suppressed activation of peribiliary fibrogenic cells, decreased biliary ductules and reduction of ECM synthesis. I have concluded that liver cells undergoing apoptosis cannot exclusively play a pivotal role in BDL-induced cholestatic liver injury, however, that ASK1 signaling tightly controls the development of not only early necro-inflammation but also subsequent peribiliary fibrosis/fibrogenesis under conditions that induce acute inflammatory processes and the subacute-to-chronic fibrogenic response.

In conclusion, our serial *in vivo* studies can provide new evidence of potential mechanisms by which ASK1 signaling can critically but diversely affect the severity of those inflammatory disease processes. It is also suggested that stimulated ASK1 signaling crucially regulates the initiation/progression within each disease process, depending on not only the animal model, influenced by each target organ, but also the phase, from acute to chronic stages. Finally, the relevance of apoptotic activities relies on the characteristics of each murine model of human diseases, as well.

4. *Atherosclerosis to Metabolic Syndrome (Manifesting as DM and/or NASH) in Human Peroxiredoxin 4 (hPRDX4) Transgenic (Tg) Mice*

Oxidative stress appears to mechanistically illustrate the EC dysfunction, and the vascular inflammation and complications especially in the initiation of the metabolic syndrome/metabolic syndrome-related diseases, manifesting as atherosclerosis to NASH (1, 3). In addition to the target organs of metabolic syndrome (arteries and liver), the small intestine with its extensive surface area is the first organ to encounter nutrients, which likely plays a crucial role in the development of metabolic disorders as well (1). Our obtained *in vivo* data indicate that lipid/BA/

glucose metabolism with both local (each tissue) and whole-body functions plays a central, key role in the etiology of metabolic syndrome. Among a new family of antioxidant enzymes, peroxiredoxin (PRDX), which is ubiquitously synthesized and has been abundantly identified in various organisms (3, 16), PRDX4 is the only known secretory form located in not only the intracellular (especially the endoplasmic reticulum) but also the extracellular space, in contrast to the intracellular localization of other family members (PRDX1, 2, 3, 5 and 6). Our aim is to confirm that PRDX4 locally (intracellularly) and systemically (extracellularly) plays a pivotal, diverse role *in vivo* in the protection against the initiation and development of metabolic syndrome manifesting as visceral obesity, DM, atherosclerosis and/or NAFLD (i.e. disordered lipid/BA/glucose metabolism). Our laboratory has generated *human PRDX4 (hPRDX4)* transgenic (Tg) mice and evaluated the *in vivo* functions of PRDX4 in serial studies of various animal models for human metabolic syndrome (3), as summarized in Table 1.

I have reported for the first time that, after single high dose of streptozotocin (STZ) (SHDS)-injection-induced type 1 DM model, Tg mice showed significantly less hyperglycemia and hypoinsulinemia and a much faster response on the glucose tolerance test than treated WT mice, despite no marked differences in the insulin tolerance test (17). A histopathological observation demonstrated that the Tg mice were significantly more resistant to serious SHDS-induced injury than WT mice, displaying accelerated reconstruction of the islets while maintaining a high level of not only hPRDX4 but also endogenous mouse PRDXs expressions, including mouse PRDX4. The overexpression of PRDX4 plays a protective, key role against SHDS-induced injury by inhibiting EC dysfunction, proinflammatory cytokines and cytotoxic T-cell infiltration, as well as by preventing β-cell-derived reactive oxygen species (ROS) generation or scavenging the generated ROS, particularly the extracellular pool of ROS (17). In fact, the insulin-secreting β-cells in the Tg islets, where hPRDX4 is specifically expressed, were significantly more protective against SHDS-induced insulitis and apoptosis than those of WT mice. Additionally, the Tg islets were more likely to activate the proliferation of β-cells, since the specific expression of hPRDX4 might be able to accelerate replication of pluripotent cells to repair and remodel islets (17).

Next, our laboratory revealed that PRDX4 protected against atherosclerotic progression in HcD-induced atheromatous and vulnerable plaque formation by suppressing oxidative damage and apoptosis. A detailed histopathological observation showed that hypercholesterolemia-induced MΦ-rich atherosclerosis was significantly more reduced, although more SMC-rich plaques were evident in Tg and *apoE*-KO mice (*hPRDX4*$^{+/+}$/*apoE*$^{-/-}$) than *apoE*-KO mice (18). Their atheromatous formation was characterized by a thicker fibrous cap, a greater amount of collagen, and fewer central necrotic lipid cores than *apoE*-KO mice, reminiscent of the structure of human stable plaques. Plaque vulnerability is known to be the most critical factor in cardiac attack and subsequently potential death (1, 2, 5). Our laboratory can confirm that the overexpression of hPRDX4 plays a crucial role in (i) ameliorating atherosclerotic progression by reducing local and systemic oxidative stressors, decreasing the size of the central necrotic core via repressing MΦ apoptosis, and suppressing inflammatory cell migration through the decrease of EC apoptosis; and (ii) reducing possible markers of plaque instability via thickening the fibrous caps and enhancing the collagen-rich ECM by repressing SMC apoptosis (18). Not only that, but PRDX4 can also protect against the development of NASH, type 2 DM, and metabolic syndrome by ameliorating ROS-induced injury in a nongenetic mouse model by feeding Tg mice a high-fructose diet (HFrD) after injecting STZ (19). Our collecting data

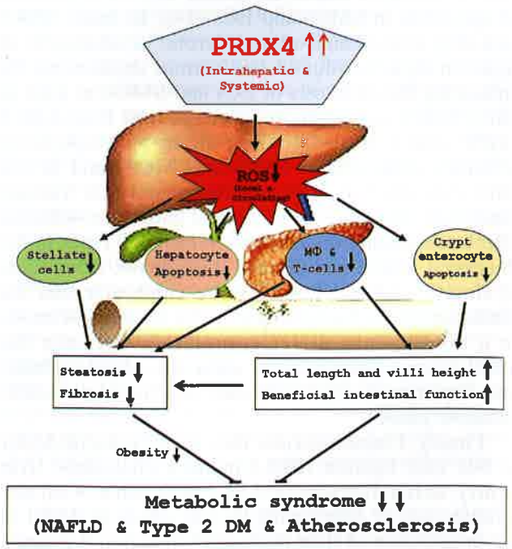

Figure 2. Summary of the critical roles of PRDX4 in mice models of metabolic syndrome.
This diagram depicts the critical, protective roles of PRDX4 in the mouse metabolic syndrome models.
PRDX4, peroxiredoxin 4; ROS, reactive oxygen species; MΦ, macrophage; NAFLD, nonalcoholic fatty liver disease; DM, diabetes mellitus.

indicated that PRDX4 overexpression especially in Tg mice plays critical, key roles in (i) ameliorating the local (intrahepatic) and systemic (circulating) expression of oxidative stressors, (ii) preventing the initiation of NAFLD and insulin resistance by reducing hepatic TG accumulation, and (iii) protecting against the development of obesity, NASH, and type 2 DM by suppressing chronic inflammation, apoptosis, and fibrogenesis, with its unique intracellular and extracellular effects (19), as summarized in Figure 2.

Finally, we have found that PRDX4 can induce beneficial effects on not only the hepatic but also the intestinal function by protecting against oxidative stress-induced injury in a methionine- and choline-deficient high-fat (MCD+HF)-induced NAFLD mouse model (20). An accumulating body of our data have confirmed that PRDX4 overexpression on Tg mice plays pivotal roles in (i) reducing local (intrahepatic and intraintestinal) and systemic (circulating) ROS; (ii) affecting beneficial intestinal function by suppressing crypt enterocyte apoptosis and inflammation and by protecting against the shortening of the total length and villi height; and (iii) synergistically reducing the severity of NAFLD, and subsequently metabolic syndrome (20), as summarized in Figure 2. I can also provide the first evidence that the small intestine as well as the liver are spared by the antioxidant properties of PRDX4 and that the overexpression of PRDX4 beneficially affects the intestinal function while preventing the progression of NAFLD. In addition to the target organ (the liver), the small intestine is also involved in a key role in the etiology of NASH, which is one aspect of human metabolic syndrome, manifesting as visceral obesity, dyslipidemia, type 2 DM, and atherosclerosis. Since the intestine is the first organ to encounter nutrients and serves as gatekeeper at the pathophysiological interface between the body and the diet, it can play a varied but crucial role in the metabolic processing of nutrients along with efficient lipid and bile acid (BA) absorption, partly via enterohepatic circulation (1, 2, 20).

Concluding remarks on our unique animal models ranging from atherosclerosis to metabolic syndrome

Not only atherosclerosis but metabolic syndrome are complex, multifactorial diseases, orchestrated by diet type affecting glucose/lipid/BA metabolism, ROS, insulin resistance, inflammatory cell infiltration, cytokine levels and/or apoptotic activities in various organs, translocation of either intestinal bacteria or microbial cell components, and other factors. There are a growing body of our and others' evidences supporting the innate links between these metabolic diseases and various factors, such as inflammation, matrix, apoptosis and oxidative stressors, which have not only local but also whole-body functions. These unique genetically modified animal models will be useful for studying the associations of the local and systemic properties with lipid/BA/glucose metabolism and may also be promising novel methods for studies of human metabolic syndrome. Finally, our laboratories more recently established a novel model for HcD-induced atherosclerosis and NASH using the world's smallest Microminipigs^{TM} (μMPs; Fuji Micra Inc., Shizuoka, Japan) (21, 22). Unlike rodents or rabbits, swine represent a promising, useful experimental animal model, since their lipoprotein metabolism as well as their anatomy, physiology, and feeding and sleeping habits are quite similar to those of humans. Thus, one of our future perspectives is to clarify the pathogenic and molecular mechanisms during the initiation and development of metabolic syndrome, particularly in those μMPs. We ultimately aim to perform a translational research, from bench to bedside, and from animals to human.

Abbreviations

Apo, apolipoprotein; ASK, apoptosis signal-regulating kinase; BA, bile acid; CA, cholic acid; DKO, double knockout; DM, diabetes mellitus; EC, endothelial cell; ECM, extracellular matrix; HcD, high-cholesterol diet; HF, high fat diet; HFrD, high fructose diet; H_2O_2, hydrogen peroxide; hMMP-12, human matrix metalloproteinase 12; hPRDX4, human peroxiredoxin 4; HR, histamine receptor; IL, interleukin; KO, knockout; LDL, low-density lipoprotein; MΦ, macrophage; MCD+HF, methionine- and choline-deficient high fat diet; MMP, matrix metalloproteinase; mPRDX4, mouse peroxiredoxin 4; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NFκ-B, nuclear factor κ-B; PRDX, peroxiredoxin; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymerase chain reaction; SHDS, single high dose of streptozotocin (STZ); SMC, smooth muscle cell; STZ, streptozotocin; Tg, transgenic; TG, triglyceride; TNF, tumor necrosis factor; WT, wild-type; μMPs, microminipigs.

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Disclosure Statement

The author declares no conflicts of interest in association with this study/review.

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