

主論文の要旨

**Omentin attenuates angiotensin II-induced abdominal  
aortic aneurysm formation in apolipoprotein  
E-knockout mice**

〔オメンチンは、アポリポプロテイン E 欠損マウスにおいて、  
アンジオンテンシン II 誘導性の腹部大動脈瘤形成を抑制する〕

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## **【Introduction】**

Abdominal aortic aneurysm (AAA) is an increasing and life-threatening disease. Although recent progress of surgical and endovascular stent graft therapies dramatically improves prognosis of AAA, their complications including peri-graft leakage and recurrence of AAA are still unsolved problems. Inflammation and proteolytic degradation of extracellular matrix in the aortic wall are pivotal steps of the progression of AAA. Obesity contributes to an increased risk of AAA. However, the molecular link between obesity and AAA development is incompletely understood.

Recent evidences have shown that dysregulation of adipose-derived secreted factors, also known as adipokines, under conditions of obesity contributes to the pathogenesis of cardiovascular disorders including AAA. Omentin is a circulating adipokine, which is downregulated in obese complications. Here, we examined whether omentin could modulate angiotensin (Ang) II-induced AAA formation in apolipoprotein-E knockout (apoE-KO) mice.

## **【Methods and Results】**

ApoE-KO mice were crossed with transgenic mice expressing the human omentin gene in fat tissue (OMT-Tg mice) to generate apoE-KO/OMT-Tg mice. ApoE-KO/OMT-Tg and apoE-KO mice were subjected to continuous Ang II-infusion by using osmotic mini pumps. ApoE-KO/OMT-Tg mice exhibited a lower incidence of AAA formation and a reduced maximal diameter of AAA compared with apoE-KO mice. ApoE-KO/OMT-Tg mice also showed attenuated disruption of medial elastic fibers evaluated by elastic van Gieson staining in response to Ang II compared with apo-E KO mice. ApoE-KO/OMT-Tg mice also displayed reduced expression levels and activities of matrix metalloproteinase (MMP) 9, MMP2 in aortic walls compared with apoE-KO mice. Furthermore, apoE-KO/OMT-Tg mice showed increased infiltration of MOMA2-positive macrophages and CD45-positive lymphocytes, which is accompanied by increased levels of pro-inflammatory mediators including interleukin (IL)-6 and monocyte chemotactic protein (MCP)-1,

Treatment of human monocyte-derived macrophages with omentin protein attenuated expression of MMP9, IL-6 MCP-1, and MMP9 activation evaluated by gelatin zymography after stimulation with lipopolysaccharide (LPS). Treatment of human vascular smooth muscle cells (VSMCs) with omentin protein reduced expression and activation of MMP2 after stimulation with tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ).

Omentin treatment increased phosphorylation levels of Akt in human macrophages and VSMCs. The suppressive effects of omentin on MMP9 and MMP2 expression were reversed by inhibition of PI3-kinase/Akt signaling by using LY294002 in macrophages and vascular smooth muscle cells, respectively. Furthermore, the suppressive effects of omentin on MMP9 and MMP2 expression were reversed by neutralizing antibody of

integrin  $\alpha V\beta 3$  in macrophages and VSMCs, respectively

Finally, systemic administration of omentin using adenovirus expression system attenuated AAA formation and disruption of medial elastic fibers in response to Ang II in apoE-KO mice. In contrast, continuous administration of LY294002, which is an inhibitor of PI3-kinase/Akt signaling, reversed the suppressive effects of systemic omentin administration on AAA formation in response to Ang II in apoE-KO mice.

### **【Discussion】**

Our study provides the first evidence that omentin can attenuate aortic aneurysm formation in an established model of AAA. Elevated circulating levels of omentin derived from adipose tissue led to a significant reduction of incidence and dilatation of AAA after Ang II infusion in apoE-KO mice, which was accompanied by reduction in disrupted elastin integrity and expression of MMPs and pro-inflammatory genes in aortic walls. Systemic delivery of omentin reduced the dilatation and elastic disruption of AAA after Ang II infusion in apoE-KO mice. Treatment of human macrophages with omentin protein at a physiological concentration resulted in decreased expression of MMP9 and pro-inflammatory mediators. Furthermore, treatment of human VSMCs with omentin protein reduced MMP2 expression. These data suggest that omentin can act as an adipokine that reduces the development of AAA, at least in part, through suppression of MMPs expression and inflammatory response in macrophages and VSMCs.

AAA is characterized by inflammation and matrix degradation of vessel wall, leading to aneurysm growth and rupture. Expression levels of MMPs are increased in aortic tissue from AAA patients. In an Ang II-induced AAA model, both MMP2 and MMP9 are activated and are involved in AAA development. MMP9 deficiency has been reported to suppress elastase-induced AAA formation. Disruption of MMP2 has been shown to attenuate AAA formation in a CaCl<sub>2</sub>-induced AAA model. Our *in vivo* data demonstrated that omentin reduced expression levels of MMP9 and MMP2 in vascular walls after Ang II infusion. Thus, it is conceivable that omentin could reduce the incidence and progression of AAA formation partly through suppression of MMP9 and MMP2 production.

PI3-kinase/Akt activation has been reported to attenuate inflammatory response of macrophages. We have previously demonstrated that omentin suppresses expression of inflammatory genes in macrophages through activation of PI3-kinase/Akt. In agreement with these data, our *in vitro* data showed that omentin reduced MMP9 and MMP2 expression through the PI3-kinase/Akt signaling pathway in human macrophages and VSMCs, respectively. We and others have shown that omentin promotes PI3-kinase/Akt signaling cascades in various types of cells including cardiac myocytes and endothelial cells. Thus, it is likely that PI3-kinase/Akt is essential for the protective features of omentin for the cardiovascular system. We have also shown that integrin  $\alpha V\beta 3$  is involved

in omentin-induced PI3-kinase/Akt activation and survival in cardiac myocytes. Our current findings indicate that omentin attenuates MMP9 and MMP2 expression through its ability to activate PI3-kinase/Akt via integrin  $\alpha$ V $\beta$ 3 in macrophages and VSMCs, respectively. These data suggest that the integrin  $\alpha$ V $\beta$ 3-PI3-kinase/Akt regulatory axis can be crucial for cardiovascular protection by omentin.

Recent clinical studies show that low circulating levels of omentin are closely associated with cardiovascular disease, in particular, atherosclerosis. Plasma omentin concentration is significantly decreased in patients with coronary artery disease (CAD). In addition, omentin levels are negatively associated with the presence and angiographic severity of CAD in patients with metabolic syndrome and in post-menopausal women. Furthermore, low omentin level is associated with an increase in a marker of atherosclerosis, carotid intima-media thickness in healthy of major causes of AAA.

### **【Conclusion】**

Our present study shows for the first time that fat-derived omentin attenuates the occurrence and development of AAA formation by suppressing MMPs expression and activity through integrin  $\alpha$ V $\beta$ 3/PI3-kinase/Akt signaling in the vascular wall. Thus, omentin could be a novel therapeutic target for prevention or treatment of AAA.