SUPPLEMENTAL MATERIAL

Data S1

Supplemental Methods

Human Sample Collection in Patients With Coronary Artery Disease (CAD)

This study was received prior approval from the Ethics Committees of Showa University and Tokyo University of Pharmacy and Life Sciences. Informed consent was obtained from a total of 98 participants prior to their enrollment. Blood was drawn from 70 patients with acute coronary syndrome (ACS), admitted within 4 hours after onset to Showa University Hospital for emergency coronary catheterization (42 men, 28 women; aged 22–88); 28 healthy volunteers (17 men, 11 women; aged 21–56) were also recruited. ACS patients included 53 with acute myocardial infarction and 17 with unstable angina pectoris. Plasma KP-10 level was measured by enzyme-linked immunosorbent assay (ELISA; Phoenix Pharmaceuticals) after extraction with Sep-Pak C18 cartridge (Waters Associates) as described before.[1]

Buffered 10% formalin-fixed paraffin-embedded human coronary artery specimens from archive collections of the National Cerebral and Cardiovascular Center were used for immunohistochemistry. Serial cross-sections (3–4 μm) of coronary arteries from 14 patients with CAD (aged 60–87) and 4 patients with dilated cardiomyopathy (as non-CAD examples) (aged 19–39) were stained with polyclonal rabbit antibody against human KP-10 or its receptor GPR54 (LifeSpan BioSiences) as described previously.[2,3]

Supplemental Results

Expressions of KP-10 and GPR54 in Coronary Artery Lesions and Plasma in CAD Patients

Faint expressions of KP-10 and GPR54 were observed in the endothelium of normal coronary arteries from non-CAD patients (Figure S2A: a, b). KP-10 also was abundantly expressed in adventitia (Figure S2A: a). In stenotic and obstructive coronary arteries from CAD patients, both KP-10 and GPR54 were expressed at high levels in the atheromatous plaques (Figure S2A: c–h). Both KP-10 and GPR54 expressions became greater in accordance with the severity of atheromatous plaques (Figure S2A: c–h). In contrast, plasma KP-10 level tended to be reduced in CAD (ACS) patients compared with healthy volunteers (Figure S2B). We speculate that plasma KP-10 may be rapidly exhausted in thrombus formation in the coronary arteries within 4 hours after onset of ACS, because KP-10 is also known to act as an anti-coagulant and thrombolytic agent.[4]

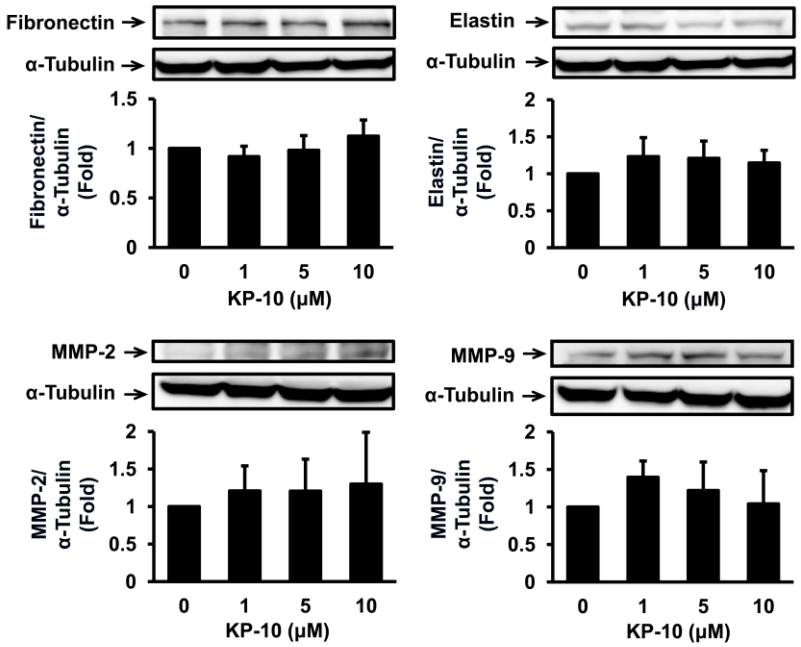

Figure S1. Effects of KP-10 on extracellular matrix expression in human aortic smooth muscle cells (HASMCs). HASMCs were incubated with the indicated concentrations of KP-10 for 24 hours and subjected for immunoblot analyses of fibronectin, elastin, metalloproteinase (MMP)-2, and MMP-9. Representative images showing protein expression (upper panels) with densitometry following normalization relative to α-tubulin (lower panels). Data are shown as means±SEM from 4 independent experiments.

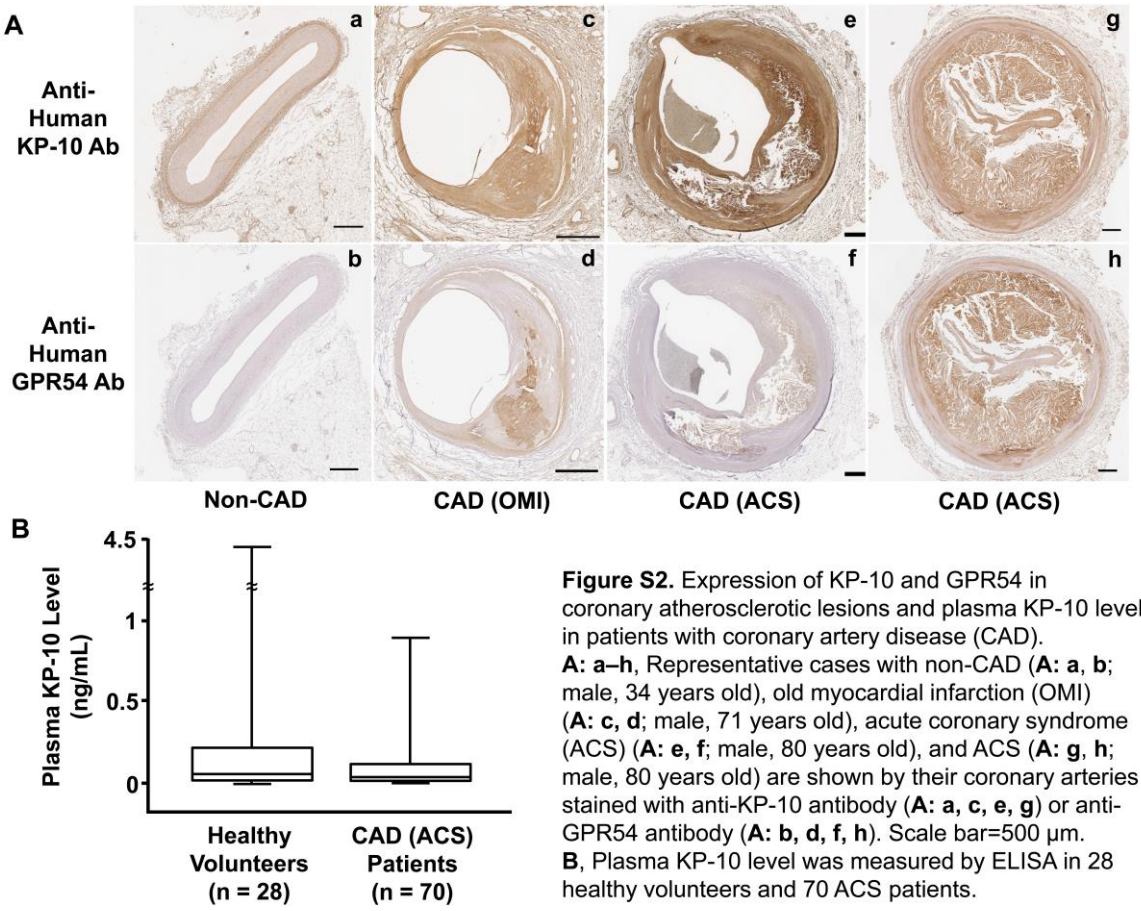

Figure S2. Expression of KP-10 and GPR54 in coronary atherosclerotic lesions and plasma KP-10 level in patients with coronary artery disease (CAD).
A: a–h, Representative cases with non-CAD (**A: a**, **b**; male, 34 years old), old myocardial infarction (OMI) (**A: c, d**; male, 71 years old), acute coronary syndrome (ACS) (**A: e, f**; male, 80 years old), and ACS (**A: g**, **h**; male, 80 years old) are shown by their coronary arteries stained with anti-KP-10 antibody (**A: a, c, e, g**) or anti-GPR54 antibody (**A: b, d, f, h**). Scale bar=500 μm.
B, Plasma KP-10 level was measured by ELISA in 28 healthy volunteers and 70 ACS patients.

Supplemental References:

1. Sato K, Koyama T, Tateno T, Hirata Y, Shichiri M. Presence of immunoreactive salusin-α in human serum and urine. *Peptides.* 2006;27:2561–2566.
2. Watanabe K, Watanabe R, Konii H, Shirai R, Sato K, Matsuyama TA, Ishibashi-Ueda H, Koba S, Kobayashi Y, Hirano T, Watanabe T. Counteractive effects of omentin-1 against atherogenesis. *Cardiovasc Res.* 2016;110:118–128.
3. Watanabe R, Watanabe H, Takahashi Y, Kojima M, Konii H, Watanabe K, Shirai R, Sato K, Matsuyama T, Ishibashi-Ueda H, Iso Y, Koba S, Kobayashi Y, Hirano T, Watanabe T. Atheroprotective Effects of Tumor Necrosis Factor-Stimulated Gene-6. *JACC Basic Transl Sci.* 2016;1:496–509.
4. Qureshi IZ, Kanwal S. Novel role of puberty onset protein kisspeptin as an anticoagulation peptide. *Blood Coagul Fibrinolysis.* 2011;22:40–49.