Proprotein Convertase Subtilisin/Kexin Type 9 Is Associated with Degenerating Adipocytes in Abdominal Aortic Aneurysm

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Abstract: Abdominal aortic aneurysm (AAA) is a common disease among the elderly. Recently, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have been indicated as useful therapeutic tools for the treatment of cardiovascular diseases. The aim of this study was to elucidate the role of PCSK9 in the pathogenesis of AAA. We used fluorescence immunohistochemistry to assess the expression of PCSK9 in aortic tissues resected from 24 patients with AAA. Histological examination showed that PCSK9 expression in the adventitia region of the aneurysms was decreased in AAA samples. In the same region, the expression of CD36 increased. We hypothesized that CD36 expression might upregulate the transport of fatty acids into cells such as the adipocytes, and subsequently cause degradation of the adventitia in the aortic wall, contributing to AAA development.

Key words: abdominal aortic aneurysm, PCSK9, adipocyte, CD36

1 INTRODUCTION

Abdominal aortic aneurysm (AAA) is an inflammatory cardiovascular disease common among the elderly¹², and whose pathogenesis is not fully understood¹³. Surgical repair is currently the only option for preventing AAA rupture⁴ and, notably, endovascular treatment improves the surgical outcome in AAA treatment⁵⁶. Recently, we found that metabolic abnormalities, especially serum dyslipidemia, are associated with AAA progression and mortality⁷⁸. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted enzyme that increases the degradation of surface low-density lipoprotein receptors (LDLRs), decreasing the removal of LDL-bound cholesterol from the blood. Therefore, PCSK9 inhibitors are promising therapeutic tools for the treatment of cardiovascular diseases. The aim of this study was to elucidate the role of PCSK9 in the pathogenesis of AAA.

2 EXPERIMENTAL PROCEDURES

2.1 Sample Collection

We studied aortic samples from 24 patients who had undergone elective open surgery to repair AAA between April 2010 and November 2016 at the Division of Vascular Surgery, Hamamatsu University School of Medicine, Hamamatsu, Japan. The study protocol was reviewed and approved by the hospital Ethics Committee of Clinical Research and all patients provided informed consent. Aortic tissue was intraoperatively removed from the patients, diagnosed by preoperative three-dimensional multidetector computed tomography (3D-MDCT) imaging. During the surgery, longitudinal tissue strips were collected from the aorta, from the nearby distal portion of the bifurcation of the renal artery to the region of maximal dilation of the aneurysm.

Ten aorta tissue samples, collected from routine autopsies in the Department of Pathology, Hamamatsu University Hospital, were used as controls. These samples were col-
lected from the mid-portion of the abdominal aorta, between the renal artery and the bifurcation. We excluded from the study autopsy specimens from patients with collagen disease and/or aortic aneurysm or dissection and conducted our analyses on samples from ten patients. Tissue samples obtained from autopsies were fixed in formalin. Patients providing the control tissue samples were all male, aged 68–82 years old (mean age 73.3 ± 4.9 years), with 5 having died of cancer, 3 of cerebral stroke and 2 of cardiac infarction.

The clinical characteristics of all patients are presented in Table 1. There were no differences in the demographic and clinical data from patients with AAA and patients who had undergone autopsy.

2.2 Immunofluorescence

To analyze the cellular distribution of PCSK9, we performed immunofluorescence staining of the excised tissues using a rabbit anti-NARC-1 polyclonal antibody (recognizing PCSK9, 1:100; Bioss Inc., Woburn, MA, USA) and a goat anti-LDLR polyclonal antibody (1:100; Santa Cruz Biotechnology Inc., Dallas, TX, USA). The cells were then stained with appropriate secondary antibodies and counterstained with 4,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei.

The expression of PCSK9 and cluster of differentiation 36 (CD36) in adipocytes was analyzed by staining the cells with goat anti-PCSK9 polyclonal antibody (1:100; Novus Biologicals, Littleton, CO, USA), mouse anti-CD36 monoclonal antibody (1:100; Novus Biologicals, Littleton, CO, USA), and rabbit anti-Perilipin polyclonal antibody (1:100; OriGene Technologies Inc., Rockville, MD, USA). The slides were then stained with appropriate secondary antibodies and counterstained with DAPI.

Histological images were analyzed using an inverted microscope (BZX-700, KEYENCE, Osaka, Japan) and fluorescence intensity was quantified using the Hybrid cell count BZ-H2C software (KEYENCE).

Table 1 Demographic and clinical data from 24 patients with AAA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \text{Sex} )</th>
<th>( \text{Age (years)} )</th>
<th>( \text{Complication} )</th>
<th>( \text{Ever smoker} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72.1 ± 7.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>2</td>
<td></td>
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<tr>
<td>CKD</td>
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<td></td>
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<tr>
<td>HL</td>
<td>3</td>
<td></td>
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</tr>
<tr>
<td>HT</td>
<td>9</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ever smoker</td>
<td>24</td>
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</table>

DM, diabetes mellitus CAD, coronary artery disease COPD, chronic obstructive pulmonary disease CKD, chronic kidney disease HL, hyperlipidemia, hypertension HT.

2.3 Statistical Analysis

The results were analyzed using the StatView software (version 5.0; SAS Institute, Cary, NC, USA) and expressed as the mean ± standard deviation (SD). Differences between groups were statistically evaluated using unpaired t-test; p values less than 0.05 were considered statistically significant.

3 RESULTS

3.1 Expression of PCSK9 in the aneurysm wall of patients with AAA

First, we investigated the expression of PCSK9 in the

![Fig. 1](image.png)  
**Fig. 1** Expression of PCSK9 in the aneurysm wall of patients with AAA. (A) Representative histology images showing PCSK9 expression (white spots) in control and aneurysm tissues. Scale bars = 200 μm. (B) Quantification of PCSK9 fluorescence intensity in control and aneurysm tissues. The results are presented as the mean ± SD; **p < 0.001 by unpaired t-test.
tissue samples and found that this protein was expressed in the aortic walls of patients with AAA, while it was hardly seen in control samples (Fig. 1A and B).

3.2 Distribution of PCSK9 in AAA wall

Next, we examined the details of the expression of PCSK9 in the aortic walls, and found that PCSK9 was expressed in various regions of the AAA walls, such as the intimal plaque region, media, and adventitia (Fig. 2). Histological examination showed that PCSK9 expression in atherosclerotic plaques and degenerative media was significantly increased in AAA samples, as expected. Interestingly, in AAA samples the expression of PCSK9 decreased in the adventitia region, which showed accumulation of hypertrophic adipocytes, thinning of collagen fibers, and an irregular collagen structure.

3.3 CD36 expression in hypertrophic adipocytes depends on PCSK9

We have recently shown that adipocytes abnormally accumulate in the human AAA wall, and the hypertrophy they cause is associated with AAA development. Therefore,
we investigated whether PCSK9 expression plays a role in the accumulation of hypertrophic adipocytes in the adventitia region. We analyzed the expression of PCSK9 in the adventitia, along with that of CD36, which is associated with fatty acid translocation and adipocyte metabolism.

In the adventitia of AAA samples, the fluorescence intensity associated with CD36 significantly increased, while that of PCSK9 decreased compared to control tissues (Fig. 3A and B). Specifically, we observed a negative correlation between PCSK9 and CD36 expression (Fig. 3C and D), suggesting that CD36 is regulated by PCSK9.

4 DISCUSSION

We have recently demonstrated that adipocytes abnormally accumulate in AAA walls\(^9,10\). Additionally, in studies on animal models, we have shown that hypertrophic adipocytes in the adventitia that undergoes rupture are significantly more abundant compared with those in the adventitia that does not undergo rupture\(^11\). These data indicate that the presence of hypertrophic adipocytes in the aortic wall is associated with AAA development. However, the mechanism underlying the accumulation of these adipocytes during AAA development has not been clarified\(^9,11\).

The primary role of the adipocytes is the storage of excess energy as triglycerides (TGs), and the supply of fatty acid and glycerol to other tissues. Adipocytes secrete various biologically active substances called adipocytokines, which are vital in glucose and lipid homeostasis\(^9\). Excess fat accumulation in the adipose tissue causes the aberrant production of adipocytokines, and is a risk factor for cardiovascular diseases. Importantly, hypertrophic adipocytes are associated with AAA pathogenesis\(^9\-\(^11\).

Among the factors relevant for adipocyte metabolism, CD36 plays an important role, participating in fatty acid uptake and in TG storage and secretion. CD36 is a multi-ligand cell surface receptor expressed in several cells and tissues, such as macrophages, heart, liver, and adipocytes (Fig. 2). CD36 contributes to muscle lipid utilization, adipose energy storage, and fat absorption in tissues with important lipid fluxes by binding long-chain fatty acids and facilitating their transport into the cells.

PCSK9 uniformly expressed in various tissues (Fig. 1). Therefore, the protein should play a great variety of roles in homeostasis of lipid metabolism. We have previously demonstrated that PCSK9 expressed by vascular smooth muscular cells in the aortic wall is related to the development of acute aortic dissection (AAD), implicating PCSK9 in the loss of structural integrity of the aorta and subsequent imbalanced vasoconstriction. Current findings based on lipid molecular analysis have made a major contribution to the understanding of the pathogenesis of cardiovascular diseases\(^12,13\). Recently, the inhibition of PCSK9 through monoclonal antibodies has been indicated as a novel therapy for cardiovascular diseases\(^14\).

PCSK9 and CD36 are both involved in the complex regulation of lipid and adipocyte metabolism. Although PCSK9 exerts a direct effect on cholesterol homeostasis, recent findings demonstrate that PCSK9 directly interacts with, and decreases, endogenous cell surface CD36, thereby reducing internalization of fatty acids in adipocytes. CD36 expression plays a role in hypertrophic adipocytes accumulation in the aortic adventitia.

Fig. 4 Role of PCSK9 in AAA pathogenesis. (A) PCSK9 directly interacts with, and decreases, endogenous cell surface CD36, thereby reducing internalization of fatty acids in adipocytes. (B) CD36 expression plays a role in hypertrophic adipocytes accumulation in the aortic adventitia.
PCSK9 and CD36 (Fig. 3D), suggesting that PCSK9 regulates CD36 protein levels and function in adipocytes.30

In this study, PCSK9 expression decreased and CD36 expression increased in the adventitia region of aneurysms (Fig. 3). CD36 expression may excessively upregulate the transport of fatty acids into adipocytes, and subsequently lead to the accumulation of hypertrophic adipocytes and cause degradation of the adventitia in the aortic wall, contributing to AAA rupture (Fig. 4). As of now, we do not know what causes the increased expression of PCSK9 in the aortic adventitia. It is possible that the accumulation of hypertrophic adipocytes in AAA walls induces the expression of PCSK9 and CD36. Therefore, though PCSK9 inhibitors are indicated as a novel treatment for cardiovascular diseases, they may not be useful for preventing AAA development or rupture. Additional studies are necessary to investigate the usefulness of PCSK9 in AAA treatment. Future assessments of lipid metabolism in aortic tissue may provide more information on the development of AAA and potentially allow for early pharmacological and/or nutritional intervention prior to the formation of an aortic aneurysm. There is at least one limitation in this study. Because AAA tissue samples from patients can only be obtained at the time of the surgical repair of the aneurysms, our findings reflect a single time point. The use of an animal model would enable investigation of the pathological processes involved in AAA over time. In future, we plan to investigate the role of PCSK9 in AAA pathogenesis using a hypoperfusion-induced AAA rat model, which can mimic the degeneration of the adventitia in human AAA.

5 CONCLUSION

Our findings indicate that the inverse correlation between PCSK9 and CD36 in hypertrophic adipocytes may be associated with AAA development.

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