学 位 論 文 の 要 旨

氏名　　長崎　雅幸

学 位 論 文 名	Apoptotic Cell Death, Elastin Loss, and Elastic Fiber Fragmentation Are Involved in the Pathogenesis of Medial Calcification in the Human Aorta
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著 者 名	Masayuki Nagasaki, Shozo Yano, Ken-ichi Matsumoto, Teiji Oda, Atsushi Nagai

論 文 内 容 の 要 旨

INTRODUCTION

It has been recognized that vascular calcification is associated with an increased risk of mortality and cardiovascular events such as heart failure, myocardial infarction, limb ischemia, and post-angioplasty dissection. The blood vessel wall is composed of three layers: the intima, the media, and the adventitia. The media consists of vascular smooth muscle cells (VSMCs) and elastic fibers. Blood vessel calcification is classified according to the site of calcification into atheromatous plaque calcification, localized to the intimal or subintimal area, and Mönckeberg type calcification in the medial layer of large arteries. Vascular calcification, especially medial calcification, is associated with an increased risk of cardiovascular events and mortality.

VSMCs play a pivotal role in the pathogenesis of aortic medial layer calcification. VSMCs undergo apoptosis due to ischemia or elevated stress, resulting in the production of numerous apoptotic bodies. The classical belief is that phosphate and calcium ions may be passively deposited around these apoptotic bodies to serve as nuclei of calcification. However, recent studies have established a mechanism whereby VSMCs undergo transformation into chondrogenic or bone-forming cells, actively taking up phosphate and calcium to generate calcification. Furthermore, fragmented elastin is involved in the calcification process in the aortic medial layer. Although these mechanisms are believed to be involved in the formation of

medial calcification, their existence in the human aorta has rarely been studied. Therefore, we conducted research with the aim of clarifying the pathological findings of medial calcification using human aortic specimens.

MATERIALS AND METHODS

This clinical study, designed as a single-center, retrospective, observational study, conformed to the ethical guidelines outlined in the Declaration of Helsinki. The study protocol was reviewed and approved by the Research Ethics Committee of Shimane University.

From 2009 to 2011, thoracic and/or abdominal aortic aneurysms were diagnosed at Shimane University Hospital. Subjects consisted of 6 cases of aortic aneurysm. All specimens contained calcified areas (CA), which were defined as areas containing visible calcification. Normal areas (NA) were defined as normal-looking and normal-feeling areas, as judged by a surgeon. Transitional areas (TA) were defined as areas located between CA and NA. For each case, an NA or TA was included as a control of CA. The staining was performed for the expression of elastic fiber (Elastica van Gieson: EVG), vascular smooth muscle actin (SMA), macrophages (CD68), osteoblast markers (Alkaline Phosphatase: ALP, Osteocalcin: OCN), and apoptosis (TdT-mediated dUTP nick end labeling: TUNEL). The staining intensity of each field of view was quantified according to standard scoring criteria, and the scores were compared between normal, calcified, and transitional areas of human aortic tissue sections. Each staining score (NA, CA, and TA) was analyzed using the Kruskal-Wallis test. Bonferroni/Dunn's test was used as a post-hoc test, and $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In the NA of human aortic tissue sections, the elastic fibers show an orderly arrangement, and elastin is highly expressed. In contrast, in the CA, the elastic fibers are ruptured and have a disordered arrangement with markedly reduced elastin expression compared to the NA and TA. However, no difference was found between the NA and TA. SMA results showed a similar tendency to EVG, although the difference did not reach statistical significance. On the other hand, significant levels of apoptotic cell death were observed in the CA and TA by TUNEL staining. CD68 was negative in the NA and TA, and the signal was very weak in the CA. In addition, ALP and OCN were not positively stained in any of the specimens.

In this study, a significant loss of elastin expression and fragmentation of elastic fibers were observed in the CA of the aortic wall, implying that these changes may be related to the late phase of the calcification process in the aortic medial layer. Since apoptosis was observed in both the CA and TA of the medial layer, we speculate that VSMC apoptotic cell death is probably related to the early stage of medial layer calcification. The findings of this study suggest that

apoptotic cell death, reduced elastin expression, and the fragmentation of the elastic fibers are involved in the pathogenesis of calcification in the human aortic medial layer.

According to our histopathological analysis, the elastic fibers are arranged in an orderly manner, and elastin is highly expressed in the normal area. In contrast, in the calcified area and transitional area of the aneurysm, the elastic fiber arrangement is disordered and ruptured, and the elastin expression is significantly decreased. Elastic fiber disruption leads to the loss of connectivity and contractility in aortic tissue, potentially contributing to the development of aneurysms and subsequent rupture. Regarding calcification, a study revealed an inverse correlation between calcium accumulation, as evaluated by CT imaging, and the expansion rate of infrarenal abdominal aortic aneurysms. These findings suggest that aortic calcification reinforces the fragility of the aneurysmal aortic wall. Recently, however, another study reported that the Agaston score, calculated as the degree of aortic calcification from a CT image, was associated with the progression of acute type A aortic dissection. Thus, the roles of aortic calcification remain uncertain, and further study is necessary to clarify the significance of aortic calcification.

Decreased expression of elastin may be associated with loss of VSMCs. Loss of VSMCs is one of the known characteristics of aortic aneurysms. In aortic aneurysms, inflammatory cell infiltration and enhanced degradation of the extracellular matrix have been observed at the transition from a normal to an aneurysmal state. In the maximum expansion of the aneurysm, smooth muscle cells decrease, and elastic fibers disappear. Our results were consistent with this, where decreased expression of elastin and SMA was observed from normal to calcified areas. Since apoptosis was observed not only in the calcified area but also in the transitional area, apoptotic cell death is most probably related to the early stage of medial calcification.

Since we could not detect osteoblast-specific molecules such as ALP and OCN, our data showed no evidence for the involvement of osteoblastic trans-differentiation of VSMCs in the calcification process of aortic aneurysm in this study. Our current findings suggest that VSMC apoptosis, rather than transdifferentiation, is involved in the development of human aortic calcification. However, as shown in a previous study, the expression of extracellular matrix proteins, including tenascin-X, is markedly altered in aortic calcification, suggesting a phenotypic change in VSMCs. Further investigation is necessary to elucidate the pathological process of calcification in the human aortic medial layer under various conditions.

CONCLUSION

Apoptotic cell death, elastin loss, and elastic fiber fragmentation may contribute to the pathogenesis of medial calcification in human aortic aneurysms.