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審査研究科	人間総合科学研究科
学位論文題目	THE ROLE OF EXTRACELLULAR MATRIX IN MECHANICAL STRESS-INDUCED AORTIC REMODELING AND AORTIC ANEURYSMS （メカニカルストレスによる大動脈のリモデリングと大動脈瘤発症における細胞外マトリクスの役割に関する研究）
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論文の内容の要旨
Abstract of thesis

（目的　Purpose）

The extracellular matrix (ECM) plays a critical role in the maintenance of the aortic wall and the fragmentation of elastic fibers is frequently associated with aortic diseases, including aortic aneurysms.
The first part of the thesis is to directly address whether a decrease in quantity or quality of elastic fibers *in vivo* predispose the aorta to mechanical stress-induced remodeling and aneurysm formation using various mutant mice with the compromised elastic fibers. The second part of the thesis is to search the molecular markers of different subtypes of aortic aneurysms in humans, focusing on the ECM, signaling molecules, and smooth muscle cell markers. The author performed histological and molecular characterization of thoracic aortic aneurysm (TAA), including annuloaortic ectasia (AAE), ascending aortic aneurysm (aTAA) with and without bicuspid valve (BAV), and abdominal aortic aneurysm (AAA) from human patients.

（対象と方法　Materials and Methods）

Eight-week-old male wild type (C57BL/6), heterozygous for an *Eln*-null allele ($Eln^{+/-}$), heterozygous and homozygous for a *Fbln5*-null allele ($Fbln5^{+/-}$ and $Fbln5^{-/-}$, respectively), and homozygous for a *Thbs1*-null allele ($Thbs1^{-/-}$) were used to perform transverse aortic constriction (TAC) surgery to induce pressure overload in the

ascending aorta. After 5 weeks, ascending aorta were harvested for histological analysis. All protocol were approved by the Animal Experimentation Committee of University of Tsukuba.

The full-thickness specimens of human aneurysmal aortic tissues were collected in accordance with the research protocol approved by the Clinical Ethics Committee of University of Tsukuba Hospital (#27-217) with an informed consent paper from each patient for sample collection before operation. The 4-mm ascending aortic wall tissues were punched from patients undergoing coronary aortic bypass (CABG) surgery as control. Comparison among 5 groups, including AAE, aTAA with and without BAV, AAA and CABG, were performed by histology, Western blot and quantitative polymerase-chain reaction (qPCR) analysis.

（結果 Results）

The maximal ascending aortic diameter in systole was significantly smaller in $Eln^{+/-}$ and $Fbln5^{-/-}$ mice compared to wild-type (WT) mice. TAC induced dilatation of the ascending aorta in $Eln^{+/-}$ and $Fbln5^{-/-}$ mice, comparably to that of WT mice, however, TAC did not induce aneurysm or dissection in the mutants. Thbs1 was clearly upregulated in the ascending aorta of $Eln^{+/-}$ mice after TA. In contrast, $Thbs1^{-/-}$ mice with normal elastic fiber structure showed decreased survival after TAC (38%) compared with WT mice (100%), and 50% of death was due to aortic rupture (3 ruptures). Necropsy and histological analyses found evidence of aortic diseases (3/7 aneurysms, 2/7 dissection) with abnormal collagen deposition and elastic fiber fragmentation. Thb1 expression in TAA was significantly upregulated compared with control aortic samples.

Human aneurysm study revealed that the changes in the medial layers of AAA were consistent with atheroma, fragmentation and partial loss of elastic fibers, calcification b, intra-aortic wall hemorrhage, and collagen accumulation. Among TAA, aortic wall degradation was more severe in AAE than aTAA and the aortic root was more severe than the ascending aorta in Marfan syndrome (MFS) with *fibrillin-1 (FBN1*) mutations.

By qPCR analysis, *TGFB1* and *THBS1* were upregulated in aneurysm tissues and in the aortic wall of MFS. However, Western blots revealed that the THBS1 expression was significantly higher in AAA than TAA, and in aneurysmal lesions than non-aneurysmal lesions within the same TAA patients.

（考察 Discussion）

$Eln^{+/-}$ and $Fbln5^{-/-}$ mice showed narrowing of the aortic lumen during development. However, TAC did not induce aneurysm, suggesting pressure overload alone was not sufficient to initiate aneurysm formation in these mutants. It was suggested that tension is not the only determinant of the aortic wall remodeling and that alteration of biomechanical signaling in vascular SMCs may be required for full development of aneurysms.

Hbs1 is an adhesive glycoprotein that mediates cell-cell and cell-ECM interactions and Thbs1 is highly upregulated in the ascending aortas of TAC-treated wild-type mice. $Thbs1^{-/-}$ mice exhibited extracellular response of pressure overload and ascending aorta of $Thbs1^{-/-}$ mice exhibited loss of smooth muscle cells (SMCs), elastic fiber fragmentation, collagen accumulation and calcification. Since Thbs1 has recently been shown to contribute to the development of aortic aneurysms in mice, the author’s results indicate the opposite effect if Thbs1 in response to overload, which is protective against mechanical stress-induced maladaptative remodeling of the aorta. The lack of Thbs1 in vascular SMCs or fibroblasts may alter cell functions in response to the increased mechanical stress and induce maladaptive remodeling. It is possible that the presence of inflammation and fibroblast activation in TAC-induced vascular remodeling may require Thbs1 to control these changes by acting on different cell types.

The severe aortic wall degradation (in AAA and AAE) was associated with high inflammatory activity and elevated collagen accumulation, which may be related to the increased mechanical stress. It is of note that most aneurysm patients have hypertension for a long time. The medical treatments that target the mechanical stress reduce blood pressure, heart rate, and cardiac contractility. Angiotensin-converting enzyme inhibitor could limit inflammatory consequence and reduce proteolysis in the aortic wall, leading to decreased stiffness and greater collagen turnover. Angiotensin II receptor blockers also could inhibit the aortic wall remodeling process in response

to overload pressure in TAC mice model.

TGF-ß pathway was upregulated in both TAA and AAA, especially in MFS and aTAA with BAV. Tbbs1 was also elevated highest in AAA. By comparing the level of Thbs1 and TGF- ß1 among subtypes of aortic aneurysm and how they are related to the severity of aneurysms may help identifying the new therapeutic targets for human aortic aneurysms.

The author concludes that Thbs1 contributes to aortic aneurysms pathology but Thbs1 is protective in the mechanical stress-induced aortic wall remodeling and that further analysis is required to elucidate the role of Thbs1 as a potential therapeutic target for treating thoracic aortic aneurysms.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The author found an involvement of Thbs1 in the remodeling process of ECM of aortas after mechanical stress. Although Thb1 may be one of the molecules involved in the development of aneurysms, the author could not produce an aneurysmal mouse model with mutations of *Elastin* or *Fibulin5* The author could not identify the role of Thbs1 as positive, negative or dual, in the aneurysm formation.

The findings of the heterogeneous upregulation of genes among the aneurysm samples from patients with aneurysms provide an important data, which are useful to treat patients with arterial diseases.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on January 10, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.