論　文　概　要

○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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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 my thesis research is to directly address whether a decrease in quantity or quality of elastic fibers in vivo predisposes the aorta to mechanical stress-induced remodeling and aneurysm formation using various mutant mice with the compromised of elastic fibers.

The second part of my thesis research is to search for molecular markers of different subtypes of aortic aneurysms in humans, focusing on the ECM, signaling molecules, and smooth muscle cell markers. I 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 (Material and method):

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 aortic aorta. After 5 weeks, ascending aortas were harvested for histological analysis. All protocols were approved by the Animal Experimentation Committee of the 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 (approved number #H27-217) 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 artery bypass (CAD) surgery

as controls. Comparisons among 5 groups, including AAE, aTAA with and without BAV, AAA and CAD, were performed by histology, Western blot and qPCR analysis.

結果 (Result):

TAC induced dilatation of the ascending aorta in *Eln*$^{+/-}$ and *Fbln5*$^{-/-}$ mice, which was comparable to that of wild-type mice, however, TAC did not induce aneurysm or dissection in the mutants. In contrast, *Thbs1*$^{-/-}$ mice with normal elastic fiber structure showed decreased survival after TAC (38%) compared with wild-type mice (100%), and 50 % of death was due to aortic rupture (3 ruptures). Necropsy and histological analyses found evidence of aortic diseases (3/7 aneurysm, 2/7 dissection) with abnormal collagen deposition and elastic fiber fragmentation.

Human aneurysm study revealed that the changes in the medial layer of AAA were consistent with atheroma, fragmentation and partial loss of elastic fibers, calcification, 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 MFS.

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 that pressure overload alone was not sufficient to initiate aortic aneurysm formation in these mutants.

Thbs1 is an adhesive glycoprotein that mediates cell-cell and cell-extracellular matrix (ECM) interactions and Thbs1 is highly upregulated in the ascending aortas of TAC-treated wild-type mice. *Thbs1*$^{-/-}$ mice exhibited exacerbated response to pressure overload and the ascending aorta of *Thbs1*$^{-/-}$ mice exhibited loss of SMCs, elastic fiber fragmentation, collagen accumulation and calcification. Since Thbs1 has recently been shown to

contribute to the development of aortic aneurysms in mice, my present results indicate the opposite effect of Thbs1 in response to pressure overload, which is protective against mechanical stress-induced maladaptive remodeling of the aorta. The lack of Thbs1 in VSMCs or fibroblast 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-β pathway was upregulated in both TAA and AAA, especially in MFS and aTAA with BAV. THBS1 was also elevated highest in AAA. By comparing the level of THBS1 and TGF-β1 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 aneurysm

結論 (Conclusion):

Thbs1 contributes to aortic aneurysm pathology but Thbs1 is protective in the mechanical stress-induced aortic wall remodeling. Further analysis is required to elucidate the role of Thbs1 as a potential therapeutic target for treating thoracic aortic aneurysms.