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審査組織	グローバル教育院
学位論文題目	Identification of novel biomarker spectra specific for thoracic aortic aneurysms by Raman microspectroscopy combined with multivariate data analysis （ラマン分光法と多変量解析による胸部大動脈瘤に特異的なバイオマーカースペクトル探索に関する研究）

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論文の要旨
Abstract of thesis (Note: about 1000 words)

Thoracic aortic aneurysms (TAAs) are abnormal dilatations of the thoracic segments of the aorta and are often associated with life-threatening complications such as aortic rupture and dissection. The maximum aortic diameter is currently used as a criterion for elective surgical repair. However, a report shows that patients with only small dilatation could also develop acute dissection, indicating an unmet need for diagnostic tools that accurately evaluate the integrity of the aneurysmal wall.

General risk factors of aortic aneurysms are smoking, hypertension, inflammation, hyperlipidemia, aging and connective tissue disorders, all of which weaken the aortic wall. In case of connective tissue disorders, TAAs are observed in patients carrying mutations in the extracellular matrix (ECM) genes. In addition, mutations in genes involved in the generation of contractile forces in smooth muscle cells (SMCs) have been identified in familial TAAs and dissection. These mutations lead to weakening of the aortic wall,

reducing the contractility of SMCs, and activation of signaling pathways such as TGFβ and angiotensin II.

Among various ECM proteins in the aortic wall, fibulin-4 (FBLN4) and FBLN5 are associated with elastic fibers and are essential for elastic fiber formation in vivo. Mutations of *FBLN4* and *FBLN5* have been shown to cause autosomal recessive cutis laxa type I and some patients with *FBLN4* mutations developed ascending aortic aneurysms. The applicant's laboratory previously generated SMC-specific *Fbln4* knockout mice ($Fbln4^{SMKO}$) and showed that they develop aortic aneurysms that resemble human conditions. They also generated *Fbln5* knockout mice ($Fbln5^{KO}$) that develop aortic elongation and tortuosity. Lack of *Fbln4* in mice resulted in abnormal collagen maturation in addition to abnormal elastic fibers in the aorta, which could be attributable to the decreased LOX-mediated cross-linking of both elastin and collagen fibrils.

Raman microspectroscopy provides a marker-independent and non-destructive imaging method, which recently evolved in the biological and biomedical fields. Raman microspectroscopy uses the detection of molecular vibrations to distinguish various components of bulk tissues. It has been reported that ECM molecules such as elastin and collagen fibers have distinct Raman spectra.

The purpose of the applicant's study is to identify the signal differences associated with the aneurysm phenotype by Raman microspectroscopy combined with multivariate data analysis (MVA). The goal of this study is to build a platform for identification of the Raman signals of TAAs by MVA, which will lead to an efficient diagnose of the various stages and types of TAAs in patients. The applicant performed Raman microspectroscopy to profile murine and human TAA tissues without staining and identify the molecular features combined to MVA with the spectral data sets. For tissues, the applicant prepared murine and human aortic tissues separated between ascending and descending aortas. After measuring of the aortic tissues, MVA was performed, including true component analysis (TCA), principal component analysis (PCA), and multivariate curve resolution (MCR).

By TCA, the applicant generated Raman images and compared with those by routine histology and immunostaining. PCA was employed to detect biochemical differences in murine and human TAAs in comparison to control samples. The applicant implemented MCR analysis to decompose Raman spectra for identifying TAA-specific Raman signals. By using Raman imaging of the aorta with MVA, the applicant successfully extracted spectral components related to the ECM, including elastic fibers, collagen fibers, proteoglycans, as well as lipids and cellular components in murine and human aortic tissues. In $Fbln4^{SMKO}$, the applicant found that collagen and aggrecan were much more intense compared with WT and $Fbln5^{KO}$. Remarkably, these images were comparable to the images obtained by immunohistochemistry.

The applicant carried out PCA on elastin and collagen fibers and detected a separation between control and TAAs in both murine and human tissues. The applicant then performed decomposition of the spectra by MCR focusing on elastin and collagen fibers, and identified specific Raman spectra in the aneurysm-related murine ascending and human aortic tissues. In elastic fibers, the component 1 (C1) appears to be derived from abnormal proteins associated with aneurysmal lesions, which is detectable only in aTAA and $Fbln4^{SMKO}$. Although the comparison of Raman spectra and accurate assignment to authentic proteins are needed, 1613 cm^{-1} band in C1 could be a characteristic marker for an aneurysm,

since the band doesn't overlap with amide I or phenylalanine. Finally, the applicant performed MCR on collagen fibers and found that the component C6 (C6) was specific to the aneurysm lesions. C6 spectrum appears to be amino acid residues, including phenyl alanine, tyrosine, tryptophan, cysteine, aspartic and glutamic acid, and was detected in mice and humans and increased in TAA lesions. The applicant thus proposed that C6 may serve as a diagnostic Raman marker derived from the aneurysm lesions.

審査の要旨
Abstract of assessment result (Note: about 150 words)

【批評 Review】

Recently, several applications for marker-independent Raman imaging have been reported; however, the imaging itself does not provide sufficient information to identify Raman marker spectra associated with cardiovascular diseases. Combining with spectral decomposition methods, Raman techniques have the potential to extract target signals from big data sets. By increasing the components of Raman spectra and improving the resolution, Raman imaging can evaluate the vessel wall integrity and biochemical components of the diseased vessel wall. The applicant gives light on the potential for the novel label-independent diagnosis by Raman microspectroscopy and allows for the detection of molecular alterations based on spectral information in both murine and human TAAs. Furthermore, a combination of Raman measurements and artificial intelligence to identify molecular tissue patterns specific to (pre-)aneurysm will be a robust diagnostic tool for monitoring patients with a high risk for aneurysm rupture/dissection.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 30 March, 2020. 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】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.