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審査組織	グローバル教育院
学位論文題目	Establishment of a Novel iRFP-Incorporated *in vivo* Murine Atherosclerosis Imaging System （生体内での動脈硬化を可視化する新技術の開発）

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Abstract of thesis

Methods: Her key technology includes the murine atherosclerosis imaging system targets the transgenic macrophages expressing Near Infrared Fluorescent Protein **(**iRFP- excitation 690 nm / emission 713 nm**)** in the atheroma, which has not been published before in atherosclerosis imaging. LDL receptor-deficient mice, *LDLR*$^{-/-}$ is a well used hypercholesterolemic model for atherosclerosis by feeding high cholesterol diet (HCD). *LDLR*$^{-/-}$ were reconstituted with bone marrow cells from beta-actin promoter derived iRFP transgenic mice (*iRFP→LDLR*$^{-/-}$) so that monocytes or macrophages in atherosclerotic region would be labeled with iRFP and used throughout the experiment. *iRFP→LDLR*$^{-/-}$ mice fed a normal diet (ND) and *LDLR*$^{-/-}$ mice transplanted with wild-type (WT) BM cells were used as controls. Atherosclerosis was induced by special non-fluorescent HCD. Atherosclerosis was compared among the three differently induced mouse groups; only ND fed ND group, ND and HCD alternatively fed HCD/ND group, and only HCD group. The live *in vivo* imaging system IVIS was used as the imaging device. All the IVIS images were acquired with excitation/emission wavelengths of 625/720 nm and 710/760 nm and with an exposure time of 1 second. *In vivo, ex vivo* IVIS® imaging were carried out every 2 weeks to visualize the plaque progression. Oil Red O staining, immuno-histochemical analysis and FACS analysis were employed for conformation. After 8 weeks, differently HCD fed three mice groups were imaged for atherosclerotic lesions. Then, the mice were

sacrificed, and *ex vivo* imaging of the aortas was performed, followed by ORO staining. ORO staining of the aortas and aortic valves showed that the different HCD feeding patterns induced different extents of atherosclerosis in each group, and the observed *in vivo* iRFP signal areas demonstrated a signal distribution corresponding to the actual plaque-positive areas. Notably, a significant correlation was observed between the iRFP *in vivo* signal area and the *ex vivo* ORO-positive plaque area of the aortas. It was suggested that iRFP-based imaging system can be used to visualize macrophage-rich plaque burden *in vivo.*

IVIS images were acquired every two weeks to observe the thoracic signal in the three mice groups. From the fourth week of induction, thoracic signals were observed in the HCD/ND and HCD groups. The thoracic iRFP signal progression was clearly observed in the same mice at week 6 and week 8 after induction. Overall, these results indicate that established imaging system could clearly capture the time course of disease progression in individual mice in a relatively straightforward fashion. This system also allows differences in plaque burden to be quantified among mice in the same group.

Conclusion: Established iRFP incorporated new murine atherosclerosis imaging system is able to noninvasively image atheroma positive areas in thoracic aorta and to generate longitudinal data of thoracic atherosclerosis lesion progression, validating the ability of the system to be effectively used in atherosclerosis disease progression and drug development studies.

Abstract of assessment result

【Review】

This iRFP incorporated new murine atherosclerosis imaging system she established is a novel and fantastic technology to visualize atherosclerosis. Atherosclerosis has been studied using many murine models for decades, but analytical procedure was so troublesome and difficult. This astonishing cutting edge methodology will be a tremendous help for atherosclerosis researchers. It can be expandable to clinics and potentially other infiltrative disorders. The project consists of establishment of in vitro methodology especially about strength and quantitativeness of the iRFP signal and in vivo application to atherosclerosis experiments. The study was carefully organized, well done step by step, obtained the beautiful linearity, feasibility and effectiveness, deserving a mile stone-class of atherosclerosis research. Usage of macrophage specific promoter for iRFP gene or photoacoustic system was discussed as future experiments, and she very well responded to questions. The signal from macrophage in the entire atherosclerotic lesion might limit significance of information whereas it might help understanding of new aspects such as apoptosis, efferocytosis, or xanthoma.

【Result】

The final examination committee conducted a meeting as a final examination on 23 01, 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.

【Conclusion】

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