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学位論文題目	Contribution of PDGFRα-positive cells in maintenance and injury responses in mouse large vessels （マウス大血管の恒常性維持と損傷反応におけるPDGFRα 陽性細胞の関与について）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, the author described the contribution of PDGFR alpha (PDGFR*a*)-positive cells in maintenance and injury responses in mouse large vessels. The content is summarized as follows:

（目的　Purpose）

Vascular diseases such as atherosclerosis and restenosis is proliferative maladaptive remodeling of the vessel wall, or more specifically, neointima formation.

The study of neointima in vascular remodeling has long been considered an important key to understand vascular diseases. Recent studies arguing that stem cells antigen-1-positive (Sca1+) or glioma-associated oncogene homolog 1-positive (Gli+) progenitor cells in the adventitia are important for proliferative changes in adult vessels. Therefore, it is necessary to explore the cellular dynamics involving adventitial progenitors and their contribution to vascular injury.

（対象と方法　Materials and Methods）

In this study, the author utilized a CreER mouse line that preferentially labels adventitial PDGFR*a*+ cells and examined cellular behavior during homeostasis and injury response in large arteries. Carotid artery ligation and wire

injury of the carotid artery were conducted as previously described with modification.

After draining the blood, wire guide (0.38 mm/0.15 inch fixed core) was inserted in the incision and moved back and forth 10 times into the main carotid. After wire insertion, the main carotid clamp was released to allow the blood flow to flush out the vascular wall cell debris and the incision at the external carotid artery (ECA) was closed by ligating the ECA incision after the bifurcation time point.

Tissues were collected at each time point after wire injury surgery, and serial sections were prepared for further analysis of distribution of vascular progenitor cells. The sections were stained with several kinds of antibodies (anti-*a*SMA, -PDGFR*a*, -CD31, -CD34, -Sca-1, -VE-cadherein, -CD68 antibodies) and analyzed under a fluorescence microscopy.

（結果 Results）

Firstly, the author found that PDGFR*a* was strongly expressed in the adventitia in the aorta and no expression was detected in the medial or intima layer. And the author treated PDGFR*a*-CreER; R26tdTomato mice with tamoxifen and harvested the carotid artery and ascending aorta. The author found that tdTomato+ cells in the adventitia layer was Sca1 positive and CD34 positive at 10-day after tamoxifen administration. And the author found that tdTomato+ cells in the media express SMC markers (*a*SMA and SM-MHC), but not endothelial cell marker CD31. Similarly, the author detected tdTomato+; Sca1+; CD34+ cells in the adventitia and tdTomato+; *a*SMA+ and tdTomato+; SM-MHC+ SMCs in medial layers of the ascending aorta, but not CD31 at 10-day-chase, suggesting that PDGFR*a*+ labeled adventitial progenitor cells and subsets of differentiated SMCs is distinct each other. The author thought that PDGFR*a*+ adventitial cells may not differentiate into SMCs within a short period time, therefore the author investigated the long-term survival of PDGFR*a*-labeled cells and their progeny. The author found that adventitial PDGFR*a*+ cells were still positive for stem cell markers, Sca1 and CD34, and they also existed at a portion of medial SMCs that were maintained for 2 years, suggesting PDGFR*a*+ cells might be involved in homeostasis of adventitial progenitor cells and SMCs in the vessel wall.

Secondly, the author employed two types of neointima in the carotid artery: a complete ligation which induces neointima due flow cessation and resultant changes in flow share stress, and wire injury for a more severe response. Upon carotid artery ligation, the author showed PDGFR*a*+ cells were slowly recruited to neointima and exhibited an immature SMC phenotype at 56 days after injury. In contrast, in a more severe wire denudation-injury, the author showed that PDGFR*a*+ cells were recruited to neointima within 14 days and fully differentiated into SM-MHC-positive SMCs.

Thirdly, the author performed transverse aortic constriction (TAC) injury to induce aortic wall thickening by pressure overload. At 28 days after TAC, the author found markedly thickened adventitia of the ascending aorta, and abundant tdTomato+ cells in the adventitia, indicating adventitia hyperplasia. To further analyze the aortic remodeling with TAC, the author performed immunostaining and found that tdTomato+ cells in the adventitia were positive for Sca1, and the cells at the boundary between adventitia and media strongly expressed Sca1. In addition, the author found tdTomato+ cells in the adventitia were *a*SMA+ but not SM-MHC+, suggesting that these cells appear to be activated fibroblasts derived from PDGFR*a*+ cells. Upon pressure overload, the author also found PDGFR*a*+ cells developed adventitial fibrosis, suggesting a role for PDGFR*a*+ cells as a reservoir of adventitial cells and a subset of medial SMCs.

（考察　Discussion）

The author has shown that PDGFR*a*-Cre labels adventitial progenitors and SMCs in large arteries, context-dependent contribution of PDGFR*a*+ cells in injury responses, and PDGFR*a*+ cells respond to pressure overload and develop adventitial fibrosis. FACS analysis would be useful for further characterization of four distinct populations in the neointima: Sca1+/*a*SMA+/tdTomato+, Sca1-/*a*SMA+/tdTomato+, Sca1-/*a*SMA-/tdTomato+, and Sca1+/*a*SMA-/tdTomato+ cells. This study underscores heterogeneity of adventitial progenitors and their differential response and mobilization upon injuries. Further injury time points and molecular characterization of PDGFR*a*+, Sca1+ or Gli1+ derived cells will be crucial to understand a homeostasis of vascular cells in detail.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The author clarified the importance of adventitial progenitors utilizing a CreER mouse line that preferentially labels adventitial PDGFR alpha (PDGFR*a*)+ cells in large arteries using a couple types of injured mouse models.

It is highly appreciated that precise PDGFR*a*+ cell expression-chase study made it possible to analyze how the neointima forms in the normal state more than 2 years old as well as in the state with vascular dysfunction.

Overall, it can be said that the author made a great contribution in understanding dynamics of vascular cells, especially PDGFR*a*+ cells as reservoir of adventitial cells during homeostasis and injury responses.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on May 14, 2021. 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 Human Biology.