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審査研究科	人間総合科学研究科
学位論文題目	MAIR-II deficiency ameliorates cardiac dysfunction post-myocardial infarction by suppressing TLR9-mediated pro-inflammatory macrophage activation（MAIR-II 欠損は TLR9 による炎症性マクロファージの活性化を抑制し、心筋梗塞後の心機能障害を改善する）
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Saori Yonebayashi describes the role of myeloid-associated immunoglobulin-like receptor II (MAIR-II) for the activation of pro-inflammatory macrophages in a murine model of post-myocardial infarction (post-MI), and beneficial effects of MAIR-II deficiency on post-MI cardiac outcome. The summary is as follows:

In Chapter 1, the author provides an extensive literature review in relation to the inflammatory response in post-MI cardiac dysfunction. Previous studies showed that mitochondrial DNA (mtDNA), released both intracellularly and extracellularly after MI, stimulates toll-like receptor 9 (TLR9), and leads to NF-κB activation and transcriptional activation of pro-inflammatory cytokines. However, the role of TLR9 has been controversial among studies. The author also emphasizes that the coordinated regulation of pro-inflammatory (M1) and anti-inflammatory (M2) macrophages is crucial for healing and cardiac remodeling, but its underlying mechanism is not fully understood.

To gain more insight into these issues, the author focuses on MAIR family. MAIR family is a group of paired activating and inhibitory receptors that consists of 9 genes in mice and 8 in humans (CD300 family), expressed on a variety of myeloid-lineage cells. MAIR-II (CD300c2) is one of the activating MAIR family members, expressed on macrophages and B cells, and associates with DAP12 and FcRγ to transmit activation signals. The role of MAIR-II in infection and pulmonary fibrosis has previously been reported, but none on MI.

In Chapter 2, the author states that the main purpose of this study is to identify a novel role of MAIR-II in post-MI inflammation. Specifically, the author made an attempt to determine how MAIR-II positive cells respond post-MI as well as the effects of MAIR-II *in vivo*, using WT and MAIR-II deficient mice. The author also aimed to elucidate MAIR-II's molecular mechanism in macrophages *in vitro*.

In Chapter 3, the author describes the methods. The author utilized MI mouse model generated by permanent left coronary artery ligation. Post-MI survival was compared between wild type (WT) and MAIR-II deficient (*Cd300c2*$^{-/-}$) mice, using Kaplan-Meier survival curve analysis. Cardiac remodeling was evaluated by echocardiography, and infarct size was determined by Masson trichrome staining of the tissue sections. Expression level of MAIR-II and cytokine genes was examined by quantitative PCR (q-PCR), and expression of MAIR-II and macrophage markers on the cell surface was tested by flow cytometry. To elucidate molecular mechanism of MAIR-II, pro-inflammatory and anti-inflammatory cytokines were measured in bone marrow-derived macrophages (BMDMs) prepared from MAIR-II deficient or WT mice, after stimulation by a TLR9 agonistic ligand CpG-oligodeoxynucleotide (ODN)1668 or a simultaneous stimulation by ODN1668 and a TLR9 antagonist ODN2088.

Chapter 4 describes the results. The author observed infiltration of MAIR-II^{+}CD11b^{+} myeloid cells into the infarcted myocardium, which peaked at days 3 to 5 after MI. When post-MI survival was compared, MAIR-II deficient mice had a significantly higher survival rate up to 14 days following MI when compared with WT mice. Moreover, the size of infarct was smaller, and cardiac function was better in the MAIR-II deficient mice.

Next, the author examined the effect of MAIR-II deficiency on post-MI macrophage infiltration. In MAIR-II deficient mice, infiltration of pro-inflammatory macrophages was significantly decreased, while that of anti-inflammatory macrophages was significantly increased, on day 3. On the other hand, significant effect on the infiltration of neutrophils and hematopoietic stem cells were not observed. In the heart homogenates from MAIR-II deficient mice, mRNA levels of pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, CCL3 and CXCL, and fibrotic markers such as TGFβ and collagen type I, were significantly reduced.

Finally, to address the molecular mechanisms behind the beneficial effects of MAIR-II deficiency on post-MI cardiac function, the author focused on TLR9 and its ligand, mtDNA. In the mice that underwent MI operation, elevated levels of circulating mtDNA were detected when compared with those underwent sham operation. The author then tested the TLR9 response against its agonist ODN1668 *in vitro*, using BMDMs from MAIR-II deficient or WT mice. Lower mRNA and/or protein expression of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and CCL3, as well as higher expression of anti-inflammatory cytokine IL-10, was observed in MAIR-II deficient BMDM, when compared with WT BMDM. When TLR9 signal was inhibited by treating the BMDMs with TLR9 antagonist ODN2088 simultaneously with ODN1668, expression of these cytokines/chemokines were comparable between both BMDMs,

In Chapter 5, the author discusses that this study revealed a novel role and mechanism of MAIR-II in MI. When MAIR-II is deficient, post-MI inflammation is attenuated and cardiac remodeling is alleviated, which result in longer survival, smaller infarct size, and improved cardiac function. In relation to its potential mechanism, the author identified that MAIR-II increases pro-inflammatory cytokine and chemokine production via CpG-ODN-TLR9-mediated macrophage activation. Based on these findings, the author discusses that macrophage activation via MAIR-II and TLR9 may cause excessive post-MI inflammation, thereby increasing cardiac necrosis and enlarged infarct

size. In view of these findings, the author suggests that decreasing the MAIR-II levels in the early stages of post-MI may prevent worse cardiac consequences over time, as a prospective molecular target for MI treatment. On the other hand, the author notes that some of the previous studies reported protective effect of TLR9, and because the direct molecular mechanism of MAIR-II in TLR9-mediated signaling in MI remains unclear, further investigations are required.

In Chapter 6, the author concludes that this study revealed a novel role and mechanism of MAIR-II in MI. Following MI, MAIR-II enhances TLR9-mediated signaling of pro-inflammatory cytokine production, which leads to adverse cardiac remodeling and poor prognosis

In Chapter 7, the author describes future prospects, suggesting that MAIR-II is a promising molecular target for future MI therapy. At the same time, the author also notes that that because a variety of cells are involved in post-MI inflammatory processes, the roles of cells other than macrophages and other MAIR family members require further investigations.

審査の結果の要旨

Abstract of assessment result（Note: about 150 words）

（**批評　General Comments**）

In this dissertation, the author investigated the role of MAIR-II in the inflammation and cardiac remodeling after myocardial infarction (MI), using MAIR-II deficient mice. The author reported that MAIR-II deficiency resulted in suppression of post-MI inflammation and better cardiac outcomes. Furthermore, by focusing on macrophages and TLR9, the author proposed a fascinating molecular mechanism that MAIR-II is required to enhance TLR9 signals to induce proinflammatory cytokine production in macrophages.

This study tested a unique and original hypothesis, the experimental processes are sound, and the findings are clear. The interpretation of the results and future prospects are appropriately discussed. In addition, the author provided an extensive literature review in Chapter 1, which is informative and insightful.

In the future, it would be interesting to test whether the significance of MAIR-II and TLR9 interaction can be generalized to other conditions, for example murine lupus, where the importance of type I interferon signals caused by nucleic acids from dying cells is strongly implicated.

In conclusion, this study adds novel information on MAIR family biology as well as the pathogenesis of post-MI inflammation, and is highly evaluated.

（**最終試験の結果　Assessment**）

The final examination committee conducted a meeting as a final examination on December 22, 2020. The applicant provided an overview of dissertation, and 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.