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学位論文題目	DNAM-1 expressing inflammatory monocytes in the acute kidney injury （急性腎障害における炎症性単球上の DNAM-1 の役割）

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論文の要旨
Abstract of thesis

In this doctoral dissertation, Vo Van Anh describes the role of DNAM-1 expressed on inflammatory monocytes in the exacerbation of acute kidney injury. The summary is as follows:

Purpose

Acute kidney injury (AKI) is a life-threatening syndrome, commonly seen in hospitalized patients. AKI could result in death or chronic complications even after recovery. Inflammation following the initial injury exaggerates the disease progression, in which myeloid cells such as monocytes and neutrophils, play critical causative roles. Thus, targeting myeloid cell activation and migration gives a protective effect in AKI. DNAM-1 is a co-stimulatory immunoreceptor, expressed on human and mice NK cells, $CD4^+$ T cells, $CD8^+$ T cells, subsets of macrophages, monocytes and B cells. Initial studies by Akira Shibuya showed that DNAM-1 activates the cell-mediated cytotoxicity and cytokines production of NK cells and $CD8^+$ T cells. However, its contribution to myeloid cells, including monocytes, are not fully understood. Therefore, this study aimed to elucidate the following points; first, expression pattern of DNAM-1 on myeloid cells in human and mice peripheral blood in comparison with that in the spleen, which was already reported; second, the function of DNAM-1 expressing on myeloid cells; third, involvement

of DNAM-1 in acute kidney injury, the disease condition wherein myeloid cells play the key roles. The applicant also intended to research the mechanism by which DNAM-1 performs its disease regulation.

Materials and methods

DNAM-1 expression on immune cells was analyzed by flow cytometry. First, blood from healthy donors was used to isolate human immune cells, while mice blood and spleens were used to prepare murine cells. Then, isolated cells were fluorescently labeled using antibodies specific for differential cell surface markers and DNAM-1. Therefore, DNAM-1 expression on different populations of cells could be acquired. The physiological function of DNAM-1 in inflammatory monocytes was examined using an *in vitro* adherence assay. 5(6)-Carboxyfluorescein *N*-hydroxysuccinimidyl ester (CFSE)-labeled inflammatory monocytes were incubated with CD155 – the stress-induced ligand of DNAM-1 – transfected cells. After washing, remaining CFSE positive adhered cells were quantified using Keyence microscopy. A murine model of unilateral warm ischemia-reperfusion injury (IRI) resembles AKI in human. It was made to explore the *in vivo* physiological function of DNAM-1. In brief, mice were anesthetized, and their right kidneys were cut-off while the left kidneys were clamped to induce ischemia. After a particular time, releasing clamps initiated the reperfusion of blood, and kidney injury progressed. The renal dysfunction was measured by plasma creatinine and blood urine nitrogen. To investigate whether DNAM-1 promoted AKI via regulating monocytes migration from blood to tissue, several strategies were used. First, monocytes accumulated in kidneys after IRI were quantified by flow cytometry. Next, inflammatory cytokines produced by sorted monocytes from organs were measured by quantitative PCR. The systemic cytokines increased after IRI was detected in the plasma by cytometric bead array.

Results

Firstly, this study comprehensively investigated the DNAM-1 expression pattern in peripheral blood cells, in comparison with that of the previously reported splenocytes. It revealed that the expression of DNAM-1 is selective on the inflammatory monocytes but not patrolling monocytes; which was conserved in both human and mouse, suggesting DNAM-1 is an evolutionary molecule in inflammatory monocytes. Neutrophils and eosinophils also express DNAM-1 at a moderate level, which was not seen in splenocytes; however, lymphoid cells express DNAM-1 at the level as much as observed in spleen, suggesting cell type-specific and tissue-specific characteristics of DNAM-1 expression. DNAM-1 deficient (CD226-/-) inflammatory monocytes has attenuated ability to adhere to CD155, DNAM-1 ligand, expressed on transfected cells *in vitro* compared to wild type (WT) cells. Supportively, inhibiting DNAM-1 and CD155 interaction by monoclonal antibodies also lowered the adhered cell number in the adherence assay compared with isotype control. These data suggested that DNAM-1 – CD155 interaction is required for inflammatory monocytes mobility characteristics, particularly the adhesion behavior. DNAM-1 deficient mice showed milder renal dysfunction induced by IRI in comparison with WT mice, indicating DNAM-1 exaggerated acute kidney injury. Furthermore, the accumulation of immune cells following ischemia-reperfusion including monocytes and neutrophils were reduced in CD226-/- mice, suggesting that DNAM-1 promoted inflammation in AKI.

Discussion

Although DNAM-1 expression on lymphoid cells was consistent between organs, it was diverse in myeloid cells, depending on cell types and tissue specificity. Myeloid cells are highly heterogeneous with multiple subsets in an organ. So, DNAM-1 could be one of those molecules which determine expression patterns and the physiological distinction between subgroups, to be specific in monocytes sub-populations in this study. It is of interest to identify which master regulator determines DNAM-1 expression, developmental window and functional requirement of DNAM-1 on myeloid sub-types. DNAM-1 function on monocytes' migration was reported in human cells *in vitro*, but this was the first to describe DNAM-1 involvement in mouse monocytes' adherence, which is corroborating with previous data of human cells, suggesting DNAM-1 knockout mice as a useful tool to investigate physiological function of DNAM-1 in murine disease models. Even though CD155 transfected cells were used for the functional *in vitro* assay, primary cells, including endothelial cells, also express CD155. Also, the expression was reported to upregulate in stress-induced conditions, including tissue damage. Therefore, DNAM-1 expressing inflammatory monocytes might interact with CD155 expressing on endothelial cells for the trans-endothelial extravasation after IRI induction in this study. There exists a correlation between attenuated kidney injury and the decrease of myeloid cells percentage and number in the injured site. Whether DNAM-1 promoted IRI induced inflammation by directly regulating myeloid cells migration is unclear, despite the dependency of monocytes adhesion on DNAM-1 *in vitro*. Therefore, other possibilities should be examined such as DNAM-1 regulated inflammatory cytokines production or DNAM-1 indirectly controlling myeloid cell migration via different cell types. Though this study showed that DNAM-1 possesses a pathogenic effect in the acute phase of kidney injury, it is unknown what would happen at the late stage of this model. For instance, whether chronic kidney inflammation such as fibrosis, could be altered by DNAM-1 deficiency. Also, it is of interest to investigate DNAM-1 involvement in other ischemia-related diseases such as brain stroke and heart attack.

審査の要旨
Abstract of assessment result

【批評 Review】

In this thesis research, Vo Van Anh found the expression of DNAM-1 on inflammatory monocytes and showed that DNAM-1 knockout mice have milder acute kidney injury indicating the exacerbating role of DNAM-1 in acute kidney injury. Research design is interesting and feasible, data are clear and convincing, discussion and future plans are reasonable and the manuscript was written in an appropriate style and quality.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 03/06/2019. 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.

【結論 Conclusion】

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