氏　　名	VUONG CAT KHANH
学位の種類	博士（医学）
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審査研究科	人間総合科学研究科
学位論文題目	Functional analysis of human adipose tissue-derived mesenchymal stem cells isolated from chronic renal failure patients involved in wound healing（慢性腎不全患者脂肪組織由来間葉系幹細胞の創傷治癒における機能解析）
主　　査	筑波大学教授　医学博士　高橋　智
副　　査	筑波大学教授　博士（医学）　山縣　邦弘
副　　査	筑波大学准教授　博士（医学）　小原　直
副　　査	筑波大学准教授　博士（医学）　上杉　憲子

論文の内容の要旨
Abstract of thesis

【背景・目的　Background/Purpose】

Chronic renal failure (CRF) results in a delay in wound healing because of its complications such as uremia, anemia, and fluid overload. Mesenchymal stem cells (MSCs), which can be isolated from adipose tissues, an abundant source in the body, are considered to be a candidate for wound healing because of the ability to recruit many types of cells. However, it is still unclear whether the MSCs derived from early stage CRF patients (CRF-AT-MSCs) have the same function in wound healing as healthy donor-derived MSCs (nAT-MSCs). In the present study, The applicant analyzed the influence of uremic toxins on the function of MSCs derived from adipose tissue (AT-MSCs) in wound healing. From the *in vitro* influence of uremic toxins, the applicant continued to evaluate the wound healing function of AT-MSCs isolated from CRF patients, which are affected by uremic toxins *in vivo*.

【対象と方法　Materials and methods】

Firstly, the applicant isolated MSCs from adipose tissues of healthy donors (nAT-MSCs) and examined the function under the treatment of uremic toxins in normoxic and hypoxic conditions. The expression of reactive oxygen species (ROS) were measured by staining AT-MSCs with 2',7'-dichlorodihydrofluorescein diacetate (H2-DCFDA). The gene and protein levels were measured by quantitative reverse transcription polymerase (qRT-PCR) and Western blot. Then, AT-MSCs from early stage CRF patients (CRF-AT-MSCs) were characterized by morphology, growth

curve, fluorescence-activated cell sorting (FACS) and differentiation assay. Inhibition of ROS was performed by treatment with N-acetyl-L-cysteine (NAC) and inhibition of prolyl-hydroxylase domain protein 2 (PHD-2) was carried out by treatment with IOX2 and small hairpin RNA target PHD-2 (shPHD-2). The flap mouse model was used as in vivo wound healing assays.

【結果・考察　Results】

The applicant found that uremic toxins induced elevated ROS expression in nAT-MSCs, resulting in the reduced expression of HIF-1 α under hypoxic conditions. The impaired hypoxic induction of nAT-MSCs under the uremic conditions led to the dysfunction of nAT-MSCs in wound healing. Consistent with the uremic-treated AT-MSCs, CRF-AT-MSCs in mid-stage CRF showed a definite imbalance of redox state and high expression of ROS. Of note, the elevated ROS expression in CRF -AT-MSCs induced prolyl hydroxylase 2 (PHD-2) expression, which caused the inhibition of HIF-1 α expression. In addition, a transplantation study clearly revealed that nAT-MSCs promoted the recruitment of inflammatory cells and recovery from ischemia in the mouse flap model, whereas CRF-AT-MSCs had defective functions and the wound healing process was delayed.

審査の結果の要旨
Abstract of assessment result

【批評　General Comments】

In the present study, the applicant found that ROS-PHD-2 pathway was involved in the impaired wound healing function of CRF-AT-MSCs. Therefore, this pathway may be considered as a therapeutic candidate to modify the function of CRF-MSCs before using in clinical treatment for wound healing delay complication. Pretreatment CRF-AT-MSCs with ROS or PHD-2 inhibitor or the combination could be effective to improve the wound healing activity of AT-MSCs. However, further studies should be continued to clarify the underlying mechanism before clinical application.

The present study provides critical information for developing a therapeutic strategy in AT-MSCs therapy in CRF. The applicant indicates that MSCs from CRF patients need modifying in order to inhibit the expression of ROS-PHD2 pathway before they can be applied in the clinical setting. Special attention and further studies should be paid to the pathological stage of CRF and underlying mechanism to apply for the transplantation.

【最終試験の結果　Assessment】

The final examination committee conducted a meeting as a final examination on June 12, 2017. 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.

【結果　Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.