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学位論文題目	Glucocorticoids Impair the Tissue Regenerative Function of Mesenchymal Stem Cells (ステロイド治療は間葉系幹細胞の組織修復能を低下させる)

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論文の要旨
Abstract of thesis

Glucocorticoids are widely used as one of popular anti-inflammatory drugs, whereas it is shown that the strong immunomodulatory action causes undesirable effects including osteonecrosis of the femoral head (ONFH). In order to treat these disorders, mesenchymal stem cells (MSCs) are considered as promising cells, because of their multi-differentiation potential and the ability to produce cytokines that recruit various types of cells for tissue regeneration. However, little information has been known regarding the effect of glucocorticoid therapy on the regenerative function of MSCs. Based on these backgrounds, in this thesis, the applicant aimed to investigate (1) the proliferative and self-renewal abilities of glucocorticoids on the function of bone regeneration in MSCs, (2) the importance of wnt/β-catenin signaling *in vitro*, and (3) the contribution of Dkk-1, an antagonist of wnt/β-catenin signaling *in vivo*.

(1) The proliferative and self-renewal abilities of glucocorticoids on the function of bone regeneration in MSCs

To uncover the glucocorticoid action on the nature of bone marrow (BM) derived-MSCs, the applicant conducted colony formation assay using primary BM that was aspirated from traumatic or

steroid-induced ONFH patients, and found that BM derived from steroid-induced ONFH patients contained a lower number of colony-forming unit fibroblasts than that from traumatic ONFH patients. Moreover, the applicant showed less and unstable proliferative ability of BM-MSCs derived from steroid-induced ONFH patients, compared with BM-MSCs derived from traumatic ONFH patients.

(2) The importance of wnt/β-catenin signaling *in vitro*

In order to investigate the differences in the characteristics between BM and adipose tissue (AT) derived MSCs, the applicant next isolated and analyzed AT-MSCs derived from steroid-induced ONFH patients (sAT-MSC). Although their proliferative potential was found to be not impaired, the applicant demonstrated decreased osteogenic potential of sAT-MSCs, compared with AT-MSCs derived from traumatic ONFH patients (nAT-MSC). The applicant also illustrated that alkaline phosphatase (ALP), a regulator of the calcification of extracellular matrix, was downregulated in sAT-MSCs and overexpression of ALP restored the osteogenic ability of sAT-MSCs *in vitro*. To identify the downregulation mechanism of ALP in sAT-MSCs, the applicant focused on wnt/β-catenin signaling, because it plays an important role in bone development. The applicant found that Dickkopf1 (Dkk-1), one of the known antagonists of wnt/β-catenin signaling, was upregulated in sAT-MSCs, where, in this situation, the impaired osteogenic potential was rescued when Dkk-1 shRNA (shDkk-1) was transfected. In accordance with these observations, the applicant showed the reduced ability of osteogenic differentiation and the increased expression of Dkk-1 leading to the downregulation of ALP in nAT-MSCs, where it was chronically exposed to dexamethasone *in vitro* to mimic the microenvironment of sAT-MSCs.

(3) The contribution of Dkk-1, an antagonist of wnt/β-catenin signaling *in vivo*

In addition to the osteogenic differentiation ability *in vitro*, the applicant clarified that sAT-MSCs possessed lower bone regenerative ability compared with nAT-MSCs using the murine calvarial defect model. Based on this result, the applicant also revealed that the reduced bone regenerative capacity in sAT-MSCs was caused by elevated Dkk-1 along with the impaired osteogenic differentiation ability *in vitro*. To prove the presence of transplanted AT-MSCs in the repaired bone regions, the applicant performed immunohistological analysis using anti-human osteipontin (hOPN) antibody, a marker of mature osteoblast, and found hOPN-positive cells in the repaired bone region with nAT-MSCs, mock-transfected nAT-MSCs, and shDkk-1-transfected sAT-MSCs. On the other hand, the applicant observed no positive cells in sAT-MSCs and mock-transfected sAT-MSCs at the transplanted regions. Finally, the applicant found that the level of Dkk-1 in plasma was elevated in steroid-induced ONFH patients compared with traumatic ONFH patients.

In this thesis, the applicant demonstrated that the proliferative potential of BM-MSCs was impaired by chronic glucocorticoid treatment, whereas it did not affect proliferation in AT-MSCs derived from steroid-induced ONFH. Intriguingly, the applicant uncovered that the downregulation of

wnt/β-catenin signaling due to highly expressed Dkk-1 impaired the osteogenic ability of sAT-MSCs, observed that Dkk-1 was elevated in the plasma from steroid-induced ONFH patients, and suggested that Dkk-1 induction is a key promoter of AT-MSCs as a useful therapy source. (653 words)

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant provided several lines of evidence regarding the effect of glucocorticoids on the function of bone regeneration in MSCs, and found potential biological significance and contribution of Dkk-1 as one of the known antagonists of wnt/β-catenin signaling in the bone regenerative capacity of AT-MSCs *in vitro* and *in vivo*. However, it still remains unclear how Dkk-1 is induced in response to and regulated by glucocorticoid. To address these questions, further studies will be required using the cell culture methods that the applicant successfully developed.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 5th March, 2018. 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 Doctor of Philosophy in Human Biology.