Glucocorticoids Impair the Tissue Regenerative Function of Mesenchymal Stem Cells

著者	Kato Toshiki
内容記述	この博士論文は内容の要約のみの公開（または一部非公開）になっています
year	2018
その他のタイトル	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
学位授与大学	筑波大学 (University of Tsukuba)
学位授与年度	2018
報告番号	12102甲第8768号
URL	http://hdl.handle.net/2241/00153830

School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

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)

Last or Family Name	First	Middle
Kato	Toshiki	

Student Number

201330533

Primary Academic Advisors

Affiliation: Faculty of Medicine, Division of Regenerative Medicine and Stem Cell Biology

Name: Osamu Ohneda

Abstract

【Background】

Glucocorticoids are popular anti-inflammatory drug, on the other hand, the strong immunomodulatory effect causes undesirable effects including osteonecrosis of the femoral head (ONFH). Mesenchymal stem cells (MSCs) are considered as promising cells to treat ONFH due to their multi-differentiation potential and the ability to produce cytokines that recruit various types of cells for tissue regeneration. However, the effect of glucocorticoid therapy on MSCs has not been fully elucidated yet. The present study aimed to investigate the effect of glucocorticoids on the function of bone regeneration in MSCs. Moreover, I aimed to characterize the function of MSCs derived from steroid-induced ONFH patients who received high-dose and long-period glucocorticoid therapy and elucidated the underlying molecular mechanism.

【Results】

1) BM-MSCs derived from steroidal ONFH patients have less proliferative and self-renewal ability compared with BM-MSCs derived from traumatic ONFH patients

To clarify the effect of glucocorticoid on the character of bone marrow-derived MSCs (BM-MSCs), colony formation assay using primary bone marrow aspirate from traumatic or steroid-induced ONFH patients was performed, and I found bone marrow derived from steroid-induced ONFH patients contained less number of BM-MSCs than traumatic ONFH patients. In addition, BM-MSCs derived from steroid-induced ONFH patients (sBM-MSCs) showed less and unstable proliferative ability compared with BM-MSCs derived from traumatic ONFH patients.

2) The osteogenic potential of AT-MSCs derived from steroidal ONFH patients are impaired by inhibition of wnt/β-catenin signaling

In order to investigate the differences in the characteristics between bone marrow and adipose tissue, I next isolated and analyzed adipose tissue derived MSCs from steroid-induced ONFH patients (sAT-MSC). Their proliferative potential was not impaired; however, the osteogenic potential of sAT-MSCs was decreased compared with AT-MSCs derived from traumatic ONFH patients (nAT-MSC). Therefore, I investigated the expression of osteogenic genes and alkaline phosphatase (ALP), which regulated the calcification of extracellular matrix, was downregulated in sAT-MSCs during osteogenesis. To confirm whether ALP is responsible for the impairment of osteogenesis in sAT-MSCs, ALP-overexpressing sAT-MSCs were established and overexpression of ALP recovered the impaired osteogenic ability in sAT-MSCs.

To identify the downregulation mechanism of ALP in sAT-MSCs, I focused on wnt/β-catenin signaling that plays an important role in bone development. I found that Dickkopf1 (Dkk-1), one of the known antagonists of wnt/βcatenin signaling, was upregulated in sAT-MSCs, where, in this situation, the impaired osteogenic potential was rescued when Dkk-1 shRNA (shDkk-1) was transfected. Consistent with these results, nAT-MSCs chronically exposed to dexamethasone in vitro, which mimicked the microenvironment of sAT-MSCs, also showed reduced osteogenic differentiation ability and increased expression of Dkk-1 that downregulated ALP.

3) Lowered bone regeneration capacity of sAT-MSCs is reversed by shDkk1

The abnormal expression of osteogenesis-related factors in sAT-MSCs suggested that sAT-MSCs might have less bone regenerative capacity than nAT- MSCs, possibly because of the overexpression of Dkk-1. Therefore, I examined the bone regenerative capacity of nAT-MSCs, sAT-MSCs, nAT-MSCs transfected with mock, sAT-MSCs transfected with mock, and sAT-MSCs transfected with shDkk-1, using the critical-sized calvarial defect mouse model. nAT-MSCs and mock-transfected nAT-MSCs could facilitate bone formation, whereas sAT-MSCs showed impaired bone regenerative capacity. In contrast, shDkk-1-transfected sAT-MSCs showed significant increases in bone regenerative capacity compared with the sAT-MSCs transfected with mock. To prove the presence of transplanted AT-MSCs in the repaired bone regions, I performed immunohistological analysis using anti-human osteipontin (hOPN) antibody, which is a maker of mature osteoblast, and found there were 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, no positive cells were observed in sAT-MSCs and mock-transfected sAT-MSCs at the transplanted regions. Of note, plasma Dkk-1 levels were elevated in steroid-induced ONFH patients.

【Discussion】

In the present study, I demonstrated that chronic glucocorticoid treatment impaired the proliferative potential of BM-MSCs whereas proliferation in AT-MSCs derived from steroid-induced ONFH was not affected. On the other hand, the osteogenic ability of sAT-MSCs was impaired through the downregulation of wnt/β-catenin signaling due to highly expressed Dkk-1. Interestingly, the elevated level of Dkk-1 was also observed in the plasma from steroid-induced ONFH patients. These results suggested Dkk-1 expression would be the key promoter of AT-MSCs as a useful therapy source.