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学位論文の題目	Aging-affected MSC functions and severity of periodontal tissue destruction in a ligature-induced mouse periodontitis model (マウス歯周病モデルにおける歯周組織破壊の進行と加齢に伴う間葉系幹細胞機能の低下)
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学位論文内容の要旨

Introduction

Mesenchymal stem cells (MSCs) are multipotent adult stem cell and contribute as stem cell niches throughout the body. They play a role in repair and regeneration of lost or damaged tissues by their multi differentiation ability and immunomodulatory property. At the cellular level, aging results from the accumulation of molecular and cellular damage over time which is characterized by functional impairment of repair and regeneration mechanism. Aging also effects on MSCs by decreasing stem cell population, reduced differentiation potential and most importantly, impair immunomodulation function. On the other hand, periodontal disease is one of the major causes of tooth loss, characterized by inflammation and alveolar bone loss, and the prevalence of the periodontal disease is known to increase along with aging. Currently, little is known about how aged MSCs affect the host response to the local inflammatory condition and tissue destruction in periodontitis. In this study, we examined the relationship between the severity of periodontal tissue destruction and aging-induced deterioration of MSC functions in young and aged periodontitis model mice.

Materials and methods

A periodontitis model was developed in 5-(young) and 50-week-old (aged) C57BL/6J mice, by ligating their mandibular first molars with 5-0 silk threads. Mice were sacrificed at 0, 3, and 10 days after ligation for further experiments. The severity of periodontal tissue destruction was evaluated by micro CT and histological analysis (HE and TRAP staining). The local inflammatory cell infiltration was assessed by IHC staining with the CD3e antibody for pan T cells and B220 antibody for B cells. Then, the inflammatory cytokine expression was measured by RT-PCR. MSC population *in vivo* was evaluated by IHC staining with PDGFRα antibody. Aging-induced phenotypic changes in MSCs were clarified by CFU-f assay, *in vitro* wound-healing assay, cell surface antigen analysis, and senescence detection assay. Moreover, *in vitro* differentiation was evaluated by osteogenic genes (*Alp*, *Ocn*) and adipogenic genes (*Pparγ*, *Lpl*) for RT-PCR, and Alizarin Red-S staining for calcium deposition and

Oil red O staining for lipid droplet formation. The expression level of FAS-L and apoptotic T cells were detected by flow cytometry analysis.

Results

Micro-CT analysis showed significantly severer bone defect in depth (mesial, distal) and area in the aged mice compared to young mice at day-3 and day-10 after ligation (two-way ANOVA, Tukey test, n=3). HE stained sections confirmed the increase of periodontal ligament space in the aged mice compared to young mice at day-10 after ligation. Furthermore, aged mice showed less dense alveolar bone compared to the young ones. The number of $TRAP^+$ osteoclasts were significantly higher in the aged than in the young mice at day-10 after ligation (two-way ANOVA, Tukey test, n=3). Of note, aged mice showed a remarkably higher number of $CD3^+$ T cells compared to young ones at day-3 and day-10 after ligation. Similarly, the number of $B220^+$ B cells (day-3, day-10) were significantly higher in aged than in young mice (two-way ANOVA, Tukey test, n=3). Interestingly, percentage of $PDGFR\alpha^+$ MSCs, was significantly lower in aged compared to young mice after ligation (two-way ANOVA, Tukey test, n=3).

In *in vitro* data, MSCs from aged mice showed a significant decrease in the colony-forming ability compared to MSCs from young mice. The migration speed of aged MSCs was slower than young MSCs at 48 hours after scratching (unpaired Students' t-test, n=3). The expression of MSC surface markers (Sca-1, CD 90, and CD 146) was lower in aged MSCs than young MSCs. Moreover, aged MSCs showed higher cell senescence-associated β-galactosidase activity compared to young MSCs (unpaired Students' t-test, n=3). Additionally, aged MSCs revealed lower osteogenic gene expression (*Alp, Ocn*) and fewer mineral depositions than young MSCs. Conversely, aged MSCs showed higher adipogenic gene expression (*Pparγ*, *Lpl*) and more lipid droplet formation than young MSCs (one-way ANOVA, Tukey test, n=3). Besides, MSCs from aged mice showed lower FAS-L expression compared to that of young mice. Annexin V^+ apoptotic $CD4^+$ T cells were lower when co-cultured with MSCs from aged mice. (one-way ANOVA, Tukey test, n=3).

Discussion and summary

These results indicated that aging-associated dysfunction of MSCs might fail to suppress immune reaction and induce immune tolerance in inflammatory condition. Therefore, severe bone destruction may occur in periodontitis model of aged mice. This is the first study showing that aging-induced impairment on functions of MSCs, potentially correlated with progression of periodontal deterioration. These findings are novel in biological science, providing useful knowledge age-related pathogenesis and regeneration in dentistry.

論文審査結果の要旨

Mesenchymal stem cells (MSCs) are known to play important roles in the repair of lost or damaged tissues and immunotolerance, which are involved in homeostasis of periodontal tissue. However, the function of MSC is impaired by several factors including aging, resulting some influences on local inflammatory condition and tissue deterioration. Because little is currently known about their relations, this study aimed to clarify the relationship between age-related functions of MSCs and local tissue destruction in disease condition using an experimental periodontitis.

In this study, the aged (50-weeks-old age) mice were prepared for ligature-induced periodontitis model, which may contain age-related impaired MSCs. The periodontitis lesions were compared to the lesions of young (5-weeks-old age) model using micro computerized tomography (micro-CT) and immuno-histological analysis. Furthermore, bone marrow-derived MSCs were investigated if the cells are functionally impaired, by cell colony forming ability, proliferative and migrative abilities, expression of MSCs surface markers, tendency of senescence, differentiation lineage to osteoblast or adipocyte, and immunomodulatory property.

Severe bone loss associated with increased osteoclast activity and inflammatory cell accumulation in aged mice compared to young mice were observed. Although accumulation of inflammatory cells was increased, cells positive for platelet-derived growth factor receptor α which is known as one of MSCs markers, were significantly lower in aged mice compared to young mice. *In vitro* analysis of the functions of bone marrow-derived MSCs showed that the markers for MSCs (Sca-1, CD90, CD146), colony formation, migration, and osteogenic differentiation were significantly declined in the MSCs from aged mice, compared to those from young mice. Moreover, the MSCs from aged mice were more positive for the senescence-associated β galactosidase activity and less expressed FAS-L, which is associated with immunomodulatory property than those from young mice.

These results indicated that aging-associated dysfunction of MSCs might fail to suppress immune reaction and induce immune tolerance in inflammatory condition. Therefore, severe bone destruction may occur in periodontitis model of aged mice. This is the first study showing that aging-induced impairment on functions of MSCs, potentially correlated with progression of periodontal deterioration. These findings are novel in biological science, providing useful knowledge age-related pathogenesis and regeneration in dentistry.

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This Dissertation Review Committee hereby accept this article as a doctoral dissertation in dentistry.