Periodontal Regeneration by Allogeneic Transplantation of Adipose-tissue Derived Multi-Lineage Progenitor Stem Cells in vivo.

Periodontal Regeneration by Allogeneic Transplantation of Adipose-tissue Derived Multi-Lineage Progenitor Stem Cells *in-vivo*

Abstract

Objectives: The ultimate goal of periodontal disease treatment is the reorganization of functional tissue by the application of stem cells, cytokines and extracellular matrices that can regenerate the lost periodontal tissue. Regeneration of periodontal tissues is being clinically possible by using autologous transplantation of mesenchymal stem cells (MSC). However, autologous MSC transplantation is limited due to depending on age, systemic disease and tissue quality, thus precluding their clinical application. Therefore, we evaluated the efficacy of allogeneic transplantation of pig derived adipose tissue-derived multi-lineage progenitor cells (ADMPC) in a micro-mini pig periodontal defect model.

Methods: ADMPC were isolated from greater omentum of micro-mini pig (2014DnA-036-3). Flow cytometry analysis was performed to analyze the MSC marker expression on ADMPC. Osteogenic, adipogenic and periodontal ligament differentiation ability was investigated to assess multi-lineage differentiation ability. Pig gingival fibroblast was also isolated from gingival tissue of micro-mini pig and used as control group. To investigate the immune-modulatory activity of ADMPC, cells were cultured with cytokine cocktail containing interferon (IFN)-γ tumor necrosis factor (TNF)-α and interleukin (IL)-6 for 7 days. To investigate the effect of inflammatory cytokines on cell growth of ADMPC, cells cultured with cytokine cocktail were examined for cell proliferation using cell counting kit -8 (CCK-8). To assess the immunomodulatory effect of ADMPC, real-time PCR was performed in cells cultured with and without cytokine cocktail and investigated the expression of immune suppressive factors, guanylate binding proteins (GBP4), C-X-C motif chemokine (CXCL10) and IL1 receptor antagonist, and inflammatory cytokines IL-10, IL-6, IL-17 and TNF-α. Periodontal defect models were established in a micro-mini pig and transplanted with allogeneic or autologous ADMPC. Computed tomography and histological analysis were used to evaluate the outcome of periodontal tissue regeneration.

Results: Flow cytometry analysis confirmed that ADMPC express moderate to high levels of MSC markers including CD44 and CD73. ADMPC exhibit osteogenic differentiation ability by calcified nodule formation, alkaline phosphatase staining and expression of osteogenic related genes in the induced cells. Adipogenic differentiation of ADMPC confirmed by the formation of lipid Droplets stained with Oil Red O in the induced cells. ADMPC showed high expression of immune-suppressive factors GBP4 and IL1-RA upon treatment with cytokine cocktail. Allogeneic transplantation of ADMPC induced significant periodontal tissue regeneration in micro-mini pig periodontal defect model and regeneration ability was comparable to those of autologous transplantation by μCT and histological analysis.

Conclusion: These results suggested that ADMPC has an immune-modulation and periodontal tissue regeneration ability following allogeneic transplantation. Thus, allogeneic transplantation of ADMPC has a potential to become an alternative to periodontal regeneration therapy by autologous transplantation.

Key words: Periodontal inflammation, Micro-mini pig, Allogeneic ADMPC, Periodontal regeneration, Immune-modulation