Hyaluronic Acid Induction Promotes the Differentiation of Human Neural Crest-like Cells into Periodontal Ligament Stem-like Cells

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論 文 名　：Hyaluronic Acid Induction Promotes the Differentiation of Human Neural Crest-like Cells into Periodontal Ligament Stem-like Cells
(ヒアルロン酸はヒト神経堤細胞様細胞の歯根膜幹細胞分化を促進する)

区　　分　：甲

論 文 内 容 の 要 旨

Tooth loss can occur when the PDL is damaged beyond repair, which is frequent in deep caries, trauma, and periodontitis. As periodontitis progresses, the resultant inflammation damages the structure of the PDL, making it difficult to repair lost PDL tissue and eventually leading to tooth loss. Periodontal ligament (PDL) stem-like cells (PDLSCs) are promising for the regeneration of periodontium because they demonstrate multipotency, high proliferative capacity, and potential to regenerate bone, cementum, and PDL tissue. However, the transplantation of autologous PDLSCs is restricted by limited availability. Since PDLSCs are derived from neural crest cells (NCs) and NCs persist in adult PDL tissue, we devised to promote the regeneration of periodontium by activating NCs to differentiate into PDLSCs, however, the challenge in acquiring large numbers of NC cells is a principal factor limiting their application in regenerative studies. SK-N-SH cells, a neuroblastoma cell line has been reported to have NCs-like features, so we aimed to 1) Investigate whether SK-N-SH cells could serve as an alternative differentiation model for iNCs previously established in our laboratory. And 2) Identify factors that regulate the differentiation and promote the transformation of NC-like cells into PDLSC-like cells, in order to develop a membrane scaffold that includes these factors for tissue regeneration.

SK-N-SH cells were cultured on HPDLC ECM, and differentiated into Periodontal stem-like cells SK-PDLSCs. After 2 weeks of culture, SK-PDLSCs has resulted in significant upregulation of PDL marker expression compared to SK-N-SH cells. After that, we examined the multipotency of SK-N-SH and SK-PDLSCs by investigating their ability to differentiate into osteoblasts and adipocytes. SK-PDLSCs showed higher osteogenic and adipocytic differentiation ability compared to SK-N-SH cells. From these results, SK-N-SH cells, similar to iNCs, exhibited a phenotype of periodontal ligament stem cells when cultured on ECM of periodontal ligament cells, so we concluded that SK-N-SH cells could be a substitute research model for iNCs.

Next, we compared the microarray expression of iNCs and iPDLSCs, from these results we found that the expression levels of various hyaluronic acid (HA)-related genes were upregulated in iPDLSCs and SK-PDLSCs compared with iPSCs-derived NCs and SK-N-SH cells, respectively. We confirmed microarray results by analyzing the expression levels of HA-related genes in iNCs and iPDLSCs using PCR which showed that HA-related genes were more highly expressed in iPDLSCs

than in iNCs. The expression of HA-related genes in SK-N-SH cells and SK-PDLSCs showed the same pattern: HA-related genes were more highly expressed in SK-PDLSCs than in SK-N-SH cells. Next, we purified CD44-expressing SK-N-SH cells to investigate the involvement of HA signalling in their differentiation into SK-PDLSCs. (CD44-/CD44+) SK-N-SH Cells were purified using MACS magnetic separator. After 2 weeks of culture, SK-CD44+ and SK-PDLSC-CD44+ showed significantly higher expression of CD44 than CD44− groups. Also, the expression levels of PDL-related genes were significantly increased in SK-PDLSC-CD44+compared with those in SK-PDLSC-CD44−.

To further investigate the function of HA signalling in the differentiation of SK-N-SH cells into SK-PDLSCs, we performed CD44 knockdown using siRNA. Knockdown of CD44 in SK-N-SH cells significantly inhibited their ability to differentiate into SK-PDLSCs, therefore we concluded that HA-CD44 interaction is important for NC differentiation into PDLSCs.

After that, we investigated the effect of continuous/initial low molecular HA (LMWHA) induction on the differentiation of SK-N-SH cells into SK-PDLSCs, our results showed enhanced SK-PDLSC differentiation when HA induction was performed. Therefore, we concluded that the activation of HA signaling on the initial phase of differentiation is important for NC differentiation into PDLSCs, so we tried to developing a scaffold that activates HA signaling on the initial phase of differentiation.

Finally, we applied electrospinning technique to produce a biodegradable scaffold-releasing LMWHA and induce the differentiation of NCs into PDLSCs. we fabricated 3 types of electrospun membranes with different concentration of HA (Cont-membrane, HA1 with 1.1% LMWHA, HA2 with 2.2% LMWHA). Next, SK-N-SH cells were co-cultured with these membranes to investigate their potential to induce differentiation into SK-PDLSCs. After 2 weeks of culture, the cells cultured in the presence of ECM and HA2 membrane led to a significantly higher expression of PDL-related genes than the cells cultured with HA1 or control membrane.

Our overall findings suggest that SK-N-SH cells could be applied as a new model to induce the differentiation of NCs into PDLSCs and that the LMWHA-CD44 interaction is important for the differentiation of NCs into PDLSCs.