氏　　名	EI EI HSU HLAING
授与した学位	博　士
専攻分野の名称	歯　学
学位授与番号	博甲第６１６２号
学位授与の日付	令和２年３月２５日
学位授与の要件	医歯薬学総合研究科機能再生・再建科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Role of intracellular Ca2+ -based mechanotransduction of human periodontal ligament fibroblasts (歯根膜における力学刺激に由来した細胞内カルシウム輸送が果たす役割の解明)
論文審査委員	吉田　竜介　教授　　上岡　寛　教授　　宮脇　卓也　教授

学位論文内容の要旨

[Introduction] Cellular mechanotransduction is critical for modulating normal homeostasis in physiologic functions of living organisms. Periodontal ligament (PDL) fibroblasts are thought to receive mechanical stress (MS) produced by orthodontic tooth movement, thereby regulating alveolar bone remodeling. However, the role of intracellular calcium ($[Ca^{2+}]_i$)-based mechanotransduction is not fully understood.

[Aim] This study aimed to investigate the MS-induced $[Ca^{2+}]_i$ responses both in isolated hPDL fibroblasts and in intact hPDL tissue as well as its possible role in alveolar bone remodeling.

[Materials and Methods] hPDL fibroblasts were obtained from healthy donors' premolars that had been extracted for orthodontic reasons. The oscillatory $[Ca^{2+}]_i$ activity induced by static compressive force was measured by a live-cell Ca^{2+} imaging system and evaluated by several feature extraction methods. The spatial pattern of cell-cell communication was analysed by Moran's I, an index of spatial autocorrelation. The gap junction inhibitor, 18 α-GA was used to validate the involvement of gap junctions intercellular network in the $[Ca^{2+}]_i$-based mechanotransduction of hPDL. The role of $[Ca^{2+}]_i$ upregulation in hPDL cell behavior was investigated using the Ca^{2+} -transporting ionophore, A23187.

[Results]

hPDL fibroblasts displayed autonomous $[Ca^{2+}]_i$ responses. Compressive MS activated this autonomous responsive behavior with an increased percentage of responsive cells both *in vitro* and *ex vivo*. The increased Moran's I after MS indicated that MS might affect the pattern of cell-cell communication via gap junctions. Similar to the findings of MS-mediated regulation, the A23187-mediated $[Ca^{2+}]_i$ uptake resulted in the upregulation of Rankl and Sost along with increased Sclerostin immunoreactivity, suggesting that $[Ca^{2+}]_i$ signaling networks may be involved in bone remodeling. A23187-treated hPDL fibroblasts also showed the suppression of osteogenic differentiation and mineralization.

[Discussion] Our findings clearly showed that hPDL is truly mechanosensitive at the cellular level within intact tissue. Another noteworthy finding of the live-cell Ca^{2+} imaging system is that some hPDL fibroblasts triggered $[Ca^{2+}]_i$ oscillations even under static conditions. The delayed Alizarin red positive reaction suggest that the $[Ca^{2+}]_i$ uptake in hPDL fibroblasts may be attributed to bone remodeling which occurs concurrently with mineralization.

[Conclusion]

The present study demonstrates for the first time that MS-induced $[Ca^{2+}]_i$ oscillations and transients can occur in integral hPDL tissue. The results of our study suggest that the augmented MS-mediated $[Ca^{2+}]_i$ oscillations in hPDL fibroblasts enhance the production and release of bone regulatory signals via Rankl/Opg and the canonical Wnt/β-catenin pathway as an early process in tooth movement-initiated alveolar bone remodeling.

論文審査結果の要旨

Introduction: Cellular mechanotransduction is critical for modulating normal homeostasis in physiologic functions of living organisms. Periodontal ligament (PDL) fibroblasts are thought to receive mechanical stress (MS) produced by orthodontic tooth movement, thereby regulating alveolar bone remodeling. However, the role of intracellular calcium ($[Ca^{2+}]_i$)-based mechanotransduction is not fully understood.

Aim: This study aimed to investigate the MS-induced $[Ca^{2+}]_i$ responses both in isolated hPDL fibroblasts and in intact hPDL tissue as well as its possible role in alveolar bone remodeling.

Materials and Methods: hPDL fibroblasts were obtained from healthy donors' premolars that had been extracted for orthodontic reasons. The oscillatory $[Ca^{2+}]_i$ activity induced by static compressive force was measured by a live-cell Ca^{2+} imaging system and evaluated by several feature extraction methods. The spatial pattern of cell-cell communication was analysed by Moran's I, an index of spatial autocorrelation. The gap junction inhibitor, 18 α-GA was used to validate the involvement of gap junctions intercellular network in the $[Ca^{2+}]_i$-based mechanotransduction of hPDL. The role of $[Ca^{2+}]_i$ upregulation in hPDL cell behavior was investigated using the Ca^{2+} -transporting ionophore, A23187.

Results: hPDL fibroblasts displayed autonomous $[Ca^{2+}]_i$ responses. Compressive MS activated this autonomous responsive behavior with an increased percentage of responsive cells both *in vitro* and *ex vivo*. The increased Moran's I after MS indicated that MS might affect the pattern of cell-cell communication via gap junctions. Similar to the findings of MS-mediated regulation, the A23187-mediated $[Ca^{2+}]_i$ uptake resulted in the upregulation of Rankl and Sost along with increased Sclerostin immunoreactivity, suggesting that $[Ca^{2+}]_i$ signaling networks may be involved in bone remodeling. A23187-treated hPDL fibroblasts also showed the suppression of osteogenic differentiation and mineralization.

Discussion: Our findings clearly showed that hPDL is truly mechanosensitive at the cellular level within intact tissue. Another noteworthy finding of the live-cell Ca^{2+} imaging system is that some hPDL fibroblasts triggered $[Ca^{2+}]_i$ oscillations even under static conditions. The delayed Alizarin red positive reaction suggest that the $[Ca^{2+}]_i$ uptake in hPDL fibroblasts may be attributed to bone remodeling which occurs concurrently with mineralization.

Conclusion: The present study demonstrates for the first time that MS-induced $[Ca^{2+}]_i$ oscillations and transients can occur in integral hPDL tissue. The results of our study suggest that the augmented MS-mediated $[Ca^{2+}]_i$ oscillations in hPDL fibroblasts enhance the production and release of bone regulatory signals via Rankl/Opg and the canonical Wnt/β-catenin pathway as an early process in tooth movement-initiated alveolar bone remodeling.

These findings are scientifically significant, providing useful knowledge that will promote the progress of orthodontic science. Therefore, the dissertation examination committee acknowledged the value of this thesis as a doctoral dissertation.