Abstract

Spontaneous Ca^{2+} response of dental epithelial cell lines
and dental pulp stem cells, and regulation of gene expression
by cell-cell interaction

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【Introduction】

Ca^{2+} is an intracellular messenger involved in the regulation of a wide range of biological processes. The major mechanism of Ca^{2+} signaling in non-excitable cells is release of Ca^{2+} from intracellular stores by inositol trisphosphate, which is produced intracellularly via stimulation of G protein-coupled receptors and receptor tyrosine kinases, and subsequent Ca^{2+} influx of extracellular Ca^{2+} by store-operated Ca^{2+} entry (SOCE). Stim1 and Orai1, two molecules responsible for severe combined immunodeficiency, regulate SOCE, and deficiency in these molecules is known to be associated with a unique clinical phenotype that is characterized by defective dental enamel calcification, highlighting the importance of SOCE and Ca^{2+} signaling in tooth development.

The dental cups, the basis of teeth, are formed from dental epithelial cells derived from the oral epithelium and mesenchymal cells derived from the neural crest, and tooth development proceeds through epithelial–mesenchymal interactions between these cells. It has been reported that the dental epithelial cell line (SF2 cells) promote the differentiation of dental pulp stem cells (DPSCs) into Odontoblasts. In addition, inhibition of SOCE decreased the expression of enamel matrix proteins in SF2 cells, suggesting important roles of Ca^{2+} signaling in regulating gene expressions. The purpose of this study is to elucidate the role of calcium signaling in tooth development. To investigated the role of Ca^{2+} signaling in the regulation of gene expression in SF2 cells and DPSC, the mechanisms of Ca^{2+} response in SF2 cells and DPSCs were clarified using Ca^{2+} imaging experiments, and examined the relationship between Ca^{2+} response and gene expression during the interaction between SF2 and DPSC.

【Materials & Method】

SF2 cells and DPSCs stably expressing the calcium sensor protein (G-GECO, R-GECO) were generated using lentiviral vectors and used for the experiments. Long term live cell imaging was performed using fluorescence microscopy under 37°C, 5% CO2 culture conditions to analyze the Ca^{2+} response of SF2 cells and DPSCs in single culture and their co-culture. For gene expression analysis, total RNA and cDNA were prepared from the single and co-cultured cells of SF2 and DPSC, and were used for RT-PCR.

【Results & Discussion】

Ca^{2+} imaging analysis under cell culture conditions revealed that G-GECO-expressing SF2 cells exhibited spontaneous Ca^{2+} responses. The P2Y receptor inhibitor Suramin (10 μM), the ATP-degrading enzyme apyrase (5 units/mL), and the fibroblast growth factor receptor inhibitor FIIN-2 (1 μM) decreased the frequency of spontaneous Ca^{2+} responses. In addition, 10 μM but not 1 μM gefitinib reduced Ca^{2+} responses. These results suggest the involvement of P2Y receptors and some receptor tyrosine kinases including FGF receptors.

Gene expression of pmepa1 and H19 was increased by a SOCE inhibitor, $LaCl_3$, suggesting that Ca^{2+} has an inhibitory effect on these gene expressions in SF2 cells. The reduction of H19 expression by 10 μM Gefitinib and its enhancement by 1 μM Gefitinib suggested the presence of inhibitory pathway through EGF receptor and stimulatory pathway through other RTKs. On the other hand, the gene expression of pmepa1 was suppressed by 1 μM Gefitinib and enhanced by 10 μM Gefitinib, suggesting the presence of an enhancing pathway via the EGF receptor and an inhibitory pathway via other RTKs.

R-GECO-expressing DPSC exhibited spontaneous Ca^{2+} oscillations, that were inhibited by the lysophosphatidic acid (LPA) receptor inhibitor, RO6842262 (RO). Ca^{2+} oscillations in DPSC were not observed in serum-free medium, and were restored by the addition of LPA (3-30 μM), demonstrate the important roles of LPA in these Ca^{2+} responses. Results also suggest the involvement of receptor tyrosine kinase receptors, such as FGF receptor, in these Ca^{2+} responses.

Gene expression of TGFβ3 and NOTCH3 was enhanced by RO but was not affected by $LaCl_3$ in DPSCs. This suggests that LPA may reduce the gene expression of TGFβ3 and NOTCH3 in a Ca^{2+}-independent manner.

In the co-cultured of SF2 cells and DPSCs, the Ca^{2+} responses of these cells were similar to that observed in the single culture of these cells. RT-PCR confirmed that co-culture enhanced the expression of pmepa1 in SF2 cells and TGFβ3 and NOTCH3 in DPSCs, indicating the presence of some bioactive substances released from SF2 cells and DPSCs to regulate their gene expression. The expression of TGFβ3 and NOTCH3 was suppressed by $LaCl_3$, suggesting that SF2-derived bioactive substances regulate gene expression in a Ca^{2+}-dependent manner. It was also suggested a presence of substances that are released from DPSC and enhance pmepa1

expression in SF2 cells.

【Conclusion】

This study revealed spontaneous Ca^{2+} responses in SF2 cells and DPSCs, which involve the interaction of RTKs with G protein-coupled receptors such as P2Y receptors and LPAR1. It was also suggested the presence of substances that are released from SF2 cells and DPSCs and regulate gene expression in a Ca^{2+}-dependent manner. These results will contribute to the elucidation of the molecular mechanism of gene expression regulation in the epithelial-mesenchymal interaction using SF2 cells and DPSC in the future.