論　文　要　旨

象牙質再生におけるＣＣＮ２のインビトロ研究

平成３０年度

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Abstract

The *in vitro* study of CCN2 on dentin regeneration

2019

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[Introduction]

Direct pulp capping has been used for the treatment of pulp exposed by deep dental caries or traumatic injury. Under conditions of severe damage, newly differentiated odontoblast-like cells may migrate to sites of injury, where they are thought to form reparative dentin that protects pulp tissue from stimuli.

Connective tissue growth factor/**C**ysteine-rich 61/**N**ephroblastoma overexpressed family 2 (CCN2) plays complex roles in various biological processes such as embryogenesis, angiogenesis, chondrogenesis, wound healing, tissue regeneration, etc. However, its effects in dentin regeneration remain unclear.

In this study, we aimed to investigate the in vitro effects of CCN2 on odontoblast-like cells and human dental pulp stem cells (hDPSCs) in proliferation, mineralization and differentiation, as well as its potential application in dental treatment.

[Materials and methods]

MDPC-23 cells, a type of rat odontoblast-like cell line, were cultured in DMEM supplemented with 5% FBS. hDPSCs were cultured in DMEM containing 10% FBS, 50 units/mL penicillin and 50μg/mL streptomycin. This study was divided into three independent experiments. In experiment 1, soluble CCN2 (100 ng/mL) was added into culture media; in experiment 2 and 3, CCN2 at the concentration of 1000 ng/mL was coated onto culture polystyrene, addition or coating of distilled water was served as a control. Cell proliferation was assessed using the CCK-8 assay. The cytoskeleton was stained using Alexa Fluor 568-labeled phalloidin and visualized by fluorescence microscopy. Cell differentiation and mineralization were analyzed by ALPase activity assay, real time RT-PCR and alizarin red staining. Data were analyzed using the post hoc Tukey honest significance difference test.

[Results]

*Experiment 1

Cell proliferation of MDPC-23 cells was promoted by the addition of soluble CCN2. Gene expression of rBSP, rOCN and rOPN were promoted after 48h exposure to soluble CCN2. Mineralization of MDPC-23 cells was accelerated by the addition of soluble CCN2.

*Experiment 2

CCN2 promoted MDPC-23 cells proliferation in a dose-dependent manner, with the greatest effect observed at 1000 ng/mL. ALPase activity of cells cultured on CCN2-modified surfaces continuously strengthened from day six (0.83±0.02 units/μg protein versus 0.56±0.01 units/μg protein of control) until day eight (1.04±0.14 units/μg protein versus 0.70±0.06 units/μg protein of control). Moreover, gene expression of rBSP, rOCN, rOPN, rALP, rCOL1A1, rRunx-2, rDSPP, and rDMP-1 was significantly enhanced by the presence of immobilized CCN2. Finally, mineralization of MDPC-23 cells was markedly enhanced by CCN2.

*Experiment 3

hDPSCs exhibited robust proliferative activity upon exposure to immobilized CCN2. Gene expression of hBSP, hCol1a1, and hALP in the CCN2-coated group was significantly higher than that of the control. The relative ALPase activity was also increased in the CCN2-coated group. Mineral nodule formation by hDPSCs grown on CCN2-coated surface was remarkably increased as compared to those grown on noncoated surfaces.

[Discussion]

Recently, ongoing studies have identified CCN2 protein as a growth and signaling factor that interacts with cell surface molecules and intracellular signaling pathways. Through its interaction with cell surface receptors, integrins, extracellular counterparts and extracellular matrixes, CCN2 governs inter- and intracellular communications to either directly or indirectly regulate cell proliferation and differentiation. The present study also indicated that the addition of 100 ng/mL CCN2 directly into culture media in a short culture period of 48h primarily promoted mineralization rather than odontogenic differentiation on MDPC-23 cells.

[Conclusion]

The findings indicate that CCN2 promotes proliferation, differentiation and mineralization of MDPC-23 and hDPSCs. It is proposed that CCN2 may be a promising adjunctive formula for dentin regeneration.