論　文　題　目

Dentin Phosphophoryn Promotes Odontoblast Differentiation *in vitro* and Induction of Mineralized Matrix *in vivo*

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Introduction

Vital pulp therapy is the method for the treatment of reversible pulpitis (Tziafas et al., 2000). The ultimate goal of vital pulp therapy is to rapidly regenerate dentin of excellent quality by using an external agent that possesses novel properties such as biocompatibility and bioactivity. The calcium hydroxide-based agents have been considered as the gold standard of direct pulp capping materials (Iwamoto CE et al., 2006; Tuna D and Olmez A, 2008) for several decades because of the favorable effects such as antibacterial and hard tissue stimulation (Schrüder U et al., 1985). However, these agents do not provide close adaptation to dentin and also do not promote consistent odontoblast-differentiation. Thus, clinical success rate of the therapy by using calcium hydroxide-based agents does not satisfy dental practitioners according to their desire.

Dentin phosphophoryn (DPP), a member of small integrin-binding ligand N-linked glycoproteins (SIBLING) family, is the most abundant of the non-collagenous polyanionic proteins in dentin. DPP contains numerous aspartic acids and phosphorylated serine residues that render this protein as nature's most acidic protein (Stetler-Stevenson and Veis, 1983; Sabsay et al., 1991). The molecule is called thus as it is considered to be "Phosphate carrier". Phosphorylation of all potential serine results in a highly negatively charged molecule and is a virtual sink for the binding of calcium ions. It has been reported that the RGD domain in DPP induces integrin mediated signaling events. Moreover, it has been also reported that activation of the MAP kinase signaling pathway occurs when human mesenchymal cells were stimulated with DPP.

The purpose of this study was to examine the effects of DPP on differentiation and mineralization of odontoblasts.

Materials & Methods

MDPC-23 was used in this study. This cell line is a rat odontoblast-like cells, which was provided by professor Nör, University of Michigan. The cells were seeded and maintained with Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum in a humidified

atmosphere of 5% CO_2 at 37°C. MDPC-23 was incubated in the presence of DPP with various concentrations 0.1, 1, and 10 μg/ml and without DPP as a control. The cell-morphology was evaluated using microscope and cells proliferation was evaluated by cell number counting. Next, we examined the mRNA expressions for odontoblast-related molecules such as Runt-related transcription factor 2 (Runx2), Dentin matrix protein-1 (DMP-1), Alkaline phosphatase (ALPase), Bone sialoprotein (BSP), Osteocalcin (OCN), Osteopontin (OPN) by RT-PCR and real-time PCR. Moreover, to evaluate the calcified nodule formation, the cells were incubated for 7 days with DPP and stained with 2% Alizarin-red S. Then, the nodule formation was observed with a microscope. ALPase activity was measured as the score of ALPase activity per amount of total protein contained in the cells, with a Lab Assay ALP kit.

To investigate calcification inducibility for DPP, we performed implantation experiments in vivo. We combined 1 mg DPP with 5 mg collagen scaffold. Then DPP-collagen was adsorbed with rat dental pulp cells (RPCs) at $1x10^5$ in number. After 14 days incubation, the DPP-collagen-RPCs were implanted at dorsal site of the rat. The rats were sacrificed at 14 days after implanting. Then, hematoxylin and eosin-stained sections for the specimens were prepared according to the general method. Statistical analysis was performed using Tukey-Kramer.

Results

Cell morphology and proliferation evaluation

According to the observation, it was found that the addition of DPP did not affect proliferation or morphology of MDPC-23.

DPP induced osteoblast/odontoblast gene markers

We assessed the expression of several osteoblast/odontoblast gene markers in response to DPP treatment in cells. The mRNA expression of DMP1 and ALPase in MDPC-23 was promoted by 0.1, 1 and 10 μg/ml DPP at 3 days (Fig. 3). The mRNA expression of osteorix, OCN and BSP was promoted by 1 and 10 μg/ml DPP at 3 days. Moreover, mRNA

expression of Runx2 and OPN was promoted by 10μg/ml of DPP. There was no change in the mRNA expression of Type I col by the stimulation with DPP.

DPP increased ALPase activity in MDPC-23

To further examine the function of DPP in osteogenic lineage progression, we examined ALPase activity, which is a common pheno-typic marker for osteogenesis. ALPase activity was measured over a time course of 3, 5 and 7 days. We observed the high ALPase activity in MDPC-23 was induced by 1 and 10 μg/ml of DPP at 5 and 7 days compared to the control ($p<0.05$).

DPP increased calcium deposition in MDPC-23

When MDPC-23 cells were cultured with differentiation medium containing 1 and 10 μg/ml DPP, increased Alizarin red S staining (Fig. 5A) and on quantitative analysis, the number of mineralized nodules was increased at 7 days, as compared to controls ($p<0.05$). Moreover, a similar trend was observed for alizarin red S staining, without the addition of differentiation medium containing 0.1, 1 and 10 μg/ml of DPP, increased Alizarin red Staining and on quantitative analysis, the number of mineralized nodules was Increased at 7 days, as compared to controls ($p<0.05$).

Implantation of collagen-RPCs with DPP in rat

In order to investigate mineralized-matrix inducibility for DPP, we performed implantation experiments *in vivo*. Mineralized tissue-like matrix formation was induced by composites of DPP-collagen and RPCs, and rapid degradation of collagen was observed, as compared to controls, at 14 days after implantation into dorsal sites in rats (Fig. 6). Moreover, there was absence of inflammatory cells in the HE staining.

Discussion

DMP-1 is a member of SIBLING family and a specific marker of dentin. In this study,

DMP-1 showed the increased expression in the presence of DPP. Moreover, DPP also promoted the mRNA expression of Runx2, osteorix and OCN after binding to integrin receptor on the cell surface via its RGD domain. It was speculated that these phenomenon were caused by activating the MAPK signaling pathways that culminate in a nature osteogenesis. ALPase gene expression was also promoted in the presence of DPP, validating ALPase activity assay result. This study demonstrates that DPP promotes the odontoblast differentiation and mineralization *in vitro* and that DPP induces the formation of hard-tissue like matrix and also promotes bio-degradability of the scaffold *in vivo*. From these results, it can be speculated that DPP is crucial to be an excellent biomaterial. Further investigation is required to elucidate the detail properties of DPP as a promising molecule for odontogenesis.

The findings suggested that DPP promotes the differentiation and mineralization of odontoblasts. Therefore DPP can be a promising candidate for formulating a new pulp capping material.

References

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