

# Abstract

Chemically induced epigenetic modifications enhance  
osteogenic and odontogenic differentiation *in vitro*

2020  
Graduate School of Dentistry,  
Health Sciences University of Hokkaido

SULTANA Shamima  
13L004

## 1. Introduction

Epigenetic is a pattern of gene expression, not involved in DNA sequences. DNA methylation and histone modification are two major mechanisms for the epigenetic modifications. DNA methylation and histone modification are the most effective epigenetic modifications, and DNMT inhibitors and HDAC inhibitors are well known as epigenetic modifications, respectively. Both DNMT inhibitor and HDAC inhibitor have been attempted to apply for tissue regenerations.

The odontoblasts, dentinogenic cells, in dental pulp share similar characteristics with osteoblasts, and dental pulp cells (DPCs) have a useful role in hard tissue regeneration. Although several agents have been applied for dental pulp cells differentiation, little information about the effects of DNMT inhibitor and HDAC inhibitor on DPCs has been shown thus far. Therefore, the present study aimed to explore the effects of DNMT inhibitor, 5-aza-2'-deoxycytidine (5-azadC) (Experiment 01), and that of HDAC inhibitors including MS-275, Valproic acid (VPA), Trichostatin A (TSA), and Sodium butyrate (NaB) (Experiment 02) on the osteogenic and odontogenic differentiation of DPCs to provide new avenues for exposed dental pulp repair and regeneration.

## 2. Experiment 01: *Osteogenic differentiation and calcification by 5-azadC*

### 2.1 Materials and methods:

Porcine dental pulp cells (PDPCs) were cultured with 1.0  $\mu\text{M}$  5-azadC free group in  $\alpha$ -MEM [Con(Mem)], and 1.0  $\mu\text{M}$  5-azadC added group in  $\alpha$ -MEM medium [5-azadC (Mem)]. Cellular cytotoxicity was measured by hemocytometer on the 7<sup>th</sup> day of culture. Quantitative real-time RT-PCR was used to evaluate the mRNA expression level of collagen type I alpha 2 (Col1 $\alpha$ 2), alkaline phosphatase (Alp), osteocalcin (Oc) and msh homeobox 1 (Msx1). Alizarin red staining was performed to confirm the protein expression level in the calcified state of dental pulp cells followed by flow cytometry was done to detect the percentage of positive cells at the protein expression level. In order to confirm the effect of 5-azadC induced DNA methylation on PDPCs, quantitative methylation-specific PCR (qMSP) was performed to detect methylation level. Statistical differences were evaluated and significance was set at  $*P < 0.05$ .

### 2.2 Results:

No cellular cytotoxicity was observed in cells treated with 1.0  $\mu\text{M}$  5-azadC. In the

result of qRT-PCR, the expression levels of Alp, Oc and Msx1 mRNA were significantly increased in 5-azadC(Oi) compared to Con(Mem). The significantly increased level of calcification was observed in 5-azadC(Oi) compared to Con(Mem) by the Alizarin red staining,. As a result of flow cytometry, the ALP/OC co-positive cell rate was significantly increased in 5-azadC(Oi) compared to control. The result of the MSP showed that the methylation rate of Msx1 was significantly decreased in 5-azadC(Oi). Summarizing, our data indicate that 5-azadC plays a crucial role in osteoblast differentiation of PDPCs more effectively by under calcification-inducing culture condition, suggesting DNA methylation by 5-azadC may act in hard tissue formation over the exposed dental pulp.

### 2.3 Discussion:

Altogether results demonstrated that the osteogenic differentiation of PDPCs was successfully achieved by 1.0  $\mu$ M of 5-azadC under mineralization medium. Therefore, 5-azadC may induce osteogenic differentiation, which play a vital role on the inducible effects of DNA hypomethylation on the osteogenic differentiation in PDPCs and may open new avenues for research into dental pulp repair and regeneration.

## 3. Experiment 02: Odontogenic differentiation and mineralization by HDAC inhibitor,

### MS-275

#### 3.1 Materials and methods:

Dental Papilla Cell-23 (MDPC-23) were cultured with Dulbecco's Modified Eagle's Medium (DMEM), and incubated with HDAC inhibitors [MS-275, Valproic acid (VPA), Trichostatin A (TSA), Sodium butyrate (NaB)] for 24 hours without mineralization medium. No treatments with HDAC inhibitor were considered as controls. Cell viabilities and migration assays were evaluated. Reverse transcription-polymerase chain reaction (RT-PCR) was carried out to examine mRNA expression of dentinogenesis/ osteogenesis related genes, including bone morphogenetic protein 2 (BMP2), BMP4, Collagen 1alpha 1(Col1 $\alpha$ 1), Osteocalcin (Oc), dentin sialoprotein (DSPP) and dentin matrix protein (DMP), Runt-related transcription factor 2 (RUNX2), Kruppel like factor 5 (Klf5), Msh homeobox 1 (MSX1). Their ALP activities were evaluated at 6 days. Alizarin Red staining were performed to observe the mineralization in MDPC-23 by the stimulation with HDAC inhibitors for 6 days. Statistical differences were evaluated and significance was set at  $*P<0.05$ .

### 3.2 Results:

No significant differences in the cell number were found between the control and either MS-275. All HDAC inhibitors except MS-275 at 1 $\mu$ M, showed significant effect in decreasing cell migration compared to control at 24h. In Dental Papilla Cell-23 (MDPC-23), MS-275 at a concentration of 1 $\mu$ M showed significant upregulation of Col1a1, RUNX2, DMP-1, DSPP without mineralizing medium. MS-275 at 1.0 1 $\mu$ M also exhibited significant increase in both Klf5 and MSX1 gene expression compared with the control. Furthermore, the mineralization in MDPC-23 by the treatment of HDAC inhibitors was observed by Alizarin Red staining and alkaline phosphatase activity performed after the 6-days incubations. These results demonstrated that, MS-275 at 1.0  $\mu$ M was the most effective for odontoblastic differentiation by increasing the mRNA expression of dentinogenesis /osteogenesis related genes followed by mineralization in pulp-derived cells under basal medium.

### 3.3 Discussion:

This study showed for the first time that HDAC inhibitor, specifically MS-275 has the potential to enhance dentinogenesis by influencing the cellular processes at low concentrations without mineralization medium, providing an opportunity to develop a topically placed, inexpensive bio-inductive pulp-capping material.

### 4. Conclusion:

Epigenetic modifying agents, 5-azadC and MS-275 demonstrated an ability of the calcification and the induction of gene expression for osteogenesis and dentinogenesis. Both inhibitors were the most effective inhibitors for epigenetic regulation in dental pulp cells. Thus, these compounds might become novel therapeutic candidates for regenerative biomaterials within dentin-pulp complex through epigenetic regulation.

-----