Effect of BMP-2 on Gene Expression of Enamel Matrix Proteins at the Dental Epithelial Cell Line

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Abstract: Epithelial-mesenchymal interactions play an important role in the control of ameloblasts and odontoblasts differentiation, and the bone morphogenetic proteins (BMPs) are known factors that regulate the differentiation of ameloblasts. We examined the effect of BMP-2 on the expression of the enamel matrix protein genes at the dental epithelial cell line. BMP-2 induced a 3- to 4-fold increase in amelogenin and ameloblastin mRNA expression, suggesting that BMP-2 is important for ameloblast differentiation. This finding has potential application in the tissue engineering of tooth re-constructions.

INTRODUCTION

The interactions between epithelial and mesenchymal cells are thought to play an important role in the control of proliferation and differentiation of these cells. In tooth development, epithelial-mesenchymal interactions also play an important role in the control of ameloblast and odontoblast differentiation. Amelogenin and ameloblastin, which are major proteins of the enamel matrix produced by epithelial ameloblasts, are known to play a role in the regulation of mineralization of enamel [1,2].

In recent years it has been reported that amelogenin regulates gene expression in the differentiation of some mesenchymal cells [3, 4]. The specific amelogenin gene splice products induce expression of the bone matrix proteins bone sialoprotein (BSP) and BAG-75 in culture and in an implant *in vivo* [5]. We previously demonstrated that the reuptake of full-length amelogenin protein results in increased levels of amelogenin mRNA through enhanced mRNA stabilization [6,7]. These findings indicated that amelogenin proteins are important for both ameloblast and odontoblast differentiation in an autocrine or paracrine manner.

Bone morphogenetic proteins (BMPs) are also known factors that regulate terminal differentiation of ameloblasts and induce secretion of amelogenin [8-10]. BMP-2 is one of the most potent cytokines that stimulates osteoblast differentiation and bone formation [11,12]. BMP-2 functions to regulate the expression of transcriptional factors such as Pitx or Msx to mediate epithelial-mesenchymal interactions during tooth morphogenesis [13,14]. However, the effect of BMP-2 on the gene expression of the enamel matrix proteins is not well understood.

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In the present study we examined the effect of BMP-2 on the gene expression of the enamel matrix proteins at the dental epithelial cell line. BMP-2 induced up-regulation of amelogenin and ameloblastin mRNA expression. This finding has potential application in the tissue engineering of tooth re-constructions.

MATERIALS AND METHODS

Cell Culture

HAT-7 cells, a dental epithelial cell line originating from the apical bud of a rat incisor [6,7,15], were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F-12 (GIBCO BRL, USA) supplemented with 10% fetal bovine serum and penicillin (100 units/ml)/streptomycin (100 μg/ml). All cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C. Recombinant human BMP-2 was purchased from R & D Systems Inc. (USA, MN).

RNA Extraction and Real-Time PCR Analysis

The mRNA levels of differentiation-related marker genes were determined by quantitative real-time PCR as described previously [6,7,16-19]. Briefly, total RNA was extracted at various points with ISOGEN (Nippon Gene, Japan). A 4-μg amount of total RNA was reverse transcribed into cDNA with Super Script First-Strand Synthesis System (Invitrogen, USA) according to the supplier's protocol. Normalization was performed using the housekeeping gene, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) expression as an endogenous control in the same reaction as the gene of interest. The primers for real-time PCR were designed with PrimerExpress software (AB Applied Biosystems) based on the sequence of the target gene. The primers were as follows: for amelogenin, forward: 5'-TGGGAGCCCTGGTTATATCAA-3', reverse: 5'-GCTGCCTTATCATGCTCTGGTA-3'; for ameloblastin, forward: 5'-TTCACCCAAGGGAGGAGACTT-3', reverse: 5'-CTCTCCTTTCTCAGGGCCTTTAGT-3'; for keratin 14, forward: 5'-GGACCTGAGCCGCATCCT-

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3', reverse: 5'-TCCACATCTTTGCGGTTCTTC-3'; for GAPDH, forward: 5'-GCCCCCAACACTGAGCAT-3', reverse: 5'-CCAGGCCCCTCCTGTTGT-3';

RESULTS

Effects of BMP-2 on the Gene Expression of Enamel Matrix Proteins in HAT-7 Cells

To investigate the effects of BMP-2 on dental epithelial cells, we analyzed the mRNA expression levels of enamel matrix protein genes and keratin 14 (as a control) in HAT-7 cells in the presence or absence of BMP-2. We found that 100 ng/ml of BMP-2 induced up-regulation of endogenous amelogenin and ameloblastin mRNA expression in HAT-7 cells (Fig. **1**). No significant change in the expression of keratin 14 was observed, and thus enamel matrix protein genes might be specifically regulated by BMP-2 in HAT-7 cells.

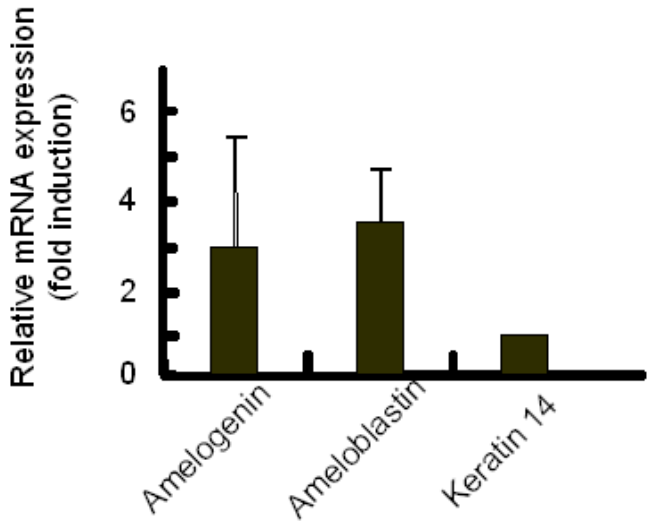

Fig. (1). Effect of BMP-2 treatment on the gene expression of enamel matrix proteins and keratin. HAT-7 cells were treated with 100 ng/ml BMP-2 for 3 days and mRNA expression measured by real time RT-PCR. Data were normalized to non-treatment gene expression. Each column represents the mean±S.D. (n=4).

Expression Time-Course of Ameloblastin Gene Expression After Treatment with BMP-2

To determine the expression time-course of the ameloblastin gene, quantitative real-time RT-PCR analysis was performed on HAT-7 cells that had been treated with BMP-2 for 0, 1, 2 or 3 days. As shown in Fig. (**2**), BMP-2 induced ameloblastin mRNA expression in a time- and dose-dependent manner. These results suggest that BMP-2 induces enamel matrix protein genes in dental epithelial cells.

DISCUSSION

BMP-2 signaling consists of a receptor complex that activates Smads and a Smad-containing complex that controls transcription of the downstream target genes, with DNA-binding factors, such as CREB-binding protein(CEP)[20]. The promoter structures of amelogenin and ameloblastin have been identified [21-23]. The amelogenin promoter contains regulatory elements of CEP. This suggests that BMP-2 activates transcription of amelogenin gene through CEP.

It has been reported that BMP-2 was detected at the apical and basal poles of preameloblasts in the developing mouse first lower molar [24], and, BMP-2, which induces ameloblast differentiation *in vitro* enamel organ culture [9]. These studies suggested that BMP-2 play a role on the ameloblasts differentiation. It has been also shown that BMP-2 and BMP-4 were expressed in the enamel knot, and they may play an important role in the control of tooth morphogenesis [25,26]. Amelogenin and ameloblastin are two major enamel matrix proteins, counting for 90% and 5-10% of enamel matrix proteins, respectively. Our results showed that BMP-2 induced amelogenin and ameloblastin mRNA expressions in the ameloblast-like cell line. These findings will provide a molecular evidence of that BMP-2 induced the differentiation of ameloblasts.

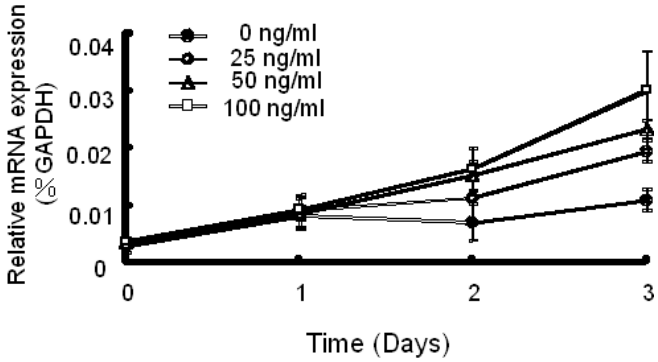

Fig. (2). The expression time-course of ameloblastin gene expression after treatment with BMP-2. Ameloblastin mRNA expression was measured by real time RT-PCR. Data were normalized to glycerol aldehyde dehydrogenase gene expression. Data represents the mean±S.D. (n=4).

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