In situ hybridization analysis of USF1 mRNA expression in odontoblasts

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Purpose: To detect and verify USF1 mRNA expression in odontoblasts.
Materials and Methods: T-USF1 clone prepared previously was used as template and the desired USF1 cDNA segment was amplified by PCR with specific primers. The segment was labeled with digoxin as a probe and in situ hybridization was performed on the mounting of odontoblasts.
Results: Evidently positive staining was shown in the cytoplasm of odontoblasts.
Conclusion: We verified the expression of USF1 mRNA in odontoblasts for the first time and provided evidence for further research. **(Int Chin J Dent 2004; 4: 15-18.)**

Clinical Significance: The results indicate that USF1 might be involved in the maturation of odontoblasts and the process of dentin formation.
Key Words: in-situ hybridization, odontoblast, mRNA, PCR, USF1.

Introduction

The proliferation, differentiation and mineralization of odontobalsts are the important components of tooth development and this process is modulated by multiple signaling molecules to act synergetically and successively.[1] To investigate the functional adjustment of USF1 to odontoblasts, we use the probe prepared by ourselves to detect whether there is USF1 mRNA expression in odontoblasts in vitro.

Materials and Methods

Amplification of USF1 cDNA Probe by PCR

With the T-USF1 plasmids we constructed previously as template, Oligo software (Takara, Dalian, P. R. China) was used to design the PCR primers adjunctively, the sequence of the upstream and downstream primers were 5TGAAACCGAAGAGGGAACAG3 (281-300) and 5GGCATCGTCACTGGTGAAAG3 (569-550), respectively. Twenty-five μL of PCR reaction system was made according to the kit instruction of BcaBest^{TM} NA PCR Kit Ver 1.1 (Takara) which contained: 12.5 μL of 2xBca lst Buffer, 2.5 μL of $MgSO_4$, 0.125 μL of Bca-optimized Taq, 1.0 μL of upstream primer, 1.0 μL of downstream primer, 0.5 μL of dNTP, 1.0 μL of plasmids template, and 6.375 μL of H_2O. The segments of USF1cDNA were amplified by PCR and the reaction conditions were as follows: at 94˚C for 2 minutes, 94˚C for 30 s, 55˚C for 1 minute, 72˚C for 1 minute, repeated in 35 cycles and then 72˚C for 10 minutes, the production of PCR was measured, collected and quantified by electrophoresis through 18 g/L agarose gel.

The Labeling of USF1 cDNA Probe

Fifteen μL of PCR production which contained 3.0 μg of target USF1 cDNA segments was labeled with digoxin DNA according to the kit instruction, and stored below 20˚C for future use.

Cell Culture and the Preparation of Cell Creeping Slides

MDPC-23[2] odontoblasts were cultured in a MEM (Gibco, Gaithersberg, MD, USA) with 10% fetal calf serum and Hela cells were cultured in DMEM (Gibco) with 10% fetal calf serum. Confluent cells were digested with trypsin and cell number was counted. At the density of $2x10^4$/mL, cells were seeded in the 24-well culture plate in which coverslips were placed beforehand, and 0.5 mL liquid was dropped to each well. Cells were cultured at 37˚C, 5% CO_2 and saturated humidity for 48 hours, then washed with PBS, and stabilized with icy methanol at below 20˚C for 15 minutes, the liquid was discarded and the coverslips were dried for future use.

In Situ Hybridization

The cell creeping slides were placed in gradient ethanol and DEPC water, then in 0.2 mol/L hydrochloric acid for 10 minutes, 5 mg/mL proteinase K for 3 minutes, 3% H_2O_2 for 30 minutes, washed with water and dried. The labeled USF1 cDNA probe was diluted with prehybridization solution to 10 mg/mL, and then put in boiled water for 10 minutes and an ice bath at below 20˚C for 10 minutes. After that, it was dropped homogeneously to the cell creeping slides, and put in wet box overnight. The following day, cell creeping slides were washed while agitated repeatedly with 2xSSC, 1xSSC, 0.5xSSC and 0.2xSSC successively, then with Buffer I for 5 minutes, repeated twice, and sealed with 2% goat serum for 30 minutes. Then it was dropped evenly with ALP coupled anti-digoxin antibody, and put in wet box incubating for 2 hours at 42˚C. After washed with Buffer I for 5 minutes, repeated three times and Buffer III for 1 minute once, the cell creeping slides was dropped with NBT/BCIP in wet box at 42˚C for staining, then washed with distilled water to terminate the reaction, dehydrated and sealed. For the negative control, prehybridization solution was used to substitute for the probe, and for the positive control, Hela cell creeping slides were used.

Results

PCR Production

As we expected, after PCR amplification and gel electrophoresis, T-USF1 plasmids produced a specific amplication production which was the size of 289 bp (Fig. 1).

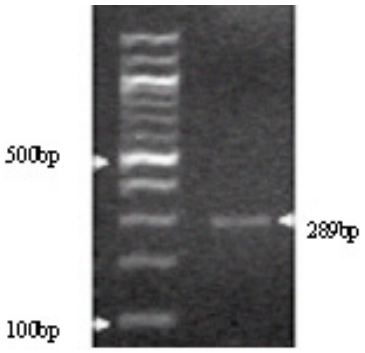

Fig. 1. Electrophoresis of USF1 cDNA segment.

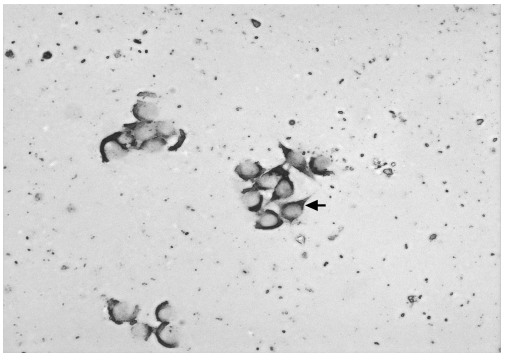

Fig. 2. USF1 mRNA expression in odontoblasts.

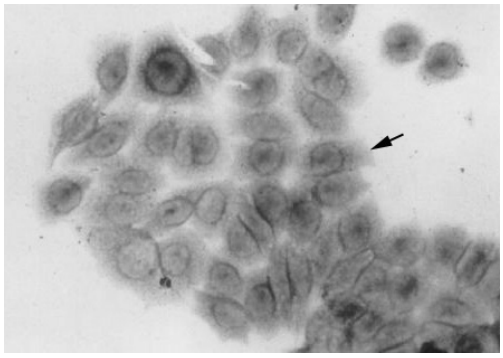

Fig. 3. USF1 mRNA expression in Hela cells.

In Situ Hybridization

There were positive blue-purple particles or balls in the cytoplasm of odontoblasts (arrow), but no staining in the nuclei (Fig. 2). There was no staining for negative control cells. For the positive control Hela cells, there was blue-purple staining in the cytoplasm (Fig. 3).

Discussion

Odontoblasts are a kind of terminal cells that are differentiated from dental papilla cells, and they can secret dentin matrix and promote its mineralization. Therefore, their dysfunction can lead to the defect of dentin formation and tooth dysplasia. Studies in recent years have confirmed that the proliferation, mineralization and extracellular matrix secretion of odontoblasts were modulated by the synergetic action of multiple hormones, extracellular growth factors and intracellular transcriptional factors.[3]

USF1 is a member of transcriptional factor bHLH-zip basic helix-loop-helix-leucine zipper family which was first purified partially form the nuclear extract of Hela cells by Sawadogo et al. in 1985. Moreover, it was found that the addition of USF1 could increase 10 to 20 times of the transcriptional activity of adenovirus MLP (major late promoter).[4] Subsequent studies successfully cloned the whole long cDNA sequence which encodes the human being 43kD USF1 and the whole long cDNA sequence which encodes the mouse USF1, and ascertained that the USF1 gene locates in human being chromosome 1 q^{22}-q^{23}, and the USF1 gene of mouse consists of 10 exons and 9 introns, with each exon coding concrete functional domain.[5] Recent findings indicate that in addition to modulate MLP function, USF1 extensively takes part in the modulation of other tissue-specific gene transcription such as BMP-4, TGFb-2, CyclinB1 and collagen etc, which is mediated by E-box (the core sequence is 5'CANNTG 3'), thus to control the cell proliferation and cell cycles.[6,7] Furthermore, it was found in an animal experiment that though USF1-null mice were viable, they had behavioral abnormalities, among the double mutants of USF1 and USF2 genes, mice were embryonic lethal, suggesting that USF proteins are essential in embryonic development.[8]

Though some data were accumulated about USF1, there is limited data in the literature regarding biological function of USF1, especially the relationship between USF1 and tooth development was not reported domesticly and overseas. In the present study, PCR primers were designed according to USF1 cDNA sequence published in Genebank and confirmed to be highly specific by Blast analysis. With

T-USF1 clone made previously as template, desired USF1 segment was prepared by PCR and labeled as probe, and then USF1 mRNA expression in odontoblasts MDPC-23 was demonstrated by in situ hybridization. Combination with the previous data about USF1, our present study indicates that USF1 probably plays a role in the modulation of growth, proliferation, differentiation and secretion of odontoblasts, which also provides experimental data for the possibility and necessity of exploring the biological function of USF1further with reverse nucleotide block or mutant transfection methods.

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