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The localization of Fos B, a member of transcription factor AP-1 family, in rat odontoblasts and pulpal undifferentiated ectomesenchymal cells

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Abstract: It has been proposed that cellular proliferation and differentiation are accomplished by AP-1 components but different components can be responsible for different functions. The aim of this study was to compare the localization of Fos B, which is a component of AP-1, in postmitotic differentiated and undifferentiated cells *via* Fos B immunoreactivity. For this purpose, maxillary incisor teeth from 10 Wistar rats were obtained and Fos-B was investigated immunohistochemically in formalin-fixed, paraffin-embedded tooth sections containing odontoblasts, which are postmitotic differentiated cells, and pulpal undifferentiated ectomesenchymal cells. No significant differences in percentage of Fos B-positive cells were observed between the two cell types ($p>0.05$). These findings suggest that Fos B, a component of AP-1 family, seems to have a negligible effect on differentiation and proliferation in odontoblasts and pulpal undifferentiated ectomesenchymal cells.

Key Words: AP-1 - Fos B - Differentiation - Odontoblasts - Mesenchymal cells - Rat

Introduction

Transcription factor AP-1 (activator protein-1) consists of Fos proteins (c-Fos, Fos B, Fra 1 and Fra 2), which are nuclear protein products of *c-fos* genes belonging to immediate early genes (IEGs), and of Jun proteins (c-Jun, Jun B and Jun D), which are nuclear protein products of *c-jun* gene [1, 7]. It is believed that AP-1 components are specific for some biological processes including cellular proliferation, differentiation, apoptosis and oncogenic transformation [2, 12]. Particularly, *c-fos* gene has been associated with the coordinated regulation of gene expression during cellular proliferation and differentiation [18].

The relationship between the immediate early genes including the *c-fos* gene producing Fos proteins as well as the entire transcription factor AP-1, and cellular proliferation and differentiation [13, 17] has been the basis for the argument that the localization of the members of AP-1 may be different in mitotic and postmitotic cells as well as in differentiated and undifferentiated cells.

In this study, the Fos B immunoreactivity in differentiated postmitotic cells and in undifferentiated mitotic cells has been compared in order to test this hypothesis from a specific perspective. Although many studies have been performed to explain the relationship between the other components of Fos protein family and the differentiation and proliferation [5, 10, 11, 16], the evidence regarding the relevance of Fos B is limited. In rat dental pulp, odontoblasts representing differentiated postmitotic cells, and pulpal undifferentiated ectomesenchymal cells representing undifferentiated mitotic cells have been chosen for the present study.

Odontoblasts are postmitotic cells of neural crest origin [15]. Pulpal undifferentiated ectomesenchymal cells with the same origin have the potential to differentiate into odontoblasts [19]. The common location and origin of these two cell types, a completed process of differentiation by odontoblasts and their inability to undergo mitosis again during their life span as well as the ability of pulpal ectomesenchymal cells to retain their ability to differentiate into odontoblasts in order to construct the reparative dentin when required have provided an appropriate context for the study.

Materials and methods

Fos B immunoreactivity was analysed in formalin-fixed, paraffin-embedded sections by immunohistochemical methods. Ten female Wistar rats (10-12 weeks, 180-220 g) were used. The rats were sacrificed under ether anesthesia and their maxillary incisor teeth

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were removed. Teeth were fixed in 10% buffered formalin for 18 hours. Following the fixation, the teeth were decalcified with 5% disodium EDTA solution adjusted to pH 7.3 with sodium hydroxide solution and placed in a cold room for 3-4 weeks. Immunoreactions were not affected by the decalcification procedure.

After decalcification, samples were rinsed with phosphate buffered saline (PBS), and the specimens were subsequently dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections (5 μm) were cut and mounted on glass slides. The sections were deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol, covered with 10 mM sodium citrate buffer (pH 6.0) and heated at 95˚C for 5 min for antigen retrieval.

The ImmunoCruz™ Staining System (sc-48 K: Fos B (102) K, Santa Cruz Biotechnology, Inc., CA, USA) was used for the immunohistochemistry protocol. Endogenous peroxidase activity was quenched by 5 min incubation in peroxidase blocking solution.

According to the standard procedure based on affinity-purified rabbit polyclonal antibody against Fos B, the sections were incubated in the primary antibody for 2 h and then in the biotinylated secondary antibody for 30 min. Negative controls were performed by substituting the primary antibody with nonimmune rabbit serum. Immunoreactivity was detected by means of horseradish-peroxidase (HRP)-streptavidin complex using diaminobenzidine (DAB) chromogen as a marker. Sections were counterstained in Mayer's hematoxylin for 5-10 sec and destained with acid alcohol. Subsequently, the sections were dehydrated in ethanol and xylene and coverslipped. All steps were carried out at room temperature in a humidified chamber. Assessments, counts and photography were performed using a Laborlux K (Leitz, Germany) light microscope.

In each rat two incisor teeth were used. Six sections were prepared from each tooth, thus a total of 12 sections were obtained for every rat. One hundred odontoblasts and pulpal undifferentiated ectomesenchymal cells were counted in each section. The percentages of Fos B-positive cells were calculated.

In order to test the statistical significance of the difference between the two cell types, paired-samples t-test was used. P values <0.05 were considered significant.

Results and discussion

Sections serving as negative controls were all unstained. In both cell types, the immunostaining was both nuclear and cytoplasmic, with predominance of the former (Fig. 1). No obvious differences in the intensity of immunostaining between the two cell types was observed.

The mean percentages of Fos B-positive odontoblasts and pulpal undifferentiated ectomesenchymal cells in rats were 64.4±29 .5% (ranging from 8.2% to 93.8%) and 66.1±30.1% (ranging from 6.7% to 97.6%), respectively (Tab. 1).

Statistical analysis revealed no significant differences in the percentage of Fos B-positive odontoblasts and pulpal undifferentiated ectomesenchymal cells ($p> 0.05$).

There is strong evidence suggesting that ectomesenchymal cells in the pulp can be differentiated into odontoblasts under appropriate conditions [19]. However, the mechanism of their differentiation is far from being clear [21].

It is well established that many agents that promote growth and differentiation by influencing cells can cause the transcription of *c-fos* genes [4]. Our knowledge regarding the role of one of the products of this gene, namely Fos B, in the differentiation of ectomesenchymal cells is very limited.

Table 1. Percentage of Fos B-positive rat odontoblasts and pulpal undifferentiated ectomesenchymal cells

Rat No.	% Fos B-positive odontoblasts	% Fos B-positive pulpal ectomesenchymal cells
1	74.1	89.7
2	93.8	97.6
3	91.4	86.3
4	37.1	24.4
5	63.4	75.9
6	8.2	6.7
7	78.0	91.6
8	28.9	54.0
9	82.6	61.8
10	87.1	73.7
Mean±SD	64.4±29.5	66.1±30.1

In the procedure used, the tissue processing might have influenced the distribution of c-Fos immunostaining. Although Fos is a nuclear oncoprotein, the delay after fixation may result in cytoplasmic reactivity and a gradual loss of nuclear localization [14]. In fresh tissue samples almost exclusive nuclear staining is observed, whereas in fixed materials both cytoplasmic and nuclear staining are seen [6].

It has been reported that the differentiation of pulpal cells is accomplished by AP-1 *via* an increase in *c-fos* expression [9]. On the other hand, there are some published data suggesting that different components of AP-1 can perform different functions [5], and even some investigators have proposed that the differentiation process in cell cultures is independent of increased levels of c-Fos [3]. On the other hand, it has also been suggested that *c-fos* expression is increased during undifferentiated growth and decreased during terminal differentiation [8]. In addition to suggestions regarding important changes in the composition of AP-1 transcription factor [2], and even the loss of *c-fos* expression [20] during ageing, maturation ameloblasts were reported to contain more AP-1 protein compared to secretion ameloblasts [12]. The relevance of the components of AP-1 for different biological processes is still unclear.

These findings demonstrate that there are no significant differences with regard to Fos B immunoreactivity between odontoblasts in rat dental pulp that are postmitotic cells with a completed process of differentiation and pulpal undifferentiated ectomesenchymal cells which retain their ability to differentiate into odontoblasts by mitosis when required. These findings suggest

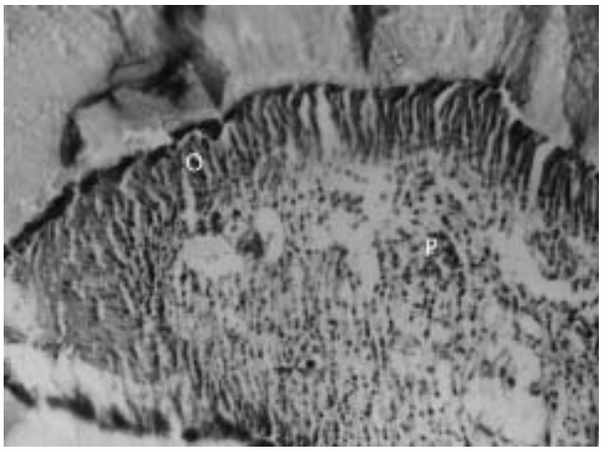

Fig. 1. Nuclear and cytoplasmic Fos B immunoreactivity in odontoblasts (O) and pulpal undifferentiated ectomesenchymal cells (P). × 160.

that Fos B, a component of AP-1 family, seems to have a negligible effect on differentiation and proliferation in odontoblasts and pulpal undifferentiated ectomesenchymal cells.

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