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Original Articles

Regulation of Apoptosis in Odontoblasts in Ageing Dental Pulp by NFkB and JAK1-STAT3 signaling pathway

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The aim of the study was to examine and compare the immunohistochemical distribution of NFkB, JAK1 and STAT3 in human odontoblastic cells depending on age, gender, tooth type, and cell topography. Ninety intact teeth of healthy individuals were enrolled in the study and arranged in three groups (n=30) regarding the patients' age. Immunohistochemistry of paraffin-embedded sections was performed using mouse monoclonal antibody NFkB p65, JAK1, and STAT3. Statistical analysis were applied ($p<0.05$) by IBM SPSS Statistics 25. In aged dental pulp, odontoblasts expressed statistically significant more NFkB ($p<0.05$). A greater expression of JAK1 and STAT3 was shown in cells ageing ($p<0.05$). The signaling pathway JAK1-STAT3 was significantly immunopositive in women, frontal teeth and root pulp ($p<0.05$). In conclusion, the activity of signaling pathway JAK1-STAT3 in dental pulp in ageing presented a direct pathway of regulation of apoptosis, however NFkB maintained the inhibitory threshold.

Key words: ageing, dental pulp, apoptosis, regulation

Introduction

The JAK-STAT signal pathway provides a fast, direct flow of information to the cell nucleus. The JAK-STAT pathway operates in the cell not only through cytokine receptors such as IL-6, but also uses other alternative receptors such as GPCRs, Toll-receptors (TLRs) and microRNA, especially in carcinogenesis [6]. Growth hormone can activate the signaling pathway JAK-STAT and the result brings a new course to its

functionality and plays an important role in ageing and carcinogenesis. Many of the signals associated with stress (oxidative, radiation, etc.), inflammation and congenital immune response work through the NFkB-dependent signaling pathway. Odontoblastic cells of the dental pulp express Toll receptors, receptors for TNFα, IL1 and other that belonged to superfamily of cytokine receptors. Pulpal odontoblast cells have a different set of adaptor proteins inside the cell, which are associated with different signaling pathways for activation of NFκB and JAK-STAT signaling pathway [7]. Transcription factor NFkB sets the inhibitory threshold in pulp odontoblasts, the effect of which it realizes anti-apoptotic effects and protects cellular life. Our available specialized literature lacks studies related to the roles of NFkB, JAK1-STAT3 in pulp odontoblasts. There are no data on the influence of ageing on the activity of NFkB, JAK1-STAT3 in human pulp cell lines, as well as in isolated human cells from bone or dental pulp. A full understanding of the gender-dependant activity of NFkB, JAK1-STAT3 signaling path remains unclear, and cells topography as well. The aim of the study is to examine and compare the immunohistochemical distribution of NFkB, JAK1 and STAT3 in human odontoblastic cells depending on the age, gender, tooth type and cell topography.

Material and Methods

Ninety intact teeth of healthy individuals were enrolled in the study and arranged in three groups (n=30) regarding the patients' age: dental germs with young pulp and dentin up to 17 years old patients, mature pulp and dentin up to 40 years old patients, and adult pulp and dentin over 41 years old patients. All teeth were freshly extracted by dental indications and the study was approved by Ethical Committee of Medical Universiy of Plovdiv (protocol 1/13.02.2020). Immediately after extraction the molars were fixed overnight in 10% buffered paraformaldehyde. The specimens were reduced in size by trimming the enamel, superficial coronal dentin and the roots up to 2 mm below the Cemento-enamel junction (CEJ). The coronal dentin-pulp specimens were decalcified in a 3% hydrochloric acid (HCl) for 6 hours and dehydrated using graded ethanol and acetone, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin. For immunohistochemistry, paraffin sections were dewaxed in xylene, rehydrated with distilled water, and then subjected to antigen retrieval (sodium citrate buffer, pH 6.0) and incubated with mouse monoclonal antibodies NFkB p65 (F-6), JAK1 (A-9), and STAT3 (F-2) (Santa Cruz Biotechnology Inc., USA). The microscopic observation of immunolabeling was done in the peripheral odontoblastic layer and in the subodontoblastic zone including 0-immunonegative, 1-weak immunopositive, 2-strong immunopositive reactions. Statistical analysis including Kruskal-Wallis and Mann-Whitney tests was performed ($p<0.05$).

Results

The expression of the biomarkers studied is presented in **Figure 1**.

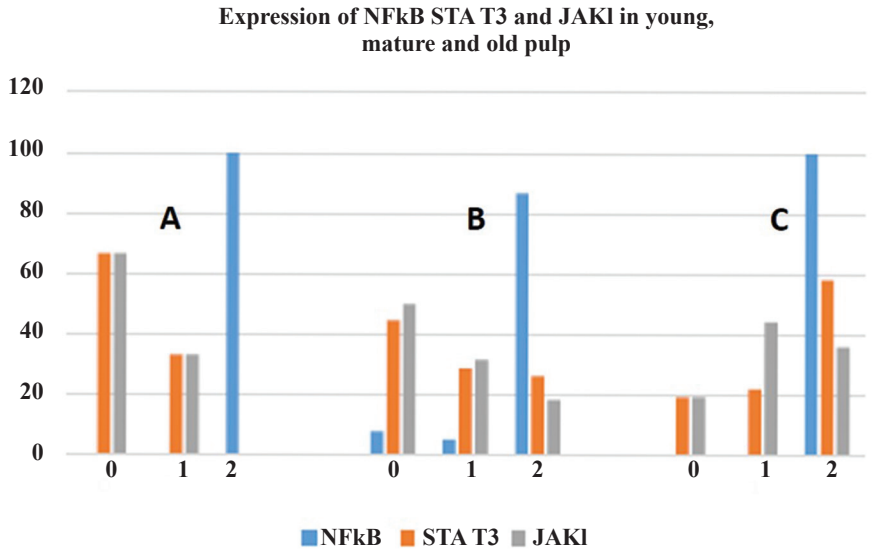

Fig. 1. Expression of biomarkers in odontoblasts in young (A), matured (B) and adult (C) pulp (0-immunonegative, 1-weak immunopositive, 2-strong immunopositive).

Age-dependant changes

The results for the expression of biomarkers in pulp odontoblasts from the three age groups studied are illustrated in **Figs. 2-4.**

The analysis of the results obtained for the expression of NFκB from odontoblasts in different ages showed that the marker was positive in all the samples studied. Only in cuts of mature pulp there was NFκB-immunonegative odontoblasts. The expression of JAK1 and STAT3 showed dynamics. The young pulp of dental germs (**Fig. 2**) was statistically significant immunonegative for STAT3 compared to an old pulp ($p<0.05$). A similar pattern was observed in the expression of JAK1.

Samples of young and mature pulp showed a negative immune reaction for the STAT3 with statistical significance ($p<0.05$) comparing to old odontoblastic cells. Our results showed that cell ageing of pulp odontoblasts increased the expression of JAK1 and STAT3, and the signaling path for the initiation of apoptosis was characterized by age-dependant activity (**Fig. 3** and **Fig. 4**).

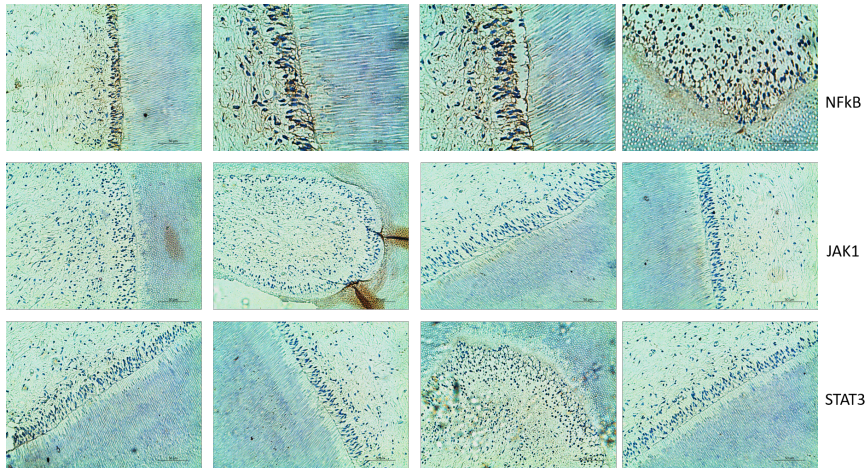

Fig. 2. Expression of NFκB, JAK1 and STAT3 from young pulp odontoblasts. Immunopositive marking for NFκB was observed in the area of the pulp horn of the young pulp tissue of dental germ 38 of a 16-year-old patient. Immune reactions for JAK1 and STAT3 were negative.

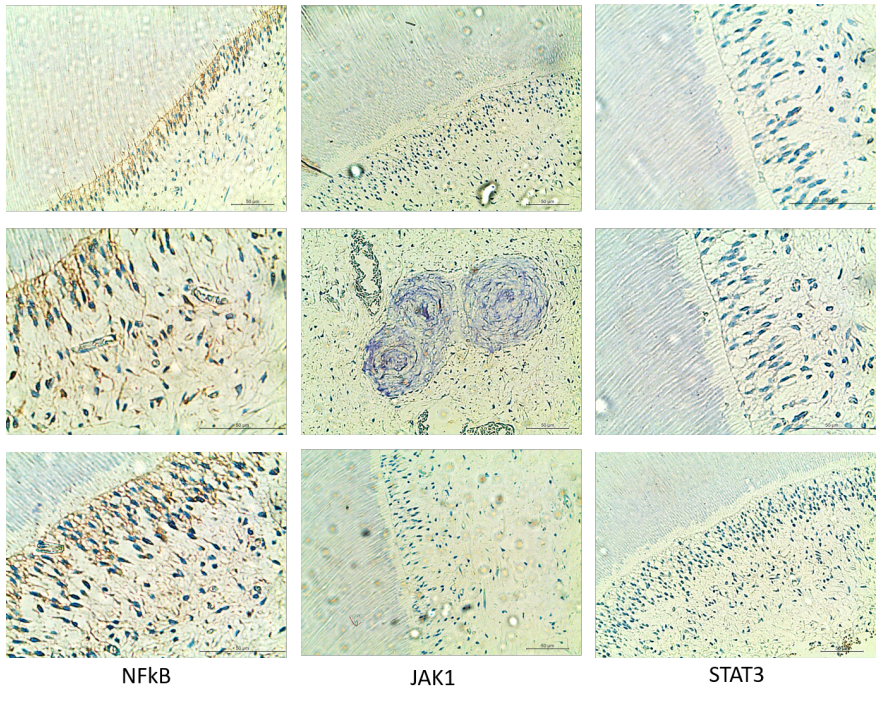

Fig. 3. Expression of NFκB, JAK1 and STAT3 from mature pulp odontoblasts in the proximal part of the pulp chamber of mature pulp tissue of a sample tooth 18 of a 20-year-old patient. Immunolabeling for NFκB was positive. Freely spaced denticle in the pulp chamber was visualized, which was immunonegative for all the markers studied. Odontoblastic cells did not express JAK1 and STAT3, objectified by the immunonegative finding.

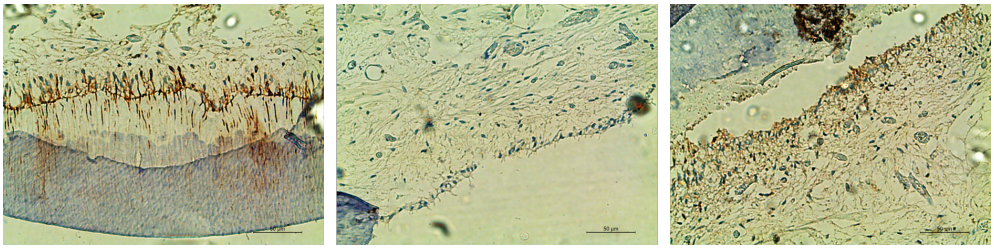

Fig. 4. Expression of NFκB, JAK1 and STAT3 from adult pulp odontoblasts of tooth 22 to 57 year-old patient. Immunopositive marking for NFκB and STAT3 and low-positive immune response for JAK1 were evident.

Gender-related changes

Analysis of results related to gender differences in the expression of biomarkers showed that the signaling route JAK1-STAT3 is significantly immunopositive in women ($p<0.05$). Explaining this result is not easy, perhaps the activity may be related to the receptor function that starts the transfer of the signal inside the cell. The receptors that activate JAK1-STAT3 are tyrosinekinasis-like type, a subgroup of membrane cell receptors associated with enzymes. Their ligands are various growth factors, cytokines, hormones. Their activity can be modulated to the concentration of ligand, negative stimulation is not excluded when there is reduced or absent growth factors, which is part of the mechanism for negative initiation of apoptosis program. A full understanding of the gender-dependant activity of JAK1-STAT3 signaling path remains unclear.

Changes according to the type of tooth (frontal/posterior, upper/lower)

In frontal teeth there was a statistically significant greater immunoreactivity for JAK1 and STAT3 ($p<0.05$). In sync with other objectives, the frontal teeth were representatives of the group of old pulp, confirming that cell ageing is the factor influencing the activity of the signaling path. The position of the tooth of the upper or lower jaw had no effect on the expression of NFκB, JAK1 and STAT3, which was confirmed in both primary and secondary data grouping.

Changes according to dental topography (crown/root)

In the analysis of the results for the expression of biomarkers according to dental topography, statistically significant immunonegative reactivity for STAT3 was revealed in the odontoblastic cells of the root pulp ($p<0.05$). This indicates different activity in terms of STAT3 positive odontoblasts in the crown and root of the tooth.

Discussion

The signaling route JAK-STAT plays a key role in the regulation of cell proliferation, differentiation, inflammatory response and apoptosis [5]. In the signaling pathway JAK1-STAT3, significantly active in the ageing of the dental pulp, a key role is played by the receptor apparatus. Odontoblasts express receptors from all families

for activation of signaling pathway JAK1-STAT3, but the tyrosine-kinaso-associated receptors in odontoblasts are of a particular importance [4]. Their ligands are various biologically active proteins including hormones. This may explain our findings that expression of JAK1-STAT3 is statistically significant immunopositive in women. The functions of NFkB are both pro-apoptic and anti-apoptic. Active NFkB in mouse cells leads to stimulation of their apoptosis and to proinflammatory cell status. [1, 2, 3]. Our results confirmed the link between NFkB expression in odontoblasts and ageing. The NFkB showed the strong regulatory effect in favor of cytoprotection and odontoblasts cells were defined as cells Type II with a potent inhibitory threshold.

Based on the limitations of this study, the high-differentiated long-lived pulp odontoblast cells also have a specific receptor function, intracellular information transfer and regulatory control of gene transcription in the nucleus. They have multilateral control over programmed cell death and use evolutionarily conservative regulation mechanisms. Clarifying the molecular map of odontoblasts would also reveal new strategies in a diagnostic and healing perspective.

Conclusion

In conclusion, the activity of signaling pathway JAK1-STAT3 in dental pulp in ageing presents a direct pathway of regulation of apoptosis, however NFkB maintains the inhibitory threshold.

References

1. **Rawlings, J. S., K. M. Rosler, D. A. Harrison.** The JAK/STAT signaling pathway. – *Journal of cell science*, 117, 2004, 1281-1283.
2. **Roskoski, Jr R.** Src protein-tyrosine kinase structure and regulation. – *Biochem. Biophys. Res. Commun.*, **324**, 2004, 1155-1164.
3. **Sahin, M., P. L. Greer, M. Z. Lin, H. Poucher, J. Eberhart, S. Schmidt, T. M. Wright, S. M. Shamah, S. O'Connell, C. W. Cowan, L. Hu.** Eph-dependent tyrosine phosphorylation of ephexin1 modulates growth cone collapse. – *Neuron*, **46**, 2005, 191-204.
4. **Schlessinger, J.** Cell signaling by receptor tyrosine kinases. – *Cell*, **103**, 2000, 211-225.
5. **Teng, Y., J. L. Ross, J. K. Cowell.** The involvement of JAK-STAT3 in cell motility, invasion, and metastasis. – *Jak-Stat*, **3**, 2014,e28086.
6. **Yu, H., H. Lee, A. Herrmann, R. Buettner, R. Jove.** Revisiting STAT3 signalling in cancer: new and unexpected biological functions. – *Nature reviews Cancer*, **14**, 2014, 736.
7. **Wullschleger, S., R. Loewith, M. N. Hall.** TOR signaling in growth and metabolism. – *Cell*, **124**, 2006, 471-484.