Dental Research Journal

Original Article

Angiogenesis in odontogenic keratocyst and dentigerous cyst: Evaluation of JunB and VEGF expression

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ABSTRACT

Background: Nowadays, different clinical behaviors of odontogenic cysts, little information about their biological agents, importance of diagnosis, and early diagnosis of these lesions have encouraged the researchers to conduct new studies. JunB acts as a regulator of vascular endothelial growth factor (VEGF) protein production and affects vessel proliferation and tissue angiogenesis. Hence, this study was conducted to compare angiogenesis through VEGF and JunB expression in odontogenic keratocysts (OKCs) and dentigerous cysts (DCs).

Materials and Methods: A total of 25 paraffin blocks of OKCs and 25 DCs were included in this experimental descriptive cross-sectional study, and immunohistochemical expression of VEGF and JunB was evaluated. Percentage and score of expression were recorded for each sample, and independent *t*-test, Mann–Whitney U, and Spearman statistical tests were run to analyze the data. The statistical significance level was set at <0.05.

Results: From 50 studied samples, 39.6% belonged to women and 60.4% belonged to men, with mean age of 34.2 ± 1.7 years. The mean percentages of JunB expression were 52.88 ± 17.35 and 74.6 ± 18.55 for DC and OKC samples, respectively. This expression was significantly higher in OKC than DC, and it had significantly higher scores as well (P = P = 0.0001 and 0.00033, respectively). The means of VEGF were 20.2% ±11.86 and 52.6% ±19.98 in DC and OKC samples, respectively. The mean VEGF expression was significantly higher in OKC than DC (P = 0.045), and it had significantly higher scores, too (P = 0.000). Furthermore, there was a significant correlation between VEGF and JunB expression in the studied samples (r_s = 0.3 and P = 0.005).

Conclusion: Based on the results of this study, it seems evaluation of angiogenesis through JunB expression can be helpful in the prediction of more aggressive behavior in pathologic lesions such as OKC.

Key Words: Angiogenesis, dentigerous cyst, JunB, odontogenic cysts, vascular endothelial growth factor

Received: July 2018
Accepted: June 2019

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INTRODUCTION

Dentigerous cyst (DC) is one of the most common developmental odontogenic cysts with good prognosis and little recurrence, which constitutes approximately 20% of oral cavity cysts. Odontogenic keratocyst (OKCs) has histopathological characteristics, specific

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Website: www.drj.ir
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www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Sadri D, Farhadi S, Nourmohamadi P. Angiogenesis in odontogenic keratocyst and dentigerous cyst: Evaluation of JunB and VEGF expression. Dent Res J 2019;16:327-32.

clinical practice and high recurrence tendency and aggressive behavior so that some resources categorize it as a part of odontogenic tumors and rename it keratocystic odontogenic tumor.[1-5] Due to the specific clinical behavior of this cyst, which affects the treatment selection, it is very important to identify and treat it timely.

The need for new studies seems necessary due to different clinical behaviors of odontogenic cysts, little information regarding their biological agents as well as the importance of diagnosis, early treatment for prevention or at least reduction of recrudescence possibility and malignity changes in them. In this regard, recent studies have demonstrated that blood vessel density of these lesions contributes to their biologic behaviors. Today, new treatments are strengthened by the reduction of blood vessel density of lesions.[6] The study of vessel density is performed by different methods of immunohistochemistry (IHC) staining and survey of indication of various vessel markers.

JunB is defined as a member of transcription factor family activator protein and a critical regulator and expression of inflammatory modulators in fibroblasts and T lymphocytes.[7,8] Quantitative analysis of PCR has shown that JunB regulates the multiple genes of tumor-related aggression and angiogenesis such as matrix metalloproteinase-2 (MMP2), MMP9, and chemokine (C-C motif) ligand 2 in 786-O cells. In addition, reduction of JunB prevents the tumor growth in xenograft tissue.[9] The other resources state that the lack of JunB expression in different types of cells with stabilized hypoxia-inducible factor leads to very weak vascular endothelial growth factor (VEGF) expression.

VEGF is a multifunctional cytokine that expresses in different situations and has a role in increasing vascular permeability and angiogenesis, thereby stimulating the proliferation and migration of endothelial cells.[10] This cytokine is produced by different kinds of cells and operates as a main regulator of physiologic and pathologic angiogenesis.[11,12]

JunB acts as a VEGF production protein regulator and influences vessel proliferation and tissue angiogenesis.[13-17] So far, JunB and effect on biologic agents have been studied in some pathologic lesions such as psoriasis,[18] melanoma, and lung carcinoma, which has provided different results regarding the relevance of JunB expression and more aggressive biologic agents in the above lesions.[19] To the best of our knowledge, this expression has not been studied in odontogenic cysts, yet. Therefore, this study was aimed to evaluate and compare VEGF and JunB expression and to investigate their correlation in OKC and DC.

MATERIALS AND METHODS

Paraffin blocks of 25 DCs and 25 OKCs registered at the Department of Oral and Maxillofacial Pathology, Islamic Azad University, Tehran branch, were included in this experimental, descriptive cross-sectional study. From the selected blocks, 4-μm sections were prepared for hematoxylin and eosin staining. An oral and maxillofacial pathologist reexamined the related slides. All samples had a definite histopathologic diagnosis, appropriate fixation, and sufficient tissue. Samples with hemorrhage, inflammation, and necrosis and those without sufficient information were eliminated from the study.

In this experimental study, Streptavidin-Biotin IHC method using JunB Antibody (C-11, Santa Cruze, CA, U. S. A) was applied based on the manufacturer's instructions to evaluate JunB expression and VEGF-VGI antibody (DAKO, Denmark).

Afterward, 4-μm sections were prepared for IHC staining. The mentioned sections were first immersed in xylene to remove the paraffin (dewax) and then in graded alcohols to dehydrate. To inhibit peroxide activity, the samples were placed in 3% hydrogen peroxide with phosphate buffer. The antigen retrieval process was performed in a microwave under the pressure of 2 atmospheres (atm) at 120°C for 10 min with exposure to the primary antibody for 30 min and then to secondary antibody (15 min), DAB (staining reaction), and Mayer's hematoxylin (background staining).

The sections of colon adenocarcinoma and normal lymph nodes were taken as a positive control for JunB expression,[20,21] and placental tissues were considered positive control for VEGF expression.[22]

After the preparation of procedure, the evaluation of tissue sections was performed by oral pathologist using optical microscope (Japan, Nikon-YS 100) through the examination of five fields of cyst wall with maximum effective staining (hot spots) at ×100 magnification. Then, nuclear and/or cytoplasmic stained cells with JunB were observed

at ×400 magnification, as follows: JunB expression in <5% of cells of cyst wall was considered (0), in 5%–45% of cells (1), in more than 45%–90% of cells (2), and in more than 90%–100% of cells (3).[19]

Evaluating the nuclear and/or cytoplasmic expression of VEGF was performed by the method proposed by Rubini *et al*.[22] In summary, the VEGF incidence by counting the stained cells included epithelial cells, fibroblasts, and endothelial cells in five microscopic fields. The mean percentage of positive cells in each sample was reported and categorized as follows:

- Score 0: 10% of cells or less showed VEGF staining
- Score 1: 10%–50% of cells showed VEGF staining
- Score 2: more than 50% of cells showed VEGF staining.

Figures 1 and 2 show the tissue sections of DCs and OKCs with JunB expression, and Figures 3 and 4 reveal the sections of these lesions with VEGF expression.

To compare the marker expression in two study groups, independent sample *t*-test was applied. Furthermore, Mann–Whitney U and Spearman tests were run to compare marker expression in two study groups and to evaluate the correlation of these two markers. The level of significance was considered <0.05.

RESULTS

A total of 50 samples, 39.6% women and 60.4% men with a mean age of 34.2 ± 1.7 years were evaluated in this study. The mean percentages of JunB expression were 52.88 ± 17.35 and 74.6 ± 18.55 for DC and OKC samples, respectively.

Furthermore, the description of JunB score is presented in Table 1.

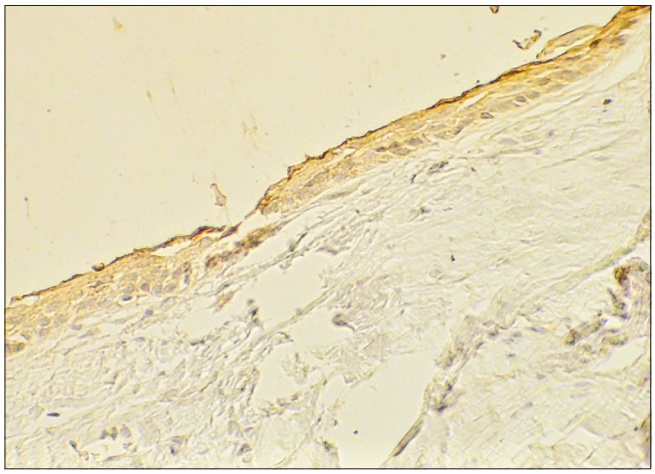

Figure 1: JunB expression in dentigerous cyst under, ×200.

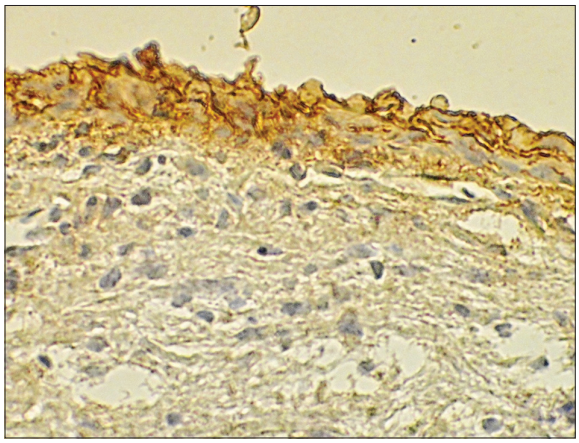

Figure 3: Vascular endothelial growth factor expression in dentigerous cyst under, ×400.

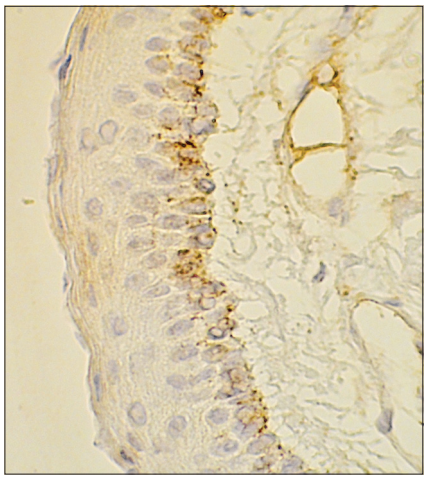

Figure 2: JunB expression in odontogenic keratocyst under, ×400.

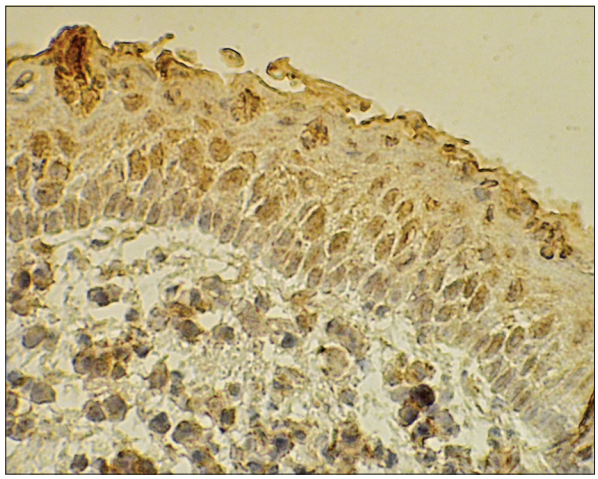

Figure 4: Vascular endothelial growth factor expression in odontogenic keratocyst under, ×400.

There is statistically significant difference in JunB expression percentage between DC and OKC ($P = 0.0001$). Moreover, the results of Mann–Whitney test showed a significant difference between the above groups in JunB score ($P = 0.0003$).

The means of VEGF were 20.2% ± 11.86% and 52.6% ± 19.98 in DC and OKC samples, respectively. The expression of VEGF in these two cysts was found to be significantly different, expression of VEGF being significantly higher in OKC than DC ($P = 0.045$). Table 2 presents the description of VEGF scores in the evaluated samples.

There was statistically significant difference between the two cysts in the terms of VEGF score ($P = 0.000$). That is OKC samples had significantly higher VEGF score than DC.

In addition, there was a significant correlation between VEGF and JunB expression in the studied samples ($r_s = 0.3$, $P = 0.005$).

DISCUSSION

There was a statistically significant difference between the two groups of DC and OKC from the viewpoint of JunB expression percentage and related scores.

Mao *et al*.[23] studied primary cutaneous T-cell lymphoma (PCL) and found a correlation between JunB expression pattern and progression of PCL. They suggested that JunB may be a critical factor involved in pathogenesis. Moreover, Schmidt D *et al*.[13] in their study on critical role of inspired JunB by NF-kappaB in VEGF regulation and tumor angiogenesis reported that JunB nonexpression caused disorder in VEGF expression. They finally concluded that tumor angiogenesis was disrupted in teratocarcinoma which did not reveal JunB. On the other hand, Kanno *et al*.[9] carried out a study on the role of JunB in cell invasion advancement and angiogenesis in renal cell carcinoma and concluded that JunB encouraged tumor invasion and increased angiogenesis. In a study on the significance of Jun transcription factors in ovarian cancer prognosis, Eckhoff *et al*.[24] reported that JunD and pc-Jun proteins were effective in carcinogenesis and tumor advancement, which suggested an important predictive role in ovarian cancer although there was no correlation between JunB expression and general survival and independent survival advancement. Moreover, JunB was not evidently associated with any clinicopathologic parameters. In a study on comparison of JunB expression profile, JunC, and S100A8 in psoriasis guttate, Park *et al*.[18] showed the reduction of JunB expression in both kinds of psoriasis compared with normal mucosa.

JunB as a member of Jun family and a biomodulator is identified in the expression of inflammatory intermediates.[7,8] PCR analysis has also shown that JunB regulates multiple genes related to tumor invasion and angiogenesis from metalloproteinase matrix family such as MMP2.9. JunB reduction significantly prevents tumor growth and angiogenesis as well.

JunB has been introduced as a cell proliferation marker, especially angiogenesis regulator, through influence on the inspiration of VEGF.[25,26] It has also been revealed that the lack of JunB in different types of cells leads to very weak VEGF expression and postponed cell growth.[10] Studies have reported JunB as an independent regulator of VEGF, and impact of angiogenesis has been considered very important in invasive growth of pathologic lesions such as malignant cells. On the other hand, significant results have been reported in other resources regarding vascular marker expression, for example, VEGF in more invasive pathologic lesions such as malignancies. Some studies have been conducted on pathologic lesions such as cysts, particularly odontogenic cysts, which have shown angiogenesis process and related agents seem necessary for the growth of pathologic lesions.[27-33] Based on the results of previous studies,[14-20] the possible impact of JunB expression

Table 1: Comparison of JunB expression score in odontogenic keratocysts and dentigerous cysts

Samples	JunB score			
	0 (%)	1 (%)	2 (%)	3 (%)
Lesion				
DC (*n*=25)	4	48	48	0.0
OKC (*n*=25)	0.0	8	80	12

DC: Dentigerous cysts; OKC: Odontogenic keratocysts

Table 2: Comparison of vascular density base on vascular endothelial growth factor score in odontogenic keratocysts and dentigerous cysts

Samples	VEGF score		
	0 (%)	1 (%)	2 (%)
Lesion			
DC (*n*=25)	41.7	58.3	0.0
OKC (*n*=25)	0.0	27.3	72.7

DC: Dentigerous cysts; OKC: Odontogenic keratocysts; VEGF: Vascular endothelial growth factor

on the invasive behavior of pathologic lesions through its influence on VEGF can be evaluated.

Moreover, JunB expression has been found to significantly increase in more advanced processes and more invasive pathologic lesions. To the best of our knowledge, there is no research regarding the evaluation of JunB expression in cysts, particularly odontogenic cysts. Hence, this study is the first one that found a significance difference between OKC and DC cysts in JunB expression.

Regarding the more invasive behavior of OKC than DC and considering JunB effect mechanism through VEGF, it seems that the evaluation of JunB expression can help predict more invasive behaviors of pathologic lesions such as OKC. However, more studies are needed to assess other effective agents involved.

CONCLUSION

Considering the results of this study indicating that JunB expression was significantly higher in OKC than DC, it seems that the evaluation of angiogenesis through JunB expression can probably help predict more invasive behaviors of pathologic lesions such as OKC.

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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