Immunohistochemical analysis of Nuclear Factor-kappa B (NF-κB) between follicular and plexiform ameloblastomas: A pilot study

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Abstract

Background: Ameloblastoma among benign tumors holds a unique position by its locally destructive and invasive nature. Tumors that originate from the odontogenic apparatus or its remnants in the jaws show diverse clinical presentations, behavior and histologic patterns. The differed biological behavior behind follicular and plexiform ameloblastomas has never attained completeness because of the lack of rhythmic correlation regarding the exact mechanism. Nuclear factor-kappa B (NF-κB) pathways play a crucial role in survival, death and differentiation during physiologic and pathologic conditions. With this background, the study has been aimed to investigate the expression of NF-κB in follicular and plexiform ameloblastomas.

Objective: The objective of this study was to analyze the immunohistochemical expression pattern of NF-κB in ameloblastoma and to compare the immunohistochemical expression pattern of NF-κB among the histological types of ameloblastoma, follicular and plexiform patterns.

Methodology: Total 20 ameloblastomas (10 follicular, 10 plexiform) were immunostained with antihuman NF-κB p65 mouse IgG monoclonal antibody, and the pattern of staining is statistically analyzed using Chi-square test with the level of significance (P < 0.05).

Results: Twelve (3 follicular, 9 plexiform) out of 20 ameloblastomas showed immunoreactivity to NF-κB p65. In ameloblastoma, only the peripheral preameloblast-like tall columnar cells showed reactivity, whereas the stellate reticulum-like cells are immunonegative. The staining pattern was membranous in the immunoreactive cells. The results were studied with the associated and inducing pathways from the literature, and a possible mechanism has been proposed.

Conclusion: The expression pattern of NF-κB was found to be higher in plexiform ameloblastoma than follicular ameloblastoma.

Keywords: Ameloblastoma, nuclear factor-kappa B, odontogenic tumors

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INTRODUCTION

Ameloblastoma is an aggressive benign epithelial odontogenic tumor; therefore, it is found exclusively within the maxillofacial skeleton or overlying tooth-bearing areas or alveolar mucosa in the edentulous areas. Despite being a benign tumor, it is locally aggressive and has a high recurrence rate. If left untreated, they often lead to extensive tissue destruction and deformity. This tumor was found even from the prehistoric era through the archaeologically obtained human skeletons. In recent years, ameloblastoma has undergone constant modifications in terminology and classification with the introduction of prospective views based on updates on current genetic studies. The proliferating activities of ameloblastoma cells vary depending on the histological type and cytological pattern. The key feature regarding this tumor is that both apoptosis and the proliferating activity of the cell are implicated in the development of ameloblastoma. The biology of tumors demonstrates relatively two distinct patterns: (1) anti-apoptotic proliferating site in the outer layer (periphery) and (2) proapoptotic differentiating site in the inner layer (center) of the tumor component. The peripheral cells of the tumor which have chosen the cell survival mode are considered as the reason behind the progression of the disease leading to expansile jaw lesion. Survivin, bcl-2 and bcl-X were observed more predominantly in the outer layer cells of ameloblastoma, but pro-apoptotic molecules such as Fas, FasL and caspase-3 are expressed in central stellate reticulum-like cells. Both clinical and histological subtypes of ameloblastoma have different biological behaviors depending on the growth pattern.

Nuclear factor-kappa B (NF-κB) was first discovered as a transcription factor in B-cells that binds to the 11-base pair sequence enhancer element controlling immunoglobulin kappa light chain expression. It is involved in numerous signaling events [Figure 1], and it is very difficult to see a physiologic or pathologic occasion without its contribution. The transcription factor NF-κB plays a vital role in regulating various mechanisms through control over the expression of various genes which are involved in immune response, oncogenesis, differentiation, proliferation, apoptosis and angiogenesis. The role of NF-κB has been studied in many tumors such as colitis-associated cancer, hepatocellular carcinoma, oral squamous cell carcinoma and in many lymphoid malignancies and shown to have an association in tumorigenesis. Previous studies have been conducted to explore the expression pattern and anti-apoptotic role of NF-κB in ameloblastoma. However, there are no studies that have attempted to find the expression of NF-κB among follicular and plexiform ameloblastomas. With this background, the immunohistochemical study is aimed to analyze the immunohistochemical expression pattern NF-κB in ameloblastoma and to compare the immunohistochemical expression pattern of NF-κB among follicular and plexiform types of ameloblastoma.

METHODOLOGY

Study samples consisting of 20 paraffin-embedded formalin-fixed tissue blocks obtained from the archives of the department comprised 10 follicular and 10 plexiform types of ameloblastoma. All the samples selected for the study were from archives. The study details have been presented in the institutional ethics committee (IEC), and ethical clearance certificate was obtained (IEC no. VDCW/IEC/148/2018).

All the 20 cases were subjected to immunohistochemistry to evaluate the expression of NF-κB. The prepared sections were covered completely with optimally diluted mouse monoclonal anti NF-κB p65 (sc-8008, Santa Cruz Biotechnology, USA) in 1:50 dilutions in phosphate buffered saline for 1½ hours. Then, the sections were washed and treated with secondary antibody tagged with poly horseradish peroxidase enzyme (Dako REAL EnVision, Denmark) for 30 min. The slides were then treated with freshly prepared 3,3′ diaminobenzidine tetrahydrochloride solution for 5 min following which it was washed and treated with Mayer’s hematoxylin, dehydrated in graded alcohol (50%, 70%, 90% and 100%), cleared with xylene and mounted using dibutyl phthalate xylene, a nonaqueous permanent mounting medium.

Interpretation of staining

The immunostained slides were observed for positivity under ×10/×40 magnifications and recorded with a high-quality photomicrograph. The positive reaction was indicated by brown precipitate in both cytoplasm and nucleus of the peripheral and central cells of ameloblastoma. All areas in each section were examined and analyzed, the intensity which was predominant in these fields was taken into consideration. The staining was scored by evaluating the positive and negative immunoreactivity of each slide. The scores for positive immunoreactivity were given as 2 and for negative immunoreactivity as 1.
Figure 1: Nuclear factor-kappa B (NF-kappa B) is the generic name of a family of transcription factors that function as dimers and regulate genes involved in immunity, inflammation and cell survival. There are several pathways leading to NF-kappa B-activation. The canonical pathway is induced by tumour necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) or byproducts of bacterial and viral infections. This pathway relies on IKK-mediated IkappaB-alpha phosphorylation on Ser32 and 36, leading to its degradation, which allows the p50/p65 NF-kappa B dimer to enter the nucleus and activate gene transcription. Atypical pathways are IKK-independent and rely on phosphorylation of IkappaB-alpha on Tyr42 or on Ser residues in IkappaB-alpha PEST domain. The non-canonical pathway is triggered by particular members of the TNFR superfamily, such as lymphotoxin-beta (LT-beta) or BAFF. It involves NIK and IKK-alpha-mediated p100 phosphorylation and processing to p52, resulting in nuclear translocation of p52/RelB heterodimers. [Copyright permission for KEGG (Kyoto Encyclopedia of Genes and Genomes) - map04064 NF-kB signalling pathway obtained from Kanehisa Laboratories, Japan. Ref.No-200501]

Statistical analysis
The Statistical Package for the Social Sciences (SPSS) software version 16.0 (IBM Corp, Chicago, IL, USA) was used. The level of significance ($P < 0.05$) was employed in all statistical comparisons. Quantitative data were recorded as mean ± standard deviation. The expressions of anti-NF-kB p65 between follicular and plexiform types of ameloblastoma were analyzed statistically using Chi-square test.

RESULTS
Among 20 cases of central ameloblastoma, 10 cases were follicular types and 10 cases were plexiform types. The positivity was predominantly observed in the peripheral tall columnar ameloblast-like cells, and the central stellate reticulum-like cells showed scattered positivity in a very few cases [Figures 2 and 3]. Among 10 cases of central follicular ameloblastoma, 3 cases (30%) showed positive immunoreactivity and the remaining
cases were negative (70%). Among 10 central plexiform cases, 9 cases (90%) showed positive immunoreactivity. The expression of NF-κB was compared between central follicular ameloblastoma and central plexiform ameloblastoma statistically using Chi-square test. The results were statistically significant between these two groups [Table 1].

**DISCUSSION**

Among the odontogenic neoplasms occurring in the jaws, ameloblastoma is unique because of its locally invasive behavior and high recurrence rate. Considerable molecular signaling variation exists among different clinical and histological patterns of ameloblastoma for which researches trying to identify the possible regulatory mechanism. Such investigations on tumor biology of ameloblastoma can lead to new non-surgical/minimal invasive therapeutic avenue.

The biological behavior (proliferation, apoptosis, matrix degradation and invasiveness) of these tumors varies within its histological types. Both apoptosis and the proliferating activity of the cells are implicated in the development of ameloblastoma. The differed biological behavior behind follicular and plexiform ameloblastomas has never attained completeness because of the lack of rhythmic correlation regarding the exact mechanism.

Ameloblastoma, irrespective of its clinical and histological variants, has two relatively distinct patterns: peripheral ameloblast like cells demonstrating anti apoptotic/proliferative pattern and central stellate reticulum like demonstrating pro-apoptotic and differentiation pattern. The expression of the molecular signals such as bcl 2 and bel X,[5] proliferating cell nuclear antigen (PCNA) and Ki 67,[17] telomerase activity and telomerase reverse transcriptase (TERT),[18] survivin,[19] Ras/mitogen activated protein kinase (MAPK),[20] Akt, phosphatidylinositol 3 kinase (PI3K) and Phosphatase and tensin homolog deleted on chromosome 10 (PTEN),[21] tumor necrosis factor (TNF) α,[22] NF-κB,[16] cycin D1,[23] FGFR2–RAS–BRAF,[24] cellular Myelocytomatosis (c- Myc),[25] and focal adhesion kinase (FAK)[26] shows the anti apoptotic/survival or proliferation behavior of the peripheral cells of ameloblastoma. The proapoptotic or cell death in the central stellate reticulum-like cells was activated through caspase-3, cytochrome c, apoptotic peptidase-activating factor 1, caspase-9, apoptosis-inducing factor and BAX.[5,27]

NF-κB is a double-edged sword. It induces both cell survival and apoptosis through the different regulatory mechanisms. NF-κB activation and its role in the survival of cancer cells are well documented in the literature. NF-κB targets many genes that facilitate inflammation (TNF, interleukin [IL]-1 and chemokines), cellular immortality (telomerase), cell survival (bcl-XL, cIAP, XIAP and cFLIP), angiogenesis (vascular endothelial growth factor, TNF, IL-1 and IL-8), proliferation (TNF, IL-1, IL-6,
cyclin D1 and c-Myc), tumor promotion (COX-2, iNOS, MMP-9 and uPA) and metastasis (ICAM-1, VCAM-1 and ELAM-1).\[28\] NF-κB regulates the downstream molecules and influences cell survival of tumor cells.

The expression of NF-κB has been reported more predominantly in the outer layer than the inner layer of ameloblastoma. However, the expression pattern among follicular and plexiform ameloblastomas has not been characterized.\[16\] In the present study, positive immunoexpression of anti-NF-κB p65 was found in 60% of the ameloblastoma cases and immunoeexpression was found in peripheral ameloblast-like cells. The immunoexpression was restricted to the cell membrane of cuboidal or columnar type of peripheral cells of the tumor islands and cords. None of the central (stellate like) cells or stromal components stained positive for NF-κB p65. There was a significant difference in the immunoexpression of anti-NF-κB p65 in plexiform ameloblastoma (90%) compared to that of follicular ameloblastoma (30%).

Neurotrophins (nerve growth factor [NGF], brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5) and neurotrophin receptors (TrK A + B + C and p75-NTR) are a class of growth factors and play a major role in numerous biological functions such as cell survival, proliferation, regulation, differentiation, apoptosis and migration not only on neuronal cell growth but also correspondingly on a wide range of nonneuronal differentiating cells.\[29\] Their role in physiologic and pathologic conditions via Ras/MAPK pathway, PI3K pathway, Phospholipase C Gamma (PLCγ) pathways, NF-κB signaling pathway and c Jun N terminal kinase signaling pathway has been established through early researches.\[30\] Positive anti-p75-NTR immunoexpression was found in the peripheral cells of follicular ameloblastoma (83.3%) comparing to that of plexiform ameloblastoma cases (10%).\[31\] The upregulation of p75-NTR could suppress NF-κB through IκB kinase.\[32\] In another study, the immunoexpression of anti-TrK A + B + C was found positive in peripheral ameloblastoma-like cells of plexiform ameloblastoma (70%) comparing to that of follicular ameloblastoma (4%) and the expression restricted to the cell membrane of cuboidal or columnar type of peripheral cells of the tumor islands and cords. This revealed a significant difference in the expression of anti-TrK A + B + C between follicular ameloblastoma and plexiform ameloblastoma.\[33\] The immunoexpression of NGF was studied between follicular and plexiform ameloblastomas. All the samples showed immunopositivity, but the intensity was strong in plexiform variant compared to follicular variant.\[34\] NGF ligand binding to p75NTR signals both pro survival and pro apoptotic effects and these functions are modulated by TrK.\[35\] In the present study, differed immunoexpression of NF-κB was possibly due to the binding of neurotrophins [NGF] with p75NTR/TrK in peripheral ameloblast-like cells of follicular and plexiform ameloblastomas.\[36\]

NF-κB activation affects hallmarks of the tumor through the transcription of genes involved in cell proliferation, survival, angiogenesis, inflammation and tumor promotion and metastasis.\[36\] There is a lack in the rhythmic correlation of the pathogenesis of ameloblastoma which causes hindrance to the nonsurgical treatment.\[37\] These findings suggest that the expression of neurotrophin receptors (p75NTR and TrK A + B + C) and neurotrophins like NGF in the peripheral ameloblast-like cells which were established through the previous study could be a possible reason behind the altered regulation of NF-κB in follicular and plexiform ameloblastomas.

CONCLUSION

By analyzing the course of literature, the expression of cell proliferating proteins (Ki-67 and PCNA), cell survival regulators (TERT and c-Myc), cell cycle regulators (cyclins), anti-apoptotic proteins (bcl-2 and bcl-X), cell proliferation enzymes (PCNA) and tumor progression molecules (MMP-9 and TIMP-1) was controlled through NF-κB signaling mechanism. The variation in the expression of NF-κB among follicular and plexiform ameloblastomas could be one of the possible reasons behind the differed biological behavior. Further studies on this pathway will lead us toward the targeted therapy with minimal surgical intervention for the betterment of the diseased.

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Conflicts of interest
There are no conflicts of interest.

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